Characterization of native fruits of the amazon region and development of an amperometric biosensor for determination of antioxidant capacity

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Caractérisation des fruits autochtones de la région amazonie et developpement d'un biocapteur ampérométrique pour la détermination de la capacité antioxydant

UNIVERSITY OF PERPIGNAN VIA DOMITIA - UVPD
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CHARACTERIZATION OF NATIVE FRUITS OF THE AMAZON REGION AND DEVELOPMENT OF AN AMPEROMETRIC BIOSENSOR FOR DETERMINATION OF ANTIOXIDANT CAPACITY

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BRAZIL-FRANCE

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Thesis presented to the Doctorate Course of the UFMA in cotutelle agreement with the UVPD, as a requirement for obtaining the Ph.D Degree.

These advisors:
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2015-2019 SCIENTIFIC PRODUCTION (Annex B)

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Deposited Patent:

NUNES, G. S.; **BECKER, M. M.**; MORAES, J. F.; MANDAJI, C. M.; SOUTO, L. A. S. Biossensor amperométrico para determinação da capacidade antioxidante, 2018. Instituição onde foi depositada: INPI - Instituto Nacional da Propriedade Industrial. País: Brasil. Natureza: Patente de Invenção. Número do registro: BR10201807511. Data de depósito: 04/12/2018. Depositante/Titular: Universidade Federal do Maranhão.

ABSTRACT

Fruit consumption has been encouraged not only by providing essential nutrients to the human body, but by being natural sources in bioactive compounds that promote health benefits and reduce the risk of developing chronic non-communicable diseases. Considering that the Brazilian Amazon has an immense fruitful diversity, but little information on its nutritional, antioxidant and bioactive properties, this work had the objective to characterize ten native fruits of the Amazon region. The characterization was initially done by the conventional bromatological analyzes, followed by the determination of the minerals Ca, Cu, Fe, Li, Mg, Mn, Na and Zn by Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES). Afterward the antioxidant capacity of the fruits was evaluated using different colorimetric methods (DPPH, ABTS, and NBT), the evaluation of the antiproliferative activity against the human colon cancer cell line (caco-2). The determination of bioactive compounds (vitamin C and total phenolic compounds) and the characterization of the profile in phenolic compounds was performed using Ultra High-Performance Liquid Chromatography (UHPLC) coupled to high resolution mass spectrometry. Considering the disadvantages of conventional methods for the determination of antioxidant capacity, this study aimed to develop an amperometric biosensor using a conventional system of three screen printed electrodes on PVC, containing Prussian Blue (PB) as electrochemical mediator. In view of this, the conditions of biofunctionalization of the xanthine oxidase enzyme (XOD) with respect to the XOD: PVA-AWP ratio, the irradiation time under the neon light and the amount of XOD used in each sensor were optimized. Afterwards, the analytical performance of the biosensor was evaluated, and the antioxidant capacity of a pure standard substance and fruit extracts was finally detected. The results of the bromatological characterization evidenced higher lipid, protein and energy contents in buriti, monguba and uxi samples. The samples of abiu, açaí, bacuri, buriti, inajá, monguba, pajurá and uxi were shown with high content and/or source in one or more minerals. In the pulps of biribá and bacuri, a high lipophilic antioxidant capacity was observed against different free radicals, whereas the fruits of abiu, inajá and monguba presented higher hydrophilic antioxidant capacity. The antiproliferative activity of the fruits of biribá, inajá, monguba and pajurá led to a significant inhibition in the cell growth of caco-2. The majority of the pulps were food of high vitamin C content, while the fruits biribá and pajurá were classified as sources. Higher concentrations of hydrophobic phenolic compounds were observed in the bacuri pulp, whereas in the abiu, bacuri, inajá and monguba samples, higher hydrophilic phenolic contents were verified. Analyzing the chromatographic composition of the extracts of biribá, inajá and monguba, it was possible to quantify a total of 11, 25 and 21 phenolic compounds, respectively, with important biological activities. The results provide information that may contribute to better understanding the Amazon biodiversity as well as the development of biotechnology for the conservation of the Amazon biome. The developed biosensor was characterized with the following optimal experimental conditions XOD:PVA-AWP of 1: 2; time of exposure to the neon light of 30 min, and enzymatic loading of 8 mU per electrode. The biosensor was stable, with fast responses, easy automation, relatively low cost, high sensitivity, low detection and quantification limits. The applicability of the biosensor was demonstrated by the in vitro analysis of gallic acid, taken as standard antioxidant, as well as of Amazonian and non-autochthonous fruits.

Keywords: Amazonian fruits, mineral composition, antioxidant capacity, bioactive compounds, amperometric biosensor.

SUMMARY

1. INTRODUCTION

The Brazilian Amazon Region is formed by a complex mosaic of endemic areas with a rich diversity of fruits species which are distributed in accordance with their biota specificities (SILVA; RYLANDS; FONSECA, 2005). It has great bioavailability of native species of the Brazilian flora with about 220 species of edible fruits plants cataloged, presenting 44% of the diversity in native fruits in Brazil (NEVES *et al.*, 2012). Although of the recognized biodiversity, Amazon fruits production contributes little to the protagonism of Brazil as the third largest fruits producer in the world (BRAZILIAN FRUIT YEARBOOK, 2015) the social and economic reality and the precariousness of health and nutrition registered in the region contrast sharply with their richness in biological resources (AGUIAR, 2006a).

The fruits comprise nutritionally important components for the human diet and have in recent years received increased attention due to epidemiological evidence regarding regular consumption of vegetables which reduces mortality and morbidity due to some chronic diseases (ALISSA; FERNS, 2012; RUFINO *et al.*, 2010). Its protective effect has been attributed to the presence of constituents like minerals and high levels of phytochemicals with antioxidant properties (KOZLOWSKA; SZOTASK-WEGIEREK, 2014; WANG *et al.*, 2013; LIU, 2013; KAHL *et al.*, 2012; NUNES *et al.*, 2011).

Antioxidants are synthetic or natural compounds capable of preventing or retarding the oxidative damage caused by oxidizing sources, especially reactive oxygen species (ROS), which can oxidize cellular biomolecules such as proteins, membranes and DNA and consequently lead to development and progression of various pathologies, such as cancer, atherosclerosis, Parkinson's, Alzheimer's and other serious diseases (RAHMAN *et al.*, 2012; HALLIWELL, 2012; FLORA, 2009).

In most cases, antioxidants derived from natural products present stability problems, due to the presence of unsaturation in their chemical structure, which make them sensitive to exposure to heat, light and the presence of oxygen (RODRIGUEZ-AMAYA, 2001). In this context, there is a need for analytical methods to perform the measurement of the antioxidant capacity at the fruit collection site, in order to minimize the losses of compounds.

Spectrophotometric, electrochemical and chromatographic methods have been used to measure the antioxidant capacity, differing in the mechanism of generation of

oxidant species and/or target molecules and in the way the final products are measured (PISOSCHI; NEGULESCO, 2011). However, in recent years the use of electrochemical biosensors has become promising tools to alternate the existing techniques in determination of the antioxidant capacity due to certain characteristics such as selectivity, low cost, ease of storage, miniaturization capacity, easy automation and portability, which combined enable *in situ* analysis (LATES; MARTY; POPESCU, 2011; PEREIRA; SANTAS; KUBOTA, 2002).

Considering the importance of the fruits for the Amazon region and the potential of some relatively less explored native species, this work has as initial objectives to characterize chemically and bromatologically 10 genuinely Amazon fruits, as well as to determine their antioxidant capacity and cellular antitumor viability. In view of the inherent limitations of conventional analytical methods, this work aimed to develop a new analytical tool based on an amperometric biosensor to determine the antioxidant capacity of real samples.

2. LITERATURE REVIEW

This chapter will provide information about the fruits studied, morphological aspects, applications in popular medicine and main scientific investigations on biological properties. In addition, a nutritional approach will be presented in relation to the presence of bioactive compounds as well as the analytical techniques used to determine them. A special focus will be given to the discussion on amperometric biosensors for the determination of antioxidant capacity in plant samples.

2.1 Amazon Fruit

The Amazon Basin has the largest tropical forest in the world, comprising approximately 611 million hectares which are distributed among nine countries in South America: Brazil, Bolivia, Peru, Ecuador, Colombia, Venezuela, Guyana, Suriname, and French Guiana. Of these approximately 370 million hectares comprise forest areas where 60% are located in Brazilian territory (RIOS; PASTORE JUNIOR, 2011).

The data on biodiversity is quite expressive. The region is estimated to house one-third of the earth's genetic stock, with more than 60,000 plant species (IBAMA, 2016).

The Amazon Forest presents several native plant species with food and pharmacological potential that are still unknown and may be threatened with extinction by the destruction of the forest, which has selected and adapted these plants for years to the climate and soil conditions of the region. (HIGUCHI; HIGUCHI, 2004).

Fruits are one of the richest nutrient's sources and antioxidant supplements. Epidemiological studies confirm that their consumption is associated with a lower incidence of certain cancers, as well as beneficial effects on cardiovascular diseases, diabetes, obesity and cataracts (SINDHI *et al.*, 2013; HAMID *et al.*, 2010; STEINMETZ; POTTER, 1996). The mechanisms of action of the nutrients and bioactive compounds present in these foods, such as minerals, vitamins and antioxidant compounds support this valorization (SANTOS; LIMA, 2008).

Chemical and pharmacological studies, *in vitro* and *in vivo*, have been reported in the literature in order to better understand the nutraceutical potential and the antioxidant benefits of native fruit from the Amazon region.

Figure 1 shows the ten Amazon fruits that were included in this work and their respective common and scientific names, and Table 1provide information on the works that evaluated the biological activities of different extracts and portions of the fruits contemplated in this study.

Among the fruits, some species have flavors widely appreciated by Brazilian and foreign consumers, while others such as abiu, bacuri, biribá, inajá, monguba, pajurá, and uxi are consumed especially by the local population and indigenous people, and little information is available regarding chemical constituents, mainly in relation to mineral elements, antioxidant capacity, bioactive compounds and antiproliferative potential in cancer cells.

Figure 1 - Amazon fruits studied in the present research.

Table 1 - Chemical and biological activities of the studied fruits

Fruit	Tissue	Chemical/biological activities	Reference
Abiu	Pulp	Antioxidant capacity	CANUTO *et al.*, 2010
	Pulp		GONÇALVES; LAJOLO; GENOVESE, 2010
	Leaf		CASTRO *et al.*, 2006
	Leaf	Antimalarial Activity	PÉREZ, 2002
Açaí	Peel and pulp	Antioxidant capacity	NEVES *et al.*, 2012
	Pulp		CANUTO *et al.*, 2010
	Pulp		RUFINO *et al.*, 2010
	Pulp	Cytotoxicity on HT-29 colon cancer cells	PACHECO-PALENCIA *et al.*, 2008
	Pulp	Antiproliferative activity in brain glioma cells	HOGAN *et al.*, 2010
	Seed	Vasodilator effect	ROCHA *et al.*, 2007
	Seed	Antihypertensive Potential	COSTA *et al.*, 2012
	Pulp		GALE *et al.*, 2014
	Pulp	Anti-inflammatory activity	SHAUSS *et al.*, 2006
	Pulp	Attenuation of the development of chemical carcinogenesis of colon	FRAGOSO *et al.*, 2013

Continued Table 1 ...

Bacuri	Pulp	Antioxidant capacity	CANUTO *et al.*, 2010
	Pulp		RUFINO *et al.*, 2010
	Pulp		GONÇALVES; LAJOLO; GENOVESE, 2010
	Seed	Leishmanicidal and genotoxic activity in lung fibroblast cells	COSTA JÚNIOR, *et al.*, 2013
	Seed	Antiepileptic action	COSTA JÚNIOR *et al.*, 2011
	Seed	Healing	SANTOS JUNIOR *et al.*, 2010
Biribá	Peel	Antioxidant capacity	YAMAGUCHI, 2015
	Pulp		BARREIROS; BARREIROS, 2011
	Seed	Insecticidal properties	MASSAROLLI; PEREIRA; FOERSTER, 2016
	Leaf and seed	Antileishmania activity	LIMA *et al.*, 2012
Buriti	Pulp	Antioxidant capacity	KOOLEN *et al.*, 2013
	Pulp		CANUTO *et al.*, 2010
	Pulp		GONÇALVES; LAJOLO; GENOVESE, 2010
	Peel and pulp	Antibacterial activity	OLIVEIRA, 2017
	Pulp	Photoprotector against UVA and UVB radiation	ZANATTA *et al.*, 2010
	Peel	Antithrombotic action	FUENTES *et al.*, 2013
	Pulp	Antimicrobian activity	KOOLEN *et al.*, 2013
	Leaf	Antitumor effects	SIQUEIRA *et al.*, 2014
Cupuaçu	Pulp	Antioxidant capacity	CANUTO *et al.*, 2010
	Seed		YANG *et al.*, 2003
	Pulp		GONÇALVES; LAJOLO; GENOVESE, 2010
	Seed	Antiproliferative activity in colon cancer cell line HCT-116 and SW-480	YANG *et al.*, 2003
Inajá	Peel and pulp	Antioxidant capacity	NEVES *et al.*, 2012
	Pulp	Acetylcholinesterase inhibitory action	FERNÁNDEZ *et al.*, 2016
Monguba	Seed	Antioxidant capacity	BARBOSA, 2016
	Peel	Hypoglycemic effect	CETTO; HEINRICH, 2005
	Stems	Potencial anti-inflammatory	CHENG *et al.*, 2017
Pajurá	Peel, pulp and seed	Antioxidant capacity	BERTO *et al.*, 2015b
	Peel, pulp and seed		SOUZA, 2016
	Pulp		MASSING *et al.*, 2018
Uxi	Peel and pulp	Antioxidant capacity	NEVES *et al.*, 2012
	Bark		SILVA; TEIXEIRA, 2015
	Pulp		GONÇALVES; LAJOLO; GENOVESE, 2010
	Bark	Antibacterial action Inhibitory effect of cholinesterases Antidiabetic potential	SILVA; TEIXEIRA, 2015
	Pulp	Potential for the treatment of diabetes	GONÇALVES; LAJOLO; GENOVESE, 2010

The abiu (*Pouteria caimito*) is a berry that has a smooth peel of 3 to 5 mm thick and contains a latex that is depreciated by the consumer. When ripe, the fruit is bright yellow, ellipsoidal to spherical in shape, 6 to 10 cm in diameter and 100 to 600 g in mass. The mesocarp is soft, gelatinous, with 1 to 5 dark brown seeds. The abiu is consumed *in natura*, especially by the local population, and has aroused the interest of the world fruit growing for being promising in the form of juices, yogurts, fruit salads, jellies and ice creams (YUYAMA *et al.*, 2013; LIM, 2006; FALCÃO; CLEMENT, 1999). In traditional medicine, all parts of the abiuzeiro are used in the treatment of diseases with astringent, emollient, hypoglycemic, fungicidal and anti-inflammatory effects (RIOS; PASTORE JUNIOR, 2011; OLIVEIRA *et al.*, 2003). Biological activities of the abiu pulp, conventionally edible portion, are still rare in the scientific literature, being available only for the the extract of the leaves (Table 1).

The açaí (*Euterpe oleracea* Mart.) is a globular or slightly globular drupe, with the diameter varying from 1 to 2 cm and weighing on average, 1.5 g. The mesocarp, about 1 mm thick is pulpy and surrounds a bulky and hard endocarp. The pulp of the fruit, after processing, is consumed pure or accompanied tapioca, fish or shrimp, is also used in the production of juices, ice cream, jellies and açaí wine (BRASIL, 2015). According to the addition of water in the pulping process, the açaí pulp can be classified as special açaí or type A açaí when it has a total solids content of more than 14%, type B (11 to 14%) and type C (8 to 11%) (BRASIL, 1999). Pulp consumption is booming in Brazil and in the international market. It is classified as a superfruit because of its functional and nutritional capacity due to the high energy content of fibers, proteins, vitamin E, minerals and fatty acids essential (SOUZA *et al.*, 2011; RIOS; PASTORE JUNIOR, 2011). Studies have revealed the potential of the inclusion of açaí in the diet as a functional food due to its antioxidant capacity coupled with nutritional and therapeutic benefits, including antiproliferative, anti-inflammatory and antihypertensive effects (Table 1).

The fruit of bacuri (*Platonia insignis*) have a variable shape with 4 to 5 cm in diameter and 5 to 6 cm in length. Inside of the hard shell has a white, aromatic and creamy pulp which contains 3 to 4 large seeds. Most of the Brazilian bacuri crop is consumed fresh, being marketed in the cultivated region or nearby, where there is still limited industrial processing for the production of ice cream, juices, and jellies (MANICA, 2000). The pulp, peel, and resin are used in traditional medicine as digestive, diuretic, antiscorbutic and cicatrization (RIOS; PASTORE JUNIOR, 2011; REVILLA, 2002),

while seed oil has been used for the treatment of diarrhea, earaches, spider bites and snakes, rheumatism, arthritis and as cicatrizing (AGRA; FREITAS; BARBOSA-FILHO, 2007). An increase in reports regarding the chemical, biological and pharmacological properties of bacuri seed can be observed in the recent literature (Table 1). However, pulp-related activities are still insufficient.

The biribá (*Rollinia ortophetala* heterotypic synonym of *Annona mucosa*) is a spherical large fruit, measuring 5 to 15 cm wide, with slight or very prominent protuberances. When completely ripe, it has a yellowish-green color. The flesh is whitish, juicy, aromatic and tasty, with a creamy texture and a delicious sweet and sour balance. Its consumption is appreciated, especially *in natura*, by the Amazonian population, as well as in the form of juices and wines after fermentation (COSTA; MULLER, 1995). The popular therapeutic use of leaves has been recorded in treatments for the cure of tumors and rheumatism and as an anti-inflammatory in arthritis. While the fruit is used to aid blood clotting, such as analeptics and antiscorbutic (RIOS; PASTORE JUNIOR, 2011; AQUINO, 2008; AGUIAR, 2006b). Little data is available in the literature on the bioactive compounds and the biological activities of the biribá pulp.

The fruits of buriti (*Mauritia flexuosa*) are subglobouse to elliptic, ranging from 4 to 5 cm in diameter and are covered by reddish-brown scales. The mesocarp is smooth and its color varies from orange to reddish orange. Buritizeiro is an extremely versatile plant, serving as animal and human food, fuel, medicinal, ornamental, among other uses (RIOS; PASTORE JUNIOR, 2011). In folk medicine, the fruit is used as an anti-flu and vitamin A deficiencies (CARNEIRO; CARNEIRO, 2011). The fruit of the buriti is classified as a functional food by virtue of its nutritional composition, especially in the portions of the pulp and oil. It contains bioactive compounds that confer, among others, antioxidant and anti-inflammatory action (FREIRE *et al.*, 2016). In this perspective, it is essential to expand the research and knowledge about new biological activities of buriti pulp.

The cupuaçu (*Theobroma grandiflorum*) is a large tree whose fruits have a white-yellow pulp. Pulp has a strong flavor and is highly appreciated by local communities and also in the international market as an ingredient in juices, beverages, ice cream, jellies and sweets (PUGLIESE, 2010). In traditional medicine, bark extracts and leaves are used in the treatment of bronchitis, diarrhea, and nephritis, while pulp and seeds have

antioxidant potential with important levels of flavonoids (TELES, 2010; OLIVEIRA *et al.*, 2009; YANG *et al.*, 2003).

The fruits of inajá (*Maximiliana maripa*) are brown, oblong-ellipsoid, with 4 to 5 cm in length and 2.5 to 3 cm in diameter. The mesocarp has the fibrous outer layer, the inner one being fleshy, with 0.3 to 0.5 cm thickness and seeds present in a number from 1 to 3. The inajazeiro palm usually produces of 5 to 6 bunch per year, with 800 to 1000 fruits per bunch. The pulp of the fruit is consumed *in natura* or in the form of porridge, being used in traditional medicine to strengthen debilitated people (SHANLEY; SERRA; MEDINA, 2010). Almond, as well as fruit pulp, can be used as raw material for the cosmetics and soap industry, and the oil obtained from the almond can reach up to 60% yield (MIRANDA *et al.*, 2001). The inajá is a fruit still not appreciated, possibly due to the insufficiency of researches and consequent devaluation of this vegetal species (BEZERRA, 2011; VILLACHICA, 1996).

The fruits of monguba (*Pachira aquatica*) present edible seeds with appreciated organoleptic characteristics. The fruit is oblong-ellipsoid, with a length of 20 to 30 cm and 10 to 12 cm in diameter, weighs 1 to 1.5 kg and has 10 to 25 seeds of irregular shape (FAO, 1986). The seeds are consumed by the local population *in natura*, cooked, toasted or in the form of flour. However, Oliveira *et al.* (2000) demonstrated that the seeds *in natura* showed toxicity to rats, possibly due to the presence of lectins and trypsin inhibitors, and therefore such antinutritional factors may limit their interest. Although it is easy to cultivate and show great yields in oil, monguba is a fruit still underutilized by Brazilians and data on its chemical composition and industrial potential is not much available (SILVA; BORA; AZEVEDO, 2010).

The fruits of pajurá (*Coupia bracteosa*) are globular drupe with 8 to 12 cm in length and 8 to 15 cm in diameter and weighing 80 to 200 g. The exocarp is dark brown with a rough surface to the touch due to numerous lenticels present. The mesocarp is thick, fleshy, brownish-yellow in color, with a granular consistency and a sweet taste. The pulp is traditionally consumed *in natura* and in the preparation of sweets (BERTO *et al.*, 2015b). There are few studies on pajurá in literature, where their approaches are mainly related to phenological and ecological aspects (FALCÃO; LLERAS; KERR, 1981). As regards the biological activities of the fruit, only records of the antioxidant capacity were found, and in relation to the nutritional composition there are few and recent studies which were used in this work for comparison purposes, despite the

complexity involved (MASSING *et al.*, 2018; SOUZA *et al.*, 2016; BERTO *et al.*, 2015a; BERTO *et al.*, 2015b).

The fruit of the uxizeiro (*Endopleura uchi* basement of *Sacchioglotis uchi*) is an ellipsoid drupe, weighing between 50 and 70 g and with a smooth exocarp of yellowish-green or dark brown when ripe. The mesocarp has an average thickness of 5 mm, with a fleshy and oily appearance. It is consumed *in natura* especially by the native population. Itis also used in the elaboration of refreshments, creams, sweets, liqueurs, ice creams, wines and oils (RIOS; PASTORE JUNIOR, 2011; SHANLEY; MEDINA, 2005; FAO, 1986). In popular medicine, bark teas are used as an anti-inflammatory to uterine inflammation, fibroids and polycystic ovaries (REVILLA, 2002) in the treatment of arthritis, rheumatism, high cholesterol and diabetes (SHANLEY; GAIA, 2004; CORRÊA, 1984). The studies in the literature on the biological activities of uxi are limited to the portion of the bark in which the antimicrobial (POLITI *et al.*, 2011) and immunological activities (MOREIRA *et al.*, 2007) have been demonstrated.

2.2 Minerals

The minerals originate from the decomposition of rocks, ores, vegetables, and animals and are metabolized by plants by absorbing them in the environment where they develop (soil, water, and air). Thus, minerals reach the human through the food chain through the consumption of fruits and vegetables (TAIZ; ZEIGER, 2009).

Essential minerals are important nutrients for the human organism that perform plastic, catalytic and regulatory functions, acting in the formation and action of the body's defense cells and antioxidant enzymes; in the structure of corporeal tissues such as bones, teeth and muscles; in the activation-regulation of enzymes; in the regulation of water and acid-base balance, and osmotic pressure; in the control of nerve impulses, muscle activity and oxygen transport (BIASEBETTI, RODRIGUES; BAZUR, 2018; AZEVEDO; CHASIN, 2003).

The minimum/maximum intake of minerals in the human body, in order to prevent deficiencies which, preclude its proper functioning and to prevent toxicity, define the amount of minerals required. Thus, the recommended intake of minerals depends on the growth phase, physiological conditions (pregnancy and lactation), nutritional status and health of the living organism, as well as the physiological requirements due to the practice

of physical exercises (KINUPP; BARROS, 2008; FRANCO, 2005, SHILS, OLSON, SHIKE, 1994).

The knowledge of the concentration of minerals in fruits is important for health professionals and for consumers in general because they indicate constituents that, due to their essentiality and/or toxicity, influence human health. In addition, there are useful information for agronomic and environmental professionals because they are related to plant health and productivity. Different analytical techniques can be employed for the determination of minerals in the most varied types of samples, such as atomic spectrometry (absorption or emission) and mass spectrometry. However, those based on atomic spectrometry are the most widely employed.

2.2.1 Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES)

The technique of instrumental chemical analysis ICP OES is an analytical tool used in quantitative and qualitative elemental determination in a wide variety of samples such as biological, environmental, geological, technological and food materials (DONATI; AMAIS; WILLIAMS, 2017; GHOSH *et al.*, 2013).

ICP OES is applied to a wide range of metallic elements and has the advantages such as low interference, good emission spectra, good detection limits, multielement analysis and wide linear range of work. On the other hand, it involves a high cost, especially in the maintenance of the equipment and gas supply (DONATI; AMAIS; WILLIAMS, 2017; GHOSH *et al.*, 2013).

The basic components of ICP OES include a nebulization system, atomization system (plasma), wavelength insulating device, radiation transducer (s), signal processor and computer system. Figure A1 in Annex A shows the technical diagram, the main components of the ICP OES system and a brief explanation of the equipment operation. A plasma, by definition, is a conductive gas mixture containing a significant concentration of cations and electrons, with a total charge close to zero (DONATI; AMAIS; WILLIAMS, 2017; GHOSH *et al.*, 2013), which is formed and maintained by a flow of argon gas in a high-frequency magnetic field on a quartz torch consisting of three concentric tubes, as shown in Figure A2, Annex A, and further explanations in the same annex.

For the quantitative determinations, it is necessary to construct the analytical curve of the metal element to be quantified, where the emission intensities of standard solutions are determined in increasing concentrations known and within the range of the analyte concentration in the sample. The results are plotted on a graph of emission intensity as a function of concentration and should be linear and with a correlation coefficient close to unity. The concentration of the analyte present in the sample is determined by the measurement of its emission intensity, the value of which shall correspond to that of the abscissa given by the analytical curve.

2.3 Oxidants and Antioxidants

Epidemiological studies have shown the strong relationship between the consumption of plants rich in antioxidant compounds and the protection against various human chronic diseases, as well as their beneficial bioactive activities, such as anti-apoptotic and anticarcinogenic action, inhibiting processes of cell proliferation (OMBRA *et al.*, 2016, PISOSCHI; NEGULESCU, 2011; BOUAYED; BOHN, 2010). The strong bioactive activity of the fruit is related to substantial amounts of antioxidant compounds, which attribute beneficial effects to human health through the inhibition or neutralization of oxidant sources (WALTER; MARCHESAN, 2011; XU *et al.*, 2017; DZIAŁO *et al.*, 2016).

The production of oxidants in living aerobic species occurs during the respiration process by the reduction of molecular oxygen in sequential steps to produce water (KRUMOVA; COSA, 2016). In this process, short-lived reactive intermediate chemical species are produced as byproducts, the so-called reactive oxygen species (ROS), as well as reactive nitrogen species (RNS). ROs are highly reactive oxidants and arise from the reduction of an electron of the molecular oxygen, with the formation of the three primary species: superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$) (RAHMAN *et al.*, 2012), as shown in Figure 2.

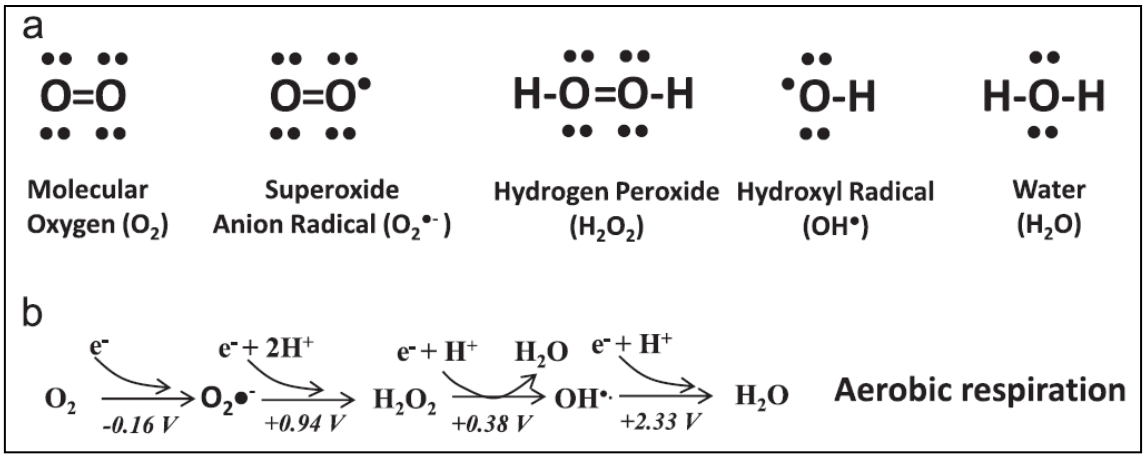


Figure 2 - Reduction of O_2 to H_2O and its ROS intermediates, (A) Lewis structures, (B) the step wise reduction of O_2 to H_2O during aerobic respiration (MAILLOUX, 2015).

In normal physiological conditions, ROS are important in energy production; defense against foreign agents such as viruses and bacteria; intercellular signaling; and programmed cell death (apoptosis) (SARMA *et al.*, 2010). However, ROs are an emerging class in which exposure to pollutants, such as tobacco, smoke, drugs, xenobiotics, radiation, and others, contributes to its overproduction, and consequently to the phenomenon of oxidative stress (BATTACHARYYA *et al.*, 2014; SINGH; SINGH, 2008).

Oxidative stress is defined by the imbalance caused by the excessive production of ROS and / or limited antioxidant defense, which implies oxidative damage to structures of biomolecules of DNA, lipids, carbohydrates and proteins, as well as other cellular components and consequently in the development and progression of several pathologies including the degenerative ones like cancer, cardiovascular diseases, cataract, decline of the immune system and cerebral dysfunctions (KAUR; KAPOOR, 2002; MARTINEZ-VALVERDE; PERIAGO; ROS, 2000; WANG; CAO; PRIOR, 1996).

In order to prevent pathological levels of ROS, the human organism has an antioxidant defense system formed by endogenous and exogenous antioxidant sources. In the condition of redox equilibrium between the production of oxidant species and the performance of the antioxidant defense, the condition of homeostasis in which the relative regulation of the physiological functioning appears becomes prominent (Figure 3) (XU *et al.*, 2017; LÜ *et al.*, 2010).

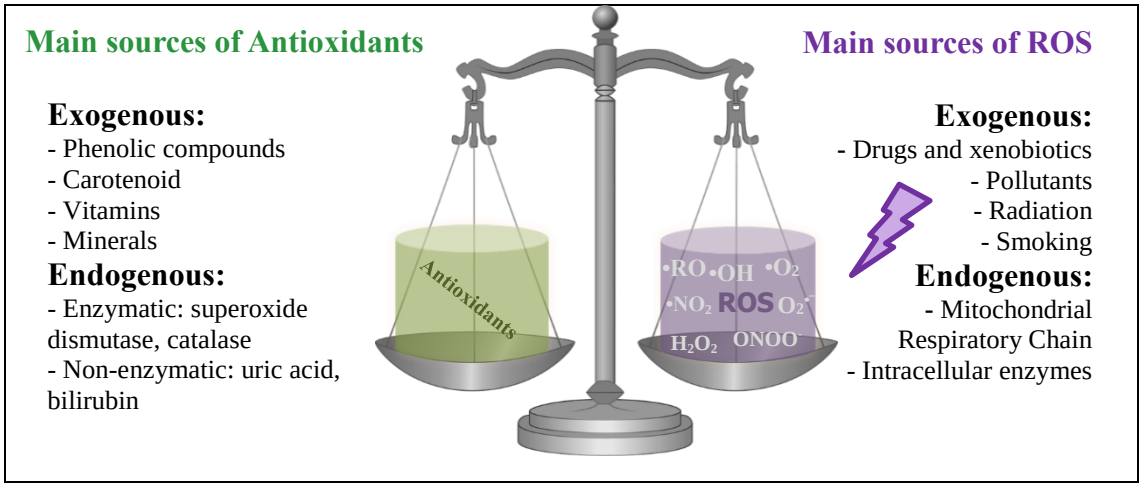


Figure 3 - Equilibrium between antioxidant compounds and ROS (Adapted from DAL, SIGRIST, 2016).

Antioxidants are present in low concentrations when compared to the oxidizable substrate, delay or inhibit the oxidation of these compounds form relatively stable inactive products (LÜ *et al.*, 2010, FLORA, 2009, RIBEIRO *et al.*, 2005). A good antioxidant has substituents donors of electrons or hydrogen to the reactive species, depending on its reduction potential; the displacement capacity of the radical formed in its structure; ability to chelate transition metals involved in the oxidative process; access to the site of action, depending on its hydrophilicity or lipophilicity and its partition coefficient (MANACH *et al.*, 2004).

Endogenous antioxidants may be enzymatic compounds (superoxide dismutase, catalase, glutathione peroxidase, among other) or non-enzymatic (uric acid, bilirubin, albumin, metallothionein, among other) (BATATACHARYYA *et al.*, 2014). However, in the face of excessive production of oxidizing species, endogenous antioxidants cannot guarantee rigorous control and complete protection of the body. This fact explains the need for exogenous antioxidants to maintain oxidative equilibrium (PISOSCHI; NEGULESCO, 2011).

Exogenous antioxidants originate from natural plant sources such as fruit, herbs, spices, and teas, as well as are found in nutritional supplements and pharmaceuticals products. These include phenolic compounds (phenolic acids, flavonoids, anthocyanins, lignans, and stilbenes), carotenoids (xanthophylls and carotenes), vitamins (vitamin E and C) and minerals (Se and Zn) (XU *et al.*, 2017).

Due to protection against oxidizing sources, exogenous antioxidant compounds from natural sources have received great attention and represent an interesting potential for many applications, such as the food, cosmetics, nutraceutical, therapeutic and medical industries. Thus, in recent years, interest in methods and variations that determine the antioxidant capacity of vegetables has increased considerably (XU *et al.*, 2017; BALMUS *et al.*, 2016; WOJTUNIK-KULESZA *et al.*, 2016; PRASAD, 2016; SALOMONE; GODOS; ZELBER-SAGI, 2016; ZHANG *et al.*, 2015; ARATHI *et al.*, 2015; LI *et al.*, 2014; SINDHI *et al.*, 2013).

2.3.1 Determination of the antioxidant capacity

Different methods in the literature for the determination of the antioxidant capacity of biological systems involve different oxidizing sources (SHALABY; SHANAB, 2013). In addition, the chemical diversity of antioxidants allows a different behavior to eliminate the reactive condition of oxidizing sources. In view of this, no assay accurately reflects the mechanism of action of all radical sources or all antioxidants of a complex system (PRIOR; WU; SCHAICH, 2005), and consequently more than one method of antioxidant capacity determination should be used to compare the mode of action of pure or crude antioxidant compounds (MOHARRAM; YOUSSEF, 2014; SHALABY; SHANAB, 2013).

Traditional analytical methods based on spectrophotometry, electrochemistry, and chromatography have been used in the determination of the antioxidant capacity in plants, each differing in relation to the mechanism of generation of oxidant sources and/or target molecules, as well as to the final detection/measurement of the products of the reaction. However, in recent years, a great deal of effort has been made to use more sophisticated and accurate bioanalytical methods such as those based on electroanalytical sensors and biosensors in order to improve the detection performance (BHATTACHARYYA *et al.*, 2014; PISOSCHI; NEGULESCO, 2011).

2.3.1.1 Colorimetric methods

In general, spectrophotometric techniques are simple, fast and inexpensive, justifying their widespread use in screening for antioxidant capacity. They are based on

the reaction of a radical, radical or complex cation with an antioxidant molecule capable of transferring hydrogen atoms (HAT) and/or electron transfer (SET) (MOHARRAM; YOUSSEF, 2014; PISOSCHI; NEGULESCO, 2011; PRIOR; WU; SCHAICH, 2005).

In this study, three colorimetric methods were adapted to microplates: DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS [2,2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid)] and NBT (nitro blue tetrazolium).

The DPPH and ABTS methods are based on the HAT and/or SET mechanism in order to neutralize the synthetic radical's $DPPH^{\bullet}$ and $ABTS^{\bullet+}$, respectively. Although some authors affirm these methods as being based on the SET mechanism, it is known that the HAT and SET mechanisms occur almost simultaneously in all systems, although a mechanism may prevail in a given test, the dominant mechanism being determined by the structure and properties of the antioxidant compounds, such as chemical accessibility, solubility and partition coefficient, employed solvent, among others (APAK *et al.*, 2013).

$DPPH^{\bullet}$ and $ABTS^{\bullet+}$ are synthetic organic radicals with purple and blue-green colors respectively, which can be reduced in the presence of antioxidants (AOX)with consequent discoloration, proportional to the antioxidant capacity of the sample (Figure 4) (PISOSCHI; NEGULESCO, 2011; SINGH; SINGH, 2008; PRIOR; WU; SCHAICH, 2005).

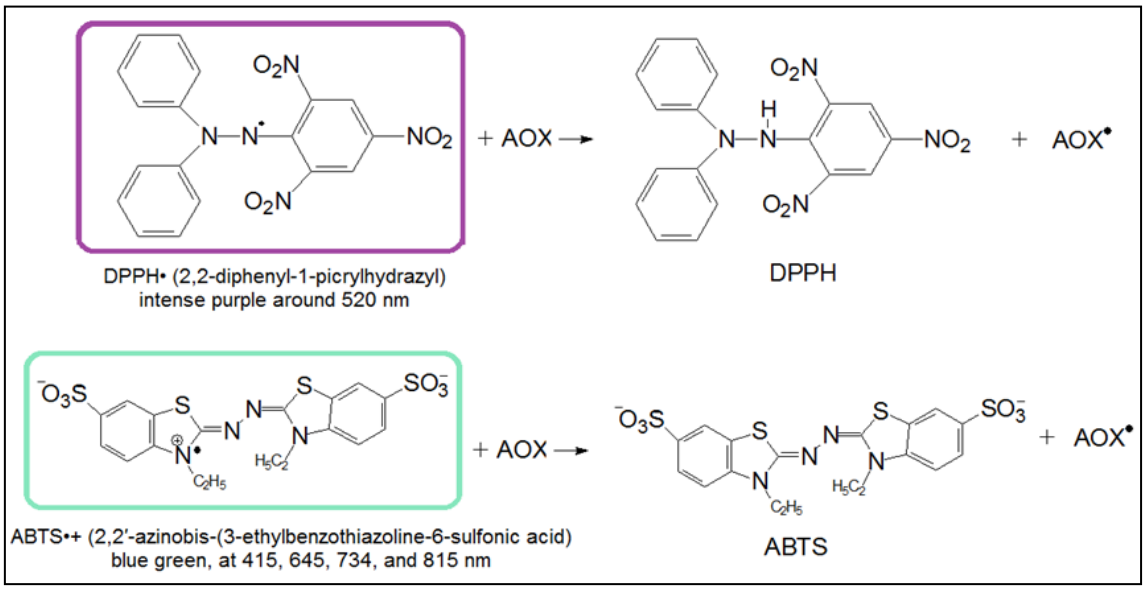


Figure 4 - Chemicals reactions involved in the DPPH and ABTS spectrophotometric assays (SINGH; SINGH, 2008; PRIOR; WU; SCHAICH, 2005).

The NBT method is based on the SET mechanism to eliminate the radical superoxide anions ($O_2^{\bullet-}$), which are present in all aerobic biological systems and are considered cytotoxic oxidants when in excess. In the NBT method, the hypoxanthine (HX) substrate in the presence of the xanthine oxidase (XOD) enzyme generates $O_2^{\bullet-}$ radicals which reduce the NBT (yellow coloring) reagent to the colored formazan product (lilac), that can be measured spectrophotometrically (Figure 5). However, the presence of the antioxidant sample scavenges the radicals $O_2^{\bullet-}$ and produces a decrease in the production of formazan and consequently a decrease in the absorbance proportional to the antioxidant capacity of the sample is observed (COUTINHO-PUIG *et al.*, 2009; SANCHEZ-MORENO, 2002).

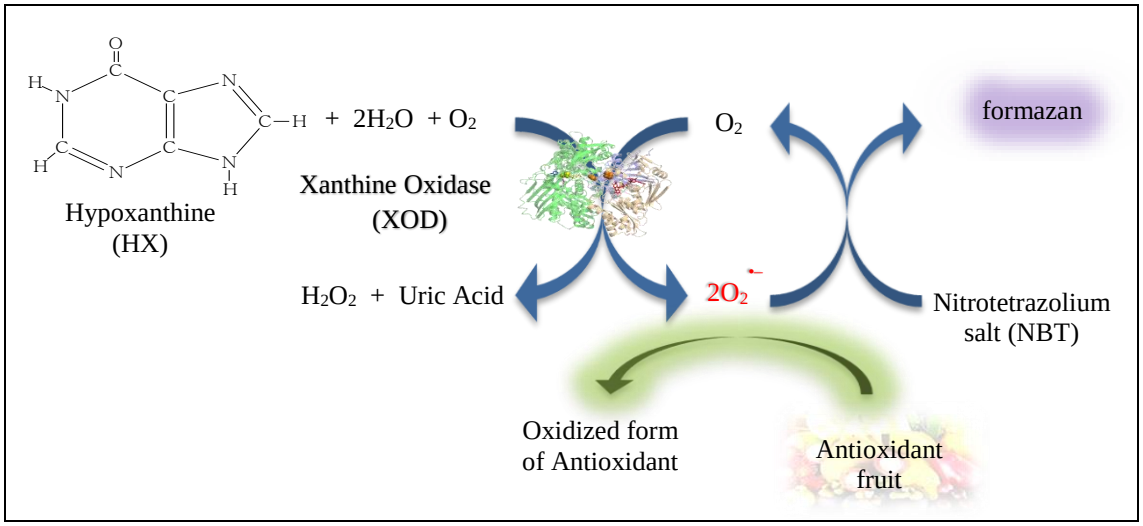


Figure 5 - Reactions involved in the measurement of superoxide radical sequestration capacity using the NBT method (BECKER *et al.*, 2019).

2.3.1.2 Enzyme amperometric biosensors

Advances in biotechnology have provided the replacement or supplementation of traditional methods with other innovative technologies such as biosensors. Biosensors are attractive and promising tools in the detection of antioxidant compounds and antioxidant capacity due to their analytical characteristics such as specificity, sensitivity, low cost, miniaturization, easy automation, time saving and manufacturing simplicity (FUSCO *et al.*, 2010; GOMES *et al.*, 2004).

By definition, a biosensor is an analytical device coupled with a biological or biologically derived entity (enzymes, antibodies, antigens, organism, animal and plant

tissue, cells, organelles, etc.) associated with a transducer that converts the biological signal in a detectable signal proportional to the analyte concentration (ROSATTO *et al.*, 2001; FATIBELLO-FILHO; CAPELATO, 1992).

Generally, in a biosensor the analyte interacts with the biological component (A) through bio-recognition processes which generate a signal, which is translated into a detectable signal by the transducer (B), filtered, amplified, analyzed and transferred to a monitor or device by the signal processing unit (C), as shown in Figure 6. The generated signal is proportional to the concentration of the analyte in the sample (PATHAK; KATIYAR; GIRI, 2008; RAITERI; GRATTAROLA; BERGER, 2002).

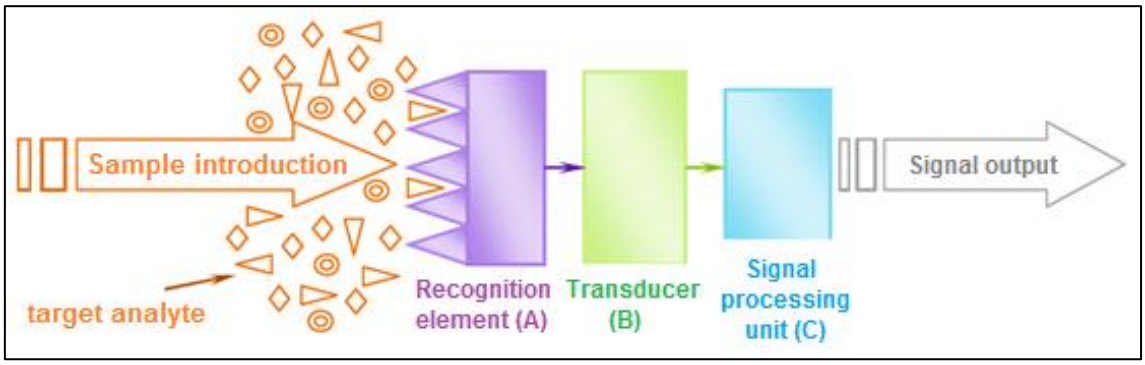


Figure 6 - Configuration of a biosensor, showing the organization of its functional components (CALIL; SILVA, 2011).

Figure 7 shows a scheme of the commonly used biosensors, in which for the same analyte of interest can be developed diverse biosensors, immobilizing different biological materials, separately in varied transducers. The biosensors can be classified according to the biological element and the employed transducers (SHAVANOVA *et al.*, 2016).

Due to their specificity and catalytic properties, enzymes have found wide application in the construction of biosensors. The majority of biosensors constructed and marketed for the most varied applications are enzymatic and amperometric (MARQUES; YAMANAKA, 2008).

The performance of a biosensor is strongly dependent on the bioactive detection layer and the quality of its association with the transducer. Because of this, the immobilization process of the biological component on the transducer is one of the main steps in the construction of a biosensor. It must guarantee the biofunctionalization and stability of the biological recognition element, providing accessibility for the target

analyte and other molecules involved in the bio-recognition event, as well as an intimate contact with the surface of the transducer in order to achieve an efficient signal transfer (MARQUES; YAMANAKA, 2008; XU; PRIETO-SIMÓN; CAMPAS; MARTY, 2008; XU; CHEN; DONG, 2006).

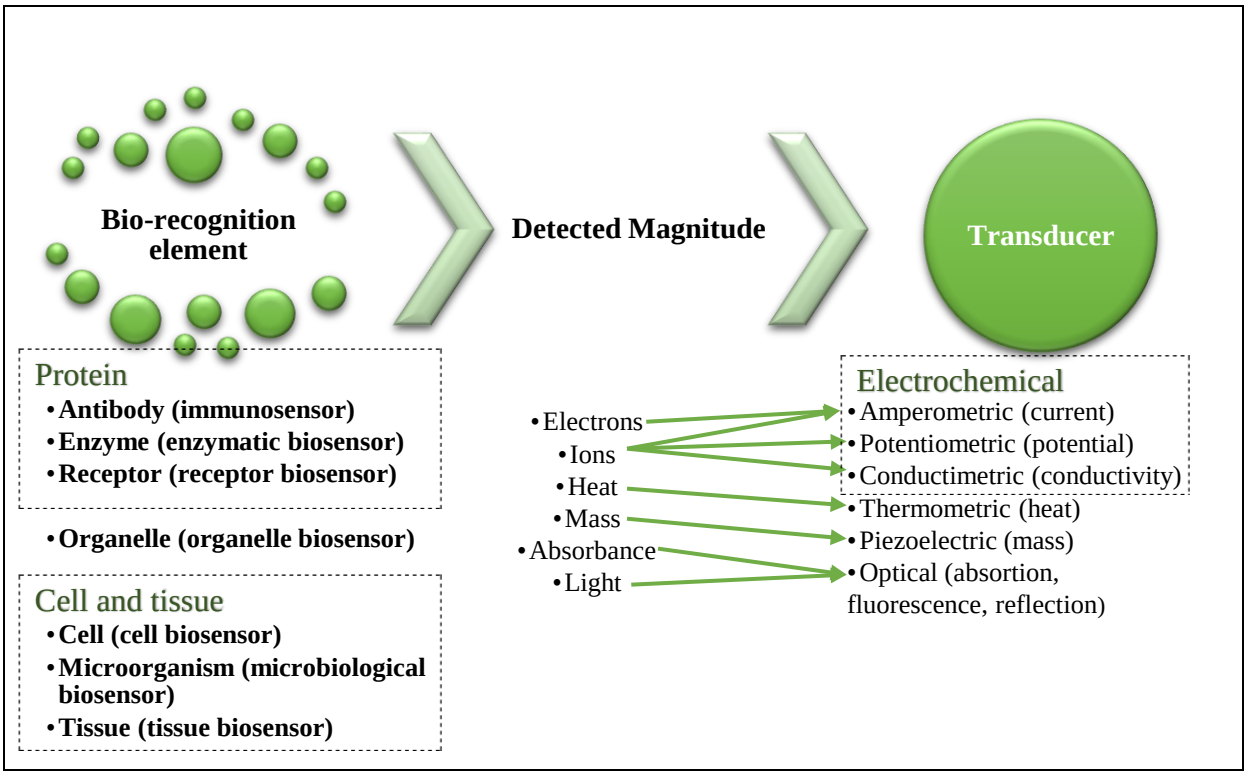


Figure 7 - Scheme of most used biosensors and their classifications according to the biological component and the transducer used (Adapted from SHAVANOVA *et al.*, 2016).

Among the factors that may affect the immobilization of biomolecules are the nature of the biomolecule (size, structural characteristics, polarity and accessibility of certain functional groups), working and storage conditions (medium pH, salinity, viscosity, and temperature) and the physicochemical and chemical properties of the transducer material. In addition, the choice of immobilization support should be closely related to the immobilization strategy, with the detection principle and should favor biocompatibility (PRIETO-SIMÓN; CAMPAS; MARTY, 2008).

In general, enzymatic immobilization techniques influence the analytical efficiency of the biosensor (sensitivity, stability, repeatability and response time) and can

be classified as physical (adsorption and entrapment) and chemical (covalent bonding and cross-linking) (GOUDA, 2000).

The entrapment immobilization technique involves the inclusion of enzymes in polymeric structures/membranes (such as polyvinyl alcohols) with pore sizes that allow the diffusion of substrates and products of the reaction of low molecular weight, but which preclude the passage/loss of the protein. Thus, the enzyme is incorporated as part of the mixture to be polymerized, in which as the polymerization proceeds, the polymer matrix forms around the enzyme, confining it in its structure (SOUZA *et al.*, 2017).

The development of enzymatic amperometric biosensors for antioxidant capacity in foods has been performed as an alternative and efficient analytical tool compared to the conventional analytical methods.

Lates *et al.* (2011) determined the antioxidant capacity of commercial beverages in the inhibition of the reactive species $O_2^{\bullet-}$ and H_2O_2 generated by the enzymatic system of xanthine (XA)/XOD using a bioreactor coupled to an amperometric H_2O_2 biosensor formed by a graphite electrode modified with polymer gel osmium-polyvinyl pyridine containing horseradish peroxidase. The H_2O_2 resulting from the enzymatic reaction was monitored amperometrically at -100 mV *vs* $Ag/AgCl/KCl_{sat}$. Authors evaluated and optimized different XOD enzyme immobilization procedures (physical adsorption, covalent bonding and glutaraldehyde crosslinking), XA substrate concentrations and the flow rates at the injection. In addition, two protocols were studied for the determination of antioxidant capacity: steady state (1), in which the antioxidant was injected into the XA flux; transient state (2) in which the substrate XA and antioxidant were simultaneously injected. Optimized conditions were established (immobilization by glutaraldehyde cross-linking, XA concentration at 0.5 mmol L^{-1}, a flow rate of 1.5 mL min^{-1}) and transient injection ensured greater contact between antioxidant compounds and ROS generated, increasing the probability of inhibiting the short-lived $O_2^{\bullet-}$ radicals. The biosensor showed a good analytical efficiency with a limit of detection (LOD) of 2.2 mmol L^{-1}, a limit of quantification (LOQ) of 7.5 mmol L^{-1}, the sensitivity of 5 mA/mol L^{-1} and the linear range of up to 50 mmol L^{-1}. The results of the commercial juice samples showed a strong correlation with the results obtained by the Folin method. Several advantages have been demonstrated such as low applied potential, simultaneous flow of antioxidant and XA, biological relevance due to the determination of the antioxidant capacity against the combined $O_2^{\bullet-}$ and H_2O_2 species. However, the biosensor involves a

bi-enzymatic system complicating the process, wherein the horseradish peroxidase enzyme employed in modifying the working electrode has limitations in connection to solid surfaces, and, moreover, osmium (costly and toxic) was employed to facilitate the electron transfer at the working electrode.

Campanella *et al.* (2003) evaluated the antioxidant capacity to inhibit the $O_2^{\bullet-}$ radicals of fruit and aromatic herbs. $O_2^{\bullet-}$ radicals were produced by the enzymatic system XA/XOD in solution, while the superoxide dismutase (SOD) enzyme was immobilized by entrapment on a kappa carrageenan membrane to catalyze the dismutation of the radical $O_2^{\bullet-}$ to H_2O_2. The generated H_2O_2 was oxidized on the surface of the platinum anode (+650 mV *vs* Ag/AgCl), with the consequent generation of an amperometric signal proportional to the $O_2^{\bullet-}$ concentration in solution. The biosensor showed simple, with a minimum of pretreatment required to the sample, with good analytical efficiency presenting a coefficient of variation (CV) ≤ 10%, and relation with classic methods to determine the antioxidant capacity. The same research group has been dedicated to the development, optimization and application of this biosensor to determine the antioxidant capacity in several types of samples (CAMPANELLA *et al.*, 2013; CAMPANELLA *et al.*, 2009; CAMPANELLA *et al.*, 2004a; CAMPANELLA *et al.*, 2004b; CAMPANELLA *et al.*, 2003; CAMPANELLA; BONANNI; TOMASSETTI, 2003; CAMPANELLA *et al.*, 2001). Although the few number of steps required and the good analytical performance, the high potential applied can admit the direct oxidation of the phenolic compounds (ROSATTO *et al.*, 2001) and bi-enzymatic systems (immobilized and/or in solution) should be avoided because they increase the cost of the process due to the number os reagents and large quantities required when using the enzyme in solution. In addition, the use of the SOD enzyme to generate H_2O_2 could be suppressed, since the dismutation of $O_2^{\bullet-}$ to H_2O_2 occurs rapidly and spontaneously, and the measurement of the antioxidant capacity against both ROs would be biologically more interesting (CORTINA-PUIG *et al.*, 2010).

The antioxidant capacity of plant samples has been attributed to the content of phenolic compounds.

2.4 Phenolic Compouds

Phenolic compounds are considered one of the most important groups of secondary metabolites of plants due to their participation in the morphological development, physiological processes and vegetal reproduction. They are responsible for color, astringency, aroma, oxidative stability, as well as a broad spectrum of biological properties, as shown in Figure 8, which are due to their variable molecular structure (DZIAŁO *et al.*, 2016; ANGELO; JORGE, 2007).

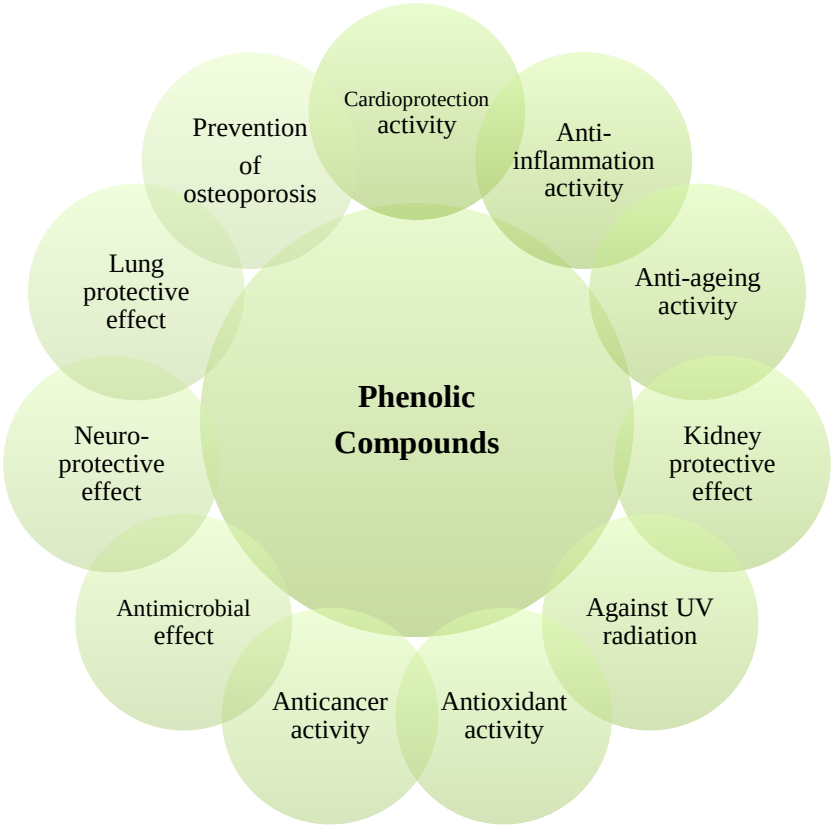


Figure 8 - Bioactivities of natural phenolic compounds (Adapted from LI *et al.*, 2014).

Phenolic compounds are defined as substances having at least one aromatic ring with one or more hydroxyl substituents, including functional groups. In plants, these compounds generally contain more phenolic rings and are therefore called polyphenols (ANGELO; JORGE, 2007; LEE *et al.*, 2005).

Phenolic compounds can be classified into flavonoid and non-flavonoid compounds, as shown in Figure 9.

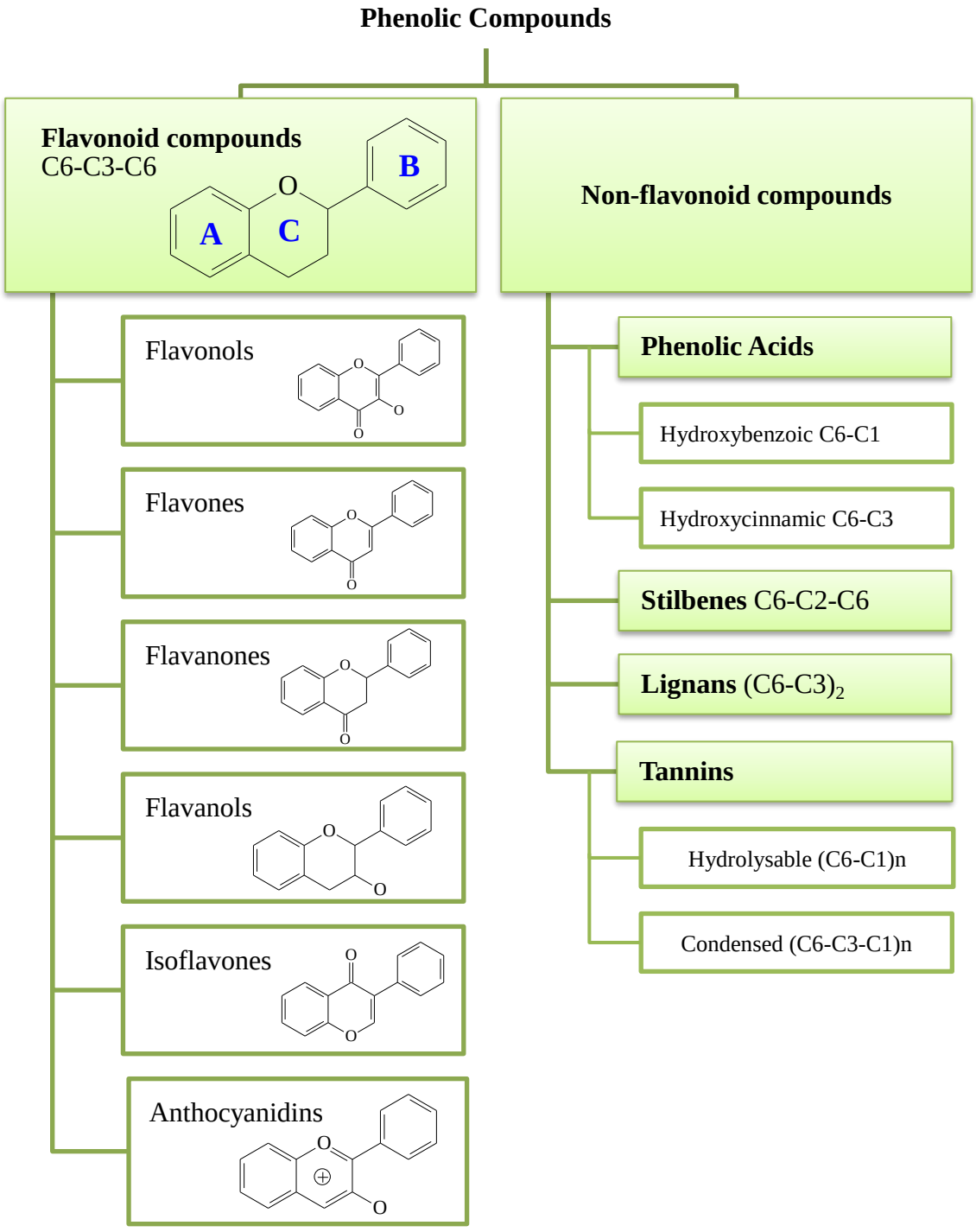


Figure 9 - Classification of phenolic compounds (ANGELO; JORGE, 2007; HOLLMAN; KATAN, 1999).

Flavonoid compounds have low molecular weight and are structurally characterized as diphenyl propane (C6-C3-C6), with two aromatic rings, called rings A and B, joined by three carbons that form a heterocyclic ring, called ring C (Figure 9).

Variations in C-ring substitutions result in important classes of flavonoids, such as flavonols, flavones, flavanones, flavanols (or catechins), isoflavones, and anthocyanidins. Substitutions of rings A and B give rise to different compounds within each class of flavonoids (ANGELO; JORGE, 2007; HOLLMAN; KATAN, 1999).

In plants, flavonoids comprise the largest and most diverse group of phenolic compounds, they act in the photoprotection, against pathogenic microorganisms, antioxidant action and enzymatic inhibition (HARBORNE; WILLIAMS, 2000). *In vivo* evidence confirms activities against free radicals, antibiotics, antiallergics, antidiarrheal agents, anti-inflammatories, among others (ARAGÃO, 2013; ROSS; KASUM, 2002).

The main non-flavonoid phenolic compounds consist of phenolic acids (hydroxybenzoic and hydroxycinnamic), stilbenes, lignans, and tannins.

The most common phenolic acids in plant tissues are hydroxycinnamic acids and include caffeic, chlorogenic, cumaric, ferulic, and sinapic acids. Tannins are also widely distributed in plants and can occur as hydrolysable tannins (formed in the path of phenolic acids with sugar polymerization) and condensed tannins (combination of flavonoids) (DZIAŁO *et al.*, 2016).

It is known that free hydroxyl groups in phenolic compounds are mainly responsible for their antioxidant capacity, but other factors such as the character of the substituents (carboxyl group or acetyl) and their position in relation to hydroxyl groups also influence this behavior, (SROKA; CISOWSKI, 2003).

2.4.1 Phenolic Compounds Determination

Several spectrophotometric methods based on oxidation-reduction reactions between phenolic compounds and metal ions have been used to estimate the total content of phenolic compounds in plant extracts. However, the use of the Folin-Ciocalteu reagent was employed in this study and is among the most widely used method (SOUSA *et al.*, 2007).

The method is based on the ability of the Folin-Ciocalteu reagent (yellow coloring) to be reduced to the phosphotungsticp hosphomolybdenum complex (blue chromophore) by reducing phenolic compounds. According to the reaction shown in Figure 10, phenolic compounds (represented by gallic acid), in alkaline media, dissociate a proton, leading to the formation of the phenolate anion that reduces the Folin-Ciocalteu

reagent. Thus, the concentration of the chromophore formed (blue staining) is proportional to the concentration of reducing substances or phenolic compounds present in the sample and can be estimated spectrophotometrically in the visible region (PIRES *et al.*, 2017).

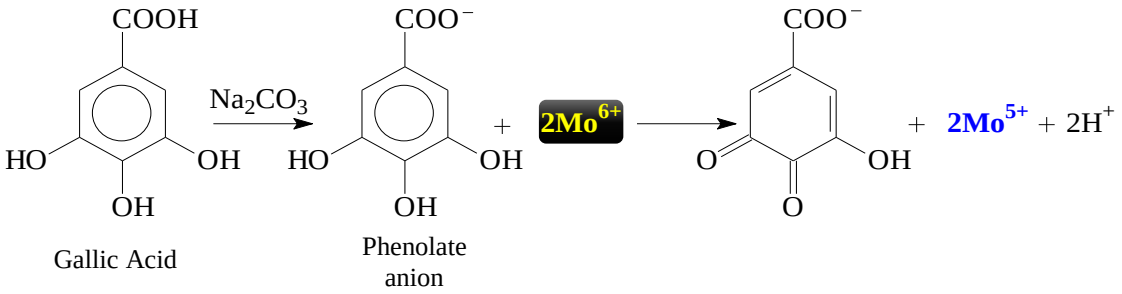


Figure 10 - Scheme of equation proposed for the Folin-Ciocalteu method (PIRES *et al.*, 2017).

The determination of phenolic substances in food is even more complex, not only because of the nature of the matrix but also due to the diversity of the existing compounds with great variability of chemical structures. In addition, phenolic substances have a range of polarity and size and can be found at different levels of concentration. Thus, the separation, determination, and identification, as well as its extraction from the samples, is not an easy process. This analysis has been done mainly using methods based on the High-Performance Liquid Chromatography (HPLC) technique coupled with mass spectrometry or tandem mass spectrometry (BARBOSA *et al.*, 2018; LUCCI; SAURINA; NÚÑEZ, 2017).

HPLC is the most versatile and widely used type of elution chromatography that allows to separate, identify and determine components from a wide variety of organic, inorganic and biological materials. Figure A3 of Annex A shows the typical components of a conventional HPLC system, as well as a brief explanation of the operation of its components.

In recent years, different manufacturers have adapted equipment that allow the miniaturization of the technique, making it Ultra High-Performance Liquid Chromatography (UHPLC). UHPLC provides the maximum chromatographic efficiency of porous particles of less than 2µm and thus gains in resolution, sensitivity, retention time, linear velocity, width and signal efficiency, thus allowing faster analysis and a lower

expenditure of solvent and samples between the analyzes when compared to the HPLC (conventional particles of 10 μm, 5 μm or 3.5 μm) (NOGUEIRA *et al.*, 2011).

3. OBJECTIVES

3.1 General Objective

To contribute to the knowledge of nutritional, antioxidant and bioactive properties of native fruits of the Amazon region and to develop an analytical efficiency tool to determine the antioxidant capacity in real samples.

3.2 Specific objectives

In the case of the fruits abiu (*Pouteria caimito*), açaí (*Euterpe oleracea*), bacuri (*Platonia insignis*), biribá (*Rollinia orthopetala*), buriti (*Mauritia flexuosa*), cupuaçu (*Theobroma grandiflorum*), inajá (*Maximiliana maripa*), monguba (*Pachira aquatica*), pajurá (*Couepia bracteosa*) e uxi (*Saccoglotis uchi*):

- To characterize bromatologically in relation to moisture content, ashes, lipids, total proteins, total carbohydrates, energy, pH and acidity in citric acid
- To determine the mineral elements including Ca, Cu, Fe, Li, Mg, Mn, Na and Zn by ICP OES and evaluate the results, considering the complexity of each matrix, the instrumental optimization, the essentiality and/or toxicity of the elements within the limits of physiological tolerance;
- To determine the antioxidant capacity of aqueous, ethanolic and hydroethanolic extracts (1: 1) by DPPH, ABTS and NBT methods;
- To evaluate the antiproliferative activity of fruit extracts against human colon cancer cell lines (caco-2) by the sulforhodamine B method (SRB);
- To determine bioactive compounds through quantification of vitamin C and phenolic compounds by titration of oxide-reduction and colorimetry, respectively;
- To identify and quantify phenolic compounds, by UHPLC coupled to high-resolution mass spectrometry, of fruits with higher antioxidant capacity and antiproliferative activity.

Regarding the new analytical tool for determination of antioxidant capacity (biosensor):

• To develop an amperometric biosensor to determine the antioxidant capacity in real samples;

• To optimize the biofunctionalization process of the biosensor;

• To characterize the analytical efficiency of the biosensor developed by means of figures of merit;

• To apply the biosensor to determine the antioxidant capacity of pure antioxidant and real samples of Amazonian and non-native fruits.

4. JUSTIFICATION

In order to prevent and cure certain diseases and/or restoring health, the consumption of natural foods has been encouraged. The mechanism of action of the nutrients present in these foods, such as minerals, vitamins, and bioactive compounds underpin this valorization (SANTOS; LIMA, 2008).

The feeding devoid of minerals and vitamins has killed billions of people who have been survived in conditions below their physical and mental potentials. Among the factors that contribute to the maintenance of this framework of multi deficiency includes lack of economic resources and the habit of valorization of industrialized foods or foods imported from other regions. Certainly, the lack of information on the nutritional value of regional foods greatly favor these factors (MANDELBAUM-SCHIMID, 2004).

With a huge diversity of plant species, the Amazon region presents thousands of native species (HIGUCHI; HIGUCHI, 2004), where the geographic dimension associated with the great vegetal biodiversity possibly explains the existence of species with unknown food and pharmacological potential (KINUPP; BARROS, 2008).

Among the natural foods, the fruits are known sources in minerals, vitamins and antioxidant compounds (SINDHI *et al.*, 2013; ALISSA; FERNS, 2012; TACO, 2011; RUFINO *et al.*, 2010; HAMID *et al.*, 2010; FRANCO, 2005; STEINMETZ, POTTER, 1996). Despite the diversity in native Amazon fruits and the strong appeal in new colors, flavors and textures, as well as the economic, nutritional and therapeutic relevance of fruits, and the fact that the nutritional content of wild fruits is generally higher than the domesticated fruits (ODHAV *et al.*, 2007), even so, there is little information on the

composition of fruits and the bioactive compounds of native fruits of the Amazon region, which favors its local devaluation as well as in the other Brazilian regions (BORGES *et al.*, 2013).

Therefore, the characterization of native fruits of the Amazon region is important, not only because it refers to new data of the constituents considered important for human health, but also because it is related to the health and productivity of the vegetable. Data on the composition of native fruits is essential to add value and encourage the national and international market and assist the food, cosmetics, pharmaceutical industries, among others. Moreover, the knowledge of the chemical composition is essential for quality control and food safety, as well as for assessing the adequacy of nutrient intake by individuals or populations (TACO, 2011).

With the current interest in determining the efficacy and use of natural antioxidants in various applications such as food technology, cosmetics industry, nutraceuticals, medicine and others, the development of methods for the determination of antioxidant capacity against biologically relevant radicals received a lot of attention. In general, spectrophotometric, electrochemical and chromatographic methods have been used for this purpose (PISOSCHI; NEGULESCO, 2011).

Conventional methods have disadvantages which may be related to the use of synthetic radicals which do not reflect the naturally occurring reactive species in biological systems; instability of the oxidizing sources; solubility and pH limited to the oxidizing source; slowness by virtue of the number of stages; expensive equipment requiring trained operator; impossibility of use in loco; low analytical performance; among others (SINDHI *et al.*, 2013; SINGH; SINGH, 2008; PRIOR; WU; SCHAICH, 2005).

In the last two decades, electroanalytical biosensors have been considered an efficient alternative to measuring the antioxidant capacity in foods, presenting a superior performance to the conventional tests (LATES; MARTY; POPESCU, 2011). Among them, the amperometric biosensors based on the ability to eliminate the O2•- e/ou H2O2 radicals generated in vitro system, are the most applied in vegetable matrices. However, a number of disadvantages have been observed, such as high working potentials that allow electrochemical interferences, high amounts of enzymes or even bi-enzymatic system that increase the cost of the total process, the absence of applications in real food samples and the use of toxic heavy metals as mediators electrochemical (CORTINA-PUIG *et al.* 2010;

CAMPANELLA *et al.*, 2013; LATES; MARTY; POPESCU, 2011; CORTINA-PUIG *et al.* 2009; CAMPANELLA *et al.*, 2009; CAMPANELLA *et al.*, 2004a; CAMPANELLA *et al.*, 2004b; CAMPANELLA *et al.*, 2003; CAMPANELLA; BONANNI; TOMASSETTI, 2003; CAMPANELLA *et al.*, 2001).

An analytical tool that presents advantages compared to the limitations of conventional methods and that can be used directly in the environment (in situ) would minimize losses of antioxidant compounds sensitive to conditions of luminosity, temperature and presence of oxygen, such as vitamin C, tannins and anthocyanins. Also, once removed from the various physical influences (pressure, temperature, light, climate, weathering and etc.), chemical (acidity, alkalinity, hydration, photodecomposition and etc.) and biological (animal or plant physiology, production environment and etc) of its environment, a sample in general suffers alteration in its natural balance (ROCHA; ROSA; CARDOSO, 2004).

In this sense, it is evident the need for an analytical tool to determine the antioxidant capacity that seeks to solve the problems presented by the existing techniques and that offers the advantages of easy construction, high precision, the sensitivity of detection and possibility of use both in the laboratory and *in loco*.

5. MATERIAL AND METHODS

The experimental work was developed in the Laboratory of the Group of Studies and Environmental Analyzes - GEEA of the Federal University of Maranhão (UFMA), in laboratories of the Federal University of Roraima (UFRR) and the Biosensors-Analyzes-Environment Laboratory (BAE) of the University of Perpignan Via Domitia (UPVD).

5.1 Reagents and Other Materials

All reagents used were of analytical purity grade and the water used was deionized (Milli-Q Millipore 18.2 MΩ cm^{-1}). Prussian blue or ferric ferrocyanide (PB) was obtained from the Gwent Group (Torfaen, United Kingdom). Poly (Vinyl Alcohol) Azide-unit pendant Water-soluble Photopolymer (PVA-AWP) was purchased from Toyo Kogyo Corporation (Chiba, Japan). Petroleum ether, sodium hydroxide (NaOH), potassium biphthalate ($C_8H_5KO_4$), phenolphthalein ($C_{20}H_{14}O_4$), sulfuric acid (H_2SO_4), potassium iodide (KI), anhydrous starch, potassium iodate (KIO_3), nitric acid (HNO_3), hydrogen peroxide (H_2O_2), yttrium oxide (Y_2O_3), quercetin ($C_{15}H_{10}O_7$), ascorbic acid ($C_6H_8O_6$), gallic acid ($C_7H_6O_5$), Folin-Ciocalteu (FC), sodium carbonate (Na_2CO_3), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), potassium persulphate ($K_2S_2O_8$), sodium chloride (NaCl), dibasic sodium phosphate (Na_2HPO_4), monobasic potassium phosphate (KH_2PO_4), dibasic potassium phosphate (K_2HPO_4), potassium chloride (KCl), ethylenediaminetetraacetic acid (EDTA), nitroblue tetrazolium (NBT), hypoxanthine (HX), enzyme xanthine oxidase from bovine milk (XOD) and absolute ethanol (C_2H_6O), Dulbecco's Modified Eagle's Medium (DMEM), Fetal Bovine Serum (SBF), non-essential amino acid (NSA) solution, penicillin ($C_{16}H_{18}N_2O_4S$), streptomycin ($C_{21}H_{39}N_7O_{12}$), amphotericin ($C_{47}H_{73}NO_{17}$), trichloroacetic acid (ATA), sulforhodamine B (SRB), acetic acid ($C_2H_4O_2$), tris ($C_4H_{11}NO_3$), were all purchased from Sigma-Aldrich Corporation (Nasdaq-Sial, Darmstadt, Germany). Methanol (CH_3OH), acetonitrile (C_2H_3N), formic acid (CH_2O_2), and acetone (C_3H_6O) were also obtained from Sigma-Aldrich, and hydrochloric acid (HCl) was from Merck (Seelze, Germany).

Standard solutions of phenolic compounds were prepared from dilutions in methanol of 53 standards of 1000 mg L^{-1} of degree of purity required by the technique, in amber glass bottles. All phenolic compounds studied were purchased from Sigma-Aldrich (Steinhein, Germany).

The materials used as glasses and plastics employed in elemental determinations were previously left in a 10% (v/v) HNO_3 bath for at least 24 h, washed with water and dried. The solutions of Ca, Cu, Fe, Li, Mg, Mn, Na and Zn were prepared from the dilution of analytical purity standards at 1000 mg L^{-1} (Merck Millipore Certipur®; Specsol®) in 2% (v/v) HNO_3.

All precision volumetric material was previously calibrated.

5.2 Equipment

The freeze drying was performed in a lyophilizer (Liotop, L101) for 48 h until complete dehydration, operating at 30 10^{-3} mmHg pressure and temperature of -50 ° C. Figure A4 of Annex A shows the photos of the equipment.

The extractions for the elementary determinations were carried out in a microwave oven (CEM Corporation, MARS Xpress 6.0, Matthews, NC, USA). For extraction of the minerals, the samples were inserted in Teflon vessels that accompany the equipment. Figure A5 of Annex A shows the photos of the equipment, especially the vessel turntable where the Teflon vessels are located.

For the elementary analyzes, an Inductively Coupled Plasma Optical Emission Spectrometry System, ICP OES (Shimadzu, ICPE-9820, Japan) under 1.2 kW of power and 10 L min^{-1} of argon flux. Figure A6 of Annex A presents photos of the ICP OES systems.

For extraction of antioxidant compounds, a sample blender (Invitrogen, HulaMixer, Carlsbad, USA) was used. Centrifugation of extracts was performed in a 12-hole centrifuge (Hettich, Routine 380 R, Tuttlingen, Germany). Spectrophotometric determinations were performed on ELISA reader (Multiskan Ex Primary, Shanghai, China) using Ascent software, version 2.6. Absorbance readings, during cell viability tests, were performed on a multiple scan spectrophotometer (Biotex Sinergy, Siafrtd, Vermont, USA).

Chromatographic determinations of the phenolic compounds were performed using a UHPLC Accela system (Thermo Fisher Scientific, San Jose, CA, USA) (Figure A7, Annex A), equipped with a quaternary pump, an auto sampler and a Ascentis Express C18 reverse phase column of porous layer (150 × 2.1 mm, partially porous particle size of 2.7 μm) supplied by Supelco (Bellefonte, PA, USA). The UHPLC system was coupled to a high-resolution mass spectrometry system Q-Exactive Orbitrap (Thermo Fisher Scientific) equipped with a heated electrospray ionization source (HESI-II) operated in negative ionization mode. The raw mass spectrometry data were processed by Exact Finder software version 2.0 (Thermo Fisher Scientific) through the application of a list of databases, comprising the 53 phenolic compounds studied and characterized.

The amperometric measurements were performed using a MicroAutolab III Type potentiostat (Metrohm, Netherlands), using the General Purpose Electrochemical System (GPES) software, version 4.9. Figure A8 of Annex A presents photos of the potentiostat, highlighting the custom connector for adaptation of the biosensor. The measurements were performed on a 10 mL dark glass cell filled with 50 mmol L^{-1} KPBS buffer containing 10 mmol L^{-1} KCl (pH 7.5) under magnetic stirring (300 rpm) at room temperature.

5.3 Characterization of Fruits of the Amazon Region

5.3.1 Sample and Sampling

Ten fruits native to the Amazon region were included in this study and data on the evaluated portion and collection sites are presented in Table 2.

The selection of the fruits bacuri, biribá, inajá, monguba, pajurá, and uxi occurred due to the scarcity of information about their chemical and biological properties in the Brazilian food tables (FRANCO, 2005; TACO, 2011; BRASIL, 2015), as well as by the devaluation of these fruits in the national market without at least knowing their potentialities (ANUÁRIO BRASILEIRO DA FRUTICULTURA, 2015). On the other hand, the fruits of abiu, açaí, buriti, and cupuaçu were also studied because there is information in the literature useful for comparison purposes with the results obtained in this research.

Table 2 - Agronomic information, classification and location of sampling of the Amazonian fruits studied

Common name	Scientific name	Family	Portion Analysed	Geographic location
Abiu	*Pouteria caimito*	Sapotaceae	Pulp	02°49’12,4”N 60°42’03,4”W
Açaí	*Euterpe oleracea*	Arecaceae	Pulp type B	02°47’10,6”N 60°45’05,8”W
Bacuri	*Platonia insignis*	Clusiaceae	Pulp	02°31'46,1"S 44°18'21,9"W
Biribá	*Rollinia orthopetala*	Annonaceae	Pulp	02°53'54,0"N 61°01'01,9"W
Buriti	*Mauritia flexuosa*	Arecaceae	Pulp	03°22'17,7"N 59°51'45,0"W
Cupuaçu	*Theobroma grandiflorum*	Sterculiaceae	Pulp	02°50’43,3”N 60°39”40,8”W
Inajá	*Maximiliana maripa*	Arecaceae	Pulp	02°46’34,7”N 60°42’17,1”W
Monguba	*Pachira aquática*	Malvaceae	Seeds	02°53'54,9"N 61°00'59,1"W
Pajurá	*Couepia bracteosa*	Chrysobalanaceae	Pulp	02°40'20,1"S 56°46'13,7"W
Uxi	*Saccoglotis uxi*	Humiriaceae	Pulp	02°36'59,9"S 56°39'28,8"W

The samples were properly packed in plastic bags, labeled, stored in refrigerated thermal boxes and transported immediately to the laboratory. In the laboratory, the samples were washed in abundant running water with the purpose of removing soil particles, dust, and other residues. They were then dipped in deionized water three times, dried at room temperature and stored at -20 ° C (freezer) until analysis. The samples, on a wet basis, were used in the bromatological and mineral analyzes and in the determination of vitamin C concentration. While a part of the samples was lyophilized and stored in vacuum packages and under light for the other analyzes (see Figure A9, Annex A).

Exsiccates of the samples were produced and incorporated into the Herbarium of the Integrated Museum of Roraima (MIRR) to resolve any doubts about the taxonomic identification of the material under study.

5.3.2 Bromatological Characterization

The values of moisture, ash, proteins, and lipids, as well as pH and acidity in citric acid, were determined in triplicate, according to the official methodologies (Table 3) adopted by the Institute Adolfo Lutz (IAL, 2008) and Association of Official Analytical Chemists-AOAC (CUNNIFF, 1998).

Table 3 - Description of the bromatological methods used

Parameter	Method	Method Code
Moisture	Direct oven drying at 105 ± 5°C	012/IV
Acidity in citric acid	Titration with NaOH	016/IV
pH	Direct measurement in pH meter	017/IV
Ash	Muffle heating at 550 ± 10°C	018/IV
Lipids	Direct extraction in soxhlet with petroleum ether extractor	032/IV
Proteins	Kjedhal classic	036/IV

SOURCE: IAL, 2008.

The carbohydrate content, in percentage, was calculated by the percentage difference, considering the sum of the values of proteins, lipids, moisture, and ashes (AOAC, 1997). The total energy value, in Kcal per 100 g of sample, was estimated considering the heat of combustion and the digestibility from protein, lipid and carbohydrate contents, using the conversion coefficient of 9 Kcal g^{-1} to lipids and 4 Kcal g^{-1} to protein and carbohydrate (MERRILL; WATT, 1973).

5.3.3 Determination of mineral contents

The conventional procedure (AOAC, 2002) was used to extract the minerals from the vegetable matrices in a microwave oven, followed by the analysis of the elements using the ICP OES technique. Masses of 1.0 to 2.0 g of the homogenized samples (wet mass) were taken directly in a digestion tube, so as not to exceed 0.5 g of the sample in dry mass. To the vessel, 5.0 mL of concentrated HNO_3, 2.0 mL of 30% (v/v) H_2O_2 and 0.5 mL of 100 mg L^{-1} Y_2O_3 were added sequentially, the latter used as the internal

standard. The mixture was subjected to a microwave oven heating program, whose operating conditions are presented in Table 4 (AOAC, 2002).

Table 4 - Digestion program in microwave oven

Process Steps	Power (W)	Duration (min)
1	250	3
2	630	5
3	500	22
4	0	15

The resulting solution was diluted to 25.0 mL and filtered on quantitative filter paper (28 μm), due to the presence of particulate matter. Blanks were prepared for each of the samples. All analyses were performed in triplicate.

The determination of the elements Ca, Cu, Fe, Li, Mg, Mn, Na, and Zn was performed by ICP OES technique, whose general operating conditions and specifications are presented in Table 5.

Table 5 - Experimental operating conditions of ICP OES

Parameters	Data
Spectrometer	Shimadzu, Modelo 9820
Software	ICPEsolution Launcher
Nebulizer	Concentric
Radio Frequency Generator	1,2 Kw
Argon flow	Auxiliary: 0.6 L min^{-1} Principal: 10 L min^{-1}
Flow of drag gas	0.7 L min^{-1}
Peristaltic pump rotation speed	20-60 rpm
Background correction	2 points
Torch configuration	Radial: Mg e Na Axial: Ca, Cu, Fe, Li, Mn e Zn
Linhas de Emissão Atômica (λ nm)	Ca (183.801); Cu (327.396); Fe (259.940); Li (610.364); Mg (383.826); Mn (257.610); Na (589.592); Zn (213.856).

The instrumental optimization for the determination of each mineral species studied was done through sensitivity, linearity, and wavelength. The analytical curves were set to seven points. In this study, the following parameters were adopted in order to characterize analytical efficiency: precision through CV, LOD, LOQ, and accuracy.

LOD represents the minimum concentration or mass of analyte that can be detected at a known confidence level (TALEUZZAMAN, 2018), which was obtained by averaging ten (10) determinations of the standard deviation of the standard blank signal (*Sbr*) and the slope of the analytical curve (*m*), multiplied by factor 3 (three), according to expression (1):

$$\mathrm{LOD} = \frac{3 \,.\, Sbr}{\mathrm{m}} \qquad (1)$$

The LOQ represents the lowest concentration where the quantitative determinations can be performed, which was calculated by means of 10 (ten) times the ratio of the average of 10 (ten) determinations of the standard deviation of the blank signal of the samples (*sbr*) and the slope of the analytical curve (*m*), according to expression (2):

$$\mathrm{LOQ} = \frac{10 \,.\, \mathrm{sbr}}{\mathrm{m}} \qquad (2)$$

The accuracy of the method indicates the agreement between the result of an assay and the reference value accepted as conventionally true (DONATI; AMAIS; WILLIAMS, 2017; GHOSH *et al.*, 2013). In the absence of certified reference material for the matrices in the analyzed analytes, the accuracy evaluation was done through standard addition experiments and calculation of the recovery indices for three samples (biribá, buriti and uxi), in two levels of concentration, with three replicates each, following the same procedure given to the samples. The concentration levels added were established through literature compilations, considering the concentration in the sample and the LOQ obtained for each metal species. Thus, the following minimum and maximum levels were used, respectively, for the fortification tests: 8.0 and 16.0 mg L^{-1} for the macrominerals Ca, Mg and Na; 1.5 and 3.0 mg L^{-1} for Li; 0.2 and 0.4 mg L^{-1} for Cu, Fe, and Zn microminerals; 0.12 and 0.24 mg L^{-1} for Mn.

5.3.4 Determination of antioxidant capacity

To determine the antioxidant capacity of the samples, the extraction method was previously defined. For this, the DPPH assay was selected and applied in buriti and uxi samples. In this step, the influence of the time, temperature, initial sample concentration and mode to the recovery of the supernatants were evaluated, when samples were submitted to sample mixer shaking and ultrasound assistance (Figure 11). Were evaluated four different time of extraction (overnight, 3 h, 1 h and 5 min), two temperature (4°C and T_{amb}), two initial sample concentration values (25 and 100 mg mL^{-1}), and three supernatant recovery procedures [centrifugation; 0.45 µm cellulose membrane filter filtration; 0.45 µm polytetrafluoroethylene (PTFE) filter filtration].

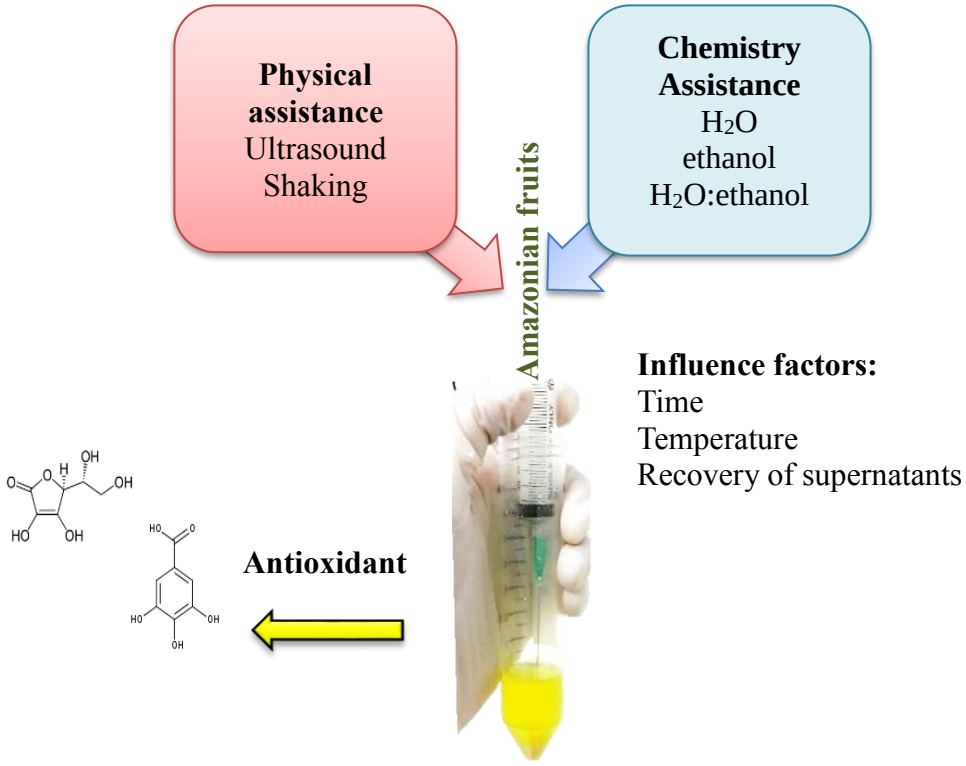


Figure 11 - Assistances and conditions evaluated for the antioxidant extraction method definition.

The best inhibition results were obtained by extraction using 100 mg mL^{-1} of lyophilized samples, followed by shaking at 4°C, protected from light, for 1 h in the sample mixer. The supernatant was collected after centrifugation for 10 min at 5000 rpm.

Extracts from lyophilized samples, in decreasing concentrations, were prepared by dilution and submitted to the miniaturized tests of DPPH, ABTS and NBT and the results expressed as the concentration of the sample (mg mL^{-1}) capable of inhibiting 50% of the oxidant sources (IC_{50}) in ascorbic acid equivalents (standard antioxidant). All assays were performed in triplicate on a micro plate reader.

5.3.4.1 DPPH method

The effect of the ethanolic extract of each antioxidant fruit on the $DPPH^{\bullet}$ radical was estimated according to the recommendations of MARINOVA and BATCHVAROV (2011), which evaluated the solvent and the sample/ DPPH reagent ratio as the main factors that significantly influence the accuracy of the DPPH assay. Preliminary tests employing extract of the aqueous and hydro ethanolic fruits (1:1) showed the formation of a suspension in the micro plates inherent in limiting the polarity of the DPPH reagent to organic solvents. For this reason, only the ethanolic extracts were evaluated for this method.

Thus, a stock solution of $DPPH^{\bullet}$ at 1.75 mmol L^{-1} was prepared by dissolution in ethanol, which was stored at 4°C under light protection until the time of analysis. From the ethanolic dilutions of the stock solution, the working solution of $DPPH^{\bullet}$ was prepared in order to obtain for the control (100%) an absorbance around 1.0 ± 0.1 unit at 490 nm.

Briefly, the reactive solution (AOX) was prepared by mixing ethanol, anti-oxidant extract (formed by sample extract) and $DPPH^{\bullet}$ working solution, in the sequence and in the amounts shown in Table 6. Ethanol was used for blank control (100%) and the solution formed by ethanol/antioxidant extract was used as AOX blank in order to eliminate interferences due to the color of the samples. The decrease in absorbance was recorded at 490 nm after incubation for 20 min at 25°C, 400 rpm and under light protection.

Table 6 - Reagent addition sequence for DPPH method

Reagents	AOX	Blank AOX	Control (100%)	Blank Control (100%)
Ethanol (µL)	200	225	225	250
Antioxidant extract (µL)	25	25	-	-
DPPH• working solution (µL)	25	-	25	-
Incubation: 20 min, 400 rpm, 25°C, under light protection. Measure at 490 nm				

A summary schematic of the DPPH assay is shown in Figure 12.

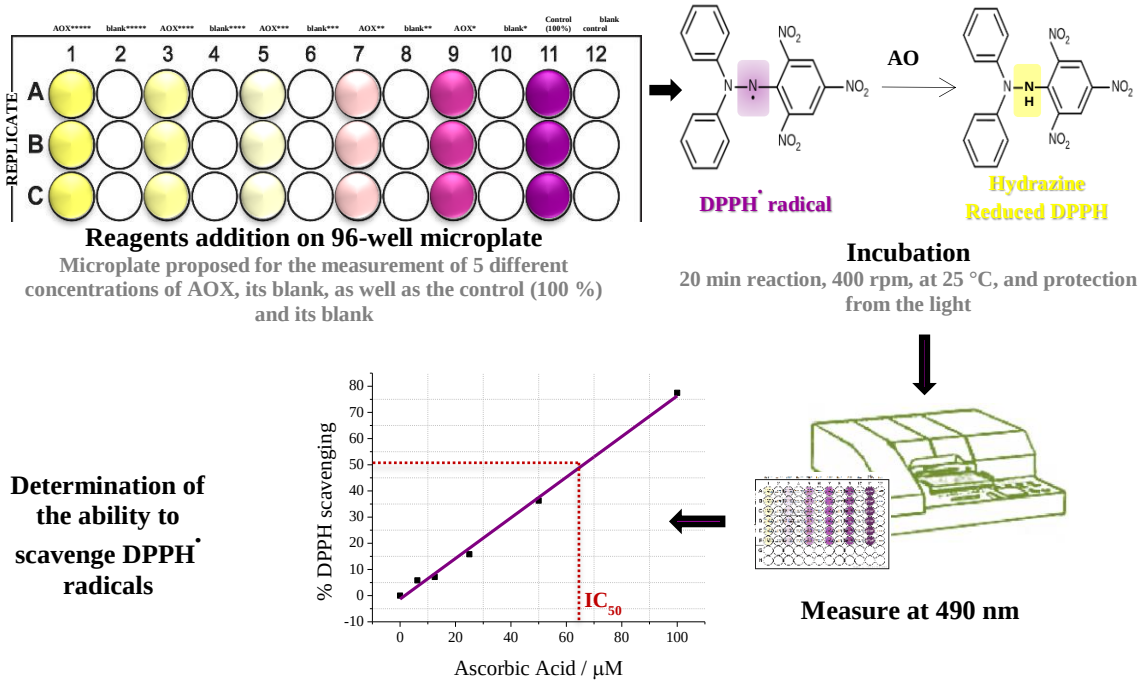


Figure 12 - Miniaturized DPPH assay scheme.

5.3.4.2 ABTS method

The ABTS assay was performed according to the procedure proposed by ARNAO *et al.* (2001) with some modifications. To allow the extraction of lipophilic and hydrophilic antioxidants from lyophilized samples, the ABTS assay was applied to three extracts of different polarities: aqueous (PBS 10 mmol L^{-1}, pH= 7.4), ethanolic and

hydroethanolic (1:1, v/v), according to the extraction procedure previously established in section 5.2.4.

A stock solution consisting of 7.0 mmol L^{-1} ABTS and 2.45 mmol L^{-1} $K_2S_2O_8$ in 10.0 mmol L^{-1} PBS buffer solution (pH= 7.4) was pre-prepared and incubated for 12 h at room temperature and under light protection to allow chemical equilibrium of the $ABTS^{\bullet+}$ cation radical generation reaction.

From the dilutions of the stock solution, using the same solvent used in the extractions, the working solution of $ABTS^{\bullet+}$ was prepared in order to obtain for the control (100%) an absorbance of 0.7 ± 0.07 units at 405 nm.

The reactive solution (AOX) was prepared by mixing the antioxidant solution (fruit extract) with the $ABTS^{\bullet+}$ working solution, as shown in Table 7. The solvent was used as the control blank (100%), while the solution formed by the solvent/antioxidant solution was used as a fruit blank in order to minimize interferences caused by the color of the samples. The decrease in absorbance was recorded at 405 nm after incubation.

Table 7 - Reagent addition sequence for ABTS method

Reagents	**AOX**	**Blank AOX**	**Control (100%)**	**Blank Control (100%)**
Solvent (μL)	-	225	25	250
Antioxidant solution (μL)	25	25	-	-
$ABTS^{\bullet+}$ working solution (μL)	225	-	225	-
Incubation: 3 min, 400 rpm, 25°C. Measure at 405 nm.				

5.3.4.3 NBT method

For the NBT assay, the method proposed by CORTINA-PUIG *et al.* (2009) was applied with some modifications. All solutions were prepared in buffer solution of 50 mmol L^{-1} KPBS (pH 7.5) containing EDTA (0.1 mmol L^{-1}) due to the required enzymatic conditions. The reagent addition sequence, as well as, the incubation steps is shown in Table 8.

Table 8 - Reagent addition sequence for NBT method

Reagents	**AOX**	**Blank AOX**	**Control (100%)**	**Blank Control (100%)**
KPBS (μL)	150	175	175	200
HX (μL)	25	25	25	25
Antioxidant extract (μL)	25	25	-	-
NBT (μL)	25	25	25	25
First stage of incubation: 5 min, 700 rpm, 25°C, under light protection				
XOD (μL)	25	-	25	-
Final stage of incubation: 15 min, 700 rpm, 25°C, under light protection. Measure at 560 nm				

The control (100%) was prepared by mixing KPBS buffer, 0.75 mmol L^{-1} HX, 0.75 mmol L^{-1} NBT, followed by homogenization (first incubation step), the addition of the solution enzyme XOD at 0.70 U mL^{-1} and the final incubation. An absorbance around 0.28 ± 0.02 units at 560 nm is desired for the control (100%).

A reaction mixture (AOX) was prepared with the reactants, in the sequence and amounts reported in Table 8. Control blank (100%) and AOX blank were prepared in the absence of the enzyme solution, the final volume of 250 μL being filled with KPBS buffer solution. The decrease in absorbance was recorded at 560 nm after final incubation stage.

5.3.5 Anticancer Cell Viability Tests

Caco-2 cells line were submitted to the treatment and determination of the cytotoxicity against Amazonian fruit extracts, using the colorimetric SRB method that constitutes a very sensitive cytotoxicity marker, as described by SKEHAN *et al.* (1990).

SRB is used for the determination of cell density, based on the measurement of cellular protein content. The assay is based on the ability of the anionic SRB dye to electrostatically bind protein components of cells that have been attached to tissue culture plates by trichloroacetic acid. SRB is a bright pink amino-xanthene dye with two sulfonic groups that bind to the basic amino acid residues under mildly acidic conditions and dissociate under basic conditions. As the binding of SRB is stoichiometric, the amount of dry extract from the stained cells is directly proportional to the cell mass (SKEHAN *et al.*, 1990).

The caco-2 cells were cultured in a stock solution formed by the DMEM reagent supplemented with 20% fetal bovine serum, 1% non-essential amino acid solution, 1% penicillin (1000 U mL^{-1}), 1% streptomycin (1000 mg mL^{-1}), and 1% amphotericin (250 U mL^{-1}), in a humidified atmosphere of 5% CO_2 at 37°C.

The 96 wells of the micro plate were filled with 100 µL of the cell solution at a density of $4x10^3$ cells per well, incubated at 37°C for 24 h for cell growth. Then the culture medium was replaced by the fruit extracts prepared in medium solution, at concentrations ranging from 0 to 1000 µg mL^{-1}, in order to expose the cells to the extracts for 72 h. Subsequently, cell growth was evaluated using the SRB method (SKEHAN *et al.*, 1990), with experiments in quadruplicate. Cells were fixed with 100 µL of 500 g L^{-1} trichloroacetic acid (1h and 4°C), washed with distilled water and stained with 4 g L^{-1} SRB for 20 min at room temperature. In order to remove the excess of the dye (unbound), five washes were performed with 50 µL of 10 mL L^{-1} acetic acid in each well. The dye-bound proteins were extracted with 100 µL of 10 mmol L^{-1} TRIS base. The readings were carried out at 492 nm. The negative control, constituted by the cells cultured in the culture medium in the absence of fruit extracts, corresponded to the value of 100% survival.

A summary scheme of the protocol used is shown in Figure 13. The effect on caco-2 cell growth was expressed IC_{50}, that is, the concentration of the sample (µg mL^{-1}) capable of inhibiting 50% of cell growth.

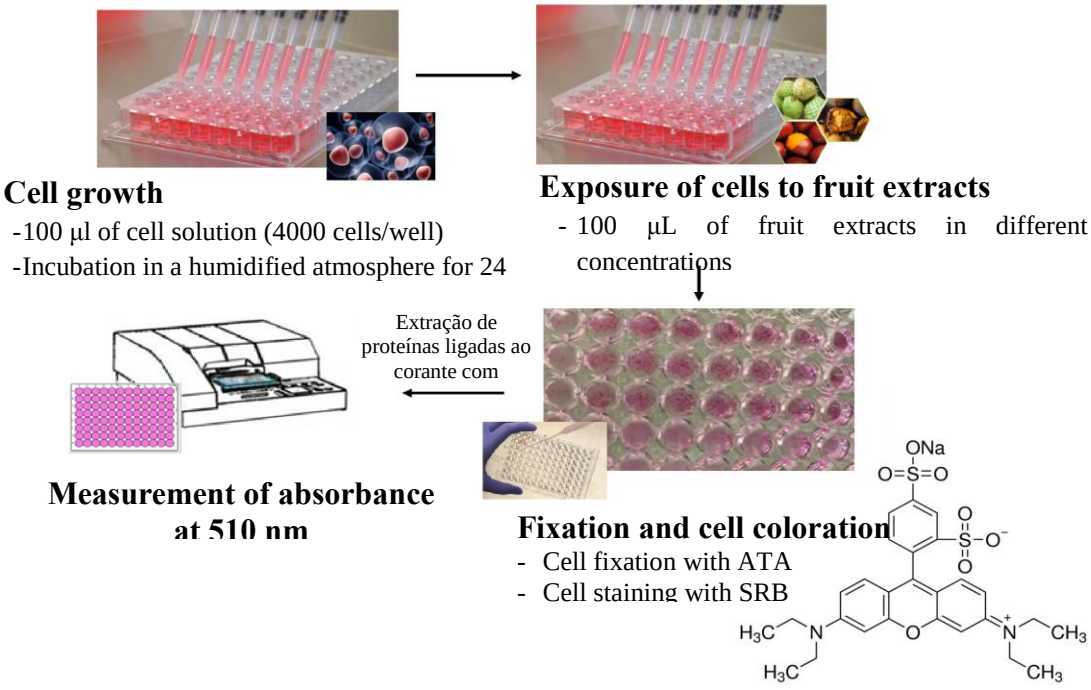


Figure 13 - Summary protocol of SRB method.

5.3.6 Bioactive compounds

5.3.6.1 Determination of ascorbic acid content (Vitamin C)

The determination of the ascorbic acid content ($C_6H_8O_6$) was performed by titration of oxide-reduction using KIO_3, according to IAL (2008). The oxidizing action of iodine (I_2), formed by the reaction between KIO_3 and KI, oxidizes $C_6H_8O_6$ to dehydroascorbic ($C_6H_6O_6$). When all of the $C_6H_8O_6$ has reacted, the excess of I_2 formed by the addition of a further drop of KIO_3 will be indicated by the starch, which acquires blue coloration in the presence of free I_2. The reactions that occur can be expressed by expressions (3) and (4):

$$KIO_3 + 5\ KI + 3H_2SO_4 \rightarrow 3\ I_2 + 3K_2SO_4 + 3H_2O \quad (3)$$

$$C_6H_8O_6 + I_2 \rightarrow 2\ HI + C_6H_6O_6 \quad (4)$$

For this, a mass of the homogenized sample was taken in an Erlenmeyer flask assuring a minimum ascorbic acid content of 5 mg, 50 mL of water and 10 mL of 20% (v/v) H_2SO_4 was added. The mixture was homogenized, and 1 mL of 10% KI (w/v), 1 mL of 1% starch solution (w/v) and titrated with KIO_3 solution (0.02 mol L^{-1} or 0.002 mol L^{-1}) to blue coloration. The analyses were performed in triplicate. The concentration of vitamin c was determined by expression (5).

$$\text{Vitamin C (mg/100g)} = \frac{100 \cdot V \cdot F}{m} \quad (5)$$

Where: V is the volume of iodate spent in the titration; F is 8.806 or 0.8806 to 0.02 mol L^{-1} or 0.002 mol L^{-1} of KIO_3, respectively; m is the mass of the sample in grams.

5.3.6.2 Determination of phenolic compounds

5.3.6.2.1 Folin-Ciocalteu method

The content of total phenolic compounds was determined by the Folin-Ciocalteu colorimetric method described by SINGLETON AND ROSSI (1965), with some modifications. In order to extract lipophilic and hydrophilic phenolic compounds, the method was applied in extracts of lyophilized samples in different polarities, following the procedure of extraction previously optimized and described in item 5.2.4. The extracts were aqueous (10.0 mmol L^{-1} buffer solution PBS, pH= 7.4), ethanolic and hydroethanolic (1:1).

The reaction medium (AOX) was prepared by mixing 125 µL of the 10% (v/v) FC reagent and 25 µL of the antioxidant extract (fruit extract), followed by the first incubation step. 100 µl of the 7.8% (v/v) Na_2CO_3 solution was added, and the increase in absorbance at 750 nm was recorded after 1 h of reaction, which occurred under the protection of light, 400 rpm, and 37°C. The blank was prepared in the absence of antioxidant extracts by adding 25 µl of the solvent in order to obtain a final volume of 250 µL. The procedure of additions and incubations of the method, adapted to the micro plates, is presented in Table 9.

An analytical curve of gallic acid in the range of 1 to 15 µg mL^{-1} was constructed, in order to obtain an absorbance of 1.0 ± 0.1 for the concentration of 15 µg mL^{-1}, measured at 750 nm. All measurements were performed in triplicate on a micro plate reader and the results expressed in mg of gallic acid equivalent (GAE) per 100 g of lyophilized sample.

Table 9 - Reagent addition sequence for the Folin-Ciocalteu method

Reagents	**AOX**	**Blank**
FC 10% (µL)	125	125
Solvent (µL)	-	25
Antioxidant extract (µL)	25	-
First stage of incubation: 8 min, 400 rpm, at 37°C		
Na_2CO_3 7,8% (µL)	100	100
Final stage of incubation: 1 h, 400 rpm, at 37°C		

5.2.6.2.2 Chromatographic analysis

The fruit extracts were submitted to qualitative analysis by UHPLC coupled to high-resolution mass spectrometry as proposed by BARBOSA *et al.* (2018) for the initial purpose of detecting the phenolic compounds present. After this preliminary analysis, samples of biribá, inajá and monguba were submitted to the quantitative determination of the phenolic compounds.

In the extraction step, a mass of 0.1 g of lyophilized sample was sonicated with 10 mL of a mixture of acetone: water: hydrochloric acid (70: 29.9: 0.1 v/v/v). Thereafter, the mixture was subjected to centrifugation at 3500 rpm for 15 min. The supernatant was collected, filtered on a 0.45 μm pore diameter nylon filter (Hatman, Clifton, NJ, USA) and stored at -4°C until analysis.

In order to obtain the separation and identification of a greater variety of phenolic compounds, two methods of elution were employed, denominated Method 1 and 2, the mobile phase of Method 1 being the mixture of a 0.1% aqueous solution of CH_2O_2 (solvent A) and the mobile phase of Method 2 is a solution of C_2H_3N also containing 0.1% CH_2O_2 (solvent B). Elution of the compounds was carried out according to the following polarity gradient: 0-1 min, 10% isocratic conditions of A; 1 to 20 min, linear gradient from 10% to 95% A; 20 to 23 min, isocratic step to 95% A; 23 to 24 min back to the initial conditions at 10% A; and from 24 to 30 min, 10% isocratic conditions of A to rebalance the column. The same elution procedure was used for solvent B (Method 2). The flow of the mobile phase was 300 $\mu L.min^{-1}$ and the injection volume (in full loop mode) was 10 μL. 53 standard phenolic compounds belonging to different families (phenolic acids, benzoic acids, cinnamic acids, phenolic aldehydes, phenolic terpenes, flavones, flavanols, proanthocyanidins and stilbenes) were monitored during the analysis. Parameters including chromatographic retention time, mass errors, isotopic patterns and spectra of product ions with normalized collision energies were used for identification and confirmation purposes.

5.3.7 Chemometric Analyzes

The Origin Pro 8.6 software was used to perform the analysis of variance (ANOVA), followed by the Tukey test in order to verify the existence of significant

differences between the concentrations, accepting significance p <0.05. To evaluate the correlation between the parameters was used the linear regression technique.

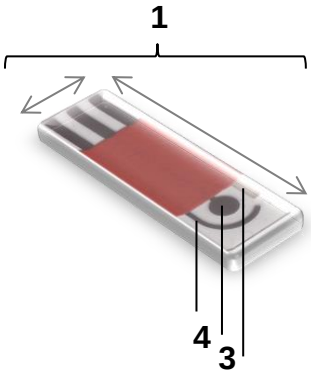
The Principal Component Analysis (PCA) was performed, where the data were previously self-staggered, in order to assign the same weight to all variables. The chemical analyses were performed in triplicate, to construct the 23x30 data matrix (twenty-three variables and ten samples, in triplicate).

5.4 Development of an Amperometric Biosensor for the Determination of Antioxidant Capacity

An amperometric biosensor able to measure the antioxidant capacity of natural or processed plant samples against the combined EROs, H_2O_2 and $O_2^{\bullet-}$ radicals, was developed in this study.

5.4.1 Electrochemical sensor

A conventional system consisting of 3 electrodes printed on a thin transparent polyvinyl chloride (PVC) plate was used, which constituted the electrochemical sensor. The sensor was screen printed on a semiautomatic printing machine (DEK 248). Figure 14 shows the sensor (1) in which the following electrodes were printed: pseudo-reference electrode (2), working electrode (3), and auxiliary electrode (4). Figures A10 and A11 (Annex A) elucidate the production of the sensors, employing a screen-printing machine.



1 – Screen printed electrode

2 – Pseudo-reference electrode

3 – Working electrode (WE)

4 – Auxiliary electrode

Figure 14 - Screen-printed electrode.

In the sensor (1), the pseudo-reference electrode (2) consisted of a straight line 5 × 1.5 mm in diameter, formed by a mixture (paste) of Ag/AgCl. The working electrode was composed of a disc of 4 mm in diameter, formed by a commercial graphite paste containing Prussian blue salt as the modifier. The auxiliary electrode, formed of a curved line with 16x1,5 mm, contained only the commercial graphite paste.

In this study, a fixed concentration of KCl was established when all solutions were prepared in phosphate buffer solution (KPBS) pH 7.5. The buffer contained 33.33 mmol L^{-1} K_2HPO_4, 16.67 mmol L^{-1} KH_2PO_4 and 10 mmol L^{-1} KCl. In addition to maintaining enzymatic activity, the buffer solution was used as the electrolyte and reflects pH and salinity conditions that approximate the physiological ones.

5.4.2 Enzymatic immobilization and optimization

The XOD enzyme was immobilized on the surface of the working electrode by entrapment with the polymer PVA-AWP. For the immobilization procedure, a homogeneous mixture containing the enzyme solution of the enzyme XOD and PVA-AWP was prepared. A volume of 3 μL of the mixture was deposited with a micropipette for viscous liquids (Gilson brand) on the surface of the working electrode. The electrode was maintained under cold neon light at 4°C (15 W), in order to allow polymerization and the consequent entrapment of the enzyme in the working electrode.

Constructing response surface diagrams optimized the immobilization procedure. In order to ensure the best conditions of bio functionalization of the sensor, the optimized variables were: XOD enzyme loading (C_{XOD}), enzyme: polymer ratio (R_{XP}) and photo polymerization time (T_P). The Box-Behnken Design program was used, considering 3 factors and 3 levels: C_{XOD} (5, 8 and 10 mU/WE), RXP [1: 2 (or 0.33); 1: 1 (or 0.50); and 2: 1 (or 0.66)] and Tp (48, 24 and 0.5 h). The statistical program constructed 15 sets of fractional factorial experiments.

5.4.3 Biosensor principle and amperometric measurements

The detection principle was based on the measurement of H_2O_2 generated as the final product of the enzymatic reaction between HX and XOD enzyme, or by spontaneous dismutation of the $O_2^{\bullet-}$ radicals. The enzymatically formed H_2O_2 was reduced on the

surface of the polarized electrode (E = -100 mV *vs* Ag/AgCl), and the generated current was proportional to its concentration. As the antioxidants inhibit the ROS, their presence induces a decrease in the cathodic current, thus allowing evaluation of the antioxidant capacity.

All amperometric measurements were performed on dark glass cells of 10 mL capacity, at room temperature and under magnetic stirring at 300 rpm. The biosensor was previously evaluated by immersing it in the cell containing 10 mL of KPBS buffer solution pH = 7.5 and then measuring the generated current at a fixed work potential of -100 mV *vs* Ag/AgCl, which corresponds to the reduction of the generated H_2O_2. The initial current intensity was recorded after the PVA-AWP swell, followed by signal stabilization, in a total time of approximately 50 min.

As a negative control, the production of ROS was used without its neutralization by antioxidants.

Briefly, a 20 µL volume of 5 mmol L^{-1} HX was added to the KPBS buffer solution and, after each addition of the HX substrate and signal stabilization (generally 60 s), the intensity of the generated current was recorded. An analytical curve of current intensity as a function of HX concentration was constructed and the angular coefficient recorded (m_a).

Then, a new analytical curve of increasing concentrations of HX was constructed, but in the presence of the antioxidant solution (standards or samples). The produced EROs (H_2O_2 and $O_2^{\bullet-}$) were captured by antioxidants present, inducing a decrease in current intensity. As a consequence, a lower slope value was obtained (m_b) and recorded. The antioxidant capacity was then expressed by the percentage of inhibition of ROS by comparing the angular coefficients obtained in the curves constructed in the absence and presence of antioxidants, according to expression 6.

$$\text{Antioxidant capacity (\%)} = 100*[1-(m_b/m_a)] \quad (6)$$

5.4.4 Analysis of real samples and reference antioxidant

Samples of non-autochthonous fruit *in natura* (strawberry, orange, and passion fruit) and pulp of Amazonian fruits (graviola, bacuri, and murici) were purchased from supermarkets in the city of São Luís, MA, Brazil. After washing the fruits, 10% (v/v) pulp

nectars and fresh fruit refreshment at 25% (v/v) were prepared, both in KPBS buffer solution.

The antioxidant capacity for each fruit was determined as described in section 5.4.3. Gallic acid was used as a reference antioxidant.

5.4.5 Electrochemical characterization and analytical efficiency of the biosensor

The following merit figures were used to evaluate the analytical efficiency of the developed biosensor: precision, expressed through CV; absolute sensitivity, expressed by the slope of the analytical curve (m); linearity, expressed as the range of concentrations at which the sensitivity (or slope) can be considered constant; and relative sensitivity, expressed by limits of detection (LOD) and quantification (LOQ). LOD values were obtained from the ratio between the standard deviation of 10 determinations (blank measurements) and the slope of the analytical curve (m), multiplied by factor 3; for LOQ, is multiplied by factor 10 (INMETRO, 2016).

In order to give greater evidence to the accuracy of the method using the biosensor, a statistical control chart was established, which determines the warning and action limits (upper and lower). The warning limit consists of the value $\mu \pm 3\sigma$ and the action limit, of the value $\mu \pm 10\sigma$, where μ = mean of the determinations and σ = standard deviation. This was done to monitor the performance of the measurements made by the biosensor (OLIVEIRA *et al.*, 2013).

6. RESULTS AND DISCUSSION

6.1 Characterization of the Amazonian Fruits Studied

In this work, we contributed to the knowledge of nutritional, antioxidant and bioactive properties of ten fruits native to the Amazon region, which was selected and purchased *in natura* in the states of Amazonas, Maranhão, and Roraima, according to the collection sites presented on the map (Figure 15), which constructed from the geographical co-ordinates as presented in Table 1.

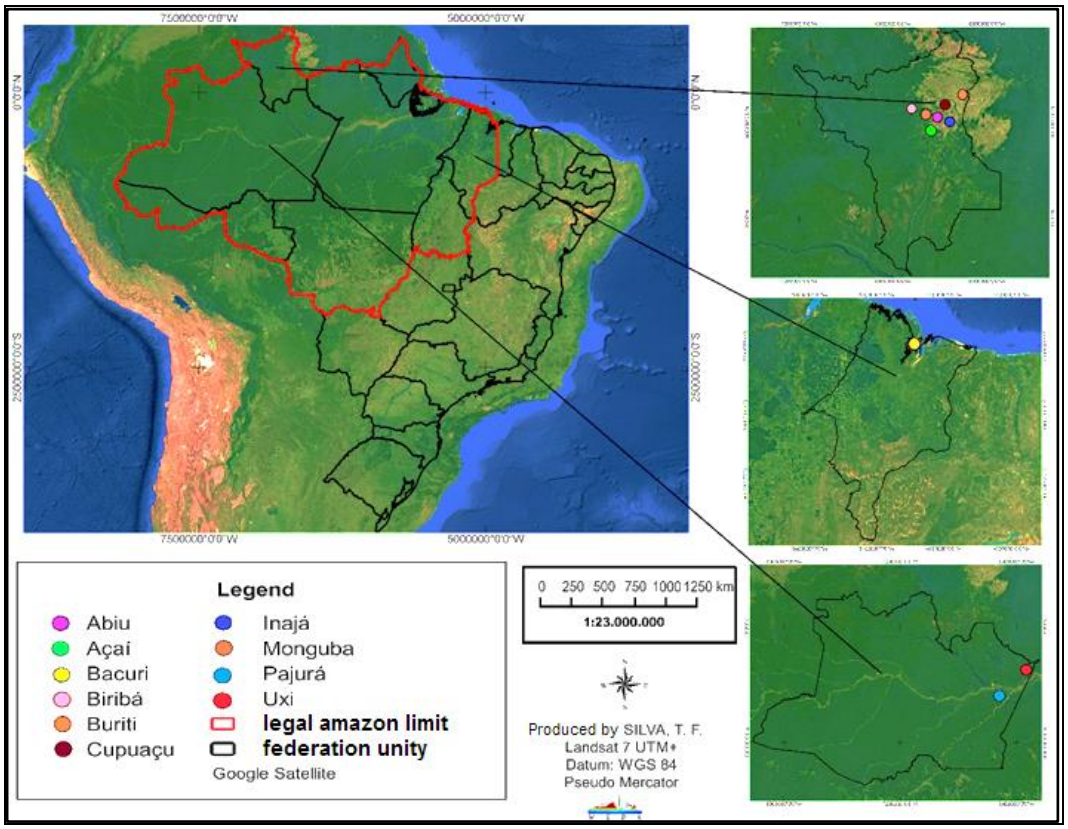


Figure 15 - Map with the geographical location of the collection sites.

Information about the period of fruiting is essential because it allows predicting the periods of reproduction of the plants, their growth cycles, the period for obtaining fruits and the seeds for silvicultural purposes, besides other characteristics of great value in the forest management, for health specialists and consumers in general (Figure 16).

Figure 16 shows information about the fruit formation period evaluated in this study, which constructed by compiling the fruiting periods presented in the literature

(BRASIL, 2015; FERCÃO; LLERAS; KERR, 1981; RIOS; PASTORE JUNIOR, 2011). The fruiting seasonality data, when confronted with information on nutritional, antioxidant and bioactive property are an indication of sources of nutrient supply to humans and other animals in the course of each year.

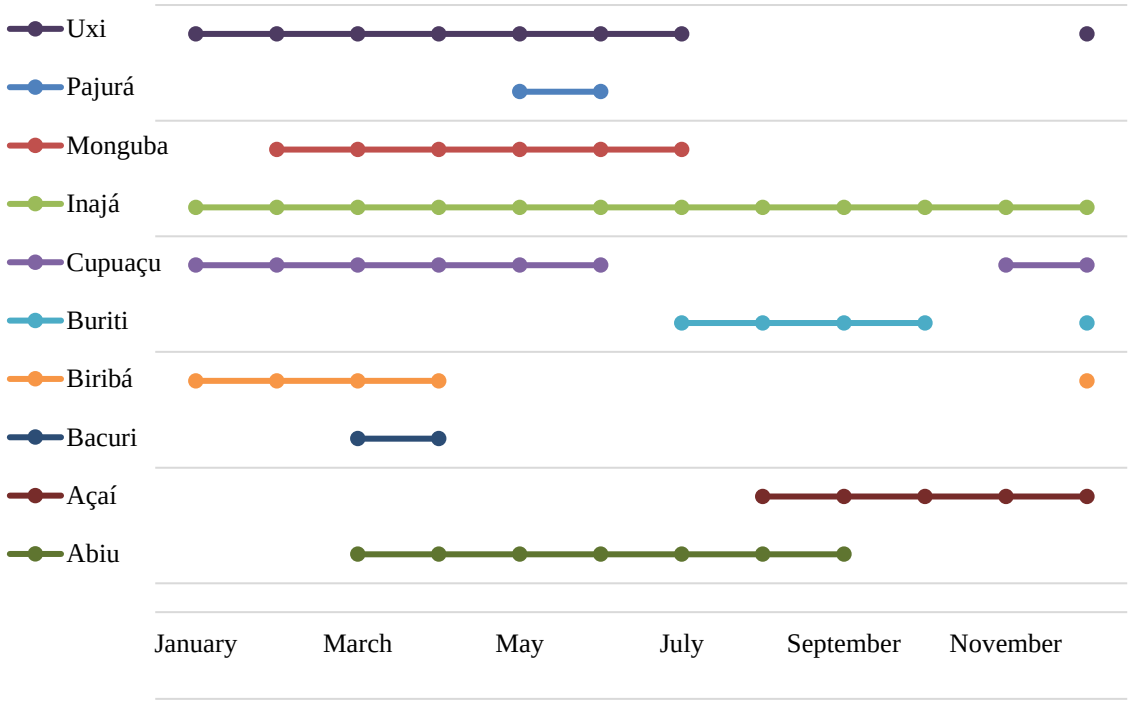


Figure 16 - Seasonality of fruiting of Amazonian fruits under study throughout the year.

6.1.1 Bromatological characterization of fruits

The bromatological characterization of Brazilian foods has been stimulated to gather information, updated, reliable and adequate to the national reality. Therefore, the knowledge of food composition is of paramount importance for assessment of nutrition and adequacy of the diet for individuals' populations, agricultural planning and the food industry (ARAGÃO, 2013).

The results obtained from the physicochemical characterization are presented in Table 10. The results showed highly accurate, with coefficients of variation (CVs) lower than 11.8%.

Evaluating the moisture factor, the fruits had contents higher than 60% in majority, except for buriti pulp (55.9%) and uxi (31.7%). Therefore, buriti and uxi pulps are less expected for the loss of quality over time, since moisture reflects the bound and free water content present in the fruit and the latter is responsible to be used as a culture medium for microorganisms that cause undesirable changes (BOLZAN, 2013). On the other hand, these data can provide to both the consumer and the industry, when choosing the fruit with high water content for the diet and formulation of products that require this characteristic. In order to compare the moisture contents with the literature, it is observed some agreement with those reported for uxi pulps (MARX *et al.*, 2002; BERTO *et al.*, 2015a), abiu (LOVE; PAUL, 2011), biribá, pajurá, bacuri (BERTO *et al.*, 2015a), açaí (NASCIMENTO, 2008), buriti (MANHÃES; SABAA-SRUR, 2011), cupuaçu (TACO, 2011), inajá (BEZERRA *et al.*, 2006) as well as for monguba seeds (OLIVEIRA *et al.*, 2000).

The ash content was less than <2.1% for all samples and the pulp of biribá was found to have the highest mineral content.

The uxi and buriti pulps and the monguba almonds presented the highest levels of lipids (18.7 to 23.3%) and proteins (2.1 to 2.4%). In the present study, a strong positive correlation ($R^2 = 0.92$) was observed between these two parameters.

Some studies have reported lipid content ranges for uxi pulp (10 to 31%) (MARX *et al.*, 2002; BEZERRA *et al.*, 2006), with some agreement with those obtained here. The nutritional classification accepted until today in Brazil (BRASIL, 1998) allows qualifying pulps of abiu, bacuri, biribá, cupuaçu and pajurá as being of low lipid content (<3 g $100g^{-1}$).

Table10 - Physical-chemical characterization of *in natura* fruits

Amazon Fruits	Moisture	Ash	Lipids	Proteins	Carbohydrates	CA*	Energy Kcal $100g^{-1}$	pH
	g $100g^{-1}$							
Abiu	71.7 (0.2)[a]	0.3 (9.3)[a]	0.3 (3.6)[a]	0.2 (4.3)[a]	27.4	0.06 (5.0)[a]	113.4	6.8 (0.6)[a]
Açaí	88.2 (1.9)[b]	0.7 (10.5)	4.3 (0.4)[b]	0.7 (7.2)	6.1	0.08 (6.6)[a]	66.3	5.7 (0.3)[b]
Bacuri	91.2 (0.2)	0.2 (11.7)[a]	0.4 (9.9)[a]	0.3 (2.1)[a,b]	7.9	0.6 (5.5)	36.2	3.7 (1.4)[c]
Biribá	89.5 (8.2)	2.1 (8.3)	0.06 (6.2)[a]	1.0 (9.2)[c]	7.4	0.2 (5.6)[a,b]	34.2	5.8 (4.6)[b]
Buriti	55.9 (0.3)	1.3 (1.2)[b]	21.0 (0.5)	2.1 (5.9)[d]	19.7	0.9 (1.1)	276.3	3.8 (8.4)[c]
Cupuaçu	82.3 (0.3)[b]	1.1 (3.1)[c]	0.6 (10.6)[a]	0.4 (2.5)[b]	15.6	1.8 (9.7)	69.2	4.1 (0.5)[c,d]
Inajá	67.5 (0.1)[a,c]	1.2 (1.0)[b,d]	4.3 (8.6)[b]	0.5 (1.5)[b]	25.5	0.07 (4.7)[a]	146.5	5.7 (0.4)[b]
Monguba	70.4 (1.2)[a]	1.4 (1.7)[b,d]	18.7 (7.0)	2.4 (5.2)	7.2	0.3 (8.7)[c]	206.5	6.7 (3.4)[a]
Pajurá	63.0 (0.5)[c]	0.9 (3.1)	0.1 (10.8)[a]	0.9 (0.8)[c]	35.0	0.2 (10.6)[a,b]	144.8	5.5 (3.8)[b]
Uxi	31.7 (2.5)	1.2 (3.8)[b,c,d]	23.3 (11.8)	2.2 (4.9)[d]	41.7	0.2 (6.9)[b,c]	384.7	4.4 (3.7)[d]

Results obtained on wet basis. In parentheses, we have the coefficients of variation (CV, %): precision of the measurements. Means followed by the same letter, in the same column, do not differ significantly from each other, by the Tukey test at the 5% probability level. * CA = Acidity in citric acid.

The protein concentrations obtained for the pulp of abiu and cupuaçu were lower than those reported by the Department of Basic Attention of the Ministry of Health (BRASIL, 2015). The same happened in relation to the inajá fruit and the monguba seed when comparing the studies developed by Mota and França (2007) and Azevedo (2008), respectively. For the fruit of açaí, biribá and buriti pulps, the results found in this study are in agreement with those presented by Yuyama *et al.* (2011), Berto *et al.* (2015a); Morton (1987), and Manhães e Sabaa-Srur (2011), respectively. For the pajurá and uxi pulps, the results were superior to those reported by Berto *et al.* (2015a).

Although they are not recognized as protein sources, the inclusion of plant proteins in the human diet from the fruits studied can have several health benefits compared to animal proteins, since they are free from saturated fats and chemical additives, and also help transit intestinal, have a high nutrient density and a lower environmental impact in their production (MOURE *et al.*, 2006).

The main role of carbohydrates is to provide energy to the body's cells. Regarding the percentage of carbohydrates in the fruits studied, the highest content was found in the uxi pulp (41.7%).

In general, a negative linear correlation was observed between the moisture content and the values of lipids, proteins, carbohydrates, and energy (R^2 = -0.74, -0.62, -0.84, -0.95, respectively). It is mainly influenced by the water content, which generally represented the highest percentage of fruit composition, followed by carbohydrate, lipid, protein and mineral contents (BOLZAN, 2013).

The caloric content was directly related to the lipid, protein and carbohydrate contents in the samples (R^2 = 0.90, 0.78, 0.64, respectively). All of them had an energy value higher than 40 Kcal $100g^{-1}$, except for pulp of bacuri and biribá, which can be classified as low-energy foods (BRASIL, 1998). These fruits may, therefore, be indicated for energy restriction diets; the others, for hypercaloric diets.

Although the highest pH value and the lowest acidity in citric acid were observed in the abiu pulp (6.8 and 0.06 g $100g^{-1}$, respectively), with a certain negative correlation (R^2 = -0.63) which led to conclusion that always a high pH value not responsible for a low acidity in citric acid. This is easily explained in analytical terms, since the pH measure indicates the amount of free H^+ ions, while the titratable acidity measures the total concentration of H^+ ions (bound and free) (IAL, 2008).

Most of the fruits contemplated here have a dense nutritional composition, which has been very important in the diet of the animal diversity of the Amazon region, within its seasonality of occurrence (Figure 16) and, so in its equilibrium; however, many of these fruits are still little used in the Brazilian diet.

6.1.2 Mineral composition of fruits

The results of the concentrations of minerals (mg $100g^{-1}$, wet mass) in fruits are presented in Table 11. The table also presents, as a figure of merit that characterizes the accuracy of the analytical method, the indices of recoveries (%); to characterize the value of precision, the coefficients of variation (CVs, %); and to characterize the sensitivity, the limits of detection (LODs) and quantification (LOQs) values, in mg L^{-1}, were presented. Thus, Table 11 shows not only the results of the minerals present in the samples but also how rigor the performance of the analytical method.

After the analysis, the results were used to perform a nutritional evaluation of the fruits, based on the Technical Report of the Ministry of Health (BRASIL, 1998). This official document classifies foods in two classes: 1) food as a source in a mineral, when 100 g of this mineral presents 15 to 29% of its Dietary Reference Intakes (DRI), and 2) food with high mineral content when 100 g more than 30% of your DRI. The classification matrices results have been studied according to the DRI as presented in Table 12.

Evaluating the results of the concentrations (Table 11), it was observed that the CV values are below 10% for most determinations, indicating the accuracy of the results.

The results of the recoveries obtained at the two concentration levels added for most mineral elements and samples are in the range of 90 to 110%, except for the Li element in the biribá and uxi samples. These results indicate the high accuracy in most of the analyzes, due to the lack of losses or contaminations during the procedures, either in the bench steps or in the final determination of the elements by ICP OES.

Table 11 - Mineral contents in Amazonian fruits studied and figures of merit of the analytical method employed

Mineral	Ca	Li	Na	Mg	Cu	Fe	Mn	Zn
LOD (mg L^{-1})	$6.5\ 10^{-2}$	$0.1\ 10^{-3}$	$1.0\ 10^{-1}$	$2.0\ 10^{-3}$	$4.0\ 10^{-4}$	$5.0\ 10^{-4}$	$7.1\ 10^{-6}$	$3.0\ 10^{-4}$
LOQ (mg L^{-1})	2.4	$0.4\ 10^{-3}$	3.5	$2.2\ 10^{-2}$	$1.7\ 10^{-3}$	$2.9\ 10^{-3}$	$1.2\ 10^{-5}$	$1.7\ 10^{-3}$
Amazonian fruits	**CONCENTRATION mg $100g^{-1}$**							
Abiu	9.5 (6.2)[a]	3.6 (13.6)[a]	44.4 (8.6)	8.3 (8.2)[a]	0.2 (7.0)[a]	0.3 (10.8)[a,b]	0.08 (7.1)[a,b]	0.3 (9.3)[c,d]
Açaí	61.5 (6.2)	15.3 (5.7)	4.1 (3.4)[a]	12.6 (4.7)	0.1 (5.8)[b,c,d]	0.8 (1.3)[c]	7.9 (3.5)	1.0 (3.9)[a]
Bacuri	7.0 (5.0)[a]	1.7 (5.0)	13.6 (5.2)	7.0 (5.0)[a]	0.2 (9.6)[a,b]	0.2 (8.1)[a]	0.02 (6.9)[b]	0.6 (8.9)[b]
Biribá	34.4 (3.8)	9.1 (3.8)	1.1 (4.5)[a]	25.8 (8.7)	0.09 (5.3)[b,d]	0.2 (4.1)[a,d]	0.1 (5.2)[a,b]	0.2 (10.2)[c]
Buriti	107.1 (1.6)	28.3 (1.6)	3.0 (5.9)[a]	84.3 (3.1)[b]	0.2 (2.2)[a,c]	0.9 (1.9)[c]	3.2 (4.1)	0.9 (2.6)[a]
Cupuaçu	17.5 (8.0)[b]	4.2 (8.8)[a,b]	1.2 (1.5)[a]	36.3 (6.2)	0.1 (5.7)[b,d]	0.3 (5.1)[a,e]	0.1 (5.6)[a,b]	0.3 (7.4)[d]
Inajá	19.8 (7.7)[b,c]	5.1 (7.5)[c]	4.2 (5.9)[a]	57.5 (8.2)	0.08 (9.1)[d]	0.5 (1.6)[b,e]	0.2 (7.0)[a]	1.1 (4.0)
Monguba	55.9 (4.1)	14.3 (3.9)	1.1 (2.1)[a]	87.5 (5.0)[b]	0.8 (6.2)	0.4 (9.9)[b,d,e]	0.2 (8.5)[a]	1.0 (8.3)[a]
Pajurá	19.2 (4.0)[b,d]	5.0 (3.9)[b,c]	68.6 (6.7)	21.3 (2.2)	0.1 (9.4)[a,b,d]	0.4 (4.8)[a,e]	0.2 (2.4)[a]	0.7 (6.6)[b]
Uxi	22.3 (6.2)[c,d]	5.5 (5.5)[c]	2.9 (10.0)[a]	40.7 (1.4)	0.3 (7.5)	1.2 (5.8)	0.7 (2.8)	0.5 (6.1)
Fortification Testing	**RECOVERY %**							
Biribá	93.1 (2.9) 103.1 (11.1)	70.0 (2.7) 112.0 (10.5)	108.6 (1.6) 108.1 (4.9)	107.3 (0.8) 109.6 (1.6)	110.5 (0.6) 106.6 (3.1)	101.5 (6.6) 102.6 (5.0)	97.2 (1.2) 103.2 (7.7)	116.5 (4.9) 109.5 (7.7)
Buriti	107.1 (9.9) 104.5 (8.9)	98.1 (2.4) 101.1 (7.1)	107.2 (4.0) 100.9 (1.3)	108.3 (1.6) 88.5 (7.0)	107.8 (5.9) 102.9 (2.1)	97.0 (8.0) 98.0 (2.5)	100.0 (11.8) 100.0 (0.0)	109.5 (7.4) 90.3 (3.3)
Uxi	102.0 (5.8) 98.1 (4.2)	85.6 (16.5) 82.8 (8.5)	102.8 (5.3) 96.6 (2.5)	110.6 (1.6) 103.6 (3.6)	106.5 (10.0) 103.5 (1.6)	107.3 (6.9) 103.2 (7.0)	100.3 (2.4) 95.4 (2.8)	112.0 (12.9) 101.5 (3.0)

Results obtained on wet basis. Ca = calcium; Li = lithium; Na = sodium; Mg = magnesium; Cu = copper; Fe = iron; Mn = manganese; Zn = zinc. In parentheses, we have the coefficients of variation (CV, %): precision of the measurements. LOD (limit of detection) and LOQ (quantification limit): sensitivity of the analytical method. Results based on the wet mass. Means followed by the same letter, in the same column, do not differ significantly from each other, by the Tukey test at the 5% probability level.

Table 12 - Recommended Daily Intake Values (DRI) in minerals for a healthy adult

Minerals	DRI (mg dia^{-1})	Samples	% DRI attended	Classification
Mg	260[a]	Buriti	32.4	Alto teor
		Inajá	22.1	Fonte
		Monguba	33.7	Alto teor
		Uxi	15.6	Fonte
Cu	0.9[a]	Abiu	22.3	Fonte
		Bacuri	17.7	Fonte
		Buriti	21.3	Fonte
		Monguba	83.0	Alto teor
		Pajurá	15.6	Fonte
		Uxi	35.7	Alto teor
Fe	8.0[b]	Uxi	15.0	Fonte
Mn	2.3[a]	Açaí	342.9	Alto teor
		Buriti	138.5	Alto teor
		Uxi	29.0	Fonte

Mg = magnesium; Cu = copper; Fe = iron; Mn = manganese. Percentage of DRI contemplated in 100 g wet mass of samples and their mineral classifications. Sources: [a]BRASIL, 2005; [b]INSTITUTE OF MEDICINE, 2006.

The highest calcium content (Ca) was obtained in buriti pulp (107.1 mg 100g^{-1}), followed by açaí and monguba. The fruit groups whose Ca concentrations did not present significant differences were: 1) abiu and bacuri; 2) cupuaçu, inajá and pajurá; 3) inajá and uxi; and 4) pajurá and uxi. With these results, it is possible to establish a balanced diet, with no overlap of fruits with the same contribution to mineral calcium, essential for bone formation and prevention of degenerative bone diseases, especially osteoporosis.

A positive correlation ($R^2 = 0.63$) was observed between calcium and magnesium (Mg) levels possibly due to the controlled and similar metabolic absorption and distribution of these chemical species in plant nutrition, together with an availability of the balanced production environment (SALVADOR; CARVALHO; LUCCHESI, 2011). Negative correlation could be expected if there was an imbalance in the absorption of the bioavailable minerals Ca and Mg, since the absorption by the vegetal in excess of one of these elements would result in the decrease of the absorption of the another with consequent decrease in the vegetal development (SALVADOR; CARVALHO; LUCCHESI, 2011).

For the lithium element (Li), the recoveries were in the range of 70.0-112.0% in the biribá fruit, while for buriti and uxi samples recovery ranges were 98.1-101.1% and 82.8-85.6%, respectively. This showed the variability of the results for the Li between the

matrices but within the acceptable limits. The results for the buriti sample, were within an optimal range of accuracy (INMETRO, 2016; BRITO *et al.*, 2003).

The reference literature related to mineral analyzes employing spectrometric methods, which are based on the IPC OES technique, reports some spectral interferences during Li analysis. This is due to the superimposition of interelementares spectra during the plasma atomization stage, especially due to the presence of sodium (Na), potassium (K), calcium (Ca), and magnesium (Mg) elements, Cl^- (chloride) and PO_4^{3-} (phosphate) ions, ethanol and glucose molecules present in the samples (SAMPSON; RUDDEL; ELLIN, 1994; MISRA; FROELICH, 2009; BOND; CANTERFORD, 1971; ZERBINATI; BALDUZZI; DELL'ORO, 2000; SHALMI *et al.*, 1994; ROCKS; SHERWOOD; RILEY, 1982; VANHOE; DAMS; VERSIECK, 1994).

In order to avoid such interferences and getting a maximum sensitivity of the method, different wavelength (λ) values were tested in the present work: 323.261, 670.784, and 610.364 nm, the latter being the wavelength which showed the better recoveries. Matrix interferences caused by the substances that make up the sample has been reported in the determination of Li (SAMPSON; RUDDEL; ELLIN, 1994; MISRA; FROELICH, 2009). Based on these observations, it can be further concluded that, for the determination of Li in food samples by ICP OES, spectral and matrix interference studies will always be necessary.

The concentration of Li found in fresh samples of Amazonian fruits ranged from 1.7 to 28.3 mg $100g^{-1}$. Compared with other fruits consumed in the same region, apricot fruit from the north (9.5 mg $100g^{-1}$), also Amazonian, is within the range of values obtained in this study. A comparison of these results was also carried out with the reported Li content for the apple, a non-autochthonous fruit that was gradually introduced in the Brazilian diet, and is also appreciated by the Amazonians and Brazilian northerners. This fruit has much higher levels of Li (35 mg $100g^{-1}$) in its composition (VAITSMAN; VAITSMAN; AZEVEDO, 1991).

The minimum daily requirement for lithium has been caused for divergence. Although Schrauzer (2002) claims to be 1 mg day^{-1}, Marshall (2015) estimates the need for larger amounts, as there are individual differences that may require greater intake for optimal health, taking into account daily energy loss, which is different for each individual. The United States Environmental Protection Agency (EPA) estimated daily dietary intake of Li between 0.6 and 3.1 mg (SAUNDERS, 1985). In the Andes, north of

Argentina, the estimated daily intake is 2 to 30 mg (CONCHA; BROBERG; GRANDÉR, 2010). In France, average daily consumption of Li of 11 μg was estimated (NOËL; LEBLANC; GUÉRIN, 2003). It is known that very different mechanisms govern high and low doses of Li and that only at very high doses, about 50 to 300 times higher than the natural dietary intake of food and water, lithium acts as a drug (NOËL; LEBLANC; GUÉRIN, 2003). In the Amazon region, basal values for Li are not yet known. In addition, considering the possible spectral and matrix interference observed at the time of mineral determination, the values reported in the present study represent data to be evaluated with the progress of the research with this mineral.

The higher Na content was obtained for the pajurá sample, followed by abiu and bacuri. The other fruits did not differ statistically ($p < 0.05$, 95% confidence) in relation to Na concentrations. The ingestion of 100 g of the edible parts of the fruits studied could provide an amount of N, which is less than 15% of the DRI. This can be considered as an excellent nutritional data, considering the current actions of public health organs around the world in search of awareness focused on the reduction of this mineral in the diet of the population. These actions are caused by an increase in the incidence of hypertension, impairment of the kidneys and heart caused by excessive ingestion of Na (WHO, 2012; BRASIL, 2010; BRASIL, 2013).

The highest Mg contents were obtained for the samples of monguba and buriti, whose contents did not present significant differences between them. In the same way, for the abiu and bacuri pulps, no significant differences ($p < 0.05$) were observed for this mineral. In mineral nutrition of plants, Mg stands out for its functions in photosynthesis, lipid synthesis and the activation of enzymes that integrate protein metabolism (FAQUIN, 2005). This fact possibly explains the positive correlation observed in the present study between Mg contents and lipid macromolecules ($R^2 = 0.74$) and proteins ($R^2 = 0.75$).

Mg is an important mineral in human health by participating in almost all anabolic and catabolic actions, such as energy and protein metabolism, glycolysis and synthesis of adenosine triphosphate. Mg deficiency is related to several diseases, such as cardiovascular diseases, pre-eclampsia/eclampsia, hypertension, stroke, diabetes mellitus, bronchial asthma, as well as its possible involvement in migraine, osteoporosis, alcoholism and immune system disorders (INSTITUTE OF MEDICINE, 2006; LUKASKI, 2004).

According to the nutritional classification referenced and accepted (BRASIL, 1998), the samples of monguba and buriti have high levels in Mg, contributing with 33.7 and 32.4% of the DRI of a healthy adult, respectively. The pulps of inajá and uxi can be considered as natural sources of this element. Therefore, the monguba seeds and the buriti, inajá and uxi pulp may contribute with Mg to attend DRI and to prevent a deficiency in this mineral species and, consequently, the development of chronic diseases (JAHNEN-DECHENT; KETTELER, 2012).

The benefits of the presence of mineral copper (Cu) in human nutrition includes help in maintenance of the bone structure, central nervous system and absorption of iron (VAITSMAN; DUTRA; AFONSO, 2001). On the other hand, there are studies that show that ingestion in insufficient amounts of copper contributes to the development of cardiovascular diseases, microbial infections and anemia's; however, its excess is related to sclerosis, asthma, hypertension, depression, seizures, liver necrosis and cardio-respiratory problems (AZEVEDO; CHASIN, 2003; GOLDHABER, 2003).

Cu contents found in inajá and monguba fruits were 0.08 and 0.8 mg $100g^{-1}$, respectively. The Tukey test revealed a certain similarity between the majorities of the other samples for this mineral species. A positive correlation ($R^2 = 0.70$) was observed between Cu and protein contents in the fruits studied. The participation of this element in the synthesis of proteins in plants may explain this result (FAQUIN, 2005).

Evaluating the mineral classification in relation to the copper intake, it is observed that the pulps of abiu, bacuri, buriti, and pajurá can be classified as sources in this chemical species, because they attend with 22.3, 17.7, 21.3, and 15.6% of the DRI, respectively. The monguba seeds and the uxi pulp showed high Cu content, contributing with 83.0 and 35.7% of the DRI, respectively. In this sense, the insertion of these fruits into the diet would be very interesting, since they would contribute to meet the daily recommendation levels and meanwhile could also prevent health damage from mineral deficiency.

The uxi sample presented the highest iron content (Fe) (1.2 mg $100g^{-1}$), attending to 15% of the DRI of a healthy adult when ingested 100 g of its pulp. Thus, this fruit can be considered a potential source of Fe. This element is part of the structure of a series of enzymes that participate in oxidation reactions during plant metabolism and is closely related to hemoprotein and Fe-S-protein (MALAVOLTA, 1980). This fact may explain the positive correlation ($R^2 = 0.61$) between Fe and protein contents.

In relation to the mineral manganese (Mn), higher levels were found in the pulps of açaí, buriti, and uxi. The uxi pulp can be classified as a natural source in Mn (29% of DRI) and the pulps of açaí and buriti showed to be fruits with high content in this micromineral, exceeding 100% of the DRI. In spite of the large amount of Mn supplied through the consumption of 100 g of the açaí and buriti pulps, even though the resulted concentrations would still lead to an amount below the maximum tolerable intake level (11 mg day^{-1}), which is quite interesting from a toxicological point of view. Additionally, it should be considered that only 3 to 5% of the ingested Mn is effectively absorbed by the organism and available for normal metabolic functions since a large part of this element ends up being excreted in the stool (INSTITUTE OF MEDICINE, 2006; MARTINS; LIMA, 2001). Therefore, the effective inclusion of these fruits in the diet would bring several benefits, such as bone formation processes, reproductive function, and carbohydrate and lipid metabolism (INSTITUTE OF MEDICINE, 2006), without causing any intoxication.

The contents obtained for Zn in the studied fruits varied from 0.2 in the pulp of biribá to 1.1 mg $100g^{-1}$ in the inajá. Although the fruits are not classified as natural sources in Zn, the concentrations obtained can contribute to reaching the DRI in Zn through mineral supplementation with other foods.

In general, the fruits evaluated here, in particular, abiu, açaí, bacuri, buriti, inajá, monguba, pajurá, and uxi, presented levels of the analyzed minerals analyzed, in sufficient amounts for a normal nutrition.

The Food Composition Table (TACO, 2011) presents data on the composition of foods consumed in Brazil, such as information on the centesimal composition, mineral (Ca, Mg, Mn, P, Fe, Na, K and Zn) and vitamin (retinol, niacin, vitamin B1 - thiamine, vitamin B2 - riboflavin, vitamin B6 - pyridoxine and vitamin C). Despite the diversity in fruit, only six fruits native to the Amazon region were considered (açaí, abiu, cupuaçu, macaúba, tucumã, and pupunha), evidence by the necessity of a database on the chemical composition of native fruits of the largest tropical forest in the world.

Studies were found in the literature involving the determination of minerals in samples of Amazonian fruits. In some, the treatment given to the samples were based on the digestion of the pulp or seeds by wet digestion; in others, the calcination was employed, followed by the dissolution of the ashes into acid. In the most cases, as in the present work, the mineral analysis was based on the atomic spectrometry technique (BERTO *et al.*, 2015a;

ARAGÃO, 2013; YUYAMA *et al.*, 2013; NAOZUKA *et al.*, 2011; YUYAMA *et al.*, 2011; ECHEVERRI; ROMÁN-JITDUTJAANO, 2011; MANHÃES; SABAA-SRUR, 2011; DUARTE, 2008; LETERME *et al.*, 2006; MARIN, 2006).

Berto *et al.* (2015a), for example, characterized different portions (peel, pulp, and seeds) of ten fruits native to the Amazon through their centesimal compositions, mineral content (Mn, Zn, Fe, Cu, Mg and Na) and compositions of fatty acids. The samples were mineralized according to the method recommended by the AOAC to obtain ashes (CUNNIFF, 1998) and the determinations performed by flame atomic absorption spectrometry. The peel and the pulp showed high moisture contents, while the seeds presented higher levels of ash, crude protein, and total carbohydrate. High lipid levels were found in the piquiá fruits (pulp and seeds), in two varieties of the umari samples (peel and pulp), in the uxi pulp and in the biribá seed. The highest mineral levels were found in the samples of bacuri-azedo pulp (Mn, Zn), cubiu small seed (Cu), piquiá outer peel (Fe), cubiu large peel (Mg), and all pajurá parts (Na). In the analysis of fatty acids, the authors identified and quantified a total of 32 fatty acids. The highest levels of palmitic acid and oleic acid were found in the peels and pulps of the 2 varieties of umari and uxi and in the pulp and almond of piquiá, while the highest levels of linoleic acid and α-linolenic acid was found in the seed of biribá and pulp of uxi, respectively. The results contributed to the composition of the Brazilian food table and assisted in food security.

Yuyama *et al.* (2013) evaluated the physicochemical characteristics and the contents of mineral elements (Na, K, Ca, Mg, Mn, Zn, Fe, and Cu) in pulps of the fruits abiu, bacuri, carambola, ingá-cipó and mapati, all coming from the Amazon region. The lyophilized samples were submitted to a wet digestion treatment and the determinations carried out in an atomic absorption spectrometer. The pulp of abiu (dry mass) presented the highest concentration in Fe; bacuri, a higher Mg content; carambola, in Zn; inga-cipó, in K, Ca and Mn; and mapati in Na. However, high values of standard deviation indicated a great heterogeneity of the samples and uncertainty associated with the mean estimate.

Leterme *et al.* (2006) evaluated the elemental content (Ca, P, Mg, K, Na, Zn, Cr, Fe, Mn, Ni, Cu, Se, and Co) in 101 food samples (tropical fruits, leaves and tubers), including the monguba fruit, produced in the rainforest and Andes regions of Colombia. The samples were submitted to two different treatments: calcination at 450°C for 6 h (for analysis of the elements P, Ca, Mg, K, Na, Zn, and Cr) and wet digestion using the acidic mixture HCl:HNO_3 (1:3) under refluxing heating for 2 h (for Fe, Mn, Ni, Cu, Co, and Se

analysis). The efficiency of the determinations was validated using certified reference material of sheets. The results showed that the monguba seed samples had a high Mg, Cu, Fe, Mn, and Zn content, being still Ca source. In addition, the levels of nickel (Ni) presented below the LOQ. Until the conclusion of the present research, no other work that has performed the mineral characterization of the monguba seeds was found in the literature.

A comparison between the mineral composition of the fruits, found here, with the data of the literature can be a rather arduous and complex task, besides not recommended. Because it is necessary to take into account some intrinsically related factors, such as genetic (gender, species, and variety), environmental factors, soil, climate, stage of maturity of the plant and fruits, and collection periods (during flowering, in fruiting stage, under water stress, etc.). The analytical technique employed and the pretreatment procedures of the samples prior to their analysis, in general, spectrometry (SILVA; SANTOS; PAIVA, 1998; HARDISSON *et al.*, 2001).

Even so, the ranges of the contents obtained for the studied samples were confronted with some data of the literature, and the following are some works already published, in which the mineral content, in certain matrices, showed some agreement with the data presented in the present research: Mn in abiu samples (TACO, 2011); Ca, Na, and Fe in açaí samples (TACO, 2011; YUYAMA *et al.*, 2011); Na, Mg, Cu, Fe, Mn, and Zn in bacuri samples (BERTO *et al.*, 2015a; YUYAMA *et al.*, 2013); Ca, Mg, and Mn in biribá samples (BERTO *et al.*, 2015a; LETERME *et al.*, 2006); Fe in buriti samples (MANHÃES; SABAA-SRUR, 2011; MARIN, 2006); Fe, and Zn in cupuaçu samples (TACO, 2011; NAOZUKA *et al.*, 2011); Mg in inajá samples (DUARTE, 2008), and Mg in uxi samples (BERTO *et al.*, 2015a; ARAGÃO, 2013).

Further work can be cited in which the levels of minerals in certain matrices are below those found in the present research: Ca, Na Mg, Cu, Fe, and Zn in abiu samples (TACO, 2011; YUYAMA *et al*, 2013); Mn, and Zn in açaí samples (TACO, 2011; YUYAMA *et al.*, 2011); Ca in bacuri samples (BERTO *et al.*, 2015a; YUYAMA *et al.*, 2013); Ca, Mg, Cu, and Mn in cupuaçu samples (TACO, 2011; NAOZUKA *et al.*, 2011), and Zn in inajá and pajurá samples (ECHEVERRI; ROMÁN-JITDUTJAANO, 2011; DUARTE, 2008; BERTO *et al.*, 2015a). On the other hand, the levels of minerals and fruits to be found in this study were below those reported by these authors: Mg and Cu in açaí samples; Na, Cu, Fe, and Zn in biribá samples; Na in buriti and cupuaçu samples;

Ca, Na, and Fe in inajá samples; Ca, Na, Mg, Cu, Fe, Mn, and Zn in monguba samples; Na, Mg, Cu, Fe, and Mn in pajurá samples; and Ca, Na, Cu, Fe, Mn, and Zn in uxi samples.

No references were found in the literature related to Cu and Mn contents in inajá pulps, nor Ca in the pulp of pajurá.

6.1.3 Antioxidant capacity of amazonian fruits

Due to the chemical diversity of antioxidants and their behavior, which may respond differently to numerous oxidizing sources, there is still no single, simple and universal method for assessing antioxidant capacity, therefore it is necessary to evaluate the antioxidant capacity of antioxidants by different methods (BHATTACHARYYA *et al.*, 2014). In the present work, three methods (DPPH, ABTS, and NBT) were chosen for this purpose, based on their different reaction mechanisms and oxidant sources. In the extraction stage of the antioxidants from the samples, the following extraction systems were tested: pure ethanol, aqueous buffer solution (50 mmol L^{-1} KPBS containing 0.1 mmol L^{-1} EDTA pH = 7.5 or PBS 10 mmol L^{-1} pH = 7.4) and hydroethanolic solution (1:1). This initial study was important, considering aspects such as safety in handling, human consumption of ethanol in food products such as beverages, wines, and liqueurs, and the previous reference of these as antioxidant extractors (SULTANA; ANWAR; PRZYBYLSKI, 2007).

The results of the antioxidant capacity, expressed as IC 50 (mg mL^{-1}, dry mass), for fruit extracts using the different analytical methods are shown in Table 13.

It is observed that all the samples exhibited relevant antioxidant qualities and that few milligrams of the extracts were sufficient to inhibit 50% of the activity of the oxidant sources. It was possible to verify that the antioxidant capacity depended on the solvent used. Lower values of IC_{50}, which correspond to a higher antioxidant capacity, were obtained in the ethanolic extracts of the biribá and bacuri samples against the $DPPH^{\bullet}$ (<7.0 mg mL^{-1}) and $ABTS^{\bullet+}$ radicals (<0.6 mg mL^{-1}).

Table 13 - Antioxidant capacity (IC_{50}) of the different Amazon fruit extracts

Fruit	IC_{50} (mg mL^{-1})				
	DPPH	**ABTS**			**NBT**
	EtOH	EtOH	PBS	PBS:EtOH	KPBS
Abiu	9.8 (6.2)[a,b]	2.3 (2.2)[a]	0.3 (3.2)[a]	0.2 (6.3)[a,b]	0.4 (16.3)[a]
Açaí	9.8 (0.6)[a,b]	0.8 (3.7)[b]	0.9 (1.1)[b]	0.6 (1.67)	0.5 (2.0)[a]
Bacuri	7.0 (3.6)[a]	0.4 (16.7)[c]	0.6 (1.6)	0.4 (2.5)[c]	6.8 (4.9)[b]
Biribá	5.4 (1.3)[a]	0.6 (1.7)[b,c]	1.7 (7.0)[c]	0.5 (3.9)	7.6 (2.6)[b]
Buriti	79.7 (8.7)	13.4 (1.6)	1.5 (1.34)	0.9 (1.1)	7.3 (2.6)[b]
Cupuaçu	27.6 (0.4)[c]	9.2 (1.9)	0.8 (1.2)[b]	0.8 (3.9)[d]	5.3 (5.5)
Inajá	13.5 (4.9)[a,b]	2.4 (4.5)[a]	0.4 (7.5)[a]	0.4 (7.9)[c]	0.5 (3.8)[a]
Monguba	20.7 (3.0)[c]	5.0 (2.0)	0.4 (2.9)[a]	0.1 (9.1)[a]	0.4 (2.9)[a]
Pajurá	10.6 (3.9)[a,b]	2.0 (2.0)	1.7 (1.8)[c]	0.2 (5.9)[b]	6.7 (3.9)[b]
Uxi	106.1 (11.7)	3.7 (0.3)	1.2 (10.4)	0.8 (3.7)[d]	16.1 (7.7)

Results obtained on a dry basis. EtOH = ethanolic extracts; PBS = extracts in phosphate buffer 10 mmol L^{-1} (pH = 7.4); PBS: etOH = hydroethanolic extracts (1:1); KPBS = extracts in 50 mmol L^{-1} potassium phosphate buffer (pH 7.5) containing EDTA (0.1 mmol L^{-1}). In parentheses, coefficients of variation, CVs (%): accuracy of measurements. Means followed by the same letter in the same column do not differ significantly from each other by the Tukey test at the 5% probability level.

In general, for the ethanolic extracts, the IC_{50} values obtained by the DPPH method were higher when compared to those found by the ABTS method, which shows the need for larger amounts of samples to inhibit $DPPH^{\bullet}$ radicals. This was due to several factors, such as greater stability of the DPPH radical (resonance effect caused by the presence of the aromatic rings in their chemical structure with consequent distribution of the electronic charge throughout the molecule) (SHALABY; SHANAB, 2013; PRIOR; WU; SCHAICH, 2005); presence of compounds that have the ability to eliminate $ABTS^{\bullet+}$ radicals, which does not always occur against the radical $DPPH^{\bullet}$ (WANG *et al.*, 2008); period of incubation to reach a steady state; concentration of the radicals employed in the method, among others (CERRETANI; BENDINI, 2010).

Aqueous extracts of abiu, açaí, inajá, and monguba resulted in a higher antioxidant capacity against $O_2^{\bullet-}$ radicals by the NBT method (IC_{50} not exceeding 0.5 mg mL^{-1}). Aqueous extracts of abiu, inajá, and monguba were better inhibitors of $ABTS^{\bullet+}$ (IC_{50} <0.4 mg mL^{-1}) radicals, which revealed higher amounts of hydrophilic antioxidants in these samples.

From the biological point of view, the NBT test represents a more concise tool, once it allows researchers to evaluate the antioxidant capacity of a sample against a specific, biologically relevant free radical. The $O_2^{\bullet-}$ radical is a ROS produced in biological systems and in food, and behaves like most radical species that are extremely reactive and cytotoxic, being related to several disorders, such as Parkinson's disease and cancer (KHODADE *et al.*, 2014; VANELLA *et al.*, 1993; KONTOS; WEI, 1986; BIRNBOIM, 1986). According to the results obtained by this method, the Amazonian fruits abiu, açaí, inajá, and monguba can be considered foods with high antioxidant potential, promising for applications in the pharmaceutical industry and in medicine, in order to offer the greatest inhibitions of the $O_2^{\bullet-}$ radical.

The hydroethanolic extracts of the abiu, monguba, and pajurá samples had the highest antioxidant activity among all extracts and assays (IC_{50} < 0,2 mg mL^{-1}). Considering the results of the ABTS method for the same samples with different polarities of extracts, the hydroethanolic solution (1:1) exhibited better extractions of the antioxidant compounds than the monosolvent systems (buffer solution or pure ethanol). Due to the heterogeneity in chemical structure and, consequently, the polarity of the antioxidant compounds, binary solvents have been reported in the literature as best extractors of antioxidants (WANG *et al.*, 2008; ZHANG *et al.*, 2007).

Depending on the fruit analyzed and the region sampled, the antioxidant capacity found may be very different from that recorded in the literature.

This is because the analysis involves matrices grown under different conditions (climate, soil and water composition) and taxonomic varieties (species, varieties and cultivars), and there were differences in the sample preparation procedures, extraction of the antioxidant compounds (solvent, temperature, time and physical procedure), concentrations of solutions of oxidizing sources, ratio between sample/ reagent volumes, duration of reaction, wavelength of the absorbance measurement, standard solutions of antioxidants (usually gallic acid, trolox, and ascorbic acid) and the way to express the results (generally in percent inhibition of the radical for a given sample concentration, mass of a reference antioxidant per sample mass or extract, or IC_{50}) (ROCHA *et al.*, 2013). In this sense, the standardization of methods for the determination of antioxidant capacity has been the subject of discussion in scientific studies that seek to unify quantities and units, as well as admit comparisons (PRIOR; WU; SCHAICH, 2005; APAK *et al.*, 2013).

Even in view of the complexity of comparisons, the antioxidant capacity obtained for the fruits studied was compared with data in the existing literature (NEVES *et al.*, 2012; CANUTO *et al.*, 2010; RUFINO *et al.*, 2010).

Neves *et al.* (2012) evaluated the antioxidant capacity by DPPH and ORAC (oxygen radical absorbance capacity) and the content of phenolic compounds by the Folin method in 8 species of freeze-dried fruits (peel and pulp) native to the Amazon, among these açaí, inajá, and uxi. Methanol was used as the solvent, trolox as a internal reference antioxidant, and the results were expressed in μmol trolox equivalents per 100 g of sample. The results showed that, among the fruit pulps studied here, the açaí sample showed the highest antioxidant capacity against the $DPPH^{\bullet}$ radical, followed by the uxi and inajá samples. Although there is a difference between the reaction time (25 min) and wavelength (517 nm) parameters used by the authors and those employed in this study, preliminary tests performed in this study showed no statistical differences between the results when using reaction times of 20 and 25 min in the samples, which shows that for these samples, a plateau was reached in the reaction time of 20 min. In addition, in general, incubation periods of 20 or 30 min have been used to reach the endpoint of the $DPPH^{\bullet}$ radical neutralization reaction (MARINOVA; BATCHVAROV, 2011). In the same way, in this study previous tests were performed at two different wavelengths (490 and 560 nm), and the results did not show significant differences, confirming the reach of the effective absorption band of the DPPH ethanolic solution. Marinova and Batchvarov (2011) listed a wide variety of scientific publications that employed the DPPH method at wavelengths in the range of 492 and 540 nm and also showed that the precision of the method is dependent, in particular, on the solvent used and the ratio volume/DPPH reagent.

Canuto *et al.* (2010) determined, in 15 samples of pulps of Amazonian fruits, the physicochemical characteristics, the contents of bioactive compounds (ascorbic acid and phenolic compounds), and the antioxidant capacity by the ABTS method, after extraction in an ultrasound system. The authors tested different solvent extraction: methanol:water (8:2 v/v) provided the best results. For $ABTS^{\bullet+}$ method, the radical was diluted with PBS buffer solution (pH = 7.4); the analytical curve was constructed with an ethanolic solution of the synthetic trolox radical, and water was used as blank control. The results were expressed in μmol L^{-1} of trolox equivalents, and the authors proposed the following

decreasing sequence of the antioxidant capacity of the fruits: açaí> buriti> abiu = bacuri = cupuaçu.

The present study provided new data on the antioxidant capacity of Amazonian fruits using the spectrophotometric methods with microplates. This strategy shows today a tendency and brings several analytical advantages such as reduction of the number of samples and reagents, greater ease, speed, and simultaneous analysis of dozens of samples (SIGUEMOTO; GUT, 2017).

In general, all the Amazonian fruits contemplated in the present study and their respective extracts, in different solvents, presented antioxidant capacity against all evaluated radicals, natural or synthetic. Thus, fruits have potential antiradical properties to be exploited in many industrial sectors, such as in the food, pharmaceutical, and cosmetic industries.

It is worth mentioning that special emphasis must be placed here on the balance provided to the biota that routinely feeds on these fruits, including the man who inhabits the Amazonian and pre-Amazonian, indigenous, quilombolas, riverside communities, etc. Over the years, these communities have been greatly benefited by the presence of these plant species through their insertion into the human and animal diet. The presence of these plant species in these ecosystems has contributed greatly to the survival of several species, as well as the natural balance throughout the different trophic chains that make up these biomes.

6.1.4 Antiproliferative effect on Caco-2 cells

There is currently a growing search for new natural sources possessing cytotoxic and antioxidant effects in the modern medical industry. The relationship between the consumption of fruits and vegetables and the reduction of the risk associated with some health disorders, such as colon cancer has been evidenced (GALLAHER, TRUDO, 2013). Due to this trend, the effects of the proliferation of human colon cancer cell line (caco-2) on extracts of the ten Amazonian fruits were evaluated at this stage of the study.

Six different dilutions of fruit extracts (0, 62.5, 125, 250, 500, 1000 $\mu g\ mL^{-1}$) were tested and, the results of the in vitro antiproliferative effects of these different concentrations of fruit extracts on the caco-2 cell line after exposure for 72 h are presented in Table 14.

Table 14 - Results of cellular anticancer viability promoted by Amazonian fruit extracts

Fruits	IC_{50} (μg mL^{-1})
Abiu	ND
Açaí	272.3 (11.8)[a,b]
Bacuri	393.8 (10.5)[d]
Biribá	233.1 (8.9)[b,c]
Buriti	349.1 (10.0)[a,d]
Cupuaçu	ND
Inajá	229.9 (6.0)[c]
Monguba	$62.5 < IC_{50} < 250.0$*
Pajurá	67.6 (8.3)
Uxi	353.3 (9.0)[a,d]

Results obtained on a dry basis. In parentheses, coefficients of variation, CVs (%): accuracy of measurements. Means followed by the same letter in the same column do not differ significantly from each other by the Tukey test at the 5% probability level. ND = inhibition did not exceed 50% at the concentrations tested. * Variation in the antiproliferative effect between repetitions over time. Results of lower IC_{50} reflect greater antitumor protection.

The results showed that the increasing concentrations of the extracts of the Amazonian fruits led to an inhibition in the growth of the cell line tested.

Biribá, inajá, monguba, and pajurá extracts presented the lowest IC_{50} values, showing thus a great ability to induce Caco-2 cells death. This behavior was confirmed by the repetition of the experiment, in different months, for the same samples of the fruits of biribá, inajá and monguba in triplicate, with four internal replicates.

An internal variability between the results obtained for the monguba sample was observed, and this may have been caused by an increase in cytotoxicity over time, throughout the test replicates in different months. A possible explanation for this behavior would be the oxidation of specific compounds, previously inhibitors of cell death, present in the composition of the fruit. Thus, for the monguba sample, the results were expressed as a concentration range that allowed significant suppression of cell growth in all the analysis (IC_{50} less than 250 μg mL^{-1}).

On the other hand, abiu and cupuaçu extracts did not cause a significant reduction on cell viability, even at the highest concentration tested (IC_{50}>1000 μg mL^{-1}), so they were discarded as potential anticancer agents. Finally, bacuri, buriti, and uxi displayed

moderate antiproliferative activity when compared to the rest of the Amazon fruits previously mentioned.

Caco2 cells have been well characterized and used in numerous anticancer studies. In this line, positive antiproliferative tests on Caco-2 cell line by employing different plant extracts have been done (MÁRMOL *et al.*, 2017). Jiménez *et al.* (2016) achieved an IC_{50} value of 250 μg mL^{-1} with rosehip extracts from *Rosa canina*, and then Gascón *et al.* (2016) found that *Pinus pinea* and *Pinus pinaster* bark extracts displayed IC_{50} values of 40 and 30 μg mL^{-1} respectively. In this work, the obtained results suggested that the anticancer effect of biribá, inajá and monguba could be comparable to that from rosehip extracts. Pajurá could be comparable with pine bark. Moreover, the antiproliferative effect of such fruits could not be limited to colorectal cancer and further evaluation on different cancer cell lines should be performed in the future. A recent study developed by Shalom and Cock (2018) demonstrated the potential of the ethyl acetate, methanolic, and aqueous *T. ferdinandiana* fruit and leaf extracts to block proliferation of some cancer cells, including Caco-2 cell line. The Caco-2 cell IC_{50} results in aqueous extracts were higher than 1000 μg mL^{-1} to fruit, and leaf (1520 and 1230 μg mL^{-1}, respectively), which shows that the majority of evaluated fruits show a higher anticancer effect.

Focusing on the antiproliferative effect of exotic fruits, Silva *et al.* (2014) evaluated the anticancer activity of hydroalcoholic extracts of açaí toward a panel of cancer cell lines, including Caco-2. Authors noticed no significant changes in cell viability after 24 and 48h incubation noted with neither of the 10, 20, 40 μg mL^{-1} of the extracts. Since, we have obtained an IC_{50} value of 272±32 μg mL^{-1} after 72h incubation, it is not surprising that at such low concentrations and short incubation time they did not observe any decrease in cell viability. Regarding the rest of the evaluated Amazon fruit extracts, to the best of our knowledge, this is the first time on Caco-2 cells towards their anticancer potential.

This preliminary screening showed that the studied Amazon fruits are promising natural sources of bioactive compounds with antiproliferative effect against colorectal cancer Caco-2 cell line. Although the mechanism of action of the studied fruits remains unclear, a recent study points a possible cytotoxic effect toward a panel of cancer cell lines (SINDHI *et al.*, 2013). Therefore, a likely correlation between antioxidant capacity and antiproliferative activity must be considered. Moreover, since phenolic compounds are usually the main responsible for the antioxidant properties of fruits and vegetables

(HERVERT-HERNÁNDEZ *et al.*, 2011), the observed antiproliferative effect, as well as the antioxidant capacity previously showed, could be related to the presence of these compounds as has been previously reported (JIMÉNEZ *et al.*, 2016).

Cancer is characterized by uncontrolled cell proliferation, and it is likely that the antiproliferative mechanism of these extracts may involve the induction of apoptosis of the caco-2 cell line (JIMÉNEZ *et al.*, 2016). In this view, it is important to continue the evaluation of the way of cell death to which the caco-2 cells are induced by extracts of the fruits studied, in order to confirm the ability to induce cell death by apoptosis. The progress of this research may lead to therapeutic strategies for the consumption of functional foods.

6.1.5 Presence of bioactive compounds in fruits

6.1.5.1 Vitamin C

The reducing ability of vitamin C in various biochemical reactions characterizes its biological function. Among other oxidizing sources, vitamin C is able to reduce ROS. Its main function is as a cofactor of numerous reactions that require reduced Cu and Fe as water-soluble antioxidants acting intra and extracellular (VANNUCCHI; ROCHA, 2012).

The vitamin C concentrations obtained in the studied fruits are presented in Table 15, as well as their classification according to the DRI, established by the Brazilian government (BRASIL, 1998).

Vitamin C contents in fresh fruits were in the range of 5.2 to 80.0 mg 100 g^{-1} for the monguba almond and the açaí pulp, respectively. In Brazil, a DRI of 45 mg of vitamin C was established for a healthy adult (BRASIL, 2005), while the Institute of Medicine (2006) lists the values of DRI per life stage group, being 90 mg for a healthy adult man and 75 mg for an adult woman (INSTITUTE OF MEDICINE, 2006).

The definition proposed in Brazil allows to qualify pulps of biribá and pajurá as sources of vitamin C (contents of 15 to 29% of DRI) and the fruits of açaí, bacuri, buriti, cupuaçu, inajá, and uxi as food of high content in this nutrient (content> 30% of DRI) (BRASIL, 1998).

Table 15 - Vitamin C contents found in the Amazon fruits analyzed

Fruits	**Vitamin C (mg 100 g^{-1})**	**% DRI ingested***	**Classificação****
Abiu	5.3 (8.2)[a]	11.9	-
Açaí	80.0 (8.8)	177.6	High content
Bacuri	28.3 (11.4)[b,c]	62.9	High content
Biribá	10.0 (1.3)[a]	22.2	Source
Buriti	21.9 (7.5)[c,d]	48.6	High content
Cupuaçu	52.6 (0.6)	116.8	High content
Inajá	37.7 (3.7)[b]	83.7	High content
Monguba	5.2 (3.1)[a]	11.5	-
Pajurá	7.7 (8.9)[a]	17.1	Source
Uxi	15.0 (13.5)[a,d]	33.4	High content

Results obtained on wet basis. In parentheses, coefficients of variation, CVs (%): accuracy of measurements. The averages followed by the same letter are not different from each other, by the Tukey test at the 5% probability level. * BRASIL, 2005. ** BRASIL, 1998.

Despite the ingestion of 100 g of açai and cupuaçu pulps contributing in more than 100% of DRI, the tolerable upper intake level, which would be related to adverse effects, such as osmotic diarrhea and gastrointestinal disorders, is 2000 mg, and will not be achieved if we consider such intake.

Comparing the results obtained with the literature data, it is observed that the vitamin C contents are within the range reported for the abiu pulp (BRASIL, 2015; CANUTO *et al.*, 2010), açaí (RUFINO *et al.*, 2010), bacuri (BRASIL, 2015), buriti (GONÇALVES, 2008), cupuaçu (BRASIL, 2015; GONÇALVES, 2008), and uxi (GONÇALVES, 2008; MARX, 2002), while the contents obtained in this study for the pulp of biribá are superior to those reported by Villachica (1996).

No records of vitamin C concentration were found for inajá, monguba and pajurá fruits. Therefore, present work is the first attempt to present data about the presence of this nutrient in such fruits. The importance of new sources of vitamin C for insertion into the diet is of great interest to the area of food science and technology, as well as for the health area since it is an essential component for the physiological functions of the human body.

6.1.5.2 Phenolic compounds

The knowledge of the content of phenolic compounds in fruits is important because it reflects the mechanism of adaptation and resistance of the plant to the environment and influences the flavor and the technological characteristics of the food, as well as the nutritive and functional potential of these fruits (ROCHA *et al.*, 2011).

In the present study, the Folin-Ciocalteu method was used to measure the reduction of this reagent by the phenolic compounds present in the Amazonian fruits through the formation of a blue complex that was monitored spectrophotometrically at 750 nm, using gallic acid as reference phenolic compound. The concentrations of total phenolic compounds in the Amazonian fruits, in dry mass, are presented in Table 16.

Table 16 - Total phenolic compounds contents of the Amazonian fruits, obtained in the different extracts

Fruits	**Phenolic Compounds (mg GAE 100 g^{-1})**		
	Fruit Extracts		
	etOH	etOH:KPBS	KPBS
Abiu	134.4 (6.8)^{a}	779.0 (7.3)^{a}	333.5 (5.2)^{a}
Açaí	95.7 (8.0)^{b}	353.2 (7.0)^{b,c}	214.8 (3.5)^{b}
Bacuri	331.5 (1.4)	531.8 (3.1)^{d}	328.3 (7.6)^{a}
Biribá	62.1 (4.1)^{c}	427.0 (7.2)^{b,e}	139.3 (9.5)^{c,d}
Buriti	28.1 (3.9)^{d}	188.6 (4.2)^{f}	170.4 (4.3)^{d}
Cupuaçu	111.3 (4.6)^{b,e}	302.9 (7.3)^{c}	241.9 (1.5)^{b}
Inajá	121.7 (3.5)^{a,e}	476.9 (3.8)^{d,e}	351.7 (2.0)^{a}
Monguba	105.0 (10.0)^{b}	751.1 (1.75)^{a}	324.1 (4.1)^{a}
Pajurá	69.9 (2.5)^{c}	800.6 (3.0)^{a}	140.0 (0.1)^{c,d}
Uxi	44.1 (6.7)^{d}	157.8 (4.0)^{f}	107.5 (0.4)^{c}

Results obtained on a dry basis. etOH = ethanolic extracts. KPBS: etOH = hydroethanolic extracts (1:1). KPBS = extracts in KPBS-EDTA buffer solution (pH 7.5). In parentheses, coefficients of variation, CVs (%): accuracy of measurements. Means followed by the same letter in the same column do not differ significantly from each other by the Tukey test at the 5% probability level.

In all samples, the extraction of the phenolic compounds was dependent on the solvent used and its polarity. In the same way, the antioxidant capacities of the studied fruit extracts also showed a strong relationship to the employed solvent. Crude phenolic

extracts usually contain complex mixtures of some classes of phenols, which are selectively soluble in the different solvents. In this sense, solvent polarity plays a key role in increasing phenol solubility (NACZK; SHAHIDI, 2006). Organic solvent-water mixtures have been reported as more efficient in extracting phenolic compounds than their respective pure organic solvents (BOEING *et al.*, 2014).

Based on the present experimental results, it was predicted that in all samples, the binary extraction allowed to obtain a higher concentration of phenolic compounds, followed by the PBS buffer, and pure ethanol extractors. The probable explanation for this behavior is the better solvation of antioxidant compounds present in fruits, such as phenolic compounds, as a result of hydrogen bonding interactions between the polar sites of the antioxidant molecules and the solvent. Ethanol was less efficient in the extraction of phenolic compounds, probably because of the presence of the ethyl radical that is longer, resulting in lower solvation of antioxidant molecules (BOEING *et al.*, 2014).

Bacuri extracts exhibited important phenolic content in different polarities, displaying diverse phenolic composition. Abiu, monguba, and pajurá fruits showed higher concentration in binary extraction, whereas abiu, bacuri, inajá, and monguba in KPBS buffer extraction. On the other hand, all polarities extract of buriti and uxi fruit presented the lower phenolic content.

Following the example of Vasco *et al.* (2008) and other scientific research (RUFINO *et al.*, 2010; SOUZA *et al.*, 2012) which classified the phenolic content measured, other studies have established some range valuation categories. In this view, the results obtained for the better extraction (EtOH:PBS) could be classified into three categories: low (<300 mg GAE $100g^{-1}$), moderate (300-600 mg GAE $100g^{-1}$), and high (>600 mg GAE $100g^{-1}$) phenolic content on dry matter.

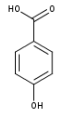
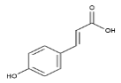
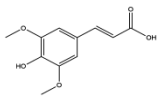
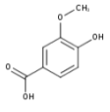
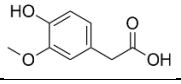
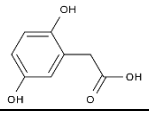
The richest fruit in phenolic compounds was abiu, monguba, and pajurá indicating that these fruits are excellent phenolic sources and well-known for their anti-atherosclerotic, anti-inflammatory, antitumor, antithrombotic, anti-osteoporosis and antiviral activities (NIJVELDT *et al.*, 2001). Açaí, bacuri, biribá, cupuaçu, and inajá fruits can be classified as having moderate phenolic content, whereas buriti, and uxi fruits, low quantities.

Despite the different extraction procedure and solvents used, as well as the environmental conditions which reflect strongly on the fruit phenolic composition, recently studies yielded close findings for total phenol contents in abiu fruit from

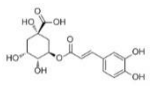
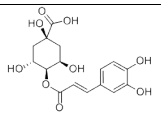
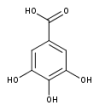
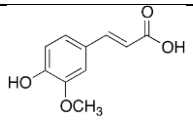
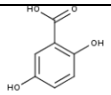
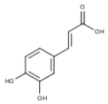
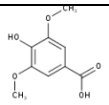
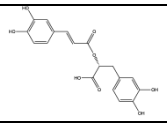
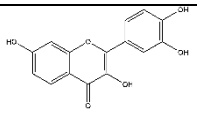
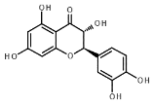
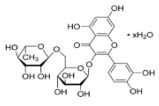
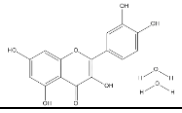
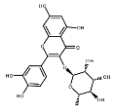
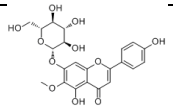
Rondônia, BR (172.3 mg GAE $100g^{-1}$ fresh weight) (VIRGOLIN *et al.*, 2017) and from Roraima, BR (900.2 mg GAE $100g^{-1}$ dry weight) (MONTERO *et al.*, 2018) in comparison to our results (220.2 mg GAE $100g^{-1}$ fresh weight or 779 mg GAE $100g^{-1}$ dry weight). In another study, involving traditionally consumed palm fruits from Amapá, BR the results after extraction steps involving acetone, methanol and water solvents, were higher to buriti (118±2 mg GAE $100g^{-1}$ fresh weight) and lower to inajá (45±2 mg GAE $100g^{-1}$ fresh weight) (SANTOS *et al.*, 2015), considering our results in fresh weight 83.15 and 155.0 mg GAE $100g^{-1}$, respectively.

By means of a detailed evaluation of the composition of these phenolic substances present in the fruits, it was possible to identify several groups of substances that contributed to their antioxidant activity. Table 17 provides some important information about substances that were monitored during qualitative analyzes. It is observed the variability of basic structures and functional groups, but it is possible to observe the presence of chromophore groups in all of them, which justifies the fact that some of these substances have a strong coloration.

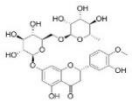
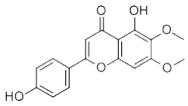
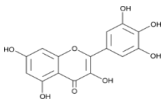
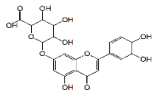
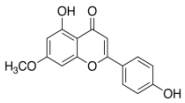
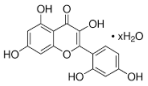
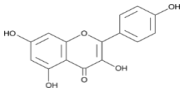
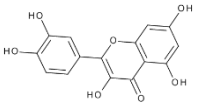
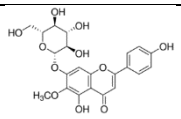
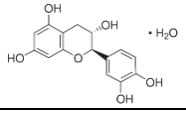
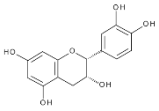
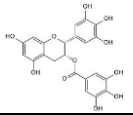
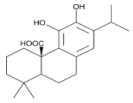
Table 17 - Chemical structures and classification of monitored phenolic compounds

Compound	**Formula**	**CAS number**	**Structure**
Phenolic acids			
4-Hydroxybenzoic acid	$C_7H_6O_3$	99-96-7	
p-Coumaric acid	$C_9H_8O_3$	501-98-4	
Sinapic acid	$C_{11}H_{12}O_5$	530-59-6	
Vanillic acid	$C_8H_8O_4$	121-34-6	
Homovanillic acid	$C_9H_{10}O_4$	306-08-1	
Homogentisic acid	$C_8H_8O_4$	451-13-8	

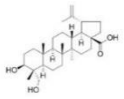
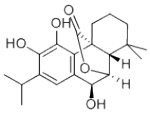
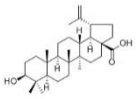
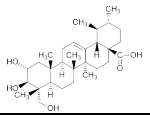
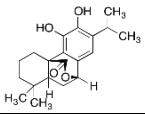
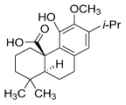
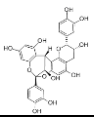
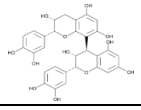
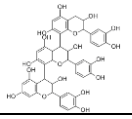
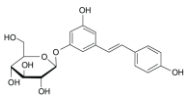
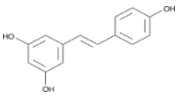
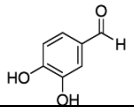
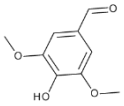
Continued Table 17 ...

Chlorogenic acid	$C_{16}H_{18}O_9$	327-97-9	
Cryptoclorogenic acid	$C_{16}H_{18}O_9$	905-99-7	
Gallic acid	$C_7H_6O_5$	149-91-7	
Ferulic acid	$C_{10}H_{10}O_4$	537-98-4	
Gentisic acid	$C_7H_6O_4$	490-79-9	
Caffeic acid	$C_9H_8O_4$	331-39-5	
Syringic acid	$C_9H_{10}O_5$	530-57-4	
Rosmarinic acid	$C_{18}H_{16}O_8$	20283-92-5	
Flavones			
Fisetin	$C_{15}H_{10}O_6$	528-48-3	
Taxifolin	$C_{15}H_{12}O_7$	480-18-2	
Rutin	$C_{27}H_{30}O_{16}$	207671-50-9	
Quercetin	$C_{15}H_{14}O_9$	6151-25-3	
Quercitrin	$C_{21}H_{20}O_{11}$	522-12-3	
Nepetin-7-glycoside	$C_{22}H_{22}O_{12}$	569-90-4	

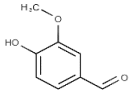
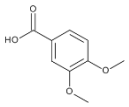
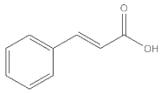
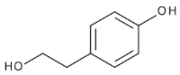
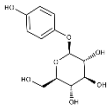
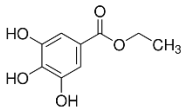
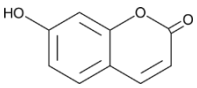
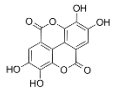
Continued Table 17 ...

Hesperidin	$C_{28}H_{34}O_{15}$	520-26-3	
Cirsimaritin	$C_{17}H_{14}O_6$	6601-62-3	
Myricetin	$C_{15}H_{10}O_8$	529-44-2	
Luteolin-7-O-B-d-glucuronide	$C_{21}H_{18}O_{12}$	38934-20-2	
Genkwanin	$C_{16}H_{12}O_5$	437-64-9	
Morin	$C_{15}H_{10}O_7$	654055-01-3	
Kaempferol	$C_{15}H_{10}O_6$	520-18-3	
Quercetin	$C_{15}H_{10}O_7$	117-39-5	
Homoplantaginin	$C_{22}H_{22}O_{11}$	17680-84-1	
Flavanols			
(+)-Catequin	$C_{15}H_{14}O_6$	7295-85-4	
(-)-Epicatechin	$C_{15}H_{14}O_6$	490-46-0	
(-)-Epigallocatechin gallate	$C_{22}H_{18}O_{11}$	989-51-5	
Phenolic Terpenes			
Carnosic acid	$C_{20}H_{28}O_4$	3650-09-07	

Continued Table 17 ...

Anemosapogenin	$C_{30}H_{48}O_4$	85999-40-2	
Rosmanol	$C_{20}H_{26}O_5$	80225-53-2	
Betulinic acid	$C_{30}H_{46}O_3$	472-15-1	
Asiatic acid	$C_{30}H_{48}0_5$	464-92-6	
Carnosol	$C_{20}H_{26}O_4$	5957-80-2	
12-Methoxycarnosic acid	$C_{21}H_{30}O_4$	3650-09-07	
Proanthocyanidins			
Procyanidin A2	$C_{30}H_{24}O_{12}$	41743-41-3	
Procyanidin B2	$C_{30}H_{26}O_{12}$	29106-49-8	
Procyanidin C1	$C_{45}H_{38}O_{18}$	37064-30-5	
Stilbenes			
Polydatin	$C_{20}H_{22}O_8$	65914-17-2	
Resveratrol	$C_{14}H_{12}O_3$	501-36-0	
Phenolic Aldehydes			
3,4-dihydroxybenzaldehyde	$C_7H_6O_3$	139-85-5	
Syringaldehyde	$C_9H_{10}O_4$	134-96-3	

Continued Table 17 ...

Vanillin	$C_8H_8O_3$	121-33-5	
Benzoic acids			
Veratric acid	$C_9H_{10}O_4$	93-07-2	
Cinnamic acids			
Trans-cinnamic acid	$C_9H_8O_2$	140-10-3	
Other phenolic compounds			
Tyrosol	$C_8H_{10}O_2$	501-94-0	
Arbutin	$C_{12}H_{16}O_7$	497-76-7	
Ethyl gallate	$C_9H_{10}O_5$	831-61-8	
Umbeliferon	$C_9H_6O_3$	93-35-6	
Ellagic acid	$C_{14}H_6O_8$	476-66-4	

The chromatographic profile (Table 18) of the Amazon fruits extracts were determined through the detection and identification of several known polyphenols. The results of the qualitative chromatographic profile evidenced a great diversity in phenolic substances, wherein 38 phenolic compounds could be separated and identified.

Comparing the evaluated methods, it can be affirmed that the chromatographic conditions established in method 1 allowed the separation and identification of a larger number of phenolic compounds, being higher in the açaí and uxi samples (28 and 30 phenolic compounds, respectively). However, some compounds were only possible to identify using the method 2 (morin hydrate and quercetin in abiu; vanillin in cupuaçu; nepetin-7-glucoside and rutin hydrate in inajá; homoplantaginin and p-coumaric acid in pajurá; umbelliferon in uxi sample).

Table 18 - Chromatographic profile of the phenolic extracts, which were identified based on the retention times of the corresponding standards, using different methods

	Phenolic compound	AB		AÇ		BA		BI		BU		CU		IN		PA		MO		UX	
		M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2
Phenolic acids																					
1	Chlorogenic acid	X						X	X	X	X			X	X	X	X	X		X	X
2	Caffeic acid	X	X	X		X	X			X	X	X		X		X	X	X	X	X	X
3	4-Hydroxybenzoic acid	X		X		X		X		X		X		X		X		X		X	
4	2,5-dihydroxybenzoic acid	X		X		X	X	X	X	X				X	X	X		X		X	
5	Ferulic acid	X	X	X		X		X	X	X	X	X		X	X	X	X	X	X	X	X
6	Gallic acid	X				X				X	X					X	X	X		X	
7	Homovanillic acid	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X	X
8	p-Coumaric acid	X	X	X		X		X	X			X	X	X			X	X		X	
9	Rosmarinic acid													X	X			X			
10	Sinapic Acid			X				X						X							
11	Sirinic Acid			X	X			X	X			X		X	X					X	X
12	Vanillic acid			X		X		X	X	X	X			X		X	X			X	
Flavonas																					
13	Cirsimaritin			X				X	X									X	X	X	
14	Quercetin		X	X						X	X	X	X	X	X	X		X	X	X	X
15	Genkwanin			X						X						X	X				
16	Hesperidin															X	X			X	X
17	Homoplantaginin			X													X	X	X	X	X
18	Kaempferol	X	X	X		X		X		X	X	X				X	X	X		X	X
19	Morin Hydrate		X	X						X	X	X	X	X	X	X		X	X	X	X
20	Nepetin-7-glycoside	X								X	X				X			X			

Continued Table 18 ...

21	Quercitrin hydrate			X						X	X					X		X	X	X	X
22	Rutin hydrate	X	X	X				X	X	X	X	X			X	X	X	X	X		
23	Taxifoline			X						X	X					X	X	X		X	X
Flavanols																					
24	(-)-Epicatechin	X	X	X		X		X	X	X	X	X	X	X		X	X	X		X	X
Phenolic terpenes																					
25	Rosmanol					X															
26	Asiatic acid															X	X			X	
27	Carnosic Acid					X															
Phenolic aldehydes																					
28	3,4-dihydroxybenzaldehyde	X		X		X		X		X		X		X		X		X		X	
29	Syringaldehyde	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X	X
30	Vanillin	X		X		X	X	X		X	X		X	X	X	X	X			X	
Benzoic acids																					
31	Veratric Acid	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X	X
Cinnamic Acids																					
32	Trans-cinnamic acid			X				X	X			X		X	X					X	
Other phenolic compounds																					
33	D - (-) - quinic acid	X	X	X				X	X	X				X		X	X	X	X	X	X
34	Ellagic acid																			X	X
35	Ethyl gallate			X	X			X	X			X		X	X					X	X
36	Arbutin			X	X							X		X		X				X	
37	4-o-caffeoylquinic acid	X						X	X	X	X			X	X	X	X	X		X	X
38	Umbeliferon			X														X			X
TOTAL		**18**	**12**	**28**	**6**	**16**	**6**	**21**	**16**	**23**	**18**	**17**	**8**	**23**	**16**	**25**	**20**	**22**	**9**	**30**	**20**

Results obtained on a dry basis. AB = abiu; AÇ = açaí; BA = bacuri; BI = biribá; BU = buriti; CU = cupuaçu; IN = inajá; PA = pajurá; MO = monguba; UX = uxi. M1 and M2 = chromatographic methods 1 and 2, respectively.

Among the Amazonian fruits evaluated in this study, inajá, biribá, and monguba were selected to be used in phenolic compounds quantitative determination (Table 19) by UHPLC based on that samples showed the highest antiproliferative activity against Caco-2 cell lines, as well as high/moderate total phenolic compounds contents.

Eleven phenolic compounds were quantified in biribá pulp, wherein homovanillic and quinic acid are at very high concentration (414,3 and 1031,6 mg kg^{-1}, respectively) ities. In the human body, quinic acid has been characterized as a pro-metabolite that leads to the induction of efficacious levels of nicotinamide and tryptophan in the gastrointestinal tract, being a source of those essential metabolic ingredients (PERO; LUND; LEANDERSON, 2009). Besides that, quinic acid has potent broad-spectrum antioxidant, anti-inflammatory, hepatoprotective, and several other medicinal properties (SOH *et al.,* 2003; PERO; LUND; LEANDERSON, 2009; XIANG *et al.,* 2001; INBATHAMIZH; PADMINI, 2013). Regarding homovanillic acid, higher content was also obtained in bibibá fruit. Homovanillic acid is a major catecholamine metabolite occurring in human biofluids, and it was reported to exhibit antioxidant capacity (ALJOVIĆ; GOJAK-SALIMOVIĆ, 2017). In psychiatry and neuroscience, brain and cerebrospinal fluid levels are measured as a marker of metabolic stress caused by 2-deoxy-D-glucose (MARCELIS *et al.*, 2006).

Greater variability in phenolic compounds was observed in the inajá pulp, including 25 compounds which belong for the classes of flavonoids, stilbenes, and phenolic acids and have been identified in this plant for the first time. Among the phenolic compounds found in inajá pulp, an important (-)-epigallocatechin gallate (EGCg) concentration was verified. Such compound possess important medicinal properties, including beneficial effects in studies of Parkinson's and Alzheimer's diseases, stroke, obesity, diabetes, inhibition of cancer proliferation, cancer chemoprevention, and antioxidant activity (STUART; SCANDLYN; ROSENGREN, 2006; DU *et al.,* 2012). EGCg is present in a limited number of plant-based foods and beverages. Arts, Putte, and Hollman (2000) determined the levels of some catechin, including EGCg in 24 types of fruits, 27 types of vegetables and legumes, some staple foods, and processed foods commonly consumed in the Netherlands, but none of the foods contained EGCg.

Table 19 - Quantification of polyphenols and phenolic acids in amazon fruits extracts (mg kg^{-1} sample on a dry basis) by UHPLC

Phenolic compound	Biribá	Inajá	Monguba
		mg kg^{-1}	
Phenolic acid			
2,5-dihydroxybenzoic acid	0,5	0,5	0,6
3,4-dihydroxybenzaldehyde/4-hydroxybenzoic acid	0,5	0,9	0,1
Caffeic acid	1,5	3,6	52,9
Chlorogenic acid	4,8	10,8	215,2
Ferulic acid	Nd	2,1	11,5
Gallic acid	Nd	Nd	1,9
Homogentisic acid	Nd	0,4	0,5
Homovanillic acid	414,3	Nd	Nd
p-Coumaric acid	0,3	1,4	11,0
Quinic acid	1031,6	34,2	571,9
Rosmarinic acid	Nd	4,1	Nd
Synaptic Acid	Nd	13,9	3,0
Flavonoids			
(-)-Epigallocatechin gallate	Nd	181.9	Nd
(-)-Epicatechin	52.1	83.1	76.2
Procyanidin-C1	172.9	176.8	196.3
Fisetin	Nd	55.9	9.6
Kaempferol	Nd	0.2	0.1
Morin hydrate	Nd	84.6	83.0
Quercetin	Nd	4.4	10.0
Rutin hydrate	4.6	0.9	21.7
Homoplantaginin	Nd	0.2	0.4
Nepetin-7-glucoside	Nd	45.1	65.1
Procyanidin-A2	Nd	6.5	29.8
Stilbenes			
Polydatin	Nd	0.3	1.2
Resveratrol	Nd	1.3	Nd
Other phenolic compounds			
Vanillin	Nd	24.2	Nd
Arbutin	Nd	0.9	Nd
Ethyl gallate	46.2	Nd	Nd

Nd = not detected.

In recent research, 10 sugar apple (*Annona squamosa* L.) cultivar peels from Thailand were evaluated (MANOCHAI *et al.*, 2018). The content of antioxidants was

notably lower (ranging from 0.4 to 32 mg kg^{-1}) than those obtained to the inajá pulp (181.9 mg kg^{-1}), and higher to the biribá pulp (<LOD) which belongs to the same botanic family. The inajá pulp showed a different stilbenes profile highlighting the resveratrol content, which inhibits the formation of free radicals and has antimutagenic activity (MALTA *et al.*, 2012).

Regarding the analysis of monguba seeds, 21 phenolic compounds were quantified, higher levels in the phenolic acids, such as 2,5-dihydroxybenzoic acid, caffeic acid, ferulic acid, gallic acid, homogentisic acid, *p*-coumaric acid, and especially chlorogenic acid. Chlorogenic acid is a compound derived from caffeic acid and it exhibits several beneficial biological properties, including antibacterial, antiphlogistic, antiviral, and inhibitory effects on carcinogenesis in the large intestine and liver (WANG *et al.*, 2015; HAO *et al.*, 2016). The chlorogenic acid values found in our study were higher than those contents reported for Gordon *et al.* (2012) in açaí pulp (0.2 to 16.4 μg g^{-1} dry basis).

Some Amazonian fruits have been evaluated for their chromatographic profile in relation to the presence of phenolic compounds (STAFUSSA *et al.*, 2018; GOMES *et al.*, 2016; MOURA, 2016; YAMAGUCHI, 2015; BERTO *et al.*, 2015b; PUGLIESE, 2010; GONÇALVES, 2008; YANG *et al.*, 2003). Considering the conventionally edible portions of the fruits contemplated in this study, there is scarce information in the literature about the presence and relative amounts of phenolic compounds in fruits of abiu, pajurá, bacuri and uxi, and no information regarding fruit pulps of biribá and inajá and the monguba seeds.

Stafussa *et al.* (2018) has been evaluated the bioactive composition and antioxidant capacity of 44 traditional and exotic Brazilian fruits, among them açaí, buriti and cupuaçu. The total content of phenolic compounds was determined according to the Folin-Ciocalteu method, the flavonoids and anthocyanins content, as well as the antioxidant capacity against the synthetic $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals were also determined by colorimetric methods. The phenolic profile analysis was performed by injecting hydroethanolic extracts from the samples to the HPLC coupled to a diode array detector. In extracts of fruit pulps the following classes of substances were identified: five hydroxycinnamic acids, with emphasis on the high content of chlorogenic acid; three hydroxybenzoic acids, with emphasis on vanillic acid in açaí; four flavonoids, the catechin being the main responsible for the content of flavonoids in the fruits, and a

stilbene, resveratrol, which presented greater content in the apple and peach fruits. The authors highlighted the most important characteristics of each pulp in relation to the composition of compounds and their pharmacological activities.

Gonçalves (2008) evaluated the potential in bioactive compounds of 16 fruit pulps (bacuri, tucumã, cupuaçu, graviola, buriti, uxi, cambuci, araça, tamarindo, maracujá doce, granadilla, carambola, camu-camu, maná-cubiu, abiu, and araça-boi) and 6 frozen fruit pulps (panã, umbu, cagaita, coquinho azedo, araça, and cambuci), all Brazilian native fruits. The levels of ellagic acid, vitamin C and flavonoids (catechin, epicatechin, quercetin, kaempferol, and cyanidin) were determined by HPLC, while the α-glycosidase and α-amylase inhibitory activity were determined by spectrophotometric methods. The antioxidant capacity was verified by the methods DPPH, ORAC and bleaching by the β-carotene/linoleic system, and the content of total phenolic compounds by the Folin-Ciocalteu method. Camu-camu, cupuaçu, coconut sour and cagaita revealed the highest levels in vitamin C. Camu-camu, cambuci, uxi, tucumã, cagaita, coquinho azedo and araçá presented higher potential antioxidants. The main flavonoids found in the fruits were quercetin and catechin. Observing the fruits that were also contemplated in the present study, it was verified that buriti pulp has quercetin and hydroxycinnamic acids and abiu hydroxycinnamic acids, while no flavonoids were quantified in the pulps of uxi, cupuaçu and bacuri.

The work contributed with information about the bioactive potential of the native fruits. Once again, we highlight the nutritional power and the trophic balance power of these fruits and their contribution to the quality of life in the Amazonian and pre-Amazonian ecosystems, where the fruits evaluated are found.

6.1. Results of the chemometric correlations analysis

A principal component analysis (PCA) was applied to characterize the ten Amazonian fruits according to their chemical composition and biological activity. In total, 23 variables were evaluated in ten (10) fruit types and the correlation matrix variables were composed of: six (6) bromatological parameters (moisture, ash, pH, acidity CA, lipids, and proteins); seven (7) minerals (Ca, Cu, Fe, Mg, Mn, Na, and Zn); vitamin C; three (3) different methods of extracting phenolic compounds (PC-etOH, PC-KPBS,

and PC-KPBS: etOH); five (5) methods for analysis of the antioxidant capacity (DPPH, NBT, ABTS-etOH, ABTS-PBS, and ABTS-PBS: etOH); and the cell viability assay.

The distributions of the variables along the major components PC1 *vs* PC2, PC1 *vs* PC3, and PC2 *vs* PC3 are shown in Figure 17, 18 and 19, respectively.

From Figure 17, it was verified that the cumulative percentage of the total variance explained by the first two components (PC1 *vs* PC2) was 51.82%. The two-dimensional chart shows the formation of three main cluster: blue, green, and red. However, it is important to note that PCA is not a classification, but can be used as the basis for it. For $ABTS^{\bullet+}$ method

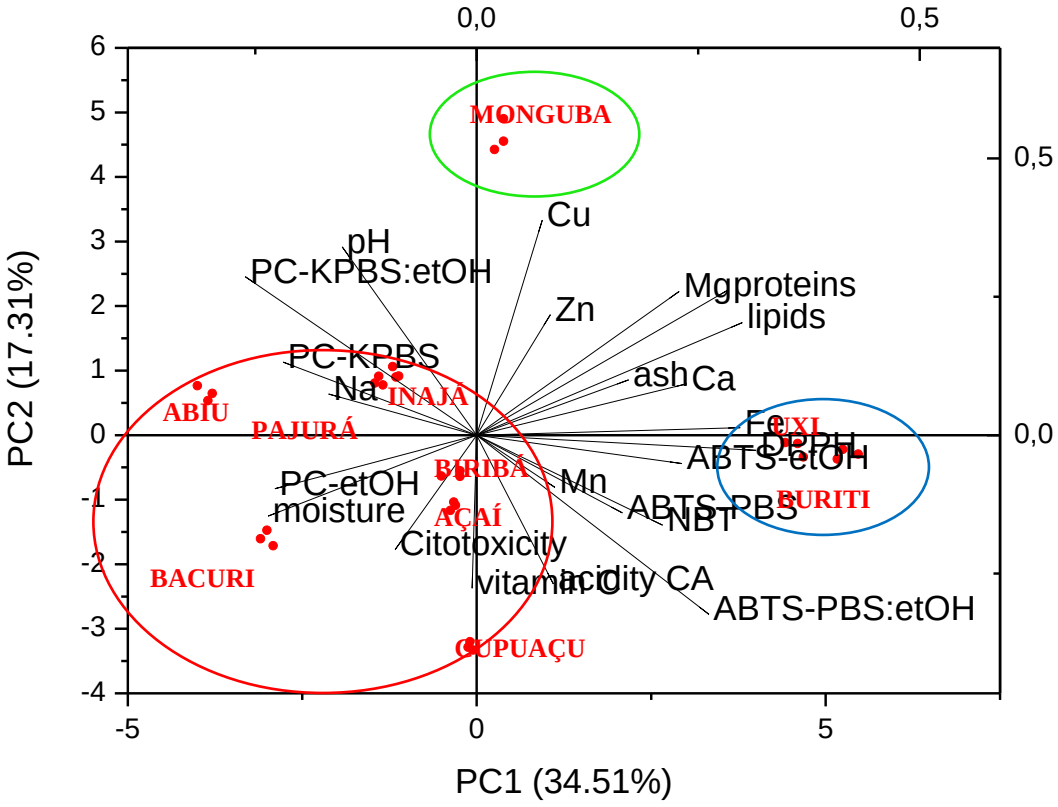


Figura 17 - PCA scatter diagram: distribution of 10 fruit, in triplicate, along principal components 1 (PC1) and 2 (PC2) using 23 variables.

The blue cluster of Figure 17 was formed by buriti and uxi fruits, which were distinguished from the others by the lower content of phenolic compounds, lower antioxidant capacity, and moderate antiproliferative effects. On the other hand, these samples were distinguished in relation to the bromatological content (high contents in lipids, proteins, and energy) and mineral (relevant contents in Cu, Mg, and Mn).

The green cluster of Figure 17 was formed solely by the almonds of the monguba. In fact, this fruit had important antiproliferative effects, besides a high antioxidant capacity against $O_2^{\bullet-}$ and $ABTS^{\bullet+}$ radicals in the hydroethanolic and aqueous extracts. In addition, the sample of monguba presented an important content in phenolic compounds in all the extracts, high contents in ashes, lipids, proteins, energy, Mg, and Cu. On the other hand, low levels of vitamin C were obtained. It is important to note that the pulp portion was evaluated in all samples, except for the monguba fruit, in which the seeds were studied. Besides the dependence of edaphoclimatic factors of the region where the vegetable is produced, the chemical composition of the seeds varies according to the botanical species, the cultivar and, like the other organs of the plant; the seeds present a very variable chemical composition (NETO; ROSSETTO, 1998), which certainly differentiates this fruit from the others studied. The composition of monguba seeds and their industrial potential had never been revealed before, as it is in the present work. It is a native fruit, economically devalued and with very little information about its chemical composition and biological activities. The presence of ant nutritional factors in the fruit, reported by Oliveira *et al.* (2000), needs to be better clarified, in view of being a fruit used as food by the local population.

In Figure 18, the distinct behavior shown by the red cluster was formed by inajá, açaí, bacuri, biribá, pajurá, cupuaçu, and abiu fruits. It was possible to determine a cumulative percentage of the total variance explained by the PC1 *vs* PC3 components of 48.40%.

Observing the dispersion of the variables along PC1 and PC3, it is possible to note in greater detail the influences of the variables on the samples formed by the red cluster of the previous figure (Figure 17), which contemplated a great part of the studied fruits. The vitamin C variable that contributed positively to the formation of PC3 is highlighted in this diagram, highlighting some samples with high content in this nutrient: açaí, cupuaçu, and inajá. Other variables contributed to the formation of PC3, which were the high levels of Mn, Zn, and phenolic compounds (aqueous extract), and for this component, the green cluster formed by the monguba samples was not evidenced. In addition, the high Na content in the pajurá sample was demonstrated by the negative contribution to PC3. Figure 19 confirms the influence of the variables on the samples of Amazonian fruits.

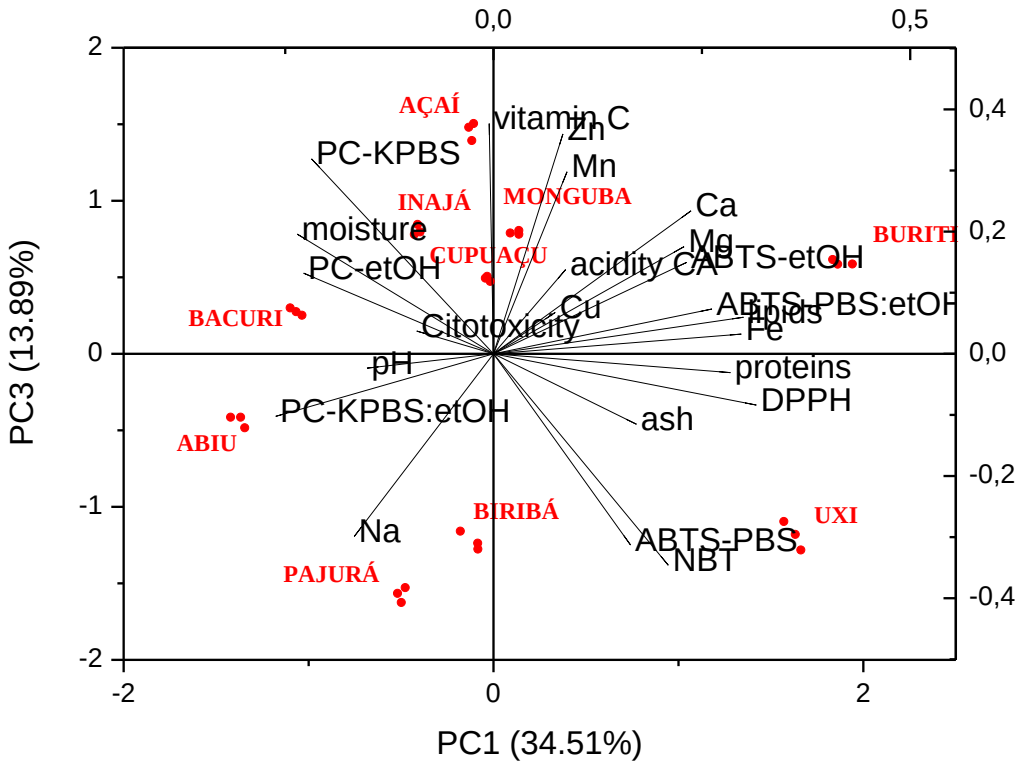


Figure 18 - PCA dispersion diagram: distribution of the 10 fruits, in triplicate, along the main components 1 (PC1) and 3 (PC3) using 23 variables.

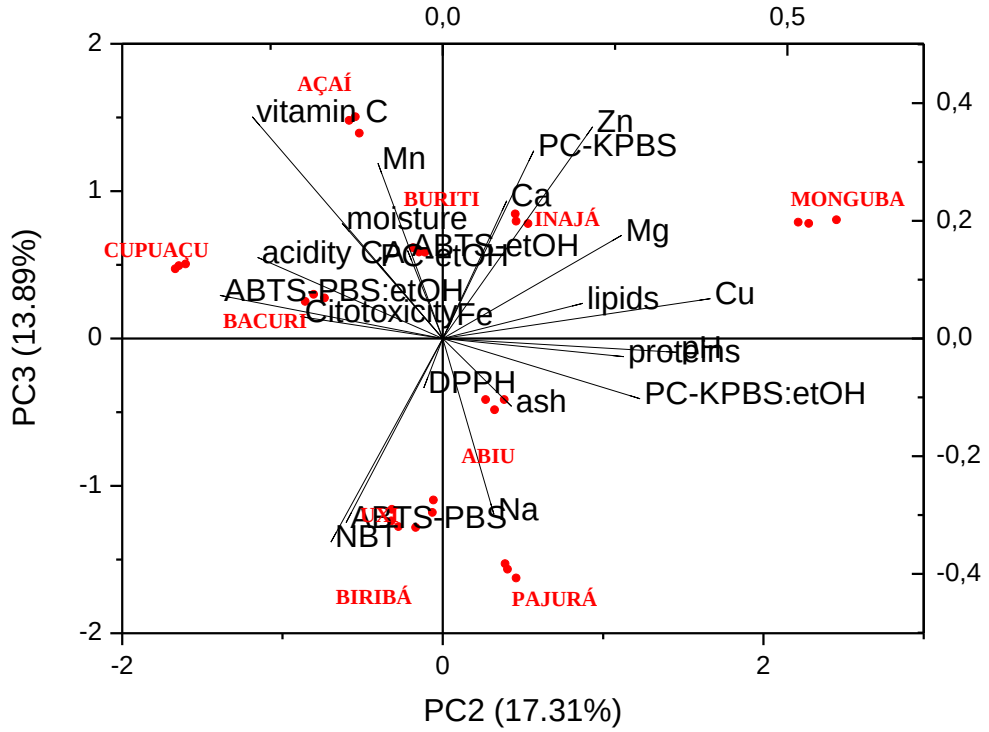


Figure 19 - PCA dispersion diagram: distribution of the 10 fruits, in triplicate, along the main components 2 (PC2) and 3 (PC3) using 23 variables.

A correlation between the results of the bioactive compounds, the antioxidant capacity and the antiproliferative activity (Table 20), showed a strong effect of the total concentration of phenolic compounds on the antioxidant capacity, such as R^2 = -0.90 for PC (KPBS) and ABTS (PBS), R^2 = -0.98 for PC (KPBS: etOH) and ABTS (PBS: EtOH), R2 = -0.74 for PC (KPBS) and NBT (KPBS). The correlation results were negative because the antioxidant capacity results were expressed as IC_{50} (and in this case, the lower the IC_{50}, the higher the antioxidant capacity), while the concentration of phenolic compounds was expressed in mg GAE $100g^{-1}$. Thus, it is suggested that, at this point in the analysis, the comparisons of the correlation coefficients (R^2) are made with modular values.

The results of the correlation suggest that the phenolic content was the main responsible for the antioxidant capacity of the fruit extracts. In the literature, the effectiveness of such compounds acting as antioxidants has been widely discussed, is considered the most responsible for the antioxidant capacity of several fruits, vegetables, and grains. The basic chemical structure of phenolic substances is ideal for the following biochemical processes: chelation of metals, because the phenolic compounds have hydroxyl and carboxyl groups, capable of binding especially to iron and copper; inhibition of lipid peroxidation by entrapment of the lipid alkoxyl radical; neutralization of ROS because of the ability of phenolic compounds to donate electrons or hydrogen atoms.

Table 20 - Correlation between the results obtained for the bioactive compounds, the antioxidant capacity and the antiproliferative activity for the Amazonian fruits studied

	DPPH	ABTS etOH	ABTS PBS	ABTS PBS:etOH	NBT	PC etOH	PC etOH:KPBS	PC KPBS	Citotoxicity	Vit. C
DPPH	1.00									
ABTS etOH	0.57	1.00								
ABTS PBS	0.27	0.15	1.00							
ABTS PBS:etOH	**0.69**	0.56	0.41	1.00						
NBT	**0.72**	0.14	**0.60**	0.57	1.00					
PC etOH	-0.47	-0.41	-0.51	-0.33	-0.21	1.00				
PC etOH:KPBS	**-0.68**	-0.45	-0.33	**-0.98**	-0.55	0.30	1.00			
PC KPBS	-0.52	-0.15	**-0.90**	-0.53	**-0.74**	**0.65**	0.47	1.00		
Citotoxicity	-0.03	0.27	-0.41	0.10	-0.15	0.17	0.00	0.31	1.00	
Vit. C	-0.14	0.01	-0.15	0.43	-0.27	0.11	-0.45	0.08	0.09	1.00

PC=phenolic compounds. etOH = ethanolic extract. KPBS:etOH = extratos hidroethanolic extract (1:1). KPBS= KPBS Buffer solution extract (pH 7,5).
DPPH, ABTS, NBT = spectrometry methods for analysis the antioxidant capacity (vide experimental part).

6.2 Development of a Biosensor: Rapid Determination of Antioxidant Capacity

The colorimetric methods described and employed in this work have been widely used in the determination of the antioxidant capacity of several matrices, whether they are composed of fresh vegetables (MONTERO *et al.*, 2018; VIRGOLIN *et al.*, 2017; WANG *et al.*, 2016; DEMIRDOVEN *et al.*, 2015; MANDAVE *et al.*, 2014; CANUTO *et al.*, 2010; RUFINO *et al.*, 2010), as well as processed foods, such as beverages (GARCÍA-LOMILLO; GONZÁLEZ-SANJOSÉ, 2014; COLINA *et al.*, 2012), teas (MILANIA; AMUZADEHB; MOETAMEDZADEGAN, 2018; KOCZKA *et al.*, 2016; AKRAM *et al.*, 2012), and olive oil (MINIOTIA, GEORGIO, 2010). It should be mentioned, however, that such methods are still expensive and relatively time-consuming, even using the microplate reading strategy, which allows for multiple simultaneous analyzes. In addition, these analytical methodologies could hardly be used in situ, since they generally require relatively large equipment, both for sample preparation and for spectrophotometric readings.

For this reason, this stage of the work initially aimed at designing an electrochemical biosensor capable of performing this task with the minimum treatment of the sample, with portability capability, and that resulted in a lower analytical cost.

6.2.1 Electrochemical characterization of the biosensor

The base sensor, used for the construction of the biosensor, comprised flexible and chemically inert support (PVC) in which three (3) electrodes, including a working electrode, pseudo-reference electrode, and auxiliary electrode, were printed through a production methodology based on semi-automatic serigraphy. Serigraphy technology is a well-established technique for manufacturing inexpensive, disposable and portable electrode systems, which enables *in situ* analysis. The process of preparation of this type of base sensor is well known and has already been described in articles (HAYAT; MARTY, 2014; LI *et al.*, 2012). Figures A10 and A11 of Annex A outline the deposition steps of the layers forming the electrochemical sensor during the screen-printing process.

At the base sensor used, the auxiliary electrode was formed by the deposition of a commercial graphite carbon paste; the working electrode, from a commercial graphite paste containing the Prussian Blue (PB) salt as an electrochemical mediator; and the

pseudo-reference electrode, from an Ag/AgCl paste. The working electrode can be considered the most important because it is where the electrochemical reactions are monitored, while the reference and auxiliary electrodes (counter electrodes) are used to complete the electronic circuit, to enable the realization of measures at fixed potential, and perform the transfer of electrons to the transducer (potentiostat) (HAYAT, MARTY, 2014).

Due to the particular characteristic of the PB mediator in catalyzing the reduction of hydrogen peroxide, screened biosensors, based on oxidase enzymes, modified by the PB inorganic compound have been developed for applications in clinical analyzes (JIANG *et al.*, 2016; BOFFI, 2015; SEKAR *et al.*, 2014) and food (ALBANESE *et al.*, 2014; WANG *et al.*, 2003). A recent search on the CAPES journal portal resulted in more than 1,000 articles on screen-printed biosensors modified with PB based on different electrode materials, modification procedures and measurement principles. Articles that deal with biosensors for the analysis of glucose, lactate, cholesterol, and other physiological analytes have been found in analytical chemistry journals (JIANG *et al.*, 2016; BOFFI, 2015; SEKAR *et al.*, 2014; HIRST *et al.*, 2013; BANERJEE; SARKAR; TURNER, 2013; CHIU *et al.*, 2009). In addition, the use of PB in the development of sensors and biosensors for food analysis has aroused the interest of many research groups and led to improved methods for the detection of biocomposites such as sucrose and galactose as well as for the determination of antioxidant capacity (ALBANESE *et al.*, 2014; VARVARI; POPESCU, 2010; WANG *et al.*, 2003; HAGHIGHI *et al.*, 2004).

The evaluation of the antioxidant capacity using the developed biosensor is based on the online monitoring of H_2O_2 produced during the oxidation process of an aqueous solution of hypoxanthine (HX) to uric acid, in the presence of the enzyme xanthine oxidase (XOD), or by the spontaneous dismutation of the $O_2^{\bullet-}$ radicals as shown in Figure 20.

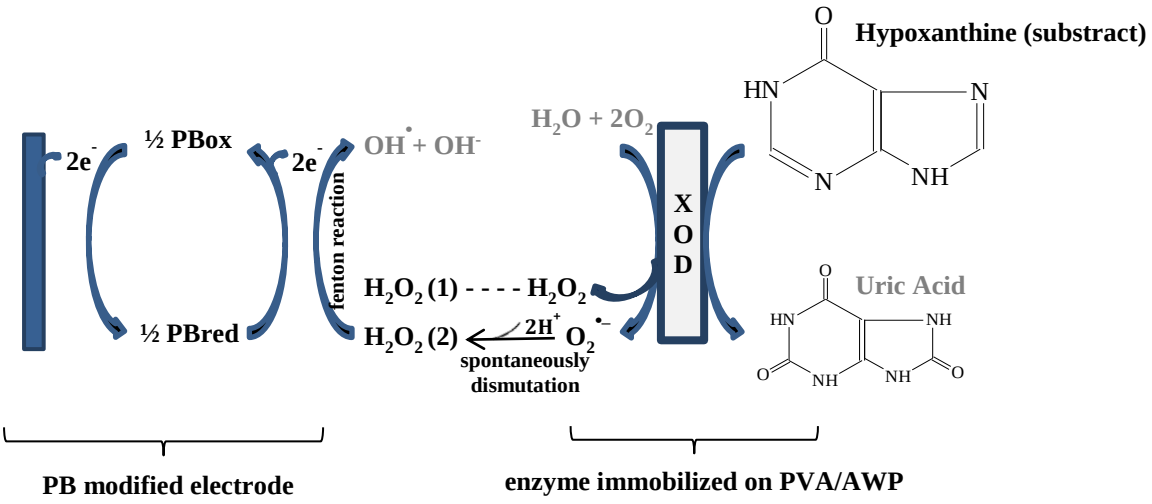


Figure 20 - Principle of detection of H_2O_2 generated by the HX/XOD enzymatic system, using the amperometric biosensor.

The generated H_2O_2 is reduced on the polarized (-100mV *vs* Ag/AgCl) WE surface, in presence of PB mediator, which has a well-known catalytic effect for the H_2O_2 reduction due to its peculiar chemistry structure (RICCI; PALLESCHI, 2005). In the presence of antioxidants, the $O_2^{\bullet-}$ radicals and/or H_2O_2 are scavenged with a decrease of the cathodic current allowing the antioxidant capacity quantification.

Many works have already demonstrated the strong influence of modifying materials on the catalytic or electrical properties of the electrodes (GARCIA *et al.*, 2015; RODRÍGUEZ; RIVAS, 2002; MOUSTY, 2004). In fact, the improvement of electronic properties can lead to lower capacitive currents and better sensor sensitivity. This influence is highlighted by the electrochemical behavior of the carbon SPE without and with PB mediator (Figure 21).

The modification of the carbon sensor with the PB mediator significantly changed the electrical properties of the working electrode, with the cathodic peak potential of +0.197V *vs* Ag/AgCl and the anodic peak of -0.073V *vs* Ag/AgCl.

With these results, favorable to the electrochemical response of the biosensor, two values of working potential (-50 mV and - 100 mV) were selected and monitored the current generated during the enzymatic reaction, over time and with increasing additions of the HX substrate (Figure 22). It was observed that higher levels of hypoxanthine in the reaction medium resulted in lower current intensities, in negative terms.

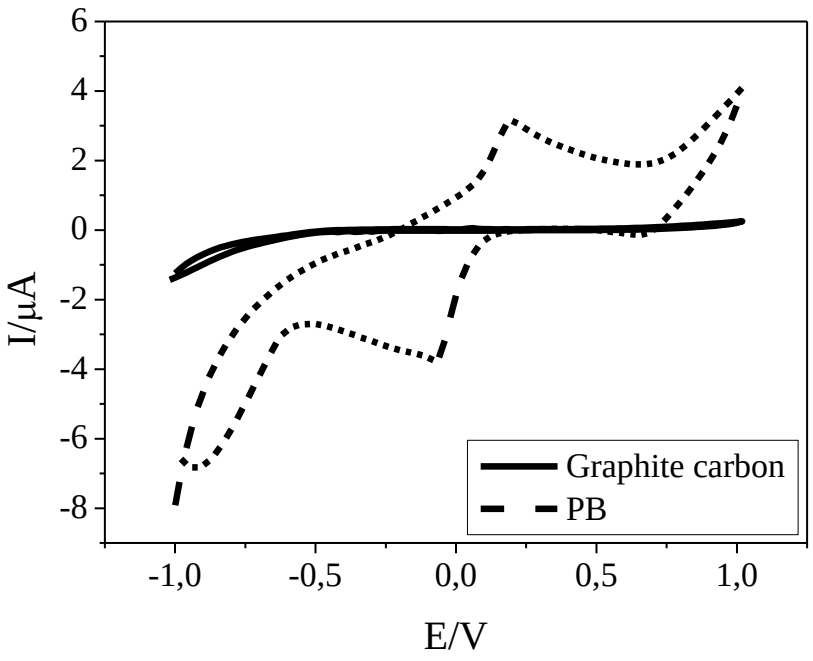


Figure 21 - Cyclic voltammograms of carbon SPE in the absence/presence of PB mediator. Electrochemical measurements performed in 50 mM KPBS buffer solution (pH 7.5) with 10 mM KCL at a scan rate of 50 mV s^{-1}.

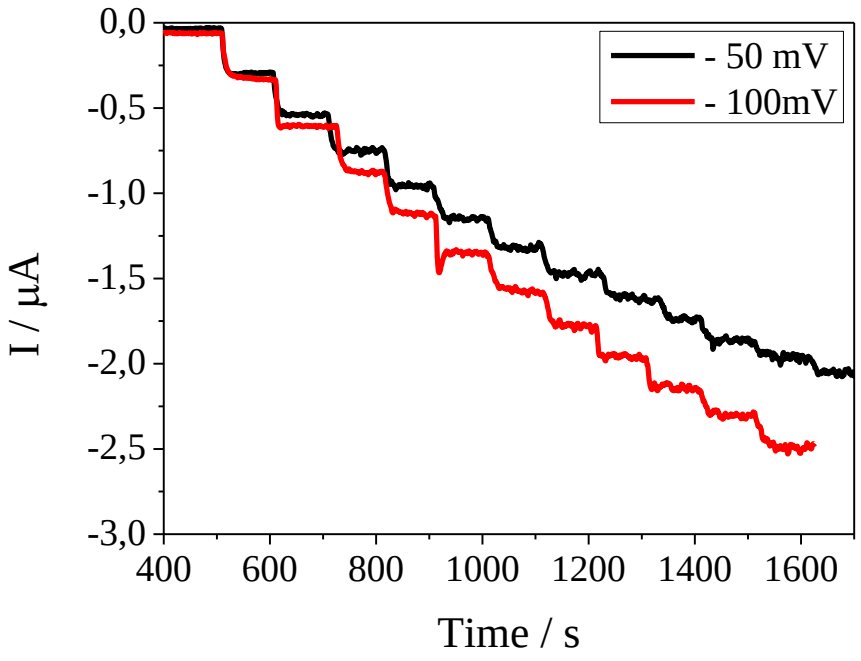


Figure 22 - Amperometric response of biosensor with the HX addition at the different applied potential.

It was also verified that, by fixing the working potential at -100 mV, the reduced form of the PB mediator was able to favor, more intensely, the reduction of H_2O_2 on the surface of the working electrode. In practice, this value of fixed working potential can be considered quite appropriate for electrochemical measurements: because it is relatively low, the possibility of external electrochemical interference is reduced, so that the use of Faraday cages during the chronoamperometric measurements (SOUZA, 2003).

By transforming the responses of Figure 22 into curves, larger linear ranges and better sensitivity of the biosensor, which was expressed by the angular coefficients, are observed when the working potential is set at -100 mV, as shown in Table 21.

Table 21 - Analytical efficiency of the biosensor in different work potentials, expressed in sensitivity and linearity

Potential (mV)	**Sensitivity (A mol L^{-1})**	**R^2**	**Linear range (µmol L^{-1})**
-50	-2.13E-8	0.992	1.0-60
-100	-2.72E-8	0.997	1.0-75

A similar effect on linear range and sensitivity in amperometric biosensors using PB-modified SPE have been obtained at -100 mV after work potential optimization study (BANERJEE; SARKAR; TURNER, 2013; BOFFI, 2015). However, a comparison of the performance of these is difficulted in view of the different target molecules and, therefore, the use of different substrates. It is worth noting that a potential range between 0 and -200 mV is desired, in view of the reduction of molecular oxygen (E<-200 mV) as well as the oxidation of antioxidant compounds, such as phenolic compounds and other electroactive substances which are frequently present in real sample, could also be oxidized to give interfering signals (E>0 mV) (ROSATTO *et al.*, 2001).

Among the desirable effects of the Prussian Blue mediating, it is possible to include the zeolitic nature of PB with a cubic unit cell of 10.2 °A and with channel diameters of about 3.2 °A which allows the diffusion through the crystal of low molecular weight molecules (such as O_2 and H_2O_2) to its catalytic center, excluding molecules with higher molecular weight (RICCI; PALLESCHI, 2005; ITAYA; ATAKA; TOSHIMA, 1982; LUDI; GÜDEL, 1973). Varvari and Popescu (2010) who proposed a PB-modified amperometric sensor for antioxidant activity evaluation at -0.1 V *vs* Ag/AgCl, KCl_{sat} confirmed this electrocatalytic effect for H_2O_2 reduction.

The PB selectivity and activity are comparable to those of a biological binding component but with all the advantages of an inorganic species, such as the low cost, high stability at certain conditions, ease of electrode surface modification, and no saturation effect for substrate (RICCI; PALLESCHI, 2005). In general, the preparation of PB through, especially, adsorption and electrodeposition is simple, which, besides being cost-effective, is highly stable in acidic and neutral media (MUREŞAN; TURDEAN; POPESCU, 2008; ADHOUM; MONSER, 2008; ALEGRET; MERKOÇI, 2007).

Once the working electrode has been characterized, it is convenient to make some comments about the pseudo-reference electrode used in the proposed biosensor system. A pseudo-reference electrode consists of an electrode that is in direct contact with the electrolyte, which does not always guarantee a thermodynamic equilibrium. In the case of the biosensor operating on a three-electrode system, having the pseudo-reference electrode formed from the Ag/AgCl mixture, no current flowed through it and therefore its potential remained unchanged during the experiment. In addition, the fixed concentration of Cl^- in the KPBS buffer solution (pH 7.5), used as a solvent in the preparation of all the solutions, and also as the electrolyte during the chrono-amperometric measurements, ensured an equally fixed potential difference between the Ag/AgCl pseudo-reference electrode and the electrolyte (INZELT, 2013).

6.2.2 Optimization of the enzyme immobilization procedure

The PVA-AWP is successfully used for the immobilization of enzymes onto the SPE surface offering several advantages such as better elasticity, low-toxicity, biocompatibility with enzymes, mechanical and long-term stability, and biodegradability (BEESABATHUNI, 2010; STOYTCHEVA *et al.*, 2016). However, the thickness of the film, depending on the polymer concentration, and the degree of crosslinking, depending on the time of UV exposure, will affect the enzyme retention properties of the polymer and its permeability for the enzyme substrates and the products of the enzyme reaction (STOYTCHEVA *et al.*, 2016).

We decided to optimize the fabrication of the biosensor in terms of the polymer concentration, irradiation time, and enzyme charge using the chemometric method. For this, the response surface methodology was applied to adjust the following factors: XOD enzyme charge (C_{XOD}), enzyme:polymer ratio (R_{XP}) and photopolymerization time (T_P)

factors for enzyme immobilization onto PB modified WE. Box-Behnken design was used applying high, central, and low values for C_{XOD} as 5.0, 8.0, and 10.0 mU per electrode, for R_{XP} as 1:2 (0.33), 1:1 (0.50), and 2:1 (0.66) representing XOD-enzyme:PVA/AWP ratios, and for T_p as 48, 24, and 0.5 hours, respectively. It should be mentioned that in relation to the activity of the enzyme per electrode, a unit (1.0 U) means that, with the amount of enzyme immobilized, 1 μmol of product is generated in one (1.0) minute reaction. 15 of the working set was generated by Statistica program and thus 15 electrodes were constructed.

Each biosensor was tested by performing the following assay: the electrode was immersed in a glass cell containing 10 mL of 50 mM KPBS buffer containing 10 mM KCl (pH 7.5) under constant stirring. The working potential of -100 mV was applied and the current monitored until the steady-state was reached. Increasing concentrations of the substrate (hypoxanthine, HX) were added, and the current intensities were recorded until the saturation level was reached, that is until the results remained the same.

For fitting of experimental data, linear (L) and quadratic (Q) models were tested. Significant **p-**value (< 0.05) was found in all factors to a linear model (Table 22), which showed 0.99887 predicted R-squared value in reasonable agreement with the adjusted R-squared value (0.99773). Also, the lack of fit *F* value of 1.792 implies the no significant value of lack of fit (p> 0.05) and represent the validity of the linear model for explanation of experimental data of the present study which was used for further model construction.

Table 22 - ANOVA analysis for C_{XOD}, R_{XP}, and T_P enzyme immobilization optimization study

Factor	**Sum of Square**	**Df**	**Mean Square**	**F**	**P**
C_{XOD} (L)	6432	1	6432.2	19.866	0.011185*
C_{XOD} (Q)	291	1	291.1	0.899	0.396711
R_{XP} (L)	112664	1	112664.2	347.965	0.000049*
R_{XP} (Q)	924	1	924.1	2.854	0.166409
T_P (L)	959762	1	959761.7	2964.240	0.000001*
T_P (Q)	25892	1	25892.4	79.969	0.000865*
Lack of Fit	1741	3	580.2	1.792	0.287985
Error	1295	4	323.8		
Total	2676986	14			

df: Degree of freedom, F: Fishers's function, p: Level of significance. *Significant values (p<0.05).

Important results of the effects of simultaneous change of C_{XOD}, R_{XP}, and T_P are given by the Pareto Chart (Figure 23) which shows the order and significance of each variable affecting on the current intensity response by the enzymatic reaction in the amperometric biosensor. The response surface diagrams for the parameters studied, shown in Figure 24, corroborate with the following conclusions.

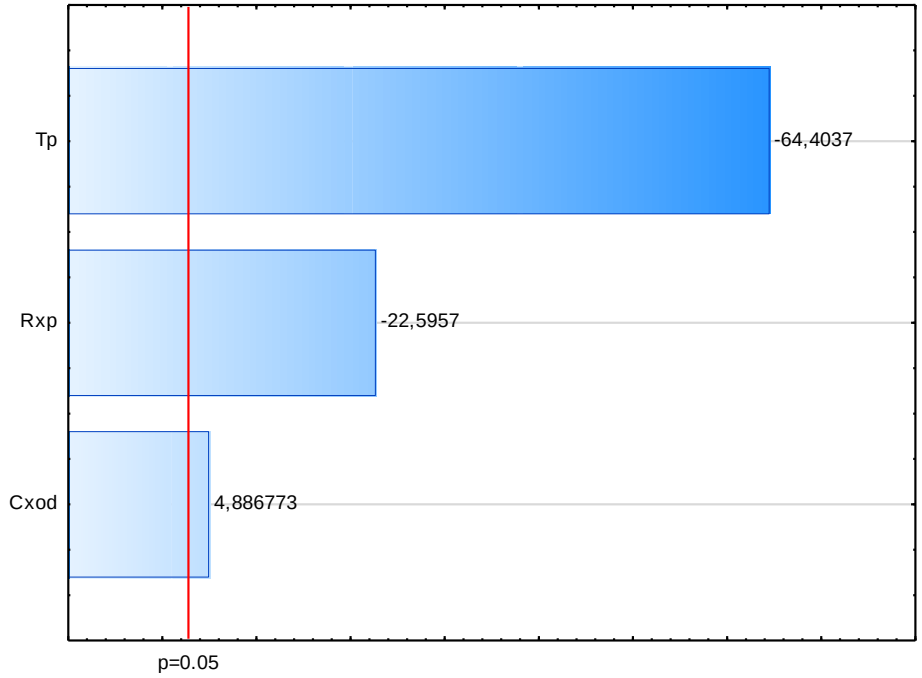


Figure 23 - Pareto chart representing the ANOVA analysis for linear components of the parameters studied. Results are significant for $p > 0.05$.

With respect to the main effect of each variable, two variables named T_P and R_{XP} affect negatively the current intensity response, where another variable named C_{XOD} affect positively the ROS production. Also, it can be observed the order of significance of influence in this process $T_P > R_{XP} > C_{XOD}$.

As shown in Figure 24A the R_{XP} and C_{XOD} variables determine the response surface. As the R_{XP} ratios decreased, the current values increased which reveal that with the same amount of enzyme higher amounts of PVA/PWA secure the crosslinking. A slight increment of C_{XOD} increases the signal.

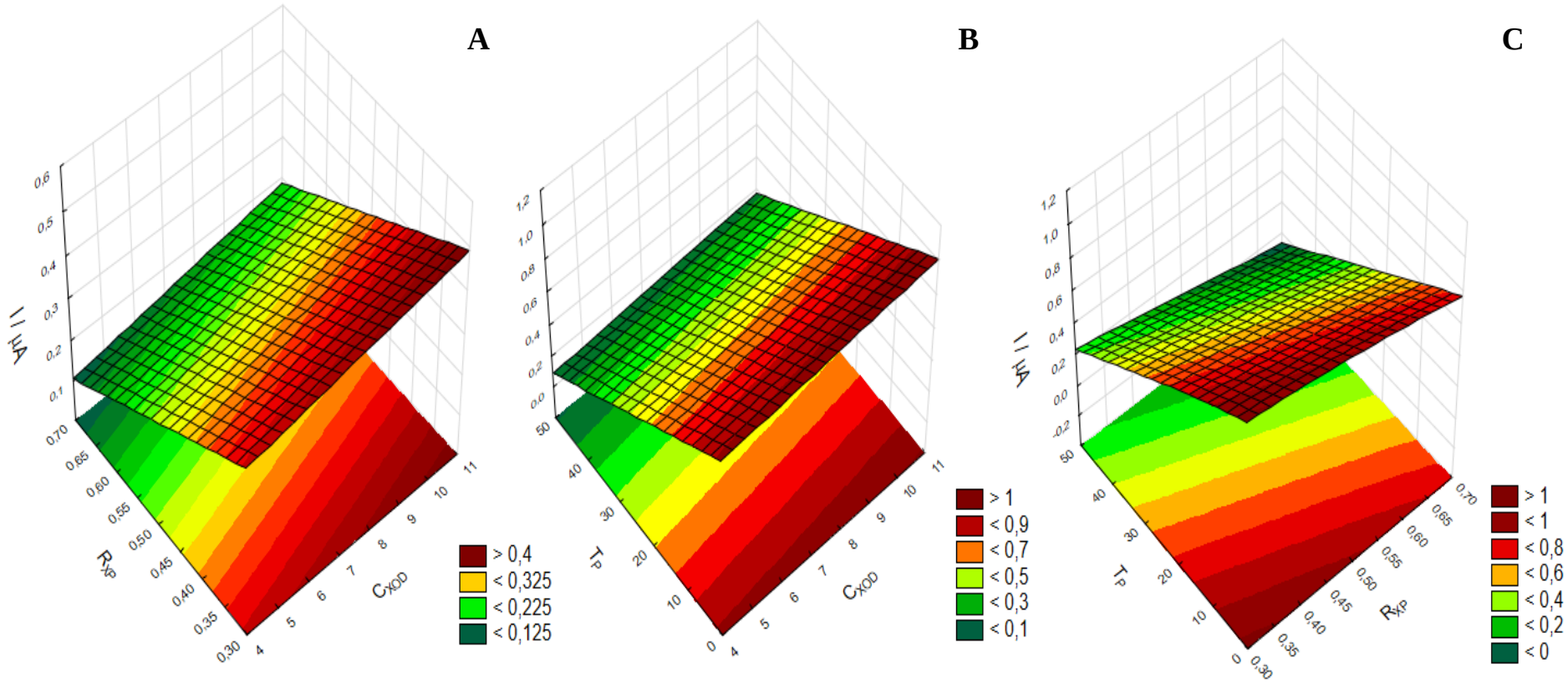


Figure 24 - Response surface diagrams showing correlations between: (A) enzyme: polymer ratio (R_{XP}) and enzyme loading (C_{XOD}); (B) photopolymerization time (T_P) and enzyme loading (C_{XOD}); and (C) enzyme: polymer ratio (R_{XP}) and photopolymerization time (T_P).

Regarding Figure 24B, is it noted that the current response increases when the T_P decreased, which shows that the polymerization time significantly influences the enzyme activity. The decrease of the enzyme activity can be due to the long light exposition. The additional times of 4 h, 2 h and 1 h were also tested, but the results did not differ statistically ($p < 0.05$) from the half-hour (0.5 h) time, and for this reason the latter was shown to be a satisfactory time to ensure the encapsulation of the XOD enzyme in the PVA-AWP structure. For stationary T_P, the signal shows a slight effect with the increased of C_{XOD} concentration. However, in 0.5 h there is no difference between the current signal at 8.0 and 10.0 mU XOD concentration ($p<0.05$).

As shown in Figure 24 C, as the R_{XP} ratios decreased, the current response values increase. In addition, as the T_P decreased the increased signal in net current values due to the maintenance of XOD enzyme activity.

The optimal enzyme immobilization conditions were chosen at low level 0.5 h and 0.33 respectively for T_P and R_{XP}, while the C_{XOD} variable which exerted a slightly positive effect on ROS production was maintained at a central level (8 mU enzymes per WE). By fixing these optimum TP and RXP conditions, the measurements using the biosensor prepared with an enzyme charge of 8 mU did not present significant differences ($p < 0.05$) in relation to the chronoamperometric responses, when compared to those obtained with the highest level of enzymatic loading (10.0 mU). It can be affirmed that the immobilization of the XOD enzyme using PVA-AWP showed to be an excellent strategy for the biofunctionalization of the printed electrode in PVC, on the occasion of the construction of the biosensor aimed at the determination of the antioxidant capacity of natural samples. In fact, the polymer showed enzymatic compatibility and ensured the entrapment of the enzyme in a reduced period of photopolymerization.

Several screen-printed biosensors have been developed successfully using the PVA-AWP photopolymer in the process of immobilization of various enzymes, such as tyrosinase, protein phosphatase and acetylcholinesterase (STOYTCHEVA *et al.*, 2016; EL-MOGHAZY *et al.*, 2016; MISHRA *et al.*, 2015; CATANANTE; ESPIN; MARTY, 2015; ESPINNOZA *et al.*, 2014).

Stoytcheva *et al.* (2016) used the PVA-AWP photopolymer as immobilization matrix of the enzyme tyrosinase in the development of an amperometric biosensor for the determination of dopamine. Different base sensors were evaluated, as well as the biofunctionalization conditions were optimized as a function of the concentration of

PVA-AWP solution (1.5%, 3% and 6% by mass) and tyrosinase (1 mg mL^{-1}) for a 1: 1 ratio, and the UV irradiation time (15, 30 and 60 min) followed by 3 h drying and rinsing with distilled water. The conditions were defined for PVA-AWP at 3% and 60 min of UV irradiation and all the base sensors evaluated showed analytical efficiency in terms of dynamic range, LOD, sensitivity, reproducibility, among others. The enzymatic charge deposited on the surface of the working electrode is essential in the construction of the biosensor because a high enzymatic charge can provide a high electrochemical response while a low charge can provide better limits of detection. Consequently, it is important to evaluate different enzymatic charges in order to obtain a significant response and high sensitivity (CATANANTE; ESPIN; MARTY, 2015). In addition, the irradiation time was higher than that obtained in this study, and the additional step of drying, with a duration of 3 h, further increased the period for construction of the device. Possibly the enzymatic specificity may be related to the need for a greater degree of crosslinking of the photopolymer in order to ensure its entrapment.

Mishra *et al.* (2015) developed an automatic flow biosensor for the detection of organophosphorus mixtures in milk. Genetically modified acetylcholinesterase (B394 and B4) were used as bio-recognition elements in the construction of two biosensors. Each enzyme was immobilized on the surface of the working electrode by entrapment in the polymer matrix of PVA-AWP. 3 μL of enzyme solution formed by 30% of enzyme and 70% of PVA-AWP were deposited on the surface of the working electrode in order to immobilize 1 mU of enzyme per electrode. Subsequently, the electrodes were exposed to neon light (15W) for 4 h at 4 ° C to promote photopolymerization between groups of azide. After drying for 72 h at the same temperature, the biosensors were ready for use. Comparing the immobilization procedure used with the one used here, there is a certain agreement of the enzyme: PVA-AWP ratio, a lower enzymatic loading and a much longer exposure period to the biosensor proposed by Mishra *et al.* (2015).

In all works, it is observed the ease and efficiency of the enzymatic immobilization when using the photopolymer PVA-AWP, as well as the savings involved in the reduction of immobilized enzyme activity losses when compared to other immobilization procedures, such as when using glutaraldehyde as a cross-linking agent (BEESABATHUNI, 2010). In addition, PVA-AWP exhibits higher photo reactivity and lower swelling properties compared to other similar, water-soluble photopolymers, such

as poly (vinyl alcohol) substituted by styryl pyridine groups (PVA-SbQ) (STOYTCHEVA *et al.*, 2016; ROUILLON; TOCABENS; MARTY, 1994).

Electrochemical biosensors based on the ability to scavenge the H_2O_2 and/or $O_2^{\bullet-}$ radicals generated by the XOD oxidative catalyze of xanthine (XA) or HX have been developed with advantages and disadvantages inherent to its method (CAMPANELLA *et al.*, 2013; LATES; MARTY; POPESCU, 2011; CORTINA-PUIG *et al.* 2010; CORTINA-PUIG *et al.* 2009; CAMPANELLA *et al.*, 2009; CAMPANELLA *et al.*, 2004a; CAMPANELLA *et al.*, 2004b; CAMPANELLA *et al.*, 2003; CAMPANELLA; BONANNI; TOMASSETTI, 2003; CAMPANELLA *et al.*, 2001). In general, these biosensors show differences in the antioxidant molecule interaction and electrode surface, different electrode materials and electroactive species, application in real samples, enzymatic system (bi-enzymatic system are commonly used with the combination of XOD and a second enzyme, generally cytochrome C/superoxide dismutase (SOD) to interact with $O_2^{\bullet-}$ radicals and produce H_2O_2), immobilization procedure and/or dissolved in solution, potential applied, number of steps, analytical performance, among others.

6.2.3 Amperometric biosensor efficiency

The analytical performance of the amperometric biosensor based on the XOD enzyme is shown in Table 23. Precision was assessed by ten (10) successive assays with 10 μmol L^{-1} of HX, in the absence and presence of the reference antioxidant. The results of the coefficients of variation, in the absence and presence of the antioxidant, were 5.3 and 13.1%, respectively, and demonstrated good repeatability in the responses.

Table 23 - Performance of the XOD-PB-modified amperometric biosensor developed

Analytical Data	**In absence of antioxidant**	**Presence of antioxidant**
Calibration curve*	$I = (-2.72x10^{-8})\ C_{HX} - 1.17x10^{-7}$	$I = (1.89x10^{-8})\ C_{HX} - 1.0x10^{-7}$
Correlation coefficient (R^2)	0.997	0.972
Linear range (μmol L^{-1})	1.0-75	-
Precision (as RSD%)	5.3	13.1
Response time (s)	< 100	< 100
Detection Limit (μmol L^{-1})	2.2	0.7
Quantification Limit (μmol L^{-1})	7.2	2.4

*I = current intensity (A) and C_{HX} = hypoxanthine substrate concentration (HX, in μmol L^{-1}); **Gallic acid 12,5 μmol L^{-1}.

The detection (LOD) and quantification limits (LOQ) were calculated by the ratio of the average of ten (10) determinations of the standard deviation of the 10 blanks measurements and the slope of the analytical curve (m), multiplied by factor 3 and 10, respectively (TALEUZZAMAN, 2018).

Once prepared, kept in refrigerated condition (4°C) and protected from light, the stability of the biosensor was tested, storing it under these conditions and verifying its response after 48 h. Relatively low variation (CV = 6.5%) was observed between the responses, and the prototype showed operational stability of at least 6 hours of uninterrupted work. Once swollen, the biosensor can be washed with KPBS buffer solution and reused, taking about 8 min for further current stabilization in order to carry out further measurements. It is important to carry out a broader study of stability over time as it is believed that the storage time may be still much longer than 48h,

An amperometric biosensor was developed for the research of medicinal plants with the potential to prevent hyperuricemia (EL HARRAD; AMINE, 2016). PB-modified electrodes were used as the base sensor and the enzyme xanthine oxidase (1.0 mg mL^{-1}), used as a bio-recognition element, was immobilized on the surface of the working electrode with the aid of the Nafion and glutaraldehyde agents. The biosensor when stored in 0.05 mol L^{-1} PBS buffer solution (pH 7.5) at 4°C exhibited stability for 3 (three) weeks, maintaining the amperometric signal close to 90% of its initial response. However, from the fourth week, a 40% decrease over the initial response was observed. Possibly, the biosensor here developed for the determination of the antioxidant capacity will present stability over three (3) weeks when its stability over time is evaluated, considering that the use of the PVA-AWP agent is subject to lower losses of the enzymatic activity when compared to the use of glutaraldehyde (BEESABATHUNI, 2010).

Piermarini *et al.* (2013) developed a biosensor to determine uric acid in 85 serum samples. A PB-modified printed electrode containing the immobilized uricase enzyme with the aid of the Nafion and glutaraldehyde agents was coupled to portable instrumentation and used in the chrono-amperometric analyzes. The data showed that about 90% of the initial activity remained unchanged for 15 days of storage.

The determination of phenolic compounds in foods using laccase enzymatic biosensors has been extensively studied. These differ in terms of the enzyme loading and nature of the laccase (lyophilized or in solution), of the immobilization procedure, the characteristics of the electrode, of the nature of the sample (pharmaceutical formulations,

plant extracts, among others), of the phenolic analyte and final analytical performance. In this scenario, we observe the disparity in biosensor stability over time, ranging from 10 (ten) to 320 (three hundred and twenty) days (RODRÍGUEZ-DELGADO *et al.*, 2015; DI FUSCO *et al.*, 2010; FERNANDES *et al.*, 2008). It is known that for the enzyme to exhibit maximum activity and contribute to the stability and reuse of the biosensor, the selection of the enzymatic immobilization method should consider the nature of the biological element, the type of transducer used, the physicochemical properties of the analyte, and the conditions of operation in which the biosensor should work (RODRÍGUEZ-DELGADO *et al.*, 2015; SINGH *et al.*, 2008).

Figure 25 shows the range of HX concentrations for which the biosensor response changes linearly with the concentration ($R^2 = 0.997$), but a non-linear adjustment can also be performed, thus increasing the range of analyses. It is observed that a concentration of 10 µmol L^{-1} resulted in a current whose intensity is suitable for the detection of small variations of current that signal the presence of antioxidants, even in small amounts.

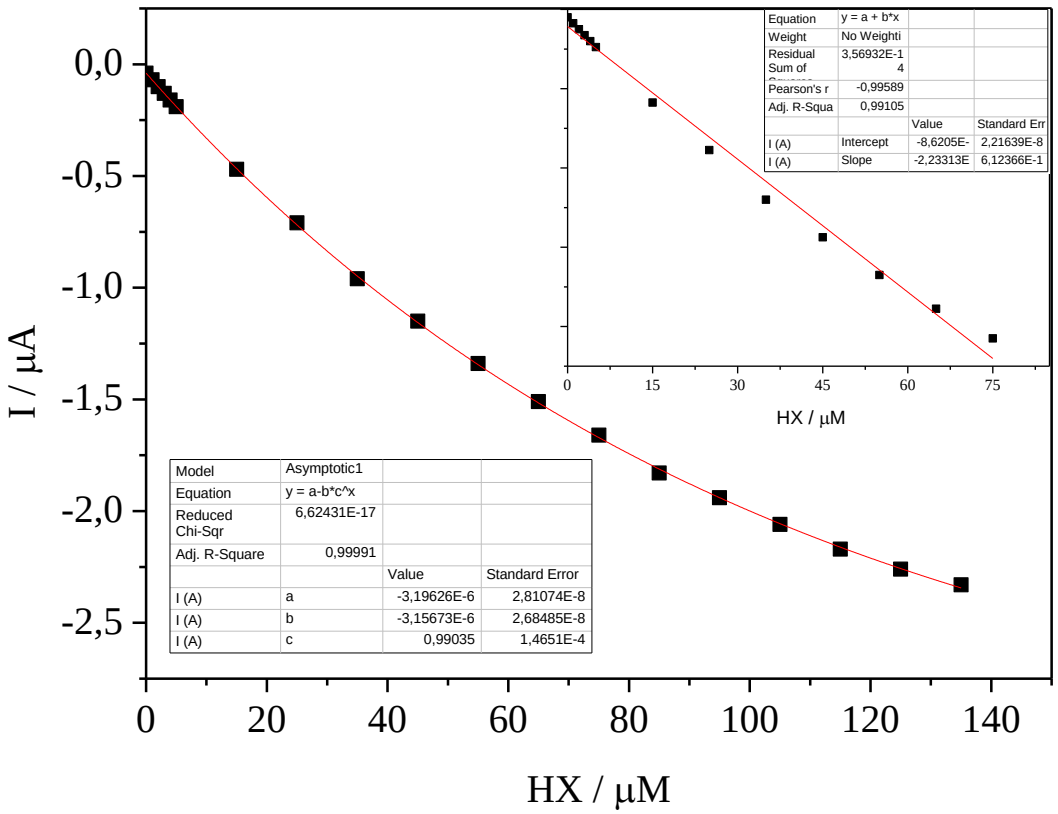


Figure 25 - The linear range of HX concentrations (1.0-75 µmol L^{-1}).

To obtain reliable results, adequate quality control is often necessary during chronoamperometric tests. The use of control charts can be an effective strategy to ensure that there is no change in a given process over time. Figure 26 shows the statistical control chart, constructed for 10 (ten) measurements of 10 μmol L^{-1} of HX, in the absence of antioxidants.

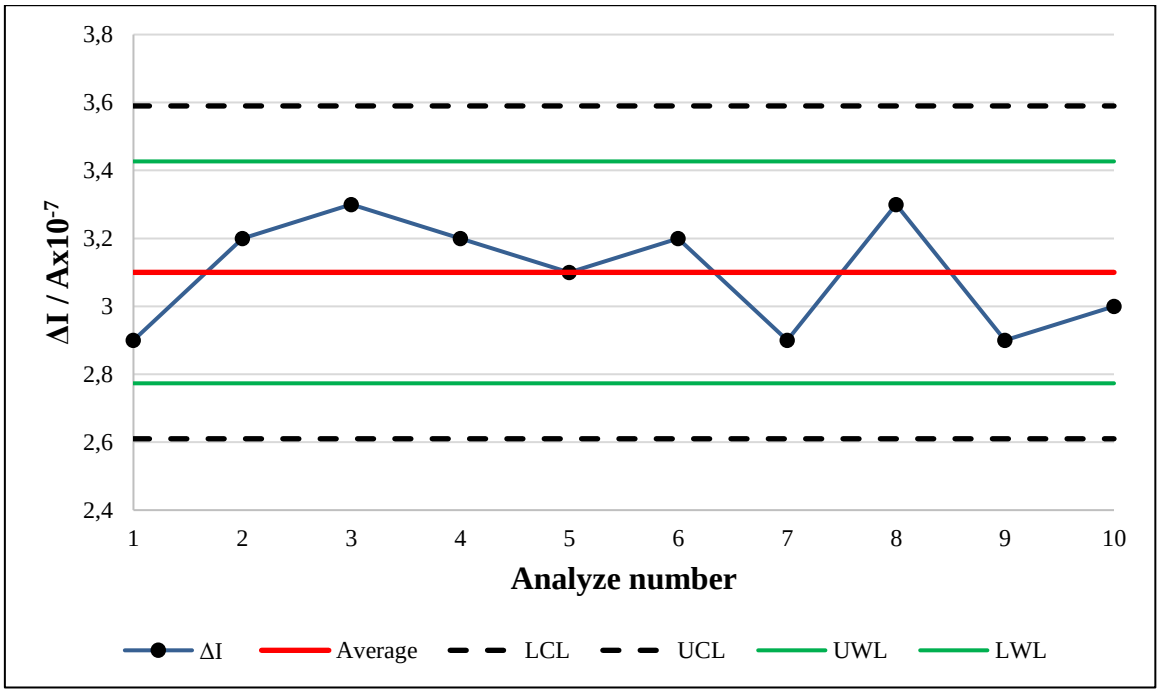


Figure 26 - Statistical control chart for monitoring and identification of variations during chronoamperometric tests with the same biosensor (n = 10). The solid red line is the intensity current variation average obtained, the solid green lines the Upper and Lower Warning Limits (UWL and LWL), and the dashed lines are the Upper and Lower Control Limits (UCL and LCL).

The x-axis data is equivalent to the sequential measurements, and those of the y-axis, the chronoamperometric responses obtained under identical conditions. It can be noticed that the biosensor did not change after repeated measurements, with minimum variations around the average current intensity (central solid line, red color). The constant value that makes up the two full lines, closest to the centerline, is the average response ± 2 times the standard deviation (Upper and Lower Warning Limits). Dashed lines were set as mean responses ± 3 times the standard deviation (Upper and Lower Control Limits). These lines were established in order to alert to the irregularities that occurred during the tests that lead to the variability of the responses. When two or more results, in sequence, exceed one of the warning lines, or when a single result exceeds one of the action lines, this means that the method can be considered statistically out of control (SUMAN;

PRAJAPATI, 2018). Within these basic assumptions, it is concluded that the developed biosensor presents a high level of reliability and repeatability in relation to the responses it provides.

Using the chart helps you detect non-standard variations, allowing you to correct them. Control charts should be reviewed periodically so that their control limits are adjusted according to optimizations and the need for the process.

Mundaca-Uribe *et al.* (2017) used the control charts approach to evaluate the stability of the biosensor built for the detection of malic acid in fruit juices over a period of nineteen (19) days. Through the control chart generated, the authors observed that the electrochemical measurements remained within the warning limits in the first 17 (seventeen) days, gradually dropping the response after this period.

Similarly, Oliveira *et al.* (2012) used the control chart as a statistical tool to evaluate the stability of the enzymatic biosensor idealized for the determination of glyphosate in aquatic environments. The control chart evidenced the efficiency of peroxidase immobilization at the electrode, with biosensor stability in a maximum time of 8 (eight) weeks of storage under controlled temperature.

The biosensor developed in this work presented good performance with high sensitivity and precision, as well as fast response time and low operation cost since it requires few reagents and in small concentrations under the conditions of the test. It also presents excellent stability of the generated signal, easy automation for macroscale preparation, simplicity of operation and manufacturing, besides using a low value of applied potential, which minimizes electrochemical interferences. Regarding the biological relevance, the biosensor presented an additional advantage, since it measures the combined antioxidant capacity against the $O_2^{\bullet-}$ and H_2O_2 ROs that are also produced by the human body, instead of using artificial radicals, as is the case of conventional colorimetric assays such as ABTS and DPPH.

Another great advantage of the prototype is the portability capability (since connected to the portable potentiostat), which gives it attributes for the determination of the antioxidant capacity *in situ*, in order to prevent losses due to degradation of these in the function of the luminosity, the variation of temperature and the presence of oxygen. This may be a promising tool for the determination of antioxidant capacity in environmental samples in a few minutes, and without requiring laborious preparation steps.

The influence of natural interferents which could affect the biosensor performances when applied in the food matrices (GA, ascorbic acid, and quercetin), as well as compounds involved in the detection scheme (HX), were evaluated. In this way, cyclic voltammetry measurements using the PB-modified electrode and the biosensor proposed was performed in conditions of absence and presence of these compounds and the results showed that at the value of applied potential selected for amperometric detection (-100 mV *vs* Ag/AgCl), no interferences were observed from neither in the substances usually found in food nor in those involved in the detection principle. In addition, the PB selectivity, the enzymatic oxidation of HX and the amperometric detection of H_2O_2 and/or $O_2^{\bullet-}$ at low potential practically assures the absence of interferences.

6.2.4 Application of the biosensor

6.2.4.1 Validation of the biosensor response by the gallic acid antioxidant

The developed biosensor was applied to the determination of the antioxidant capacity of GA, which is a strong reducing molecule found in food and herbs of great medical, pharmaceutical, and food technology interest. Elsewhere, GA is very often used as a reference (YEN; DUHB; TSAI, 2002). Amperometric measurements were performed following the protocols detailed in Section 5.4.3. The decrease in cathodic current intensity as a function of gallic acid concentration is shown in Figure 27. The antioxidant capacity (%) of gallic acid, calculated by expression 6 for the different concentrations, is shown in Figure 28.

Successive additions of 20 μl of HX (5 mmol L^{-1}) in the presence of gallic acid in increasing concentrations resulted in a decrease in the electrochemical signal, which was proportional to the concentration of gallic acid (Figure 28). GA molecules and other antioxidant species react with $O_2^{\bullet-}$ radicals and/or H_2O_2, inducing a decrease in the amount of produced H_2O_2 and a subsequent decrease in the oxidation current, that allows the quantification of antioxidant capacity.

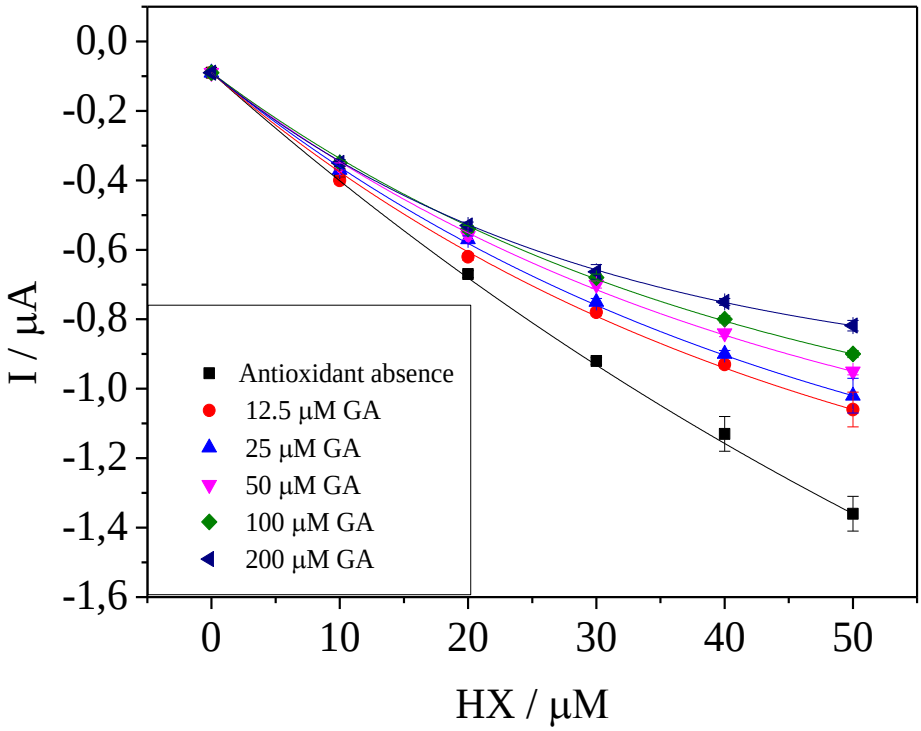


Figure 27 - Intensity of the current generated in the biosensor system, as a function of the concentration of HX added, in the absence and presence of gallic acid, in different concentrations.

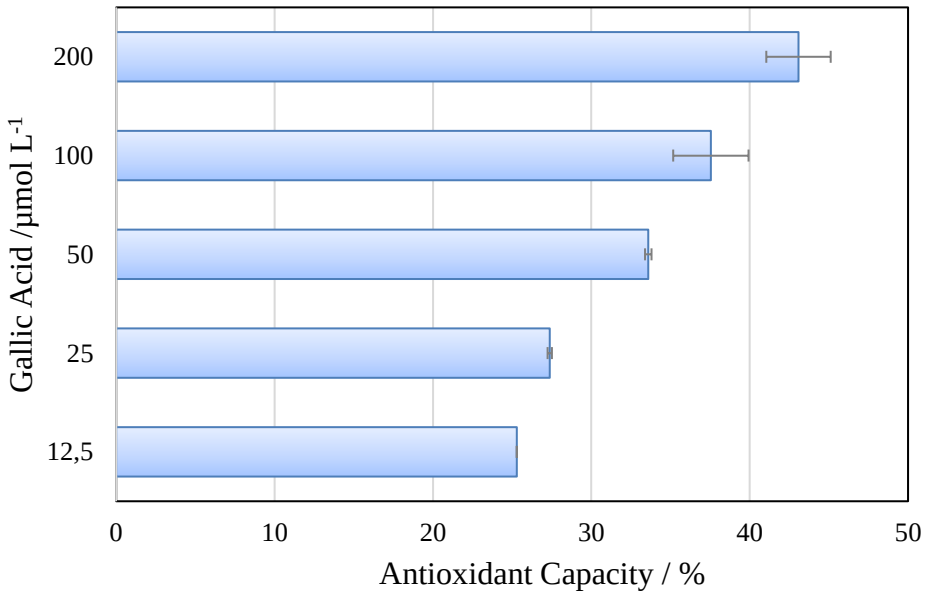


Figure 28 - The antioxidant capacity of gallic acid, as a function of its concentration, determined by the biosensor system.

With this experiment, it was possible to verify, with the developed biosensor system, a strong correlation between the reduction of the chronoamperometric response and the antioxidant concentration. Thus, combining this experiment with the merit figures already discussed (linearity, sensitivity, precision, stability), it can be concluded that the proposed biosensor was conveniently validated for analytical purposes.

In order to validate analytical and bioanalytical assays for antioxidant capacity determination, such as NBT, ORAC, ABTS, and DPPH, different pure antioxidant substances have been employed, among them gallic acid, ascorbic acid, trolox and uric acid. These substances were also used as a reference in the IUPAC technical report on methods for measuring and assessing antioxidant capacity (AKAK *et al.*, 2013).

Researches involving the development of biosensors for the determination of antioxidant capacity have been used as reference antioxidants the gallic acid (BARROSO, DELERUE-MATOS; OLIVEIRA, 2012), trolox (CAMPANELLA *et al.*, 2013), ascorbic acid (BARROSO; DELERUE-MATOS; OLIVEIRA, 2012; LATES; MARTY; POPESCU, 2011), acetylsalicylic acid (CORTINA-PUIG *et al.*, 2010), alliin (CORTINA-PUIG *et al.*, 2009), caffeic acid, coumaric acid, and resveratrol (BARROSO; DELERUE-MATOS; OLIVEIRA, 2012).

Among the reference antioxidants, the use of gallic acid as a reference for the validation of tests aimed at the determination of antioxidant capacity is highlighted by both the high antioxidant potential and the efficiency as an inducing agent of apoptosis. These properties justify the various biological and pharmacological activities inherent to this triphenolic molecule, and their interest in clinical and industrial applications (BADHANI; SHARMA; KAKKAR, 2015).

6.2.4.2 Determination of the antioxidant capacity of real samples

The developed biosensor was applied in the determination of the antioxidant capacity of commercial pulps of Amazonian fruits (nectars formed by the mixture pulp:buffer in the ratio of 10:90, v/v) and in natura pulp of non-autochthonous fruits (refreshments formed by mixing pulp:buffer in the ratio of 25:75, v/v). Amperometric measurements were performed following the protocol detailed in Section 5.4.3. In Figures 29 and 30 the curves obtained from the concentration of the enzymatic substrate HX are

presented as a function of the cathodic current intensity observed in the absence of antioxidants and in the presence of nectars and refreshments of fruits.

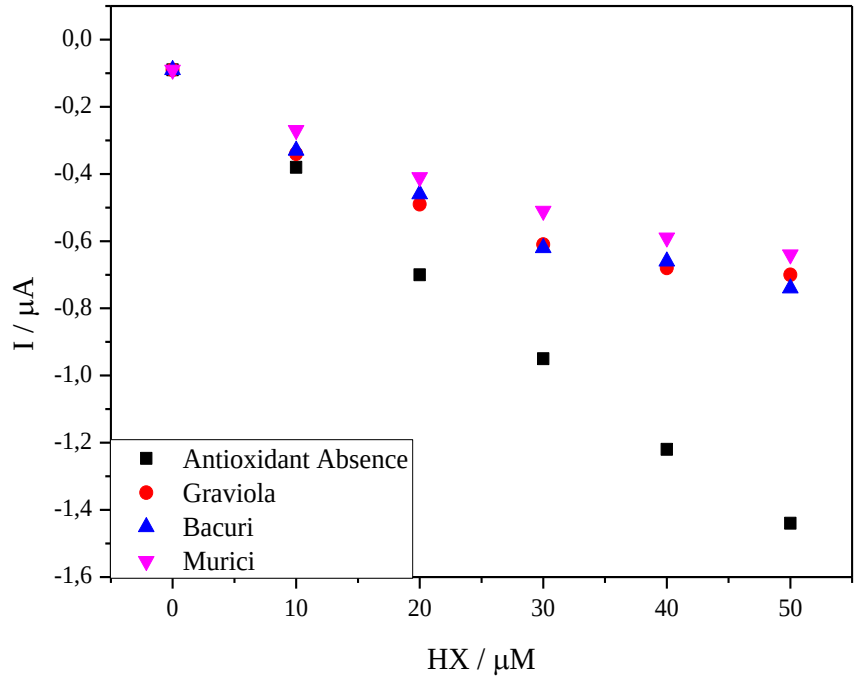


Figure 29 - Antioxidant effect of nectars of the commercial pulps of Amazonian fruits. Response of the amperometric biosensor as a function of successive additions of the hypoxanthine substrate (HX), in the absence and presence of the samples.

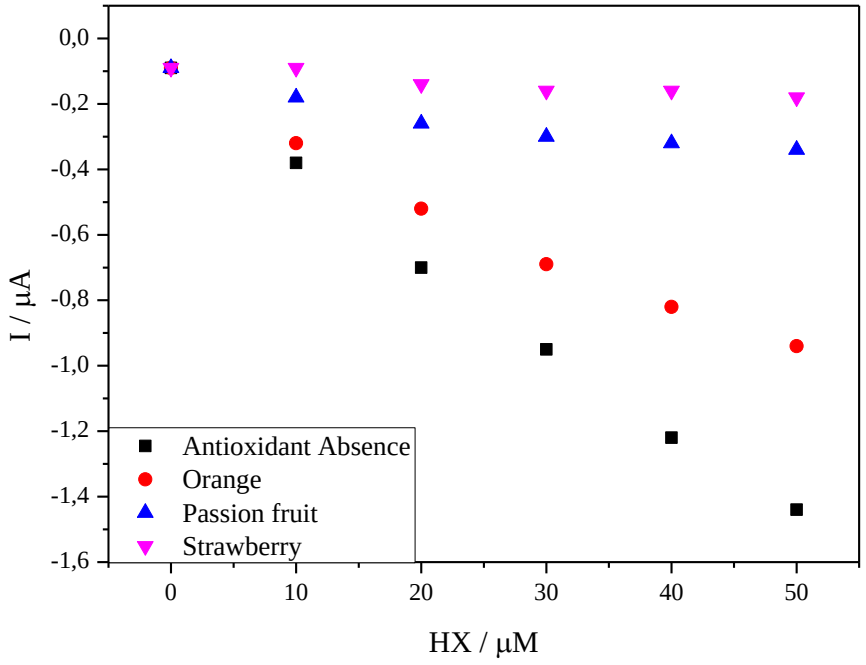


Figure 30 - Antioxidant effect of refreshments of fruits non-autochthonous. Response of the amperometric biosensor as a function of successive additions of the hypoxanthine substrate (HX), in the absence and presence of the samples.

Turning to Figures 29 and 30, apparently, the strawberry sample had a higher antioxidant capacity, due to the greater slope of the analytical curve when compared to the negative control (non-antioxidant test). However, in order for a comparison between these results to be achieved, without the samples of Amazonian fruits being underestimated in their antioxidant capacity, it is necessary to evaluate the samples for the same amount of pulp or juice. In this sense, an approximation was made, considering that in the preparation of the refreshments (25% v/v) 2.5 times more strawberry, passion fruit, and orange pulp were used than for the Amazonian fruits graviola, bacuri and murici (10% v/v nectars). Thus, it was necessary to divide by 2.5 the values of the antioxidant capacity obtained for the refreshments of the non-autochthonous fruit (Figure 32), and thus it was possible to observe that Amazonian fruits have a higher antioxidant potential. The Amazonian fruits, because they are native to the region, have the necessary adaptations to survive in the habitat and are integrated to the biogeochemical cycles present in the environment; for this reason, commonly the nutritional content of these fruits are superior to those of domesticated fruits (ODHAV *et al.*, 2007).

The reference literature shows research that evaluated the antioxidant capacity of some fruits here contemplates (BECKER *et al.*, 2019; HIDALGO; ALMAJANO, 2017; BHAT; STAMMINGER, 2015). However, the results obtained are hardly comparable, due to the different mechanisms of reaction, concentrations of extracts, potential redox, pH values, types of solvents used, the concentration of radical species, as well as the types of tests applied (APAK *et al.*, 2013).

In a previous work (BECKER *et al.*, 2019), extracts of fresh strawberries (prepared in KPBS buffer solution) produced in France had their antioxidant capacities determined using the NBT colorimetric assay. The extracts had different mass/buffer ratios and the antioxidant potential was measured against the superoxide radicals produced by the enzymatic system HX/XOD. Comparing the results obtained with the biosensor and the NBT test (93.0 and 92.4%, respectively), we can observe that there are no statistic differences ($p < 0.05$) between these. It is important to point out that the fruits came from different countries and the concentration has taken for the purpose of comparison of 10%, occurred in the volumetric ratio for the wet mass samples in the biosensor, while for the NBT assay was used 10 % in mass/volume ratio for freeze-dried French samples. Despite the differences pointed out, it is known the high antioxidant capacity of red fruits such as strawberry, which was demonstrated in both methods

through the similar inhibition of ROS in more than 90% (HIDALGO; ALMAJANO, 2017).

The results obtained, now expressed in antioxidant capacity (%) of the nectars and refreshments of the fruit are shown in Figures 31 and 32, respectively.

Nectar samples from bacuri, graviola and murici fruits inhibited more than 50% of ROS generated *in vitro* and the results did not differ statistically ($p < 0.05$) (Figure 31). From the analytical point of view, these results can be considered very interesting, since high inhibition rates have been observed, even for the diluted samples, which demonstrates once again the high sensitivity of the proposed enzymatic biosensor. Bacuri and graviola are native Brazilian fruits and murici is a cultivated fruit used by the local population as food and in popular medicine, being consumed *in natura*, in the form of juices, sweets, and ice creams.

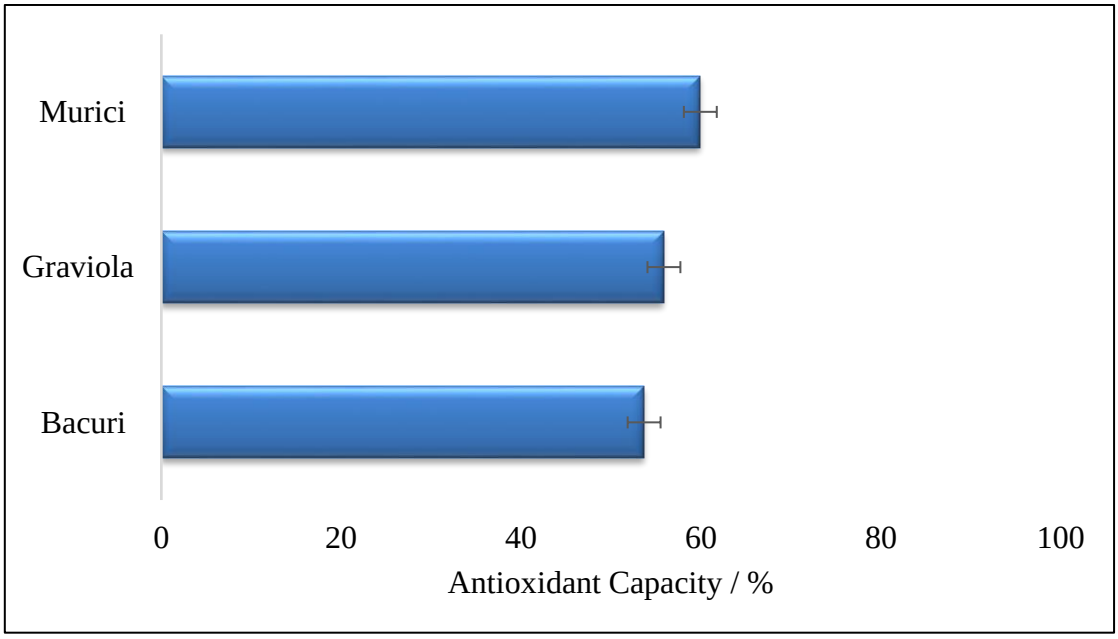


Figure 31 - Antioxidant capacity of Amazon fruit nectars evaluated by the developed biosensor.

Comparing the results for orange, passion fruit and strawberry fruits (Figure 32), it was observed that the strawberry sample presented the highest antioxidant capacity (93.0%), followed by passion fruit (82.0 %) and orange (37.9%) samples.

The orange refreshment, even being prepared from the *in natura* fruit, in which it is expected to have smaller losses related to storage, presented the lowest antioxidant capacity, even when compared to the nectars of frozen pulps. It is known that factors such

as plant variety, genetical diversity, stage of maturation, climatic and edaphic conditions, processing and food storage, among others, can strongly influence the phytochemical content (MELO *et al.*, 2008). However, in the present work, a highly sensitive and selective biosensor has shown that orange refreshments do not present high antioxidant potential.

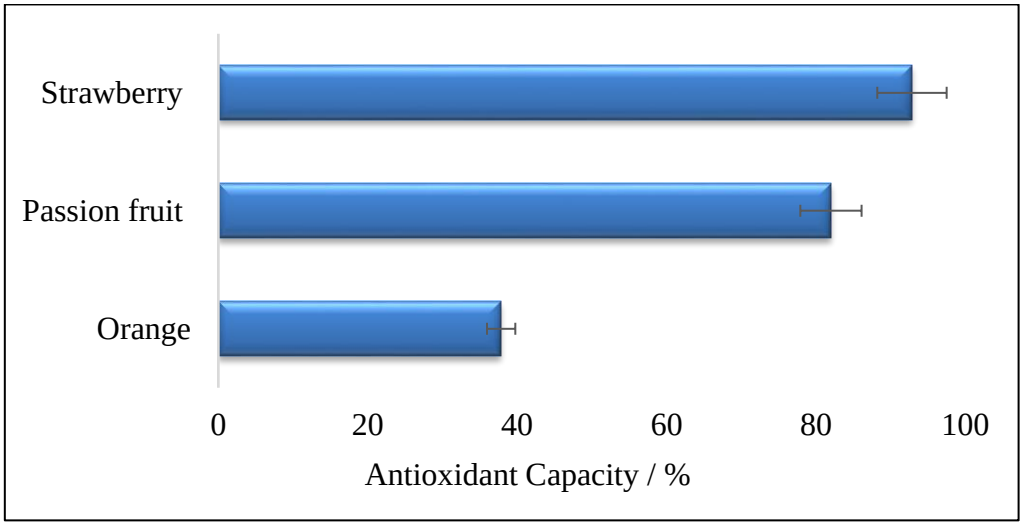


Figure 32 - Antioxidant capacity of refreshments of the non-autochthonous fruits evaluated by the developed biosensor.

The antioxidant capacity determined by the biosensor expresses the synergistic potential of the antioxidant chemical compounds present in the samples, in inhibiting the combined ROS ($O_2^{\bullet-}$ e H_2O_2). Due to the chemical diversity of antioxidants in real samples such as orange, it is not possible to decide with certainty the reason why orange nectar presented the lowest antioxidant capacity since it is necessary to identify and quantify all the antioxidant compounds present in this matrix. It is known that orange is appreciated for its high content of vitamin C, a nutrient known for its natural antioxidant properties (VANNUCCHI; ROCHA, 2012). On the other hand, scientific studies have pointed to the low content of phenolic compounds in this fruit, which are considered the major responsible for antioxidant activities in vegetables (ÁLVAREZ *et al.*, 2014). Therefore, the chemical composition of the orange, possibly formed by lower contents in phenolic compounds, contributed to its lower antioxidant potential, a result that corroborates with the statements of Riso *et al.* (2005).

Finally, it is noted that prior dilution of the samples in the assay buffer itself is necessary when using the biosensor. Due to the complexity of environmental matrices, not always the same concentration for different samples can be used, so dilutions may be necessary, which is a reality of any bioanalytical method.

It is worth mentioning that the methodology used to prepare the nectars and refreshments was also based on the inclusion of these fruits in the diet of the population in the form of beverages, in order to obtain concentrations of fruits close to the conventionally consumed fruit (nectar and refreshments). The use of buffer solution as a solvent in the extraction of analytes, as well as in the dilution of samples, has been a strategy used in studies involving enzymatic biosensors for the most diverse matrices such as infant foods, fruits, vegetables, teas and water samples (CORTINA-PUIG *et al.*, 2009; NUNES *et al.*, 2005; CAMPANELLA; BONANNI; TOMASSETTI, 2003; NUNES *et al.*, 1999). In addition to maintaining enzymatic activity, the buffer solution may reflect pH and salinity conditions that approximate the physiological in vivo and offer greater sensitivity (CORTINA-PUIG et al., 2009).

Other ways of expressing the results can be used, such as the equivalent mass of a reference antioxidant per sample mass and the required concentration of the sample resulting in a 50% inhibition of the generated ROS (IC_{50}). Evaluating the biosensors developed in other works for this purpose, it is observed that, as in the present work, the antioxidant capacity has been represented by the inhibition reflected in the slope of the analytical curve (sensitivity), obtaining the relative antioxidant capacity (CAMPANELLA *et al.*, 2013; CAMPANELLA *et al.*, 2009**;** CAMPANELLA; BONANNI; TOMASSETTI, 2003; CAMPANELLA *et al.*, 2004a; CAMPANELLA *et al.*, 2004b; CAMPANELLA *et al.*, 2003). But also the antioxidant capacity has been expressed as IC_{50} (in g L^{-1} or μg mL^{-1}) (CORTINA-PUIG *et al.* 2009; CORTINA-PUIG *et al.* 2010) and as percentage (%), calculated from the relative decrease of the peak current recorded in the absence and presence of an antioxidant (LATES; MARTY; POPESCU, 2011).

It is also interesting to mention the costs of obtaining a biosensor. If the laboratory has a suitable screen-printing machine, on 24.5 x 20 cm PVC plates, 24 electrochemical sensors can be screened at one time (see Figures A10 and A11 of Annex A). Hence, after modification of the graphite working electrode, by incorporation of the mediator, followed by the immobilization of the enzyme, the total cost of a single biosensor can

reach less than two dollars, since the amounts of reagents and pastes of graphite, Ag/AgCl and ink are negligible. During the test, due to the small volumes of buffer and HX substrate solutions, and considering the low concentration of the HX substrate, it can be inferred that a single application would have the final cost of fewer than three dollars. Thus, it is evident the economic viability and the great applicability for the biosensor developed here.

7. CONCLUSIONS

Despite the vegetal biodiversity, many Amazonian fruits are still little explored in relation to their biotechnological potential. In this study, ten native fruits that are part of the diet of the inhabitants of the Amazon were evaluated for their nutritional properties, antioxidants, bioactive composition, as well as their cytotoxicity in the human colon carcinoma cell line. In addition, an amperometric biosensor for the determination of antioxidant capacity in complex samples such as fruits was developed, considering the need for tools of better analytical efficiency and biological relevance.

The results of the bromatological analysis show the diversity in the physicochemical composition of the fruits, in which higher lipid, protein, and energy contents were obtained in buriti, monguba and uxi samples. From the nutritional point of view, and with a focus on industrial applications, these data are quite relevant.

The evaluation of the mineral composition of the fruits of abiu, açaí, bacuri, buriti, inajá, monguba, pajurá and uxi allowed to classify them as being of high content and / or source in one or more minerals (Mg, Cu, Fe and Mn) and therefore the inclusion of these fruits in the diet can contribute to nutritional corrections and to prevent diseases related to deficiencies of these minerals. The contents of Cu and Mn in inajá pulp and Ca in pajurá pulp were reported for the first time in this study.

In relation to the bioactive compounds, the pulps of açaí, bacuri, buriti, cupuaçu, inajá, and uxi were classified as foods high in vitamin C, while the fruits of biribá and pajurá showed be sources in this nutrient. This work was the first to present the concentration of vitamin C in the fruits of inajá, monguba and pajurá.

All fruits presented antioxidant capacity against different oxidizing sources, however, the pulps of biribá and bacuri deserved prominence because they presented

greater lipophilic antioxidant capacity, whereas the fruits of abiu, inajá and monguba showed a greater hydrophilic antioxidant capacity.

Increasing concentrations of Amazonian fruit extracts led to inhibition of caco-2 cell line growth. The present work was the first to show the antiproliferative effects of a large part of the fruits studied, and the continuation of these studies may lead to therapeutic strategies.

The qualitative chromatographic profile in phenolic compounds revealed a wide diversity of these, in which 38 compounds could be identified.

The quantification of these in the fruits of biribá, inajá and monguba allowed the determination of 11, 25 and 21 phenolic compounds, respectively, among them phenolic acids, flavonoids and stilbenes with important biological activities, such as beneficial effects on Parkinson's disease, Alzheimer, obesity, diabetes, and inhibition of the proliferation of some forms of cancer.

In this work, a new analytical tool was also developed: an enzymatic biosensor capable of determining the antioxidant capacity of natural or processed plant samples. The biofunctionalization conditions were optimized by chemometric modeling, its analytical performance characterized, and, finally, the prototype was used to determine the antioxidant capacity of pure antioxidant substances and actual fruit samples.

The immobilization of the XOD enzyme using PVA-AWP proved to be an excellent strategy for the biofunctionalization of the electrode in the construction of the biosensor, having been optimized, chemometrically, operational conditions leading to the reduction of electrochemical interference and in greater sensitivity and precision, as well as saving time and quantities of reagents. The prototype also showed simplicity in construction and operation, low cost, the stability of the signal generated, easy automation, a good period of continuous operation, reuse, and high capacity of portability.

It is also worth mentioning the biological relevance of this tool in relation to conventional colorimetric assays, since, by using the biosensor, the antioxidant capacity of samples against the combined EROs ($O_2^{\bullet-}$ e H_2O_2) is determined, which are present in biological systems and present cytotoxicity under conditions of oxidative stress. The bioelectroanalytical parameters, allied to simplicity in its construction, makes the proposed biosensor a promising tool for the determination of antioxidant capacity in natural or processed plant samples.

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ANNEX A

Analytical Techniques and Sample Preparation

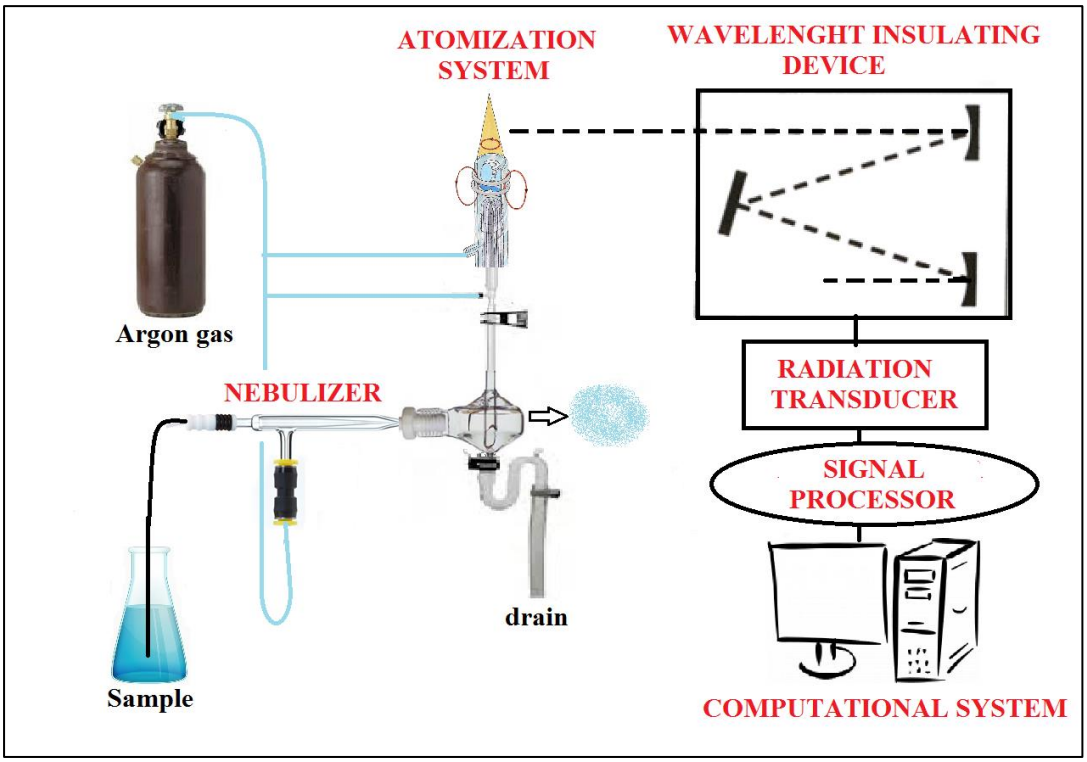

Figure A1 - Scheme of typical components of a Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES) system (Adapted from DONATI; AMAIS; WILLIAMS, 2017; GHOSH *et al.*, 2013).

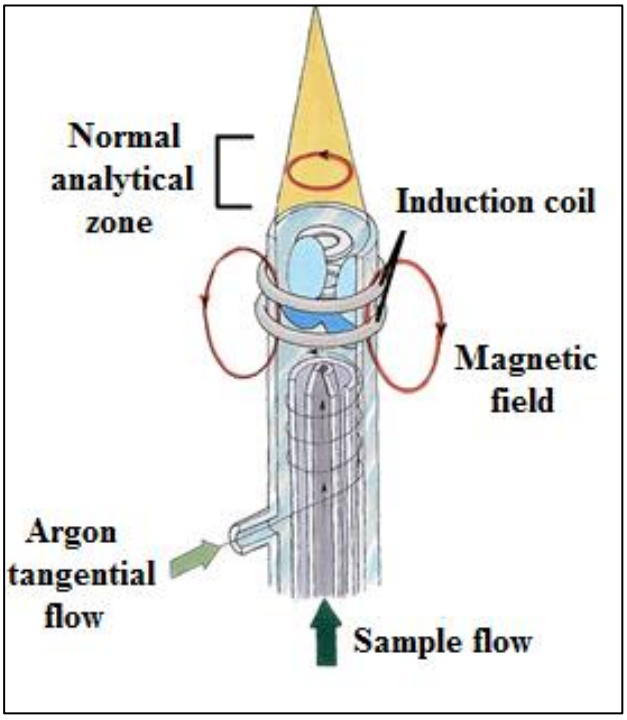

Figure A2 - Scheme of torch and plasma formed used in the ICP OES system (Adapted from DONATI; AMAIS; WILLIAMS, 2017; GHOSH *et al.*, 2013).

Summary Operation of an ICP OES system: The sample, usually in aqueous solution, is aspirated by an argon stream under high pressure (Bernoulli effect) and brought to the nebulizer system (1) where it is converted to a fine aerosol by a jet of compressed gas. There are several types of nebulizers on the market, two of which are more common: concentric nebulizers, used in this study, are well known for their simplicity in construction, reproducibility and low cost; Cross-flow nebulizers which generally pump the sample into the nebulizer with the aid of a peristaltic pump and have less clogging problems and greater resistance to corrosion and chemical attack (DONATI; AMAIS; WILLIAMS, 2017; GHOSH *et al.*, 2013).

The gas flow takes the aerosol produced in the misting system into the atomization system (2) through the argon flow of the torch central tube. In the torch, the high temperature promoted by the argon plasma allows the production of atoms and ions in the gas phase through a series of interrelated physical and chemical phenomena: desolvation, volatilization, dissociation, atomization, ionization and/or excitation, accompanied by the production of spectral lines, which is characteristic of the atomic species under study and proportional to the population of atoms present in the sample, which obeys the law of Lambert-Beer (DONATI; AMAIS; WILLIAMS, 2017; GHOSH *et al.*, 2013).

A plasma, by definition, is a conductive gas mixture containing a significant concentration of cations and electrons, with a total charge close to zero (DONATI; AMAIS; WILLIAMS, 2017; GHOSH *et al.*, 2013), which is formed and maintained by a flow of argon gas in A high-frequency magnetic field in a quartz torch, consisting of three concentric tubes, according to the scheme shown in Figure A2.

In the torch the central tube conducts the sample in aerosol form, coming from the nebulizer; in the intermediate tube flows auxiliary gas for the stabilization of the plasma; in the outer tube flows argon gas in a flow tangential to the walls in order to cool them preventing their fusion in view of the high temperatures, as well as to centralize the plasma.

The normal analytical zone is the plasma region where the determinations of emitted radiation are performed, which can be performed at right angles (radial observation geometry) or under the axial axis of the plasma (axial observation geometry). The radial geometry offers better stability and precision, while the axial geometry has lower limits of detection, being sought in the analysis of elements in trace levels.

The atomic or ionic emission of the plasma is separated at its constituent wavelengths by a wavelength insulating device (3) equipped with devices that improve selectivity and instrumental sensitivity. Isolated radiation is converted into electrical signal by a transducer, multiple transducers or by a detector arrangement (3). The electrical signals are then processed (4), producing the reading of the radiation in the form of emission intensity or concentration, in addition to a series of information necessary for analytical quality control (DONATI; AMAIS; WILLIAMS, 2017; GHOSH *et al.*, 2013).

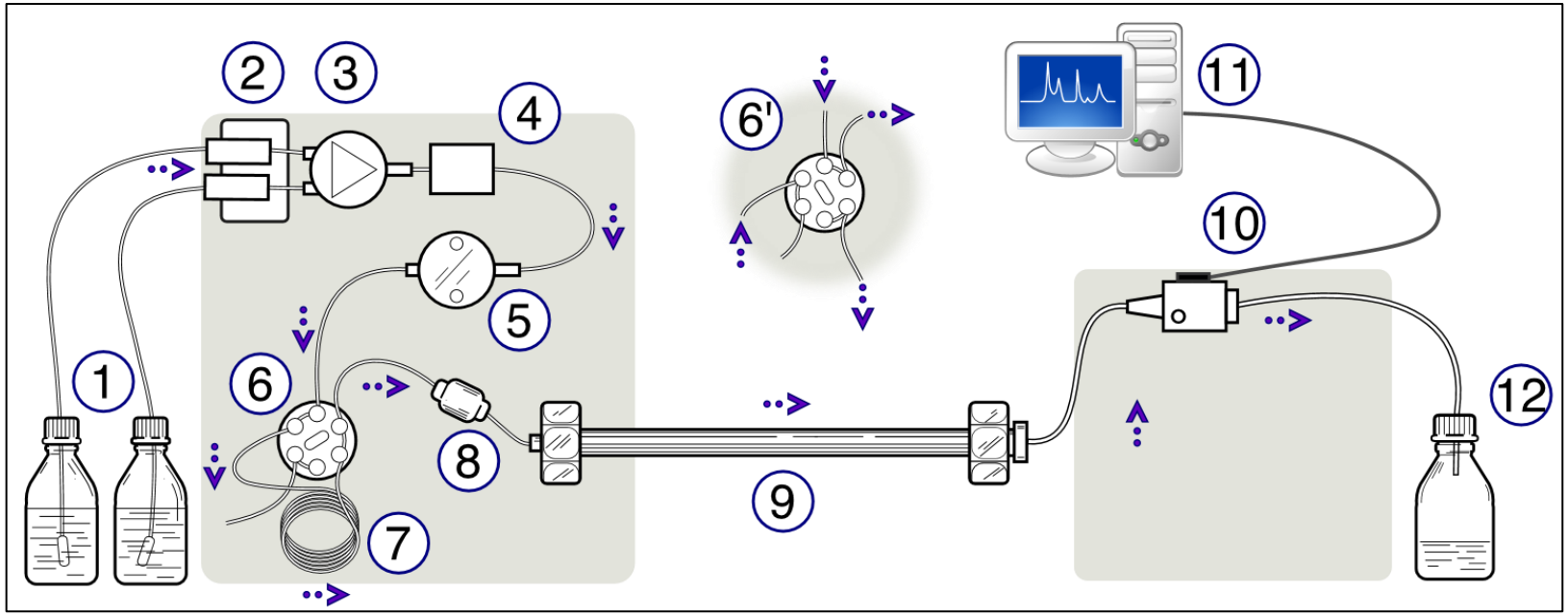

Figure A3 - Schematic representation of an HPLC unit: ① Solvent reservoirs, ② Solvent degasser, ③ Gradient valve, ④ Mixing vessel for delivery of the mobile phase, ⑤ High-pressure pump, ⑥/⑥' Switching valve in "inject/load" position", ⑦ Sample injection loop, ⑧ Pre-column (guard column), ⑨ Analytical column, ⑩ Detector, ⑪ Data acquisition, ⑫ Waste or fraction collector (Image credit:Yassine Mrabet).

Summary Operation of an HPLC System: The use of a stationary (solid) and a mobile (liquid) phase allows the sample components to be transported through the stationary phase by the flow of the mobile phase and the separation of the components of a complex mixture occur on the basis in the differences in migration speed between the components of the mobile phase (DONATI; AMAIS; WILLIAMS, 2017; GHOSH *et al.*, 2013).

Briefly, the pump (5) moves the mobile phase (1) formed by a single solvent or a mixture of solvents of constant composition (isocratic elution) or a system of solvents that differ in polarity (gradient elution). The flow of the mobile phase carries the injected sample (7) through the analytical column (9) containing the stationary phase. The components of the sample are distributed between the two phases according to their affinities, where the substances with greater affinity with the stationary phase move more slowly, whereas the substances with little affinity with this phase move more quickly. On passing through the column, the components are brought to the detector (10) which registers the signal as a function of time (or the volume of the mobile phase), which is processed (11) by the electronics of the instrument generating a series of peaks of the solute concentration as a function of the elution time (or elution volume). Peak positions on the time axis are employed in the identification of the components, while the areas on the peaks are used in the quantification by comparing the area of a standard whose purity is known with the area of the sample in question (DONATI; AMAIS; WILLIAMS, 2017; GHOSH *et al.*, 2013).

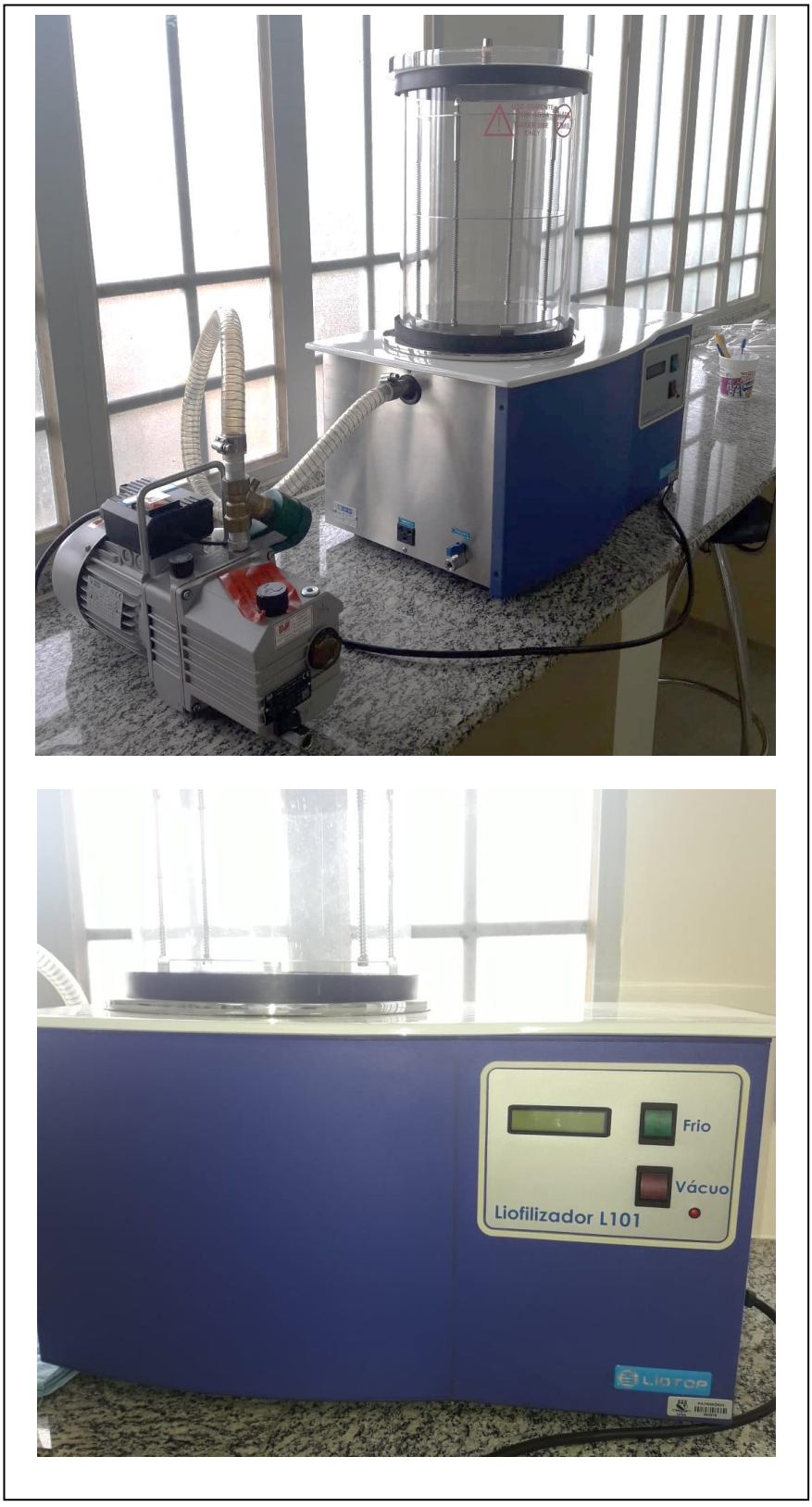

Figure A4 - Photos of the lyophilizer used in the pre-preparation of the samples.

Figure A5 - Photos of the microwave oven used in the extraction of minerals from samples of Amazonian fruits. Highlight given to the carousel containing the Teflon vessels.

Figure A6 - Photos of the Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP OES).

Figure A7 – Photos of the Ultra High-Performance Liquid Chromatography (UHPLC) system (LABBULLETIN, 2019).

Figure A8 - Photos of potentiostat, especially the custom connector for biosensor coupling.

Freeze-drying process Lyophilized samples

Figure A9 - Photo of the freeze-drying scheme of the fruit samples and vacuum storage of lyophilized samples.

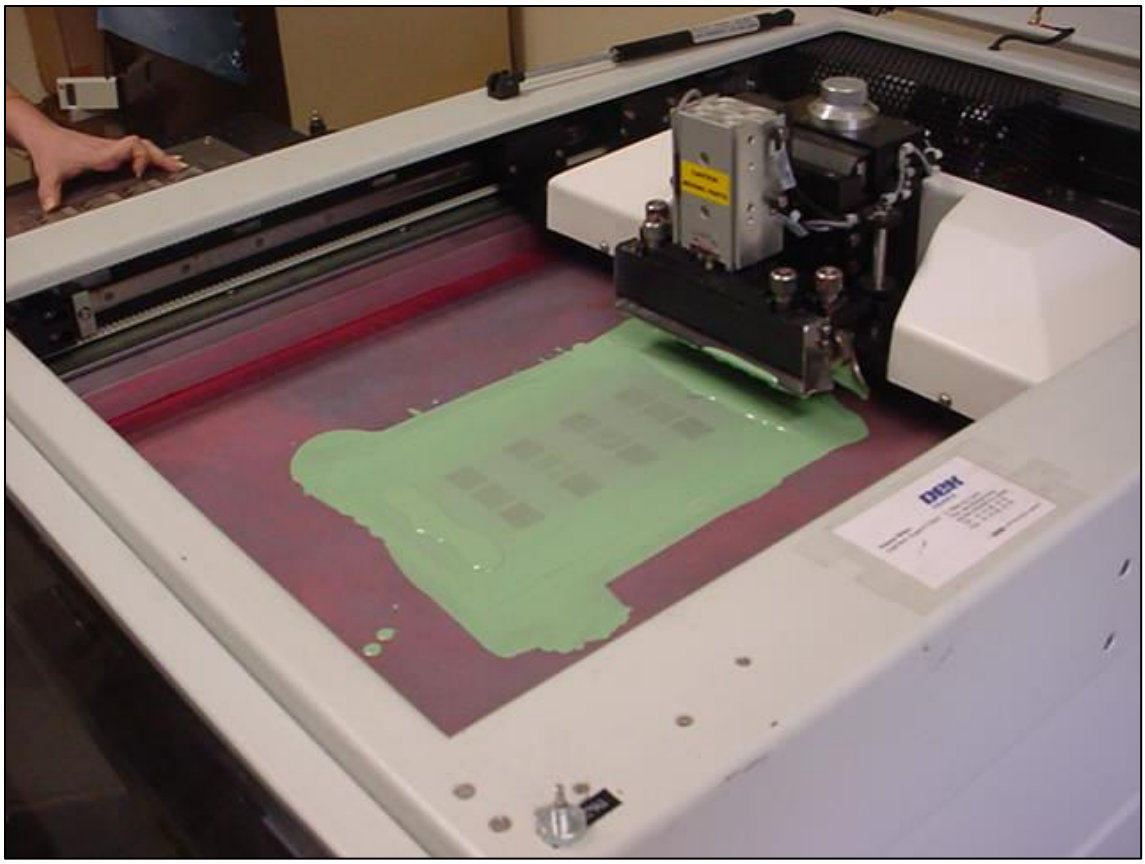

Figure A10 - Photos of the screen printing machine, at the time of production of electrochemical sensors (electrodes), from the successive deposition of layers of graphite and Ag/AgCl on a thin PVC plate.

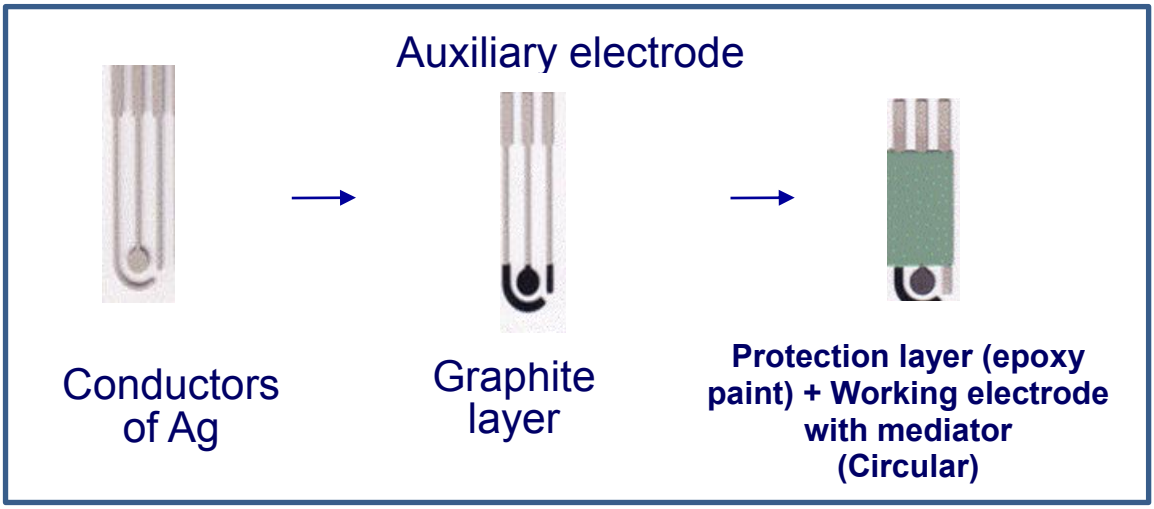

Figure A11 - Scheme of deposition of the layers of the electrochemical sensor, forming the three electrodes, using the screen printing machine.

ANNEX B

2015-2019 SCIENTIFIC PRODUCTION

Chemical variability in Amazonian palm fruits: *açaí* (*Euterpe oleracea* Mart.), *buriti* (*Mauritia flexuosa* L. f.), and *inajá* [*Maximiliana maripa* (Aubl.) Drude] (Arecaceae)

Variabilidade química em frutos de palmeiras amazônicas: açaí (*Euterpe oleracea* Mart.), buriti (*Mauritia flexuosa* L. f.) e inajá [*Maximiliana maripa* (Aubl.) Drude] (Arecaceae)

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Abstract: The bromatological composition, mineral content, bioactive compounds, and antioxidant capacity of three native Amazon Arecaceae fruits (*buriti*, *açaí*, and *inajá*) were chemically evaluated. These fruits showed high moisture contents (> 55%), and levels of ash values, total crude protein, and total carbohydrates in the range of 0.68-1.28%, 0.49-2.14%, and 6.10-26.51%, respectively. High levels of total lipids were found in *buriti* (21.0%). A wide range of mineral content was detected and the highest levels were found in the pulps of *buriti* (Ca, Cu, Fe, and Mg), *inajá* (Na and Zn) and *açaí* (Mn). All three fruits showed antioxidant activity with important levels of phenolic compounds and good or rich content of vitamin C. This study provides new data on the antioxidant activity and the nutritional composition of native Amazonian fruits. Based on this study, these fruits are suitable for use in the food and cosmetics industries, as well as in pharmaceutical compositions.

Keywords: Native Amazonian fruits. Bromatological composition. Minerals. Antioxidant capacity.

Resumo: Foram quimicamente avaliados as composições bromatológicas, os teores minerais, os compostos bioativos e a capacidade antioxidante de três frutos de palmeiras (buriti, açaí e inajá), nativos de Amazônia. Os frutos mostraram elevados teores em umidade (> 55%), níveis de cinzas, proteína bruta total e carboidratos totais na faixa de 0,68-1,28%, 0,49-2,14% e 6,10-26,51%, respectivamente. Altos teores em lipídios foram obtidos na polpa de buriti (21,0%). Uma ampla faixa de conteúdo mineral foi determinada, na qual os maiores teores estão nas amostras de buriti (Ca, Cu, Fe e Mg), na polpa de inajá (Na e Zn) e no açaí (Mn). Todos os frutos mostraram atividade antioxidante com níveis importantes de compostos fenólicos e boas ou ricas concentrações em vitamina C. Este estudo fornece novos dados sobre a atividade antioxidante e a composição nutricional de frutos nativos amazônicos. Com base neste trabalho, estes frutos são promissores para utilização nas indústrias de alimentos e de cosméticos, bem como em composições farmacêuticas.

Palavras-chave: Frutos nativos amazônicos. Composição bromatológica. Minerais. Capacidade antioxidante.

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INTRODUCTION

Arecaceae, or the palm family (formerly Palmeae), is one of the largest botanical families of economic and ecological importance and among the first groups of plants that have gained significant attention regarding the risks of becoming endangered (Moore, 1979; Balick & Beck, 1990; Zambrana *et al.*, 2007). This family has a great diversity comprising 283 species in Brazil, where 147 species are native to the Amazon biome (Leitman *et al.*, 2016).

> In addition to the importance in the rainforest structure and food source for many animals and humans, the palm trees have great economic potential to ornamental plant, medicine and cosmetic industries for human (Henderson, 1995; Lorenzi *et al*., 2004) (Arasato *et al.*, 2011, p. 7630).

Although some Arecaceae species, including some from Brazil, have been analyzed for centesimal composition in past years and a plethora of literature has reported on palm fruits (Silva *et al.*, 2015; Crepaldi *et al.*, 2000; Hiane *et al*., 2003; Menezes *et al.*, 2008; Teixeira da Silva de La Salles *et al*., 2010; Coimbra & Jorge, 2011), there is still a lack of research on the industrial applications of some palm trees.

Palms fruits comprise nutritionally important foods due their protective effect attributed to the presence of constituents such as minerals and high levels of phytochemicals with antioxidant properties (Nunes *et al*., 2011; Kahl *et al*., 2012; Liu, 2013; Kozlowska & Szotask-Wegierek, 2014; Wang *et al*., 2013). Therefore, data on the composition of native fruits are essential to encourage national and international marketing; assist the food, cosmetics, bio-cosmetics and others industries; and support policies to protect the environment and biodiversity. Meanwhile, an adequate knowledge of the composition aids quality control and food safety, as well as the evaluation and adequacy of intake of individual nutrients to the population.

Açaí (*Euterpe oleracea* Mart.) is a typical Amazonian palm in Brazil. Its fruits are globular or lightly depressed drupes with a diameter around 1.5 cm and weighing 1.5 g on average. The pulp has an exotic flavor, as well as high antioxidant and anti-inflammatory properties that classified it as the new 'super fruits'. It is consumed pure or accompanied with manioc flour, fried fish, or shrimp, as well as being used yet in manufacturing of juices, ice cream, jams, jellies, *açaí* wine, and dyes. *Açaí* plays an important socioeconomic and cultural role since the fruits have a high regional consumption, and their export has increased greatly in recent years (Souza *et al*., 2011; Brasil, 2015).

Buriti (*Mauritia flexuosa* L. f.) is an Amazon palm tree with a height of 15 to 20 m and is typical of muddy riverbanks and river islands. Its fruit is subglobose to elliptical, varies from 4 to 5 cm in diameter, and are covered with reddish-brown scales. The pulp is orange colored, oleaginous, and tasty. *Buriti* fruit pulp is consumed *in natura*, as juices, and in ice cream. The unprocessed oil is used to fry foods, like fish. In popular medicine, the fruit is used, for example, as a cold remedy, in infant nutrition, and for vitamin A deficiencies (Carneiro & Carneiro, 2011; Darnet *et al*., 2011).

Inajá fruits (*Maximiliana maripa* (Aubl.) Drude) are brown, oblong-ellipsoid, 4 to 5 cm long and 2.5 to 3 cm in diameter. The mesocarp has the fibrous outer layer, the inner layer being fleshy, with 0.3 to 0.5 cm in thickness and one to three seeds present (Shanley *et al*., 2010). The pulp of the fruit is consumed *in natura* or in the form of porridge and has been used in traditional medicine for the strengthening of debilitated people. However, it is not a highly appreciated fruit, possibly due to insufficient research and the consequent devaluation of the species (Villachica *et al*., 1996; Bezerra, 2011).

"Works related to the composition and quality of fruits and oils of native palm trees are important to add value to species still little explored in the region and consequently encourage the creation of new markets" (Santos *et al.*, 2017, p. 2). The aim of this work is to perform bromatological analysis, mineral composition determination and antioxidant capacity analysis of *buriti*, *inajá*, and *açaí* pulps collected in the Amazonian biome,

some of which have not been evaluated to date, in order to determine their potential use as foods and for other industrial purposes.

MATERIALS AND METHODS

REAGENTS

Analytical grade chemicals were employed in the preparation of all solutions. Deionised water (Milli-Q Millipore 18.2MΩ cm^{-1}) was used in all experiments. All plastics and glassware were cleaned by soaking in dilute nitric acid (1:9). The standard solutions of analytes for calibration procedure were produced by diluting a stock solution of 1,000 mg/L of the investigated elements (Ca, Cu, Fe, K, Li, Mg, Mn, Na, and Zn; from Merck Millipore Certipur®, Specsol®). The others reagents used were: nitro blue tetrazolium (NBT, N6876), hypoxanthine (HX, H9377), xanthine-oxidase enzyme (XOD from bovine milk, X4376), petroleum ether, phenolphthalein, sodium hydroxide (NaOH), sulfuric acid (H_2SO_4), potassium iodide (KI), dry starch, potassium iodate (KIO_3), nitric acid (HNO_3), hydrogen peroxide (H_2O_2), gallic acid, quecetin and oxide yttrium (Y_2O_3), all purchased from Sigma-Aldrich Corp (Nasdaq-Sial, Darmstadt, Germany).

SAMPLE COLLECTION

Three Amazon palm fruits were collected and used in the present study: *açaí* (*Euterpe oleracea*), *buriti* (*Mauritia flexuosa*), and *inajá* (*Maximiliana maripa*), at complete physiological maturity, were collected in Roraima state (02° 47.177' N; 60° 45.096' W; 3° 22' 17.7" N; 59° 51' 45.0" W; 02° 46.579' N; 60° 42.285' W, respectively). Fruit samples were refrigerated in the laboratory of the of Environmental Studies and Analysis Group (GEAA) at the Federal University of Maranhão, washed in deionized water and stored at -20 °C until the time of analysis.

BROMATOLOGICAL ANALYSIS

Moisture content (MC), total ash (TA), hydrogen potential (pH), acidity in citric acid (CA), crude protein (CP), and total lipids (TL) were performed according to the Association of Analytical Methods (AOAC methods) (Cunniff, 1998). Total carbohydrate (TC) was determined by the following equation: [TC = 100 – (MC + TA + CP + TL)]. Total energy value (TEV) was estimated as the at water conversion values of 4 kcal/g of protein and carbohydrates and 9 kcal/g of lipid, according to Merrill & Watt (1973). All analyses were performed in triplicate.

ANTIOXIDANTS

Antioxidant capacity

The samples were washed, pulped and mixed in a stainless steel mixer. An amount of approximately 200 g of the pulp was filtered in vacuum Buchner funnel with qualitative filter paper, followed by a new filtration with a quantitative filter paper (1.2 μm). A portion of 500 μL of the obtained extract was diluted to 1 mL in 50 mM Potassium Phosphate-Buffered Solution (K-PBS) containing 0.1 mM ethylenediamine tetra-acetic acid (EDTA) (pH 7.5). From this solution, dilution was performed in decreasing concentrations in K-PBS (pH 7.5).

A reaction mixture was prepared with 50 mM K-PBS (pH 7.5), 25 μM HX, 50 μM NBT, the antioxidant fruit extract (distilled water for the blank) and 0.2 $U \cdot mL^{-1}$ XOD, which was added last. The increase in absorbance for 15 min was recorded at 560 nm in a Beckman DU520 UV-Vis Spectrophotometer (Beckman Coulter France, S.A., Roissy CDG, France). Stock solutions of NBT, HX and XOD were prepared in K-PBS (pH 7.5). All spectrometry assay measurements were performed in triplicate.

The method used was established by Cortina-Puig *et al*. (2009), where the $O_2^{\bullet-}$ radicals and uric acid were generated in vitro by the HX/XOD system. The $O_2^{\bullet-}$ radicals reduce the NBT reagent (yellow color) into formazan (purple color), which is measured spectrophotometrically at 560 nm. The presence of radical scavengers (the antioxidant sample) generates inhibition (competitive) in the formation of formazan

leading to the decrease of its production rate and consequently of the absorbance.

The % radical scavenging activity (RSA) of the plant extracts was calculated using the following formula:

RSA% = 100 x [(Abs. control – Abs. sample)/Abs. control]

Where: Abs. control is the absorbance of formazan without the sample; Abs. sample is the absorbance of formazan with the sample.

Biocompounds

Determination of total phenols

The total phenol content was determined by adopting the method of Pueyo & Calvo (2009) and Berker *et al*. (2010). In buckets, we added 100 μL of pulp etanolic extracts (1:1), 630 μL deionized water, 20 μL of HCl (1 mol L^{-1}) 150 μL $K_3Fe(CN)_6$ (1% m/v), 50 μL sodium dodecyl sulphate (1% v/v) and 50 μL $FeCl_3.6H_2O$ (0.2% m/v). The absorbance reading was done after 30 minutes at 750 nm using a Shimadzu UV-probe spectrophotometer. The calibration curve was obtained using standard solutions of gallic acid (1, 2, 4 and 8 μg mL^{-1}). The results were expressed in equivalents of gallic acid in grams per 100 g of pulp (EGA 100 g^{-1}).

Determination of flavonoid content

The concentration of flavonoids was determined by adapting the spectrophotometric procedure described in Chaillou *et al*. (2004) and Teles (2014). In buckets, we added 0.2 mL of metanolic pulp extracts (1:1), 0.2 mL methanolic solution of $AlCl_3$ (5% m/v) and completed the volume to 2 mL with concentrated methanol. After 30 minutes, the absorbance was read at a wavelength of 425 nm using a Shimadzu UV-probe spectrophotometer. The calibration curve was obtained using standard solutions of quercetin. The results were expressed in equivalents of quercetin in milligrams per 100 g of pulp (EQE·100g^{-1}).

Ascorbic acid

The vitamin C concentration was determined by redox titration using iodine solution. Masses of the homogenized sample guaranteeing a vitamin C content of more than 5 mg were added, with 50 mL deionized water, 10 mL sulphuric acid 20% (v/w), 1 mL KI 10% (m/w) and 1 mL amido 1% (m/w). The iodine generated was titrated against 0.02 mol·L^{-1} $KI0_3$.

MINERAL ELEMENTS

Digestion procedure

A mixture of 0.2-0.5 g of homogenized dry samples, 5.0 mL of concentrated HNO_3, 2.0 mL of 30% H_2O_2 (v/v) with 0.5 ml of yttrium (100 mg·L^{-1}) as internal standard, was submitted to heating in a closed microwave oven (MARSX press 6.0), which utilizes high voltages and microwave radiation to accelerate the sample acid digestion. The digestion procedure was based on the AOAC method (Jorhem & Engman, 2000), according the following steps: 3 minutes at 250 W, 5 minutes at 630 W, 22 minutes at 500 W, and 15 minutes at 0 W. The resulting solution was diluted with deionized water to 25.0 ml in a volumetric flask before being analyzed by inductively coupled plasma optical emission spectrometer (ICP-OES). Blanks were prepared in each lot of samples. All analyses were performed in triplicate.

ICP-OES operational conditions

Concentrations of three macroelements (Ca, Mg, and Na) and four microelements (Fe, Mn, Zn, and Cu) were determined for the selected fruits. The measurements for simultaneous determination were carried out with an ICP-OES (Shimadzu, model 9820), equipped with a concentric nebulizer and allowing choice of the minitorch configuration between the radial or the axial mode in an integrated unit. Yttrium was used as an internal standard at a concentration of 2 mg·L^{-1}. Operating conditions are summarized in Table 1.

Table 1. Operating conditions of the ICP-OES method used during elemental analysis of the selected Amazon palm fruits.

Parameter	Value
Radio frequency power	1.2 kW
Plasma argon flow rate	10 L.min^{-1}
Auxiliary argon flow rate	0.6 L.min^{-1}
Carrier gas	0.7 L.min^{-1}
Exposure time	30 s
Solvent rinse time	30 s
Peristaltic pump rotation speed	20-60 rpm
View direction	Radial for Mg and Na; axial for Ca, Cu, Fe, Mn and Zn
Nebulizer	Concentric
Emission lines (λ nm)	Ca (183.801); Cu (327.396); Fe (259.940); Mg (383.826); Mn (257.610); Na (589.592); Zn (213.856)

Figures of merit

Calibration curves, linear working range over a wide range of analyte concentrations, multi-elemental response and method sensitivity were determined. The analytical performance of the method was evaluated considering the following figures of merit: practical linear range; precision and accuracy, sensitivity, estimated by limits of detection and quantification (LOD and LOQ, respectively); accuracy of the complete analysis by ICP-OES, since the digestion step to the spectrometric analysis itself, was estimated by the recovery indexes obtained by *buriti* sample fortification with two concentrations.

RESULTS AND DISCUSSION

BROMATOLOGICAL ANALYSIS

The results of proximate analyses for the three Arecaceae fruits studied are shown in Table 2. The *açaí* sample was prepared as a class B pulp (12.84% m/v), as it is traditionally consumed and marketed.

The studied species had levels MC ranging from 55.91 to 88.16%, similar to reported values in the literature (Darnet *et al.*, 2011; Manhães & Sabaa-Srur, 2011; Nascimento *et al*., 2008; Bezerra *et al*., 2006). In the studied palm fruits, TA contents ranged from 0.68 to 1.28 g/100 g in the pulps, and the *buriti* and *inajá* contents were statistically similar.

Buriti showed the highest TL and can be considered a rich natural sources of lipids (21.0%), which corroborates with the use of this fruit in the food, pharmaceutical and cosmetics industries. The *açaí* and *inajá* pulps have similar oil content.

A comparison of lipid composition with data from the literature is complex, due to interrelated factors such as genetics, soil, climate, and stage of maturity of the plant and fruits, collection periods. Moreover, lipid accumulation in plants depends greatly on culture conditions such carbon source, nitrogen source, C/N molar ratio, temperature, and oxygenation (Sestric, 2015; Ageitos *et al.*, 2011).

Table 2. Proximal composition of the selected Amazon palm fruits (relative standard deviation – RSD). Legends: MC = moisture content; TA = total ash; TL = total lipids; CP = crude protein; TC =total carbohydrate; TEV = total energy value; CA = acidity in citric acid. All results are presented together the respective RSD. N = 3. Means followed by the same letter in the columns do not differ significantly from each other by the Tukey test at the 5% probability level.

Amazon fruits	MC	TA	TL	CP	TC	CA	TEV (kcal·100g^{-1})	pH
	(g·100g^{-1})							
Açaí	88.16 (1.89)	0.68 (10.51)	4.33 (0.35)[a]	0.72 (7.22)	6.10	0.082 (6.62)	66.27	5.69 (0.30)[a]
Buriti	55.91 (0.32)	1.28 (1.24)[a]	21.0 (0.45)	2.14 (5.68)	19.67	0.89 (1.00)	276.27	3.84 (8.41)
Inajá	67.49 (0.13)	1.24 (1.01)[a]	4.27 (8.63)[a]	0.49 (1.46)	26.51	0.06 (4.70)	146.46	5.73 (0.36)[a]

The obtained results, nevertheless, show some agreement with the literature for *açaí* and *buriti* pulps (Brasil, 2015; Darnet *et al*., 2011; Canuto *et al*., 2010; Aguiar, 1996).

The CP contents ranged from 0.49% in the *inajá* pulp to 2.14% in *buriti*. Therefore, the intake of 100 g of *buriti* pulp contributes approximately 8% of the recommended dietary allowances (RDA) of proteins to an adult man. The CP obtained from the *inajá* pulp was lower than those recorded by Mota & França (2007), whereas for *açaí* (Yuyama *et al*., 2011) and *buriti* (Manhães & Sabaa-Srur, 2011) our data presented a certain agreement with the literature.

The three species studied had TC ranging from 6.10 to 26.51 g 100 g^{-1}. The proximate analysis of the TC content showed that *inajá* pulps are a major source of sugar.

Based on CP, TL and TC contents, the calorific values (TEV) of the fruits ranged from 66 to 276 kcal 100 g^{-1}. Only the *açaí* pulp exhibited TEV value < 100 kcal 100g^{-1}. These samples can be included in energy-restricted diets; on the other hand the *buriti* and *inajá* pulps presented high TEV and can be included in the high calorie diets.

For titratable acidity, among evaluated fruits, *buriti* showed a higher average value (0.89%) and the lowest pH (3.89). The average acidity value obtained for *buriti* pulps was higher than that observed by Santos *et al*. (2017) of 0.56%, and for *inajá* (0.07%) it was lower than their value (0.14). The pH values are statistically similar between *açaí* and *inajá*.

Data on food composition is extremely important for the development of food composition tables, consumption of balanced nutrients, assessment of the supply and food consumption of a country, verification of the nutritional adequacy of the diets of individuals and populations, evaluation of the nutritional status, and development of research regarding the relation between diet and disease, agricultural planning, and food industry inovation (Torres *et al*., 2000).

MINERAL ELEMENTS

Mineral concentrations in the palm fruits with their respective RSD, LOD, and LOQ, as well as the results of the addition tests, are presented in Table 3.

The results show that the method is precise with RSD < 10% for all samples and accurate with recuperation ranged from 88.54 to 109.50%.

Plants are a source of minerals that are essential nutrients for the maintenance of human health. The RDA is a parameter used to stipulate the nutrient levels that meet the human needs of most healthy individuals. According to these parameters, the average daily requirements for adult males (19 to 30 years of age) of the evaluated minerals are

Table 3. Levels of mineral elements (mg·100g^{-1}) in the selected Amazon fruits with respective RSD, LOD, and LOQ (mg·L^{-1}). All results are presented together the respective relative standard deviation (RSD). N = 3. Means followed by the same letter in the columns do not differ significantly from each other by the Tukey test at probability level p = 0.05.

Mineral / LOD / LOQ / Amazon fruits	Ca 6.5 10^{-2} 2.42	Na 1.0 10^{-1} 3.49	Mg 2.0 10^{-3} 2.2 10^{-2}	Cu 4.0 10^{-4} 1.7 10^{-3}	Fe 5.0 10^{-4} 2.9 10^{-3}	Mn 7.12 10^{-6} 1.2 10^{-5}	Zn 3.0 10^{-4} 1.7 10^{-3}
	Concentration mg·100g^{-1} (RSD)						
Açaí	61.47 (6.17)	4.05 (3.41)[a]	12.64 (4.65)	0.12 (5.79)[a,b]	0.84 (1.26)[a]	7.89 (3.46)	0.97 (3.91)[a]
Buriti	107.12 (1.55)	2.97 (5.92)[a]	84.28 (3.12)	0.19 (2.15)[a]	0.94 (1.91)[a]	3.18 (4.10)	0.89 (2.64)[a]
Inajá	19.82 (7.74)	4.21 (5.87)[a]	57.48 (8.23)	0.08 (9.10)[b]	0.48 (1.62)	0.17 (7.04)	1.05 (4.04)
Addition Test	Recuperation % (RSD)						
Buriti	107.08 (9.90) 104.50 (8.88)	107.21 (4.04) 100.9 (1.29)	108.33 (1.63) 88.54 (6.99)	107.83 (5.92) 102.90 (2.10)	97.00 (8.02) 98.00 (2.53)	100.00 (11.79) 100.00 (0.00)	109.50 (7.43) 90.3 (3.33)

as follows: Na = 1.3 to 1.5 g/day; Ca = 1 g/day; Mg = 310 to 400 mg/day; Cu = 0.9 mg/day; Fe = 8 to 18 mg/day; Mn = 1.8 to 2.3 mg/day; Zn = 8 to 11 mg/day (Institute of Medicine, 2006).

Ca presented the highest macromineral contents in the majority of the samples, followed by Mg and Na. The highest micromineral contents were observed for Mn in *açaí* and *buriti* pulp, as well as Zn in *inajá* samples.

Ca plays a key role in the health of bones, and it is involved in vascular, neuromuscular, and glandular functions in the body (Institute of Medicine, 2006). Ca levels < 10.71% of the RDA were found in the fruits, and they may be introduced into the diet of populations with a Ca deficiency.

Good results are shown for Na, because the Na concentrations showed values of the RDA < 0.32%. In the human body, Na is necessary to maintain extracellular fluid volume and plasma osmolality, but there is little evidence of any adverse effect from low dietary sodium. On other hand, adverse effects of increased sodium intake are elevated blood pressure, which is directly related to cardiovascular disease and end-stage renal disease.

Concentrations of Mg ranged from 84.28 to 12.64 in the palm fruits, which show that *buriti* and *inajá* pulps are natural sources in this macromineral.

Cu concentrations ranged from 0.08 to 0.19 in the fruits. *Buriti* showed the highest content, being classified as a natural source of this micromineral because it encompasses 21.11% of the RDA. Therefore, *buriti* consumption can help to prevent diseases associated with Cu deficiency, including normocytic, hypochromic anemia, leucopenia, and neutropenia.

The highest content of Fe was founded in *buriti* samples, which provide 11.75% of the RDA for adult males.

The results showed highest levels of micronutrients for *açaí*, followed by *buriti* and *inajá*. Although of contribution of Mn was > 100% of RDA when *açaí* e *buriti* were ingested at 100 g per day, the concentrations are below the maximum tolerable intake level (11 mg·day^{-1}) and only a small percentage, 3 to 5%, of dietary Mn is really absorbed by the body, while much absorbed Mn is excreted very rapidly into the gut via the bile and only a small amount is retained. In general, the palm fruits contain high contents of Mn and can contribute to prevent diseases related to Mn deficiency.

Inajá showed the highest content of Zn and could contribute with 13.13% of the RDA when 100 g of this fruit pulp are ingested daily.

ANTIOXIDANT CAPACITY

The results of the antioxidant activity expressed in function of the production rate of formazan for different title mass (% m/v) of analyzed fruits, and standard deviations for each analysis are shown in Figure 1. Although acerola (Barbados cherry or West Indian cherry) is not an Amazonian fruit, this fruit was used for comparative purposes, due to its high ascorbic acid content and antioxidant potential (Nunes *et al*., 2011; Lima *et al*., 2011).

All the fruits were observed to show antioxidant activity. The inhibition of $O_2^{\bullet-}$ radicals generated by the antioxidant action of the selected fruits were revealed by the smaller amount of NBT which was reduced to formazan when the reaction catalyzed by XOD processed in presence of its diluted pulps. As expected, the superoxide radical scavenging activity (RSA) was higher for *acerola* (96.39%), followed by *buriti*, *açaí* and *inajá*.

Values of RSA were: *buriti* (84.28%), *açaí* (84.21%) and *inajá* (73.60%). Evaluating the closeness of the obtained results, a one-way ANOVA test was applied, followed by Tukey's test, in order to identify significant differences among averages obtained with 20% of the fruits dilution. The ANOVA and Tukey's test showed *buriti* and *açaí* with no significant difference ($p < 0.05$) between them in relation to their antioxidant behavior.

Concentrations of vitamin C, phenolic compounds, and flavonoids in the fruits studied are presented in Table 4, as well as their antioxidant capacity for comparative purposes.

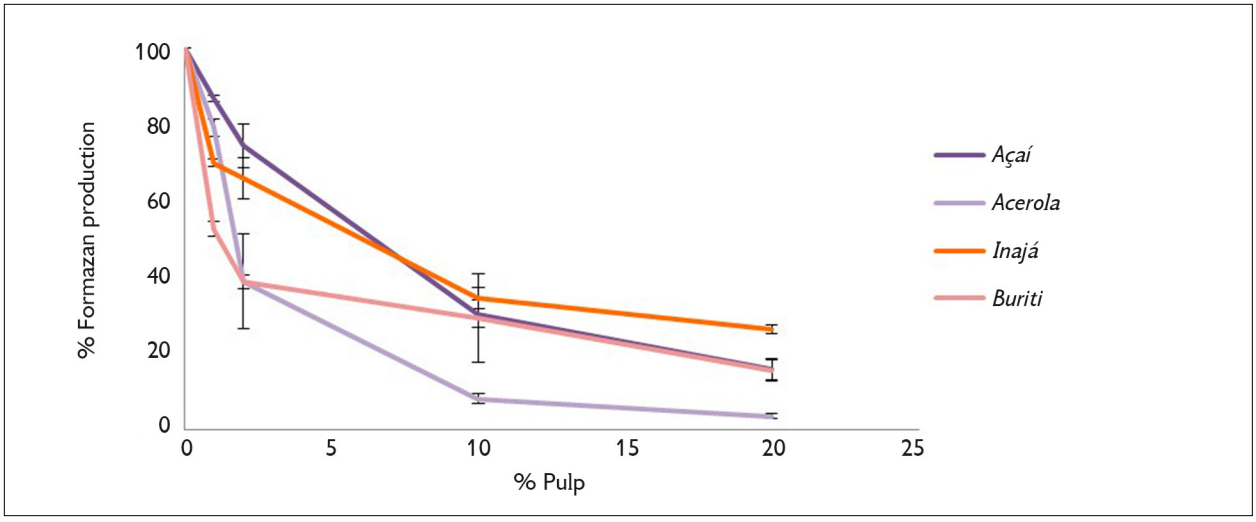

Figure 1. Antioxidant activity expressed as a function of production rate of Formazan for different title mass (%, m/v).

Table 4. Concentrations of vitamin C, phenolic compounds and flavonoids in wet mass, and antioxidant capacity observed for fruit extract at 20% (m/v). Legend: ND = undetectable signal. Means followed by the same letter in the columns do not differ significantly from each other by the Tukey test at 5% probability level

Bioactive compounds / Amazon fruits	Vitamin C mg·100g^{-1}	Phenolic compounds EAG·100g^{-1}	Flavonoids EQE·100 g^{-1}	Antioxidant capacity (RSA %)
Açaí	80,00 ± 7,07	4,86 ± 0,79^{a}	10,21 ± 1,16	84,21 ± 2,55^{a}
Buriti	21,87 ± 1,63	5,17 ± 1,60^{a}	ND	84,28 ± 2,90^{a}
Inajá	37,70 ± 1,39	3,61 ± 0,40^{a}	ND	73,60 ± 1,08

The values of vitamin C content in fresh fruits are in the range of 21.87 to 80.0 mg·100 g^{-1} for *buriti* and *açaí* pulp, respectively. The Institute of Medicine (2006) establishes the RDA of 90 mg of vitamin C for a healthy adult, which allows classifying *açaí* and *inajá* pulps as foods high in vitamin C and *buriti* as a source in this nutrient. Comparing the obtained results with data from the literature, it can be seen that the vitamin C contents obtained are within the range reported for *açaí* pulp (Rufino *et al*., 2010) and *buriti* (Gonçalves, 2008). There were no published records for vitamin C concentrations in *inajá* pulp, and therefore, this work is the first to present data on the ascorbic acid content in this fruit.

There are no significant difference ($p < 0.05$) between the phenolic compound concentrations of the palm fruits. The knowledge of the content of phenolic compounds in fruits is important because it reflects the mechanism of adaptation and resistance of the plant to the environment, and it influences the flavor and the technological characteristics of the food, as well as the nutritive and functional potential of these fruits (Rocha *et al*., 2013).

Comparing the results of the phenolic compound content with the literature, lower values were observed than those reported by Yamaguchi (2015) for extracts of hydroalcoholic residues of *açaí* fruits, while the present results were higher for *açaí* (Rufino *et al*., 2010) and *buriti* (Manhães & Sabaa-Srur, 2011).

Only *açaí* pulp showed quantifiable concentrations of flavonoids.

CONCLUSIONS

Although the three tested fruits belong to the same botanical family, their analyses confirmed the natural compositional variability of these plants, which may be related to the different genera to which they belong, as well as the edaphoclimatic conditions of their natural environments.

The fruits showed expected variations in bromatological parameters. They had good mineral contents, each being rich in one or more nutrients. From the nutritional point of view, their consumption can be recommended because of the beneficial effects of adequate contents, such as moisture, ash, lipid, protein, carbohydrate, and energy, as well as considerable mineral content, especially of microelements.

The chemical composition of *inajá* fruit is presented for the first time, and its nutritional potential revealed.

All the studied fruits may be considered promising sources of bioactive compounds having high antioxidant properties, increasing interest in them by the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of foods. Besides that, the fruits exhibit great potential for applications in the pharmaceutical, cosmetic, and food industries.

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Mineral and bromatological assessment and determination of the antioxidant capacity and bioactive compounds in native Amazon fruits

Avaliação mineral, bromatológica, capacidade antioxidante e compostos bioativos em frutos nativos amazônicos

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Abstract

The proximate compositions, mineral contents, antioxidant capacity and bioactive compounds of 7 native Amazon fruits were chemically evaluated. The majority of the fruits showed high moisture contents (> 63.02%), and ash, total crude protein and total carbohydrate contents in the ranges of 0.22–2.07%, 0.17–2.44% and 7.17–41.71%, respectively. High levels of total lipids were found in uxi (23.25%) and monguba (18.67%). A wide range of mineral contents was detected and the highest levels were found in the samples of monguba seeds (Ca, Cu, Mg, and Zn), uxi pulp (Fe, and Mn) and pajurá pulp (Na). All the fruits showed antioxidant capacity, but the pajurá revealed the highest potential, statistically similar to that of acerola ($p < 0.05$). The highest vitamin C contents were found in bacuri and cupuaçu and the highest phenolic compound contents in monguba and pajurá fruits, but flavonoids were only detected in pajurá. A statistical correlation between the Na content and antioxidant capacity was also observed. Based on the results obtained, the fruits analyzed are suitable for use in the human diet, in the food and cosmetics industries as well as in pharmaceutical compositions.

Keywords: *Native fruits; Amazon; Chemical composition; Minerals; Antioxidant capacity; Bioactive compounds.*

Resumo

A composição bromatológica, o conteúdo mineral e a capacidade antioxidante de 7 frutos nativos da Amazônia foram avaliados. Os frutos mostraram, em sua maioria, alto conteúdo de umidade (> 63,02%) e teores de cinzas, proteína bruta total e carboidratos totais na faixa de 0,22-2,07%, 0,17-2,44% e 7,17-41,71%, respectivamente. Os maiores teores em lipídios foram obtidos nos frutos de uxi (23,25%) e monguba (18,67%). Uma ampla variedade de minerais foi detectada, sendo as maiores concentrações obtidas nas amostras de sementes de monguba (Ca, Cu, Mg e Zn), polpas de uxi (Fe e Mn) e pajurá (Na). Todos os frutos mostraram atividade antioxidante, em que a polpa de pajurá revelou o maior potencial, semelhante estatisticamente à acerola ($p < 0,05$). Maiores teores em vitamina C foram obtidos nos frutos de bacuri e cupuaçu, fenólicos totais na monguba e pajurá, enquanto flavonóides foram determinados somente nos frutos de pajurá. Uma correlação positiva entre o teor de Na e a capacidade antioxidante também foi observada. Baseado nos resultados obtidos, os frutos analisados são adequados para uso na dieta humana, nas indústrias de alimentos e cosméticos, bem como em composições farmacêuticas.

Palavras-chave: *Frutas nativas; Amazônia; Composição química; Minerais; Capacidade antioxidante; Compostos bioativos.*

1 Introduction

The Brazilian Amazon Region is formed of a complex mosaic of endemic areas with a rich diversity of fruit species which are distributed in accordance with their biota specificities (SILVA et al., 2005). The region shows great bioavailability of fruit species with approximately 220 edible fruit producing plant species, representing 44% of the native fruit diversity in Brazil (NEVES et al., 2012).

Recognized sources of nutrients, fruits comprise nutritionally important foods for the human diet and in recent years have received increased attention due to epidemiological evidence regarding the regular consumption of vegetables, which reduces the mortality and morbidity due to some chronic diseases (RUFINO et al., 2010; ALISSA; FERNS, 2012; BORGES et al., 2013). The protective effect has been attributed to the presence of constituents like minerals and high levels of bioactive compounds with antioxidant properties (NUNES et al., 2011; KAHL et al., 2012; LIU, 2013; KOZŁOWSKA; SZOSTAK-WEGIEREK, 2014; WANG et al., 2013).

Data on the composition of native fruits is essential to encourage national and international marketing; assist the food, cosmetics, bio cosmetics and other industries and support policies to protect the environment and biodiversity. In addition, knowledge of the composition aids quality control and food safety as well as evaluating the adequacy of intake of individual nutrients or populations.

Information regarding the nutritional composition of Brazilian fruits is still scarce, especially those found in the Amazon Region, but on the other hand, there is an evident need for better use of its natural resources. Considering the potential benefits that knowledge regarding the nutritional composition of fruits can offer to human health, the aim of this study was to determine the physical and chemical properties, mineral contents and antioxidant capacities of seven native Amazon fruits, some of which have been studied and parameters assessed by other authors.

2 Materials and methods

2.1 Reagents

Analytical grade chemicals were employed in the preparation of all solutions. Deionized water (Milli-Q Millipore 18.2MΩ cm^{-1}) was used in all experiments. All the plastic articles and glassware were cleaned by soaking in dilute nitric acid (1:9). The standard analyte solutions for calibration procedures were produced by diluting stock solutions of 1000 $mg.L^{-1}$ of the elements under investigation (Ca, Cu, Fe, Mg, Mn, Na and Zn; from Merck Millipore Certipur®, Specsol®). The other reagents used were: nitro blue tetrazolium (NBT, N6876), hypoxanthine (HX, H9377), xanthine oxidase (XOD from bovine milk, X4376), petroleum ether, phenolphthalein, sodium hydroxide (NaOH), sulphuric acid (H_2SO_4), potassium iodide (KI), dry starch, potassium iodate (KIO_3), nitric acid (HNO_3), hydrogen peroxide (H_2O_2) and oxide yttrium (Y_2O_3), all purchased from Sigma-Aldrich Corp (Nasdaq-Sial, Darmstadt, Germany).

2.2 Sample collection

Seven native Amazon fruits were included in this study: abiu (*Pouteria caimito*), bacuri (*Platonia insignis*), biribá (*Rhollinea orthopetala*), cupuaçu (*Theobroma grandiflorum*), monguba (*Pachira aquatica*), pajurá (*Couepia bracteosa*) and uxi (*Saccoglotis uchi*).

From 1 to 5 kg of each fruit sample, in the complete physiological maturity stage, were collected during the appropriate seasonal period in the states of Amazonas, Maranhão, and Roraima. A voucher specimen of each plant was deposited in the herbarium of the Integrate Museum of Roraima. After collection, the samples were refrigerated and taken to the laboratory of the Group of Environmental Studies and Analysis (GEAA) at the Federal University of Maranhão, Brazil, where they were washed in deionized water and stored at −20 °C until analysed.

2.3 Bromatological analysis

The moisture content, total ash content, hydrogen potential (pH), acidity in citric acid, crude protein content and total lipids content were determined according to the AOAC methods (CUNNIFF, 1997). The total carbohydrate content was determined by difference, subtracting the sum of the crude protein, total lipids, moisture and ash contents from 100 (MERRILL; WATT, 1973). The total energy value was estimated according to the Atwater conversion values using 4 Kcal/g for protein and carbohydrates, and 9 Kcal g^{-1} for lipids (MERRILL; WATT, 1973). All the analyses were carried out in triplicate.

2.4 Antioxidants

2.4.1 Antioxidant capacity

The procedure used followed the method of Cortina-Puig et al. (2009) with some modifications. A reaction mixture was prepared consisting of 50 mM K-PBS containing 0.1 mM EDTA (pH 7.5), 25 µM HX, 50 µM NBT, the antioxidant fruit extract (distilled water for the blank) and 0.2 $U.mL^{-1}$ XOD, which was added last. The increase in absorbance at 560 nm was recorded for 15 min using a Beckman DU520 UV–Vis Spectrophotometer. Stock solutions of NBT, HX and XOD were prepared in K-PBS at pH 7.5. All the spectrometric assays were carried out in triplicate.

In the method, $O_2^{\bullet-}$ radicals and aciduric compounds were generated in vitro by the HX/XOD system. The $O_2^{\bullet-}$ radicals reduce the NBT reagent (yellow colour) into formazan (purple colour), which is measured spectrophotometrically at 560 nm. The presence of radical scavengers (the antioxidant sample) generates inhibition (competitive) in the formation of formazan, leading to a decrease in its production rate and consequently in absorbance.

The % superoxide Radical Scavenging Capacity (RSC) of the plant extracts was calculated using Equation 1:

$$RSC\left(\%O_2^{\bullet-}\ \text{scavenging}\right)=100-\left[\frac{A_{AOX}-A_0}{C_{100}-C_0}\times 100\right] \quad [1]$$

Where: A_{AOX} is the AOX absorbance; A_0 is the blank AOX absorbance; C_{100} is the control absorbance; and C_0 is the blank control absorbance.

2.4.2 Total phenolic compounds

The total phenolic compound content was determined according to the method of Pueyo and Calvo (2009) and Berker et al. (2010). 100 µL of ethanolic pulp extract (1:1), 630 µL deionised water, 20 µL of HCl (1 mol L^{-1}), 150 µL $K_3Fe(CN)_6$ (1% m/v), 50 µL sodium dodecyl sulphate (1% v/v) and 50 µL $FeCl_3$.6H2O (0.2% m/v) were added to a cuvette. The absorbance was read at 750 nm after 30 minutes using a Shimadzu UV-probe spectrophotometer. The calibration curve was obtained using standard gallic acid solutions (1, 2, 4 and 8 µg mL^{-1}). The results were expressed in gram equivalents of gallic acid per 100 g of pulp (GAE.100 g^{-1}).

2.4.3 Determination of the flavonoid content

The flavonoid concentration was determined by adapting the spectrophotometric procedure described by Chaillou et al. (2004) and Teles (2014). Aliquots of 0.2 mL of methanolic pulp extract (1:1) and 0.2 mL methanolic $AlCl_3$ solution (5% m/v) were added to a cuvette and the volume completed to 2 mL with concentrated methanol. After 30 minutes, the absorbance was read at a wavelength of 425 nm using a Shimadzu UV-probe spectrophotometer. The calibration curve was obtained using standard quercetin solutions. The results were expressed in milligram equivalents of quercetin per 100 g of pulp (QEE.100g^{-1}).

2.4.4 Ascorbic acid

The vitamin C concentration was determined by redox titration using an iodine solution (IAL, 2008).

2.5 Mineral elements

2.5.1 Digestion procedure

The sample digestion procedure was carried out in a closed microwave oven according to the following AOAC steps (AOAC, 2002). The resulting solution was diluted to 25.0 mL with deionized water in a volumetric flask before being analysed by ICP-OES. Blanks were prepared for each sample batch. Yttrium was used as the internal standard at a concentration of 2 mg.L^{-1} and all the analyses were carried out in triplicate.

2.5.2 ICP-OES operational conditions

The concentrations of three macroelements (Ca, Mg and Na) and four microelements (Fe, Mn, Zn and Cu) were determined in the selected fruits. The measurements were determined simultaneously in an ICP OES (Shimadzu, model 9820) equipped with a concentric nebulizer, which allowed for the choice of the minitorch configuration between the radial or axial mode in an integrated unit. The operational conditions are summarized in Table 1.

2.5.3 Performance characteristics

The analytical method performance was evaluated considering the following figures of merit according to Skoog et al. (2008): practical linear range; precision, by calculating the relative standard deviation (RSD) for each analysis under repeatable conditions; and the sensitivity, estimated by the limits of detection and quantification (LOD and LOQ, respectively).

The accuracy of the complete ICP-OES analysis was estimated through addition and recovery experiments of the analytes for two samples (biribá and uxi) at two concentration levels.

2.7 Statistical analysis

The results were expressed as the mean value with the respective RSD (%) of three replications. The statistical differences were analyzed using one-way ANOVA followed by Tukey's test at the 95% confidence level ($p \leq 0.05$). The correlation analysis was applied and expressed as Pearson's correlation coefficient (r). The statistical analysis was carried out using Statistica, 8.0.

Table 1. ICP-OES operational conditions used to determine the elements in the selected Amazon fruits.

Parameter	Value
Radio frequency power	1.2 (kW)
Plasma argon flow rate	10 (L.min^{-1})
Auxiliary argon flow rate	0.6 (L.min^{-1})
Carrier gas	0.7 (L.min^{-1})
Exposure time	30 (s)
Solvent rinse time	30 (s)
Peristaltic pump rotation speed	20-60 (rpm)
View direction	Radial for Mg and Na Axial for Ca, Cu, Fe, Mn and Zn
Nebulizer	Concentric
Emission lines (λ nm)	Ca (183.801); Cu (327.396); Fe (259.940); Mg (383.826); Mn (257.610); Na (589.592); Zn (213.856).

3 Results and discussion

3.1 Bromatological analysis

The results of the bromatological analysis of the native Amazon fruits are shown in Table 2.

In general, the analyzed fruits presented high moisture contents (> 63.02%), except for *uxi* fruit (31.72). The moisture contents were shown to be similar to those reported for the respective pulps of *abiu* (LOVE; PAULL, 2011), *biribá, pajurá* (BERTO et al., 2015), *cupuaçu* (UNICAMP, 2006) and *uxi* (MARX et al., 2002; BERTO et al., 2015). The total ash contents < 2.07% were found for all the samples and the highest value was observed for *biribá* fruit.

The total lipids contents ranged from 0.06% to 23.25% for *biribá* and *uxi* pulps, respectively. Other studies have reported 10–31% total lipids for *uxi* (MARX et al., 2002; BRASIL, 2015; BEZERRA et al., 2006) and *Monguba* seeds and *uxi* pulps can be considered as rich natural sources of total lipids (18.67 to 23.25%). This fact favours the use of their oils as raw materials for the food, pharmaceutical and cosmetic industries.

Crude proteins are primary components of living things, and the main sources of protein in human consumption tend to be animal products, which normally also have high fat and saturated fat contents. Thus the presence of a high protein level in a plant points towards a possible increase in its food value. Moreover, a protein based bioactive compound could also be isolated from the original fruits (THOMSEN et al., 1991). In the present study, the highest crude protein content was found in *monguba*, followed by *uxi* fruit. The *Monguba* fruit is still very little used by Brazilians and therefore devalued economically, but the results showed a high oil content and a significant amount of protein, showing its potential for industrial exploitation.

Carbohydrates are the main energy reserves of plant foods. In all organisms, carbohydrates make up the building blocks of cells and supply potential energy to maintain life. The total percent of carbohydrate varied greatly amongst the samples, and their values were influenced primarily by the moisture content. The highest total carbohydrate percentages were found for the *uxi* (41.71%) and *pajurá* (35.03%) pulps.

The nutritional parameter of total energy is directly related to the total lipids, crude proteins and total carbohydrate levels found in the samples. Almost all the samples evaluated presented high total energy values and only the *biribá* and *cupuaçu* pulps exhibited total energy values below 100 Kcal $100g^{-1}$. Thus these fruits could be included in energy-restricted diets whereas the others could be employed in high-caloric diets. It was observed that the Amazon fruits with higher total energy values also presented higher total lipids and lower moisture contents.

The highest pH value was 6.76 for *abiu* fruit, whilst the highest citric acid content was found in *cupuaçu* (pH 4.09 and 1.78 g of citric acid per 100g of pulp).

3.2 Mineral elements

Plants are a source of minerals that are essential nutrients for the maintenance of human health. The recommended dietary allowance (RDA) is a parameter used to stipulate the nutrient levels that meet the needs of most healthy individuals (INSTITUTE OF MEDICINE, 2006). According to these parameters, the average daily requirements for adult males (19 to 30 years) of the minerals evaluated

Table 2. Proximate composition of the selected *in nature* Amazonian fruits with their respective RSD (%).

Amazon fruit	Moisture	Ash	Lipids	Crude protein	Carbohydrate	Acidity*	Total energy	pH
	(g.100 g^{-1})						(kcal.100 g^{-1})	
Abiu	71.73 (0.21)	0.33 (9.34)	0.32 (3.57)	0.17 (0.00)	27.44	0.056 (5.04)	113.37	6.76 (0.60)
Bacuri	91.20 (0.15)	0.22 (11.69)	0.38 (9.94)	0.34 (0.00)	7.85	0.56 (5.46)	36.22	3.60 (1.35)
Biribá	89.46 (8.24)	2.07 (8.33)	0.06 (6.15)	1.04 (9.17)	7.37	0.16 (5.56)	34.16	5.80 (4.56)
Cupuaçu	82.32 (0.25)	1.09 (3.09)	0.57 (10.6)	0.40 (2.50)	15.63	1.78 (9.71)	69.22	4.09 (0.51)
Monguba	70.35 (1.20)	1.37 (1.65)	18.67 (6.98)	2.44 (5.22)	7.17	0.34 (8.69)	206.47	6.70 (3.37)
Pajurá	63.02 (0.53)	0.91 (3.10)	0.11 (10.83)	0.93 (0.00)	35.03	0.16 (10.63)	144.82	5.51 (3.79)
Uxi	31.72 (2.48)	1.15 (3.80)	23.25 (11.77)	2.17 (4.90)	41.71	0.24 (6.85)	384.74	4.44 (3.74)

**Acidity in citric acid.* All results are presented together with the respective *Relative Standard Deviation* (RSD). N = 3.

are as follows: Na: 1.3 to 1.5 g/day^{-1}; Ca: 1 g/day^{-1}; Mg: 310 to 400 mg/day^{-1}; Cu: 0.9 mg/day^{-1}; Fe: 8 to 18 mg/day^{-1}; Mn: 1.8 to 2.3 mg/day^{-1} and Zn: 8 to 11 mg/day^{-1}.

Table 3 shows the mineral concentrations (mg 100 g^{-1}) found in the native Amazon fruits with their respective RSD (%), LOD and LOQ (mg L^{-1}).

The highest Ca, Cu, Mg and Zn contents were found in the *monguba* fruit, representing 5.6%, 83.0%, 21.9% and 12.4% of the RDA (INSTITUTE OF MEDICINE, 2006) for these minerals, respectively. The *Monguba* fruit can be classified (BRASIL, 1998) as a food very rich in Cu, followed by the *uxi* (35.7%), *bacuri* (17.7%), *pajurá* (15.6%) and *abiu* (22.3%) fruits. Copper functions as a component of several metalloenzymes which act as oxidases in the reduction of molecular oxygen. Symptoms associated with its deficiency include normocytic, hypochromic anemia; leucopenia; and neutropenia; and osteoporosis in copper-deficient infants and growing children. Copper toxicity is generally rare except in individuals genetically susceptible to an increased risk of the adverse effects from an excess copper intake. Therefore these fruits can be included in the diet to improve human health (INSTITUTE OF MEDICINE, 2006). *Monguba* fruit can also be considered an excellent source of Mg.

The highest contents of Fe and Mn were found in *uxi* pulp, with 15% and 29% of the RDA for these minerals, respectively. Fe is a critical component of several proteins, including enzymes, cytochromes, myoglobin and hemoglobin, the latter of which transports oxygen throughout the body. Iron deficiency anemia is the most common nutritional deficiency in the world (INSTITUTE OF MEDICINE, 2006) and *uxi* pulp could be used to prevent and/or treat this problem. Of the world's estimated 7 billion people, 1.6 billion suffer from iron deficiency (WHO, 2008, 2009). In turn, Mn is involved in the formation of bone and in specific reactions related to the amino acid, cholesterol and carbohydrate metabolisms. Although Mn deficiency may contribute to one or more clinical symptoms, a clinical deficiency has not been clearly associated with poor dietary intakes by healthy individuals (INSTITUTE OF MEDICINE, 2006).

Table 3. Minerals contents (mg.100 g^{-1}) (wet weight basis) in the samples studied, with their respective RSD (%), LOD and LOQ (mg.L^{-1}), and recoveries (%).

Mineral	Na	Ca	Mg	Cu	Fe	Mn	Zn
LOD	0.1044	0.065	0.0020	0.0004	0.0005	7.12 10^{-6}	0.0003
LOQ	3.49	2.42	0.022	0.0017	0.0029	1.2 10^{-5}	0.0017
Abiu	44.35	9.49	8.29	0.20	0.29	0.08	0.27
	(8.58)	(6.16)	(8.20)	(6.94)	(10.77)	(7.14)	(9.29)
Bacuri	13.58	7.03	7.01	0.16	0.20	0.02	0.64
	(5.22)	(4.95)	(4.97)	(9.63)	(8.14)	(6.86)	(8.90)
Biribá	1.12	34.42	25.83	0.09	0.22	0.11	0.18
	(4.48)	(3.84)	(8.73)	(5.33)	(4.13)	(5.15)	(10.16)
Cupuaçu	1.24	17.48	36.27	0.11	0.32	0.12	0.34
	(1.54)	(8.00)	(6.21)	(5.65)	(5.05)	(5.58)	(7.35)
Monguba	1.14	55.89	87.53	0.75	0.44	0.20	0.99
	(2.08)	(4.11)	(4.97)	(6.18)	(9.85)	(8.45)	(8.32)
Pajurá	68.56	19.22	21.33	0.14	0.37	0.22	0.69
	(6.72)	(4.01)	(2.24)	(9.36)	(4.79)	(2.42)	(6.55)
Uxi	2.79	22.27	40.65	0.32	1.20	0.67	0.51
	(9.95)	(6.24)	(1.39)	(7.46)	(5.79)	(2.80)	(6.08)
			RECOVERIES %				
Biribá	93.13	108.63	107.29	110.50	101.50	97.22	116.50
	(2.85)	(1.63)	(0.82)	(0.64)	(6.62)	(1.21)	(4.86)
	103.10	108.1	109.6	106.60	102.60	103.20	109.50
	(11.14)	(4.91)	(1.61)	(3.11)	(5.00)	(7.74)	(7.66)
Uxi	102.0	102.75	110.63	106.50	107.25	100.28	112.00
	(5.79)	(5.30)	(1.60)	(9.99)	(6.92)	(2.35)	(12.94)
	98.10	96.6	103.6	103.50	103.20	95.40	101.50
	(4.18)	(2.45)	(3.63)	(1.57)	(7.01)	(2.84)	(2.96)

All results are presented together with the respective *Relative Standard Deviation* (RSD). N = 3.

The highest Na content was found in *pajurá* fruit, although this amount only represents 4.5% of the RDA when 100 g of the fruit is ingested by an adult man. In general, most of the results for mineral contents were similar to those reported in the literature (BERTO et al., 2015; SMITH et al., 2014; LOVE; PAULL, 2011; CANUTO et al., 2010; SILVA, 2008; UNICAMP, 2006; AGUIAR, 1996).

3.3 Antioxidants

Figure 1 shows the results obtained for antioxidant capacity expressed as a function of the production rate of formazan for different masses (%, m/v) of the fruits analyzed, and the standard deviation for each analysis, except for the *uxi* pulp, which showed a smaller antioxidant capacity than the others and was not included. Although *acerola* is not an Amazon fruit, this fruit was used for comparative purposes, due to its very high ascorbic acid content and antioxidant potential (NUNES et al., 2011; LIMA et al., 2011).

It was observed that all the fruits showed antioxidant capacity. The inhibition of $O_2^{\bullet-}$ radicals generated by the antioxidant action of the selected fruits was revealed by the smaller amount of NBT reduced to formazan when the reaction catalyzed by XOD proceeded in the presence of the diluted pulps. As expected, the superoxide radical scavenging capacity (RSC) was highest for *acerola* (96.39%), but the *pajurá* fruit presented a very similar result (95.93%).

The RSC values for the other fruits were as follows: *cupuaçu* (80.45%), *abiu* (79.33%), *bacuri* (78.35%), *monguba* (75.74%) and *biribá* (75.55%). In order to evaluate the closeness of the results obtained, a one-way ANOVA test was applied followed by Tukey's test, so as to identify significant differences between the average values obtained with 20% dilutions of the fruits (Table 4). The ANOVA showed significant differences ($p < 0.05$) in the antioxidant capacities of the fruits studied. According to Tukey's test, the fruits could be separated into two groups: the first formed by the *acerola* and *pajurá* fruits, which presented no significant difference ($p < 0.05$) in their antioxidant activities; and the second composed of the other fruits, presenting statistical similarity between them in relation to their antioxidant behaviour.

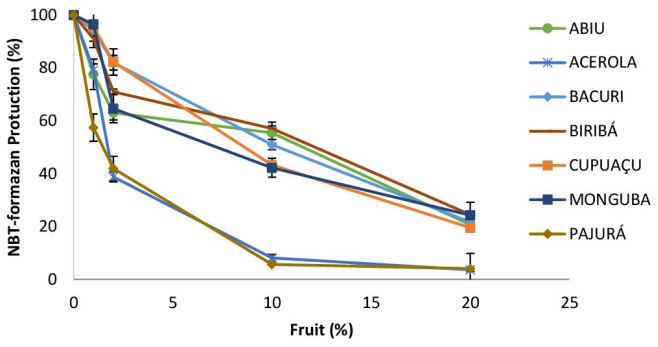

Figure 1. Antioxidant capacity expressed as a function of the production rate of formazan for different concentrations.

Interestingly, but not intentionally, a significant correlation ($R^2 = 0.84$) between the sodium concentration and antioxidant capacity was observed (Table 5). Normally, the cultivation system (CARDEÑOSA et al., 2016), colour and the ascorbic acid/anthocyanin/polyphenol compound contents (CARDEÑOSA et al., 2016; SUMCZYNSKI et al., 2015) are the main parameters imposing a significant influence on the antioxidant capacity in vegetables and fruits, but almost no scientific publication has reported the effect of Na content on this important nutritional feature. It is known that agricultural conditions such as soil type, growing location, climate and harvesting season directly influence the content of macroelements in agricultural crops (CARDEÑOSA et al., 2016; ROP et al., 2009). Specifically, regarding the Amazon fruits here evaluated, some of them were collected in locations in which the soils have a relatively saline character and the weather has striking tropical characteristics, such as the sampling points of Maranhão state. From the biological point of view, sodium plays a key role in biochemical processes that prevent the imbalance between the production of reactive oxygen species and the antioxidant defense system (SARKADI et al., 2006).

Table 6 shows the concentrations obtained for vitamin C, phenolic compounds and flavonoids in the fruits studied, as well as the antioxidant capacity for comparative purposes.

The vitamin C contents of the fresh fruits were in the range from 5.20 to 52.59 mg 100 g^{-1} for the *Monguba* and *Cupuaçu* fruits, respectively. The Institute of Medicine (2006) has established an RDA of 90 mg of vitamin C for a healthy adult, which allows one to classify the *bacuri* and *cupuaçu* pulps as high vitamin C content items, according to Brasil (1998), while *uxi* can be classified as a source of this nutrient. Comparing the results obtained with the literature data, it can be seen that the vitamin C contents obtained were within the ranges reported for *abiu* (BRASIL, 2015; CANUTO et al., 2010), *bacuri* (BRASIL, 2015),

Table 4. Average percent formazan production, F value and Tukey's test.

ABIU	ACEROLA	BACURI	BIRIBÁ	CUPUAÇU	MONGUBA	PAJURÁ	F
20.67 ± 2.75^b	3.61 ± 0.64^a	21.66 ± 0.90^b	24.45 ± 0.25^b	19.56 ± 1.38^b	24.26 ± 4.90^b	4.07 ± 5.76^a	48.99

F = value calculated (ANOVA; F critical value = 3.11 for 10 and 12 degrees of freedom and 95% confidence level). Means with the same letter are not statistically different at 5% significance in Tukey's test.

Table 5. Pearson's correlation - results between the antioxidant capacity, bioactive compounds and sodium content for the fruits studied.

	Vitamin C	Phenolic compounds	Flavonoids	Antioxidant capacity	Na
Vitamin C	1.00				
Phenolic compounds	-0.55	1.00			
Flavonoids	-0.25	0.68	1.00		
Antioxidant capacity	0.04	0.32	0.45	1.00	
Na	-0.42	0.40	0.81	0.84	1.00

Table 6. Vitamin C, phenolic compounds and flavonoids content, as well as the antioxidant capacity.

		Bioactive Compounds			
		Vitamin C	Phenolic compounds	Flavonoids	Antioxidant capacity
		(mg.100 g^{-1})	(GAE.100 g^{-1})	(QEE.100 g^{-1})	(RSC %)
Amazon Fruits	**Abiu**	5.34 ± 0.44^{a}	4.30 ± 0.47^{a,b}	ND	79.33 ± 2.75^{a,b}
	Bacuri	28.31 ± 3.23	5.21 ± 0.27^{a}	ND	78.3 ± 0.90^{a,b}
	Biribá	10.00 ± 0.13^{a}	5.45 ± 0.21^{a}	ND	75.55 ± 0.25^{a}
	Cupuaçu	52.59 ± 0.33	3.05 ± 0.08^{b}	ND	80.45 ± 1.38^{a,b}
	Monguba	5.20 ± 0.16^{a}	13.28 ± 1.28^{c}	ND	75.74 ± 4.90^{a}
	Pajurá	7.72 ± 0.69^{a}	14.46 ± 1.11^{c}	2.25 ± 0.10	95.93 ± 5.76
	Uxi	15.04 ± 2.03	5.56 ± 0.05^{a}	ND	23.25 ± 5.86*

ND = undetectable signal. *RSC for fruits extracts at 20% (w/v). Means followed by the same letter in the same column do not differ significantly from each other by the Tukey test at the 5% probability level.

cupuaçu (BRASIL, 2015; GONÇALVES, 2008) and *uxi* (GONÇALVES, 2008; MARX et al., 2002). There were no records of the vitamin C concentration for *monguba* and *pajurá* fruits, and therefore this paper is the first to present data on the ascorbic acid content of these fruits.

The highest phenolic compound concentrations were obtained for the *Monguba* and *Pajurá* fruits, which reflects on the flavour and technological characteristics of these fruits as well as on their nutritive and functional potentials (ROCHA et al., 2013).

Only the *pajurá* pulp showed quantifiable concentrations of flavonoids.

Evaluating the correlation between the bioactive compound composition and antioxidant capacity, a positive correlation (R^2 = 0.68) was observed between the flavonoids and the phenolic compounds, and between the flavonoids and the Na content (R^2 = 0.84) (Table 5).

In general, the antioxidant capacities were high for most of the Amazon fruits studied, but amongst them, the *pajurá* fruit was shown to have the highest antioxidant capacity against the oxidizing effects of $O_2^{\bullet-}$ radicals of physiological importance. Thus, extracts of all the fruits, but especially *pajurá*, may be considered as promising sources of bioactive compounds with high antioxidant properties, exhibiting great potential for application in the pharmaceutical, cosmetic and food industries. Up to the completion of this study, the literature reviewed had no mentioned of any other fruit with antioxidant properties similar to those of *acerola*, and thus the *pajurá* fruit was truly a great revelation.

4 Conclusions

The Amazon region remains the world location with the largest plant diversity, and it is common to find fruits with high nutritional potential. These properties were unknown even to the inhabitants of the region and to the Brazilian people as a whole. In this study, seven native fruits making up part of the diet of Amazonian inhabitants were evaluated with respect to their nutritional and antioxidant properties.

The fruits showed the expected variations for the bromatological parameters and a good mineral content, each being rich in one or more nutrients. All the fruits showed high antioxidant capacity, but the *pajurá* fruit showed the highest one, statistically equal to that of *acerola* fruit, and can therefore be explored in various application fields.

The fruits studied can be considered as valuable food supplements due to their positive influence on the nutrition status, thus increasing human productivity and longevity. The antioxidant study revealed these fruits as promising sources of bioactive compounds with high antioxidant properties, exhibiting great potential for application in the pharmaceutical, cosmetic and food industries.

These results can contribute to both composing the Brazilian Food Composition Table and to Brazilian food safety. Notably, this study is one of the first to provide a detailed evaluation of the nutritional compositions of

fruits poorly explored in the Amazon region. ICP-OES methods were used and the chemical composition of some compounds was presented for the first time for the *monguba* and *pajurá* fruits and their nutritional potential revealed. It is interesting to mention that these nutritive and antioxidant fruits are native to the Amazon region, and thus the industrial exploitation of such fruits must be supported.

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Development of a highly sensitive xanthine oxidase-based biosensor for the determination of antioxidant capacity in Amazonian fruit samples

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ABSTRACT

The paper describes the development of an amperometric biosensor using Prussian Blue (PB) modified electrodes containing xanthine oxidase (XOD). The enzyme is immobilized by photo-polymerization into an azide-unit pendant water-soluble photopolymer (PVA-AWP). The parameters of the fabrication of the biosensor, XOD:PVA/AWP ratio, crosslinking irradiation time, and XOD charge, were optimized. Operational conditions for electrode preparation were defined as 1:2 ratio of XOD:PVA/AWP; exposure time to neon light of 30 min; pH = 7.5 at room temperature and enzymatic charge of 8 mU per electrode. The biosensors showed stable, fast, simple, selective, cost-effective and sensitive (-2.72E-8 A mol L^{-1}), with a good linear range (1.0–75 μmol L^{-1}), and respectively detection and quantification limits for antioxidants of 2.17, and 7.15 μmol L^{-1}. The applicability of this biosensor was demonstrated by in vitro analysis of gallic acid as standard antioxidant and Amazonian fruits as natural sources.

1. Introduction

Molecular oxygen is a central molecule in the cellular respiration of aerobic species [1]. During metabolism, the oxygen is reduced to water in sequential steps, generating a large number of short-lived intermediates called reactive oxygen species (ROS): superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($HO^{\bullet}$) [2].

ROS play an important role to protect the body against viruses and bacteria, to cell signalling, and to program cell death [3]. However, ROS can be over producted with the exposition to pollutants, tobacco, smoke, drugs, xenobiotics, radiation, and others. The excessive accumulation of ROS leads to oxidative stress which has been implicated in oxidative damage the structure of biomolecules of DNA, lipids, carbohydrates, and proteins, as well as other cellular components. Consequently, it causes numerous disorders, like mutagenesis, carcinogenesis, cardiovascular disturbances and ageing [4,5]. Antioxidants play a key role in preventing pathological levels of ROS, avoiding or slowing down the progression of these diseases [6,9].

Antioxidants are molecules with vary widely in chemical structure and mechanisms of action that can neutralize ROS forming relatively stable inactive products [7,8]. The human organism has an antioxidant defence system, formed by endogenous (enzymes, and nonenzymatic compounds) and exogenous (carotenoids, some minerals and vitamins, and polyphenols) sources [8,9]. However, the endogenous defence against antioxidants couldn't ensure rigorous control and complete protection to ROS. This fact explains the need for exogenous antioxidants from vegetables, fruit, herbs, spices, teas, nutritional supplements and pharmaceutical products [10].

In view of the protection provided by antioxidants, the use of these compounds in nutraceutical, medicinal, therapeutic, foods and cosmetic applications have been extensively reported [9,11–18]. Consequently, development of analytical tests for antioxidant capacity has received much attention. In general, spectrophotometric, electrochemical and chromatographic methods have been explored for this purpose [10,19]. In addition, in the last two decades, electrochemical biosensors have been considered an efficient alternative for measuring the antioxidant capacity in foods, presenting a higher performance to conventional assays. Among them, the biosensors based on the ability to scavenge the H_2O_2 and/or $O_2^{\bullet-}$ radicals have been developed [20]. However these methods have several disadvantages, such as high working potentials

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that allow electrochemical interferences, high enzyme amount which makes more expensive the total process, use of combined apparatus which leads to larger preparation time, non-application in real food samples and use of toxic heavy metals as electrochemical mediators [20–36].

This paper describes the development of an innovative XOD-based biosensor and the optimization of its bio-functionalization process by chemometric modelling. The developed biosensor was then applied to determine the antioxidant capacity of standards and real samples.

2. Materials and methods

2.1. Reagents and chemicals

Xanthine oxidase (XOD) of bovine milk 0.47 U protein mg^{-1} (EC 1.17.3.2), hypoxanthine (HX), and reference antioxidants were obtained from Sigma-Aldrich Corp (St. Quentin Fallavier, France). Azide-unit Pendant Water-soluble Photopolymer (PVA/AWP) was obtained from Toyo Kogyo Co. (Chiba, Japan). All reagents were of analytical grade and were used without further purification. All solutions were prepared by using 50 mM phosphate buffer pH 7.5 (K-PBS) with 10 mM KCl.

2.2. Apparatus

Amperometric measurements were performed by using a MicroAutolab III Type potentiostat (Metrohm, Netherlands) by applying -100 mV *vs*. a pseudo-reference Ag/AgCl electrode. Operational conditions: 10 mL glass cell under magnetically stirred condition (300 rpm), room temperature, in the dark, and the electro catalytic solution of 50 mM K-PBS buffer pH 7.5 containing 10 mM KCl.

2.3. Preparation of the biosensors

Screen-printed electrodes (SPE) were produced in our laboratory using a DEK 248 printing machine. The working electrode (WE) was a 4 mm graphite disk, the auxiliary electrode was a 16 mm × 1.5 mm curved line and the Ag/AgCl pseudo-reference electrode was a 5 mm × 1.5 mm straight line. The working electrode was modified with Prussian Blue (PB or ferric hexacyanoferrate).

2.3.1. Optimization of the enzyme immobilization

A homogeneous mixture containing XOD solution and PVA/AWP was prepared and 3 µL of this mixture was carefully spread on the WE surface. The electrodes were exposed to neon light at +4 °C to allow polymerization. The following parameters: XOD charge per electrode (C_{xod}), XOD:PVA/PWA ratio (R_{XP}), and polymerization time under UV exposition (T_p) were optimized for biosensor construction through Response Surface Methodology (RSM) on the Statistica software. Box-Behnken Design was used with 3 factors and 3 levels: C_{xod} (5, 8, and 10 mU/WE), R_{XP} (1:2, 1:1, 2:1), and T_p (48, 24, and 0.5 h). The statistic program constructed by 15 sets of fractional factorial experiments. .

2.4. Biosensor principle and amperometric measurements

All amperometric measurements were performed in a dark glass cell containing10 mL of K-PBS buffer magnetically stirred (300 rpm), at room temperature. The electrodes were previously tested in 10 mL of K-PBS buffer at a working potential of -100 mV *vs* Ag/AgCl, which corresponds to the reduction of H_2O_2. The initial current intensity was recorded after the current stabilization (baseline signal). Then, a 20 µL volume of 5 mM HX was successively added to the K-PBS buffer, in order to obtain increased substrate concentrations followed by the signal stabilization time of ~60 s before current record. Hence, calibration curves with increasing HX concentrations were constructed in the absence or presence of antioxidant (standards or samples), and the curve slopes (m_a) taken to further statistical evaluations.

The addition of antioxidants reduces the radical concentration (H_2O_2 and/or $O_2^{\bullet-}$), inducing the decrease of the intensity of the current and, consequently, a lower slope value is obtained (m_b). The antioxidant capacity, expressed by the inhibition of H_2O_2 and/or $O_2^{\bullet-}$ radicals, was determined by comparing the angular coefficients, according to the following equation:

$$\text{Antioxidant capacity (\%)} = 100*[1- (m_b/m_a)]$$

Antioxidant capacity can also be expressed as inhibition percent of H_2O_2 and/or $O_2^{\bullet-}$ for a given sample present in a known HX solution, as the mass equivalent of a reference antioxidant per gram of mass of the sample or extract, and even as the sample concentration necessary for 50 % signal inhibition (IC_{50}).

2.5. Analysis of real samples and reference substances

Samples of natural non-autochthon fruits (strawberry, orange, and passion fruit) and pulp of Amazonian fruits (graviola, bacuri, and murici) were purchased at the local supermarkets of São Luís city, State of Maranhão, at Northeast of Brazil. To frozen the pulps (1), nectars containing 10 % (v/v) of the depleted/chopped fruits were prepared in K-PBS buffer, whereas natural fruits (2) were transformed to a 25 % (v/v) refreshment, by mixing them to K-PBS in a 1:3 proportion of fruit/buffer.

The antioxidant capacity for each selected fruits was then determined according described in 2.4 section. Gallic acid (GA) was used as a reference antioxidant.

3. Results and discussion

3.1. Biochemical principle of the biosensor

The evaluation of the antioxidant capacity using the XOD-biosensor is based on the online monitoring of the H_2O_2 produced during oxidation process of the aqueous HX to uric acid in the presence of the enzyme XOD or by spontaneously dismutation of the $O_2^{\bullet-}$ radicals as shown in Fig. 1. The generated H_2O_2 is reduced on the polarized (-100mV *vs*. Ag/AgCl) WE surface, in presence of PB mediator, which has a well-known catalytic effect for the H_2O_2 reduction due to its peculiar chemistry structure [37]. In the presence of antioxidants, the $O_2^{\bullet-}$ radicals and/or H_2O_2 are scavenged with a decrease of the cathodic current allowing the antioxidant capacity quantification.

Electrochemical biosensors based on the ability to scavenge the H_2O_2 and/or $O_2^{\bullet-}$ radicals generated by the XOD oxidative catalyze of xanthine (XA) or HX have been developed with advantages and disadvantages inherent to its method. In general, these biosensors show differences in the antioxidant molecule interaction and electrode surface, different electrode materials and electroactive species, application in real samples, enzymatic system (bienzymatic system are commonly used with the combination of XOD and a second enzyme, generally cytochrome C/superoxide dismutase (SOD) to interact with $O_2^{\bullet-}$ radicals and produce H_2O_2), immobilization procedure and/or dissolved in solution, potential applied, number of steps, analytical performance, among others [20–36].

Campanella *et al.* [25] evaluated the antioxidant capacity to inhibit $O_2^{\bullet-}$ radicals of plant products and teas. The $O_2^{\bullet-}$ radicals were produced by XA/XOD in dissolved solution system, while the SOD enzyme was immobilized in a gel-like Kappa-carrageenan membrane in order to catalyze the $O_2^{\bullet-}$ dismutation to H_2O_2 which were oxidized in the platinum anode at +650 mV *vs* Ag/AgCl., generating an amperometric signal. Relative Standard Deviation (RSD) ≤/10 % and a Limit of detection (LOD) value about 0.1 for relative antioxidant capacity were obtained. Despite the short number of steps required and the good analytical performance, the high potential applied allowed the direct oxidation of phenolic compounds [38]. Besides that, a bi-enzymatic system was used (immobilized and in solution) which increased the

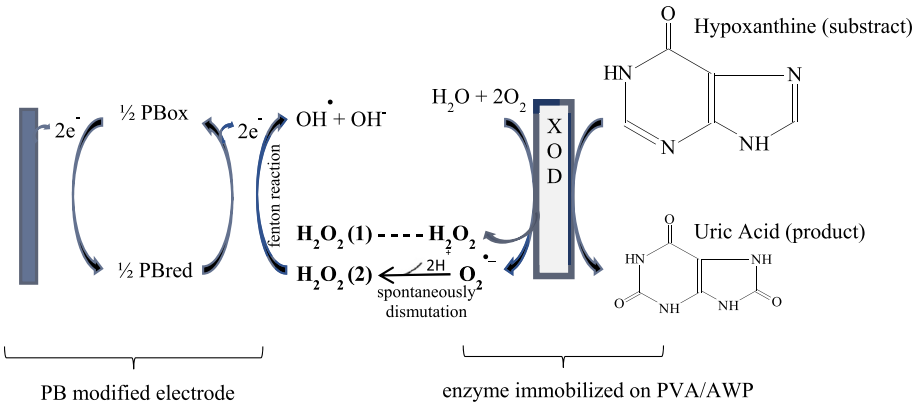

Fig. 1. Principle of detection of H_2O_2 generated by the HX/XOD enzymatic system, using the amperometric biosensor.

effective cost of its approach due to its price and the enzymes high amount necessary to dissolve in solution. In addition, the use of SOD for the generation of H_2O_2 is unnecessary once this occurs spontaneously at a fast rate and the suppression of SOD would simplify the construction and cost of the biosensor, as well as, the combined contribution measure of the antioxidant capacity against both $O_2^{\bullet-}$ and/or H_2O_2 species is more biologically interesting [39].

Lates *et al.* [20] determinate the ability of commercial beverages to scavenge both reactive species generated by the xanthine/XOD system using a bioreactor coupled with flow-through H_2O_2 amperometric biosensor at E = -100 mV *vs.* Ag/AgCl/KCl_{sat} and Os-wired horseradish peroxidase (HRP) modified WE. As immobilization procedure, the NH_2-CPG (controlled-pore glass) functionalized adsorbent coupled with glutaraldehyde crosslinking was used. LOD (2.2 mM), limit of measurement (7.5 mM), sensitivity (5 mA/M), and the linear range (up to 50 mM), were determinate. If on the one hand several advantages were showed such as the low potential applied that avoid electrochemical interferences, the simultaneous flow of antioxidant and XA subtract in the proposed bioreactor, the combined measure of the antioxidant capacity against both $O_2^{\bullet-}$ and/or H_2O_2 species, on the other hand, this approach suffers from some important shortcomings such as high cost due to the bi-enzymatic system used (XOD and HRP, to generate the oxidant species and to modify the WE, respectively), the limited binding of HRP to solid surfaces, the larger preparation steps required becoming more complicated, as well as, the expensive and toxic heavy metal used as electrochemical mediator.

3.2. Electrochemical characterization of the biosensor

Many works have already demonstrated the strong influence of modifying materials on the catalytic or electrical properties of the electrodes [40–42]. In fact, the improvement of electronic properties can lead to lower capacitive currents and better sensor sensitivity. This influence is highlighted by the electrochemical behaviour of the carbon SPE without and with PB mediator (Figs. S–1). The modification of the carbon sensor with the PB mediator significantly changed the electrical properties of the working electrode, with the cathodic peak potential of + 0.197 V *vs.* Ag/AgCl and the anodic peak of -0.073 V *vs.* Ag/AgCl

In this work, it is evident that the reduced form of PB mediator promotes a catalytic effect on the reduction of H_2O_2. and induces the reduction of the working potential to – 100 mV *vs* Ag/AgCl, a value appropriate to be applied in the subsequent electrochemical measurements. Wide linear ranges and better sensitivity are obtained in comparison with other working potentials evaluated (Fig. 2). A similar effect on linear range and sensitivity in amperometric biosensors using PB-modified SPE have been obtained at -100 mV after work potential optimization study [43,44]. However, a comparison of the performance of these is difficulted in view of the different target molecules and, therefore, the use of different substrates. It is worth noting that a

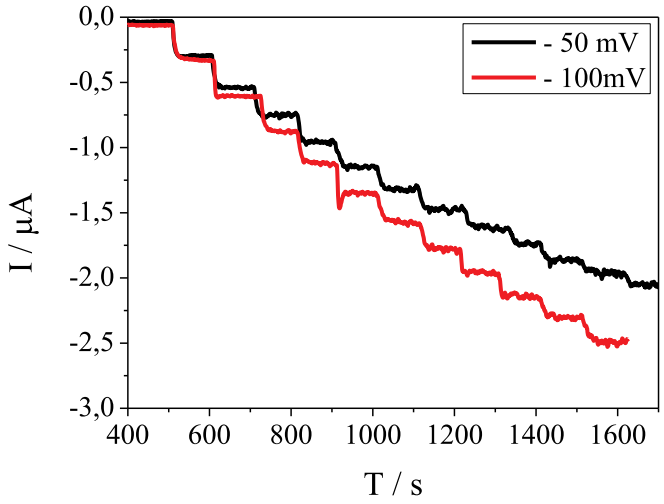

Fig. 2. Amperometric response of biosensor with the HX addition at different applied potential, and its respectively analytical performance.

potential range between 0 and -200 mV is desired, in view of the reduction of molecular oxygen (E < -200 mV) as well as the oxidation of antioxidant compounds, such as phenolic compounds and other electroactive substances which are frequently present in real sample, could also be oxidized to give interfering signals (E > 0 mV) [38].

Among the desirable effects of the Prussian Blue mediating, it is possible to include the zeolitic nature of PB with a cubic unit cell of 10.2 ˚A and with channel diameters of about 3.2 ˚A which allows the diffusion through the crystal of low molecular weight molecules (such as O_2 and H_2O_2) to its catalytic centre, excluding molecules with higher molecular weight [37,45,46]. Varvari and Popescu [47] who proposed a PB-modified amperometric sensor for antioxidant activity evaluation at -0.1 V vs. Ag/AgCl, KCl_{sat} confirmed this electrocatalytical effect for H_2O_2 reduction.

The PB selectivity and activity are comparable to those of a biological binding component but with all the advantages of an inorganic species (low cost, high stability at certain conditions, ease of electrode surface modification, no saturation effect for substrate) [37]. In general, the preparation of PB through, specially, adsorption and electrodeposition is simple, which, besides being cost-effective, is highly stable in acidic and neutral media [48–50].

3.3. Optimization of the enzyme immobilization procedure

The PVA-AWP is successfully used for the immobilization of enzymes onto the SPE surface offering several advantages such as better elasticity, low-toxicity, biocompatibility with enzymes, mechanical and

long-term stability, and biodegradability [51,52]. However, the thickness of the film, depending on the polymer concentration, and the degree of crosslinking, depending on the time of UV exposure, will affect the enzyme retention properties of the polymer and its permeability for the enzyme substrates and the products of the enzyme reaction [52].

We decided to optimize the fabrication of the biosensor in terms of the polymer concentration, irradiation time, and enzyme concentration using the chemometric method. RSM was applied to optimize C_{XOD}, R_{XP}, and T_P factors for enzyme immobilization onto PB modified WE. Box-Behnken design was used applying high, central, and low values for C_{XOD} as 5.0, 8.0, and 10.0 mU per electrode, for R_{XP} as 1:2 (0.33), 1:1 (0.50), and 2:1 (0.66) representing XOD-enzyme:PVA/AWP ratios, and for T_p as 48, 24, and 0.5 hour, respectively. 15 of the working set was generated by Statistica program and thus 15 electrodes were constructed. Each electrode was tested in 50 mM K-PBS buffer containing 10 mM KCl (pH 7.5) at a working potential of -100 mV *vs.* Ag/AgCl until steady state currents were obtained, then the net current values in 50 μM HX solution were measured.

For fitting of experimental data, linear (L) and quadratic (Q) models were tested. Significant **p**-value (< 0.05) was found in all factors to a linear model (Tables S–2), which showed 0.99887 predicted R-squared value in reasonable agreement with the adjusted R-squared value (0.99773). Also, the lack of fit **F** value of 1.792 implies the no significant value of lack of fit ($p > 0.05$) and represent validity of the linear model for explanation of experimental data of the present study which was used for further model construction.

Important results of the effects of simultaneous change of C_{XOD}, R_{XP}, and T_P are given by the Pareto Chart (Fig. 3) which shows the order and significance of each variable affecting on the current intensity response by the enzymatic reaction in the amperometric biosensor and in the RSM diagrams for depending on studied parameters (Fig. 4).

With respect to the main effect of each variable, two variables named T_P and R_{XP} affect negatively the current intensity response, where another variable named C_{XOD} affect positively the ROS production (Fig. 3). As shown in Fig. 4A, the R_{XP} and C_{XOD} variables determine the response surface. As the R_{XP} ratios decreased, the current values increased which reveal that with the same amount of enzyme higher amounts of PVA/PWA secure the crosslinking. A slight increment of C_{XOD} increases the signal. The current response increases when the T_P decreased (Fig. 4B), showing that the polymerization time significantly influences the enzyme activity. The decrease of the enzyme activity can be due to the long UV light exposition. Others T_P were tested (4.0 and 2.0 h) with similar results to 0.5 h ($p < 0.05$). For stationary T_P, the signal shows a slightly effect with the increased of C_{XOD} concentration. However, in 0.5 h there is no difference between the current signal at 8.0 and 10.0 mU XOD concentration ($p < 0.05$). As shown in Fig. 4C, as

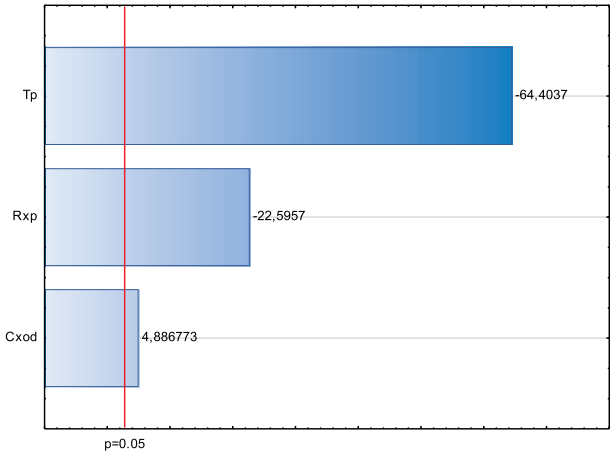

Fig. 3. Pareto chart representing the ANOVA analysis for linear components of the parameters studied. Results are significant for $p > 0.05$.

the R_{XP} ratios decreased, the current response values increase when the R_{XP} ratios decrease. In addition, as the T_P decreased the increased signal in net current values due to the maintenance of XOD enzyme activity.

The optimal enzyme immobilization conditions were chosen at low level 0.5 h and 0.33 respectively for T_P and R_{XP}, and C_{XOD} variable which exerted a slightly positive effect on ROS production was maintained at a central level (8 mU enzyme per WE) because there is no difference between a high level (10 mU). The XOD immobilization using PVA-AWP is an excellent strategy for SPE biofunctionalization in this biosensor construction and the RSM is a good method to determine the optimal conditions for enzyme immobilization.

3.4. Performance of the amperometric biosensor for antioxidant capacity screening

The performance of the XOD-PB-modified amperometric biosensor is shown in Tables S–3. The precision, in the absence of antioxidants, was evaluated with 10 μM HX for ten (10) successive assays [53]. RSD was 5.27 %, showing the good reproducibility of the tool proposed.

The detection and quantification limits were calculated by the ratio of the average of ten (10) determinations of the standard deviation of the 10 blanks measurements and the slope of the analytical curve (m), multiplied by factor 3 and 10, respectively [43].

Once prepared and kept in the conditions at 4°C and protected from light, the XOD-PB-modified electrodes shows a shelf life at least 2 days.

XOD-PB-modified electrodes presented operational stability for at least 6 hours if used in continue. Once swollen, the biosensor can be washed with K-PBS buffer and reuse, taking around of 8 min to current stabilize to new measurements. Fig. S-4 shows the range of HX concentrations for which the biosensor response changes linearly with the concentration (R^2: 0.997), but a non-linear adjustment can also be performed, thus increasing the range of analyses.

To achieve reliable results, quality control is required during all steps of the analysis including data processing and analysis. Fig. 5 shows such a control chart to 10 measurements of 10 μM HX in the antioxidant absence which is an effective method of quality control to ensure there are no changes to a process over time.

The biosensor exhibited good analytical performance for sensitivity, reproducibility, fast response time, low cost, good shelf life, signal stability, easy automation, the simplicity of operation and manufacturing.

Besides that, this biosensor presented advantages in relation to the biological relevance, once it measures the combined antioxidant capacity against the reactive oxygen species which are also produced by human body and not artificial radicals such as ABTS and DPPH used in conventional assays.

The influence of natural interferents (GA, ascorbic acid, and quercetin), as well as compounds involved in the detection scheme (HX) were evaluated. In this way, cyclic voltammetry measurements using the biosensor proposed was performed in absence or in presence of these compounds and the results showed that at the applied potential selected for amperometric detection (-100 mV *vs.* Ag/AgCl), no interferences were observed from neither in the substances usually found in food nor in those involved in the detection principle. To due the PB selectivity, the enzymatic oxidation of HX and the amperometric detection of H_2O_2 and/or $O_2^{\bullet-}$ at low potential practically assures the absence of interferences.

3.5. Determination of the GA antioxidant capacity

The developed biosensor was applied to the determination of the antioxidant capacity of GA, which is a strong reducing molecule found in food and herbs of great medical, pharmaceutical, and food technology interest. Elsewhere, GA is very often used as a reference. The addition of GA resulted in a decrease of the electrochemical signal which is proportional to its concentration. GA molecules and other

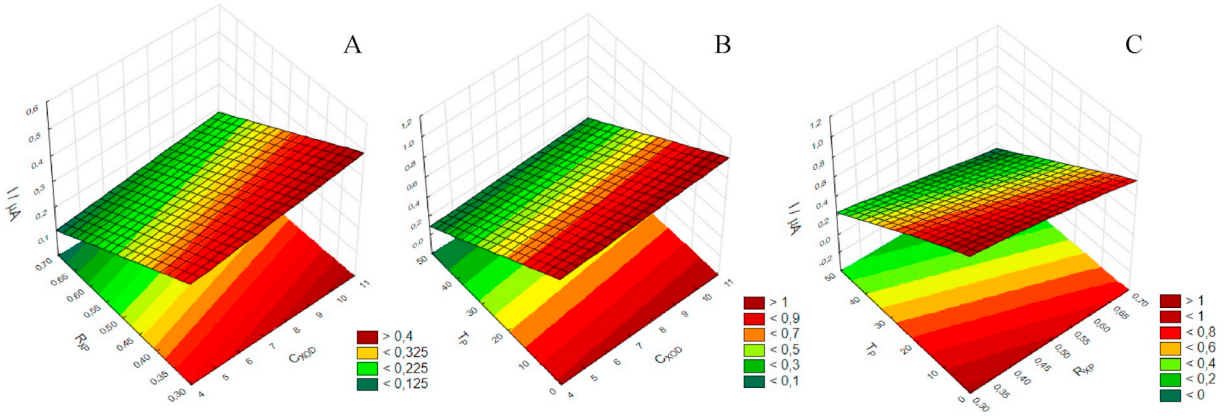

Fig. 4. Surface diagram for the current response depending on R_{XP} and C_{XOD} (A), T_P and C_{XOD} (B), T_P and R_{XP} (C).

antioxidant species react with $O_2^{\bullet-}$ radicals and/or H_2O_2, inducing a decrease in the amount of produced H_2O_2 and a subsequent decrease in the oxidation current, that allows the quantification of antioxidant capacity. Fig. 6 shows the calibration curve of the biosensor for GA in different concentration and its antioxidant capacity (%).

3.6. Application of the biosensor to the detection of the antioxidant capacity of real samples

We determined the antioxidant capacity of fresh and frozen fruit following the protocols detailed in Sections 2.4 and 2.5 (Fig. 7).

The graviola, bacuri, and murici nectar samples showed 55.9, 53.7, and 59.9 % of scavenging potential (Fig. 7B), which display a statistical similarity antioxidant capacity among those ($p < 0.05$). Bacuri and murici are Brazilian native fruit and murici is a cultivate fruit which is used by the local population as medical and food, being consumed in natura, as well as to produce juice, sweets, and ice cream.

The refreshment fruit samples results showed the strawberry as the highest antioxidant capacity refreshment fruit samples (93.0 %), followed by passion fruit (82.0 %), and orange (37.9 %), considering the inhibition of the observed slope (Fig. 7A). We have to recalculate the anti-oxidant capacity taking into account the method of preparation of the samples. The antioxidant capacities of refreshment fruit samples were respectively 37.2, 32.8 and 15.1 % for strawberry, passion fruit and orange (Fig. 7B). The highest anti-oxidant capacity was obtained for the Amazonian fruits. The lowest antioxidant capacity was obtained with the natura orange fruit.

4. Conclusion

In this paper, an enzymatic biosensor based on the XOD-PB-modified electrode was developed for the detection of the antioxidant capacity of natural or processed vegetal samples. The immobilization strategy of the XOD enzyme using PVA-AWP proved to be very efficient. The construction of the biosensor has been chemometrically optimized. The operational conditions lead to the reduction of electrochemical interferences and to a better sensitivity and precision, as well as reducing the time of analysis and quantities of reagents

The prototype also showed simplicity in construction and operation, low cost, stability of the generated signal, easy automation, good period of continuous operation and reuse, besides high capacity of portability.

A number of advantages is observed in our biosensor in comparison with previous biosensors for antioxidant activity, such as the low working potential that reduces electrochemical interference, the small amount of enzyme of the monoenzymatic system that reduces the total process and the use of PB as a mediator that provides selectivity and activity comparable to those of a biological binding component but with all the advantages of an inorganic species.

It is also worth noting the biological relevance of this tool in comparison to other tests, since, by using the biosensor, the antioxidant

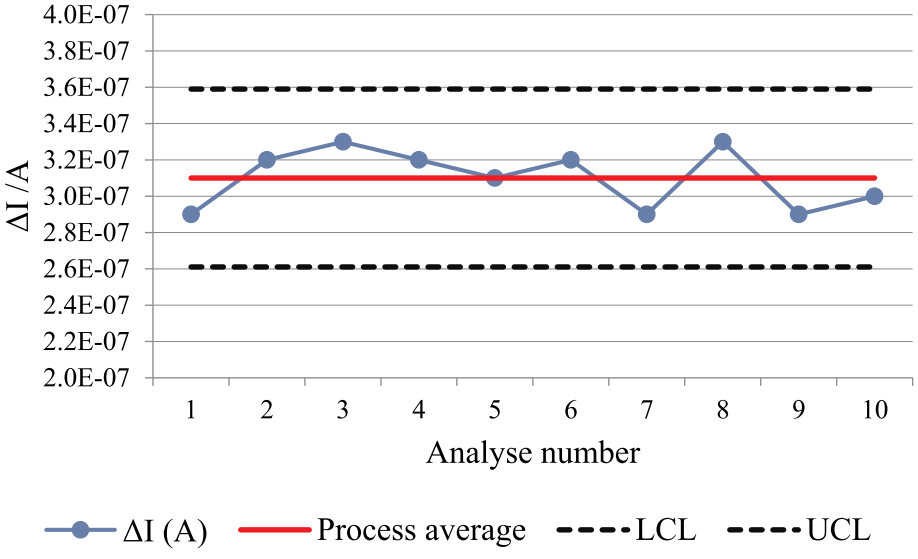

Fig. 5. In-house created control chart for H_2O_2 generation. The points are measurements and show that the sensor has not changed over many measurements. The dashed lines are 3 times the standard deviation to upper control limit (UCL) and lower control limit (LCL), and the solid line is the intensity current variation average obtained.

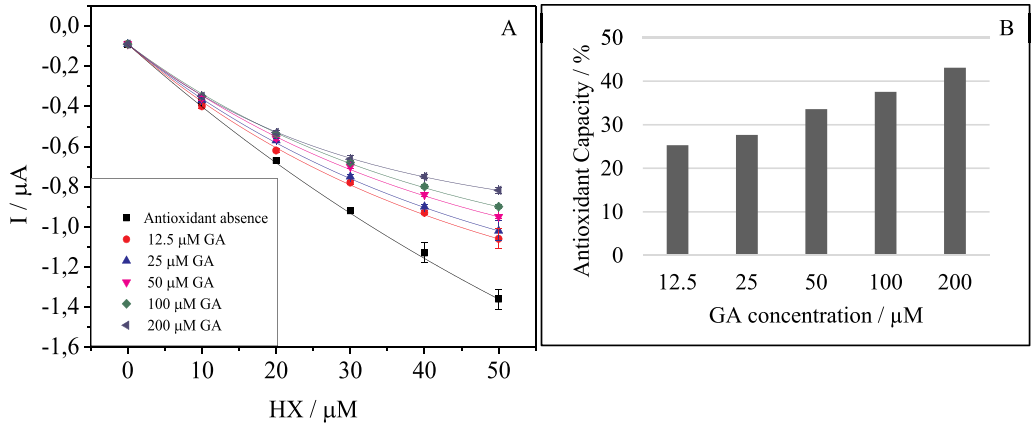

Fig. 6. Amperometric biosensor response in function of successive additions of 20 µL of 5 mM HX in the absence and presence of GA (A) and its antioxidant capacity correspondent, expressed in percentage (B).

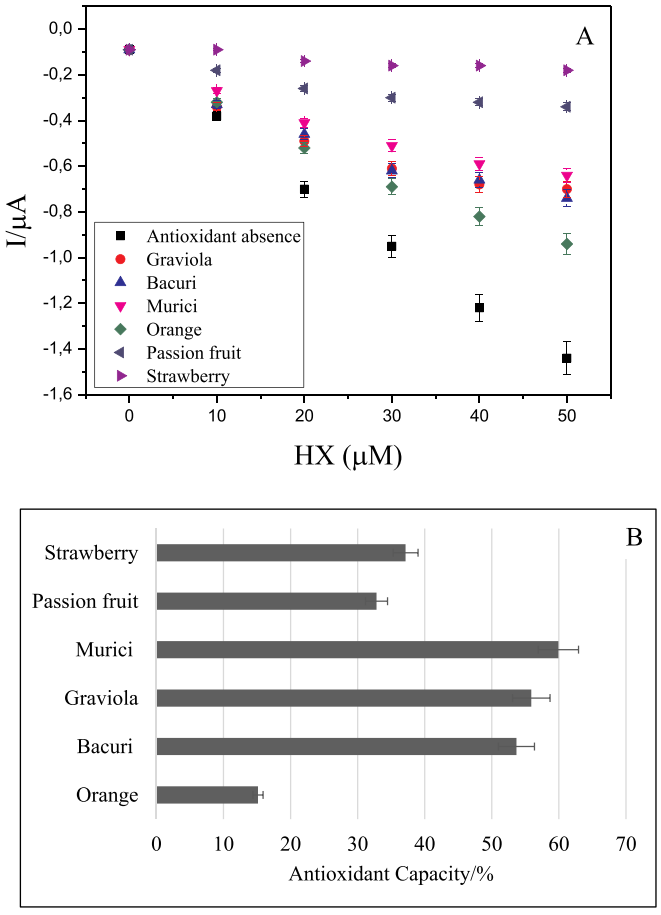

Fig. 7. Amperometric biosensor response in function of successive additions of 20 µL of 5 mM HX in the absence and presence of nectar and refreshment fruit (A) and its antioxidant capacity in percentage to 10 % v/v samples (B).

capacity of samples against the combined ROs ($O_2^{\bullet-}$ and/or H_2O_2) is determined, which are present in biological systems and exhibit cytotoxicity under conditions of oxidative stress. The bioelectroanalytical parameters, combined with the simplicity of its construction, make the proposed biosensor a promising tool for the determination of antioxidant capacity in natural or processed vegetable samples.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2019.06.002.

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Short Report

Determination of the Antioxidant Capacity of Red Fruits by Miniaturized Spectrophotometry Assays

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Natural sources with high antioxidant capacity represent an interesting potential to prevent or minimize the oxidative stress that causes many chronic diseases. The antioxidant capacity of red fruits (strawberry and cherry) was evaluated by miniaturized spectrophotometric methods 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitroblue tetrazolium (NBT). ABTS and DPPH colorimetric methods are based on the ability of antioxidants to scavenge synthetic free radicals produced *in vitro*, which have a different chemical structure from the natural reactive oxygen species generated in the human body. In this respect, the NBT method stands up because it is based on $O_2^{\bullet-}$ scavenging which is generated *in vitro* by enzymatic reaction systems. The spectrophotometric assays adapted on microtiter plates allowed a rapid, inexpensive and simultaneous analysis. Larger amounts of lipophilic and hydrophilic antioxidants were obtained from strawberry fruit, which showed the lowest 50% signal inhibition concentration (IC_{50}) values. The fruit analyzed showed promising sources of bioactive compounds with high antioxidant properties.

Keywords: antioxidant capacity, miniaturized assays, red fruits

Introduction

In the last years, there is a continuous demand for natural sources of antioxidants in order to prevent the oxidative process that has been identified as the cause of the decreasing of nutritional quality in the foodstuff, the rancidity in cosmetic products and oils, but above all, the development and progression of several human pathologies like cancer, Parkinson's and Alzheimer's.[1,2]

Antioxidants are any substances that, when present even at low concentrations, delay or inhibit significantly oxidation processes in living beings. This occurs due to their ability to hydrogen atom transfer (HAT) and/or single electron transfer (SET) to eliminate the unpaired condition of the free radical and to chelate metals resulting from the oxidation process.[3,4]

Antioxidants can be classified into two major groups: enzymatic and non-enzymatic substances. Some of these compounds, that include enzymes, low-molecular-weight molecules, and enzyme cofactors, are produced endogenously.[5] Many non-enzymatic antioxidants are obtained from dietary sources, such as vegetables, fruits, whole grains, wine and herbal infusions, considered sources of vitamins (A, E and C), phenolic compounds (gallic and caffeic acids, quercetin, and rutin), and minerals (Se and Zn).[6,7]

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Due to chemical diversity of antioxidants and its behavior that may respond in a different manner to numerous radical or oxidant sources, there is not yet a unique, simple and universal method for antioxidant capacity screening, which is why the need to evaluate the antioxidant capacity of foodstuffs by different methods.[7] Several traditional analytical methodologies, such as spectrophotometric, electrochemical and chromatographic ones, have been used, each one differing in relation to the mechanism of generation of radicals and/or target molecules as well as to the final detection/measurement of the reaction products.[2] However, in recent years, a great effort has been done in the use of more sophisticated and precise bioanalytical methods such as those based on electrochemical sensors and biosensors, in order to improve the detection performance.[8]

In general, spectrophotometric techniques are simple, rapid and not expensive, which probably explains their widespread use in antioxidant screening. In turn, spectrometric techniques rely on the reaction of a radical, radical cation or complex with an antioxidant molecule capable of HAT and/or SET.[2,9,10] The most common methods for the *in vitro* determination of antioxidant capacity in foods are based on spectrophotometric assays employing 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and nitroblue tetrazolium (NBT) as chromogens, the latter being the most efficient in the physiologic point of view.[9-11]

DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ are synthetic organic radicals with purple and blue-green colors, respectively, which can be reduced in the antioxidant presence, with the consequent decolorization (Figure 1). The antioxidant capacity can be evaluated by the decrease of absorption at certain wavelength.[2,9,10]

The DPPH and ABTS methods are based on the inhibition of synthetic-free radicals, which have a different structure to the reactive oxygen derivatives. In this perspective, NBT process is more advantageous because it evaluates the capacity for removal of superoxide radicals by antioxidants present in the sample under physiological conditions.

In NBT method, superoxide radicals ($O_2^{\bullet-}$) and uric acid are generated *in vitro* by the hypoxanthine/xanthine oxidase system. The $O_2^{\bullet-}$ radicals reduce the NBT reagent (yellow color) to formazan (purple color), which is measured spectrophotometrically at 560 nm (Figure 2).[11]

Considering the current upsurge of interest in the measurement of efficacy and use of natural antioxidants for applications in food technology, cosmetic industry, therapeutic, nutraceutical and medical usages, the aim of this work was to determine the antioxidant capacity by ABTS, DPPH and NBT miniaturized spectrophotometric methods in the red fruits (strawberry and cherry) and to compare the previously discussed spectrophotometry methods adapted to microplate reader.

Experimental

Reagents and equipment

Analytical grade chemicals were employed in the preparation of all solutions. Deionized water (Milli-Q, Millipore, 18.2 MΩ cm) was used in all experiments. Ascorbic acid (A5960), gallic acid (G7384), Folin-Ciocalteu (F9252), Na_2CO_3 (S7795), DPPH (D9132), ABTS (A1888), $K_2S_2O_8$ (P5592), NaCl (S7653), Na_2HPO_4 (S0876), KH_2PO_4 (P9791), K_2HPO_4 (P3786), KCl (P3911), ethylenediamine tetraacetic acid (EDTA, E9884), NBT (N6876), hypoxanthine (HX, H9377), xanthine oxidase (XOD) enzyme from bovine

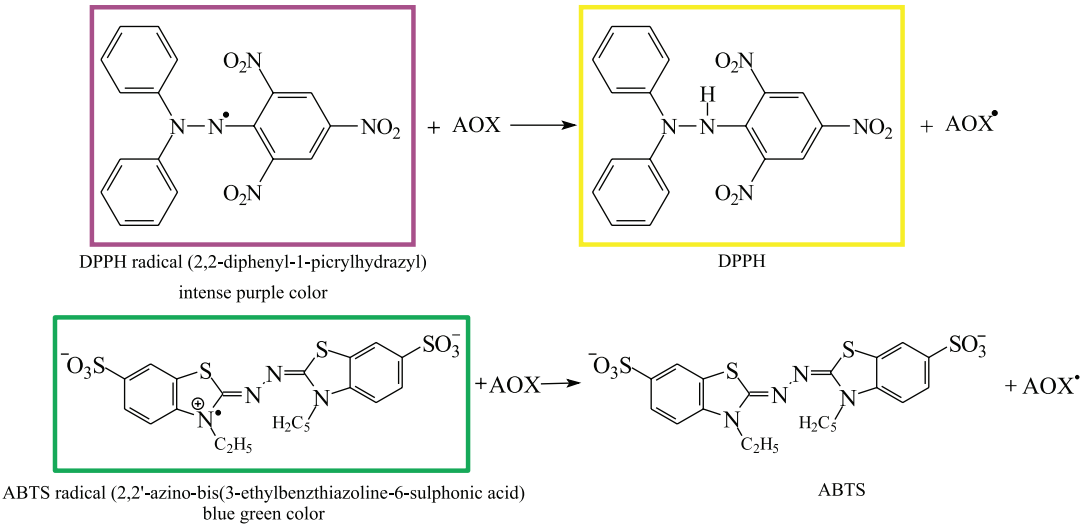

Figure 1. Chemicals reactions involved in the DPPH and ABTS spectrophotometric assays.[9,10]

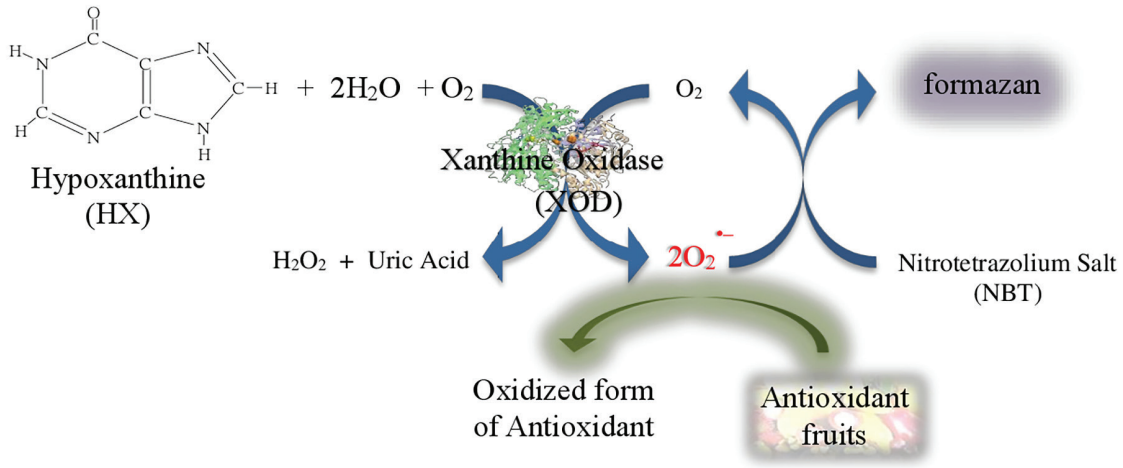

Figure 2. Reactions involved in the measurement of superoxide radical sequestration capacity using the NBT technique.

milk (X4376) were purchased from Sigma-Aldrich Corp. (Nasdaq-Sial, Darmstadt, Germany). Spectrophotometric analyses were carried out in a Multiskan Ex Primary.

Sampling

Strawberry and cherry samples presenting complete physiological maturity stage were obtained in June 2017, from a supermarket in Perpignan, France (42°41'07.4"N and 2°54'06.7"W). The fruit samples were lyophilized at the laboratory of the University of Perpignan Via Domitia, UPVD, and allowed to stand at room temperature until analysis.

Antioxidant capacity determination

Before applying the colorimetric method, the extraction procedure had to be previously defined. So, DPPH assay was selected and the influence of time, temperature, amount of lyophilized samples and the solvent was studied. Also, the final recovery of supernatants was evaluated by comparing two different procedures: shaking and ultrasound-assisted extraction.

Better inhibitions were obtained in the extraction using 100 mg mL^{-1} of the lyophilized samples subjected to shaking for 1 h on a sample mixer (HulaMixer, Invitrogen Dynal AS, Life Technologies) at ca. 4 °C and protected from light. Then, the supernatant was collected after centrifugation for 10 min at 5000 rpm (Hettich, Rotina 380 R). Lyophilized samples, in decreasing concentrations, were submitted to the miniaturized DPPH, ABTS and NBT assays.

DPPH assay

The effect of each antioxidant fruit on DPPH• radical was estimated according to recommendations of Marinova and Batchvarov[12] with some modifications. All solutions were prepared in ethanol. The stock solution was prepared by dissolving 13.8 mg DPPH with 20 mL ethanol and then stored until needed. The control (100%) solution was obtained by mixing 225 µL ethanol with 25 µL stock solution to obtain an absorbance of 1.0 ± 0.1 unit at 490 nm. Ethanolic fruit extracts (25 µL) were allowed to react with 200 µL of ethanol and 25 µL of the DPPH solution for 20 min in the dark, 400 rpm, at 25 °C. Ethanol (250 µL) was used for the blank control (100%), and the ethanol (225 µL)/fruit extracts (25 µL) was used as blank fruit to avoid interferences due to the sample's color. The absorbance decreasing was recorded at 490 nm. For all evaluated assays, absorbance measurements were performed in triplicate in a microplate reader.

Different antioxidant solution concentration was evaluated to determinate the ability to scavenge DPPH• radicals by the 50% signal inhibition concentration (IC_{50} in mg mL^{-1} that means the concentrations of samples required to scavenge 50% of free radicals), in ascorbic acid equivalents, using linear regression analysis.

A scheme review of the miniaturized DPPH assay is shown in the Figure 3.

ABTS assay

The ABTS assay was carried out according to procedure proposed by Arnao *et al.*[13] with some modifications. Initially, a stock solution formed by 7.0 mmol L^{-1} ABTS plus 2.45 mmol L^{-1} potassium persulfate solution dissolved in 10.0 mmol L^{-1} phosphate-buffered saline (PBS) buffer (pH = 7.4) was prepared and kept during 12 h at room temperature and in the dark in order to allow chemical equilibrium. In order to allow the extraction of lipophilic

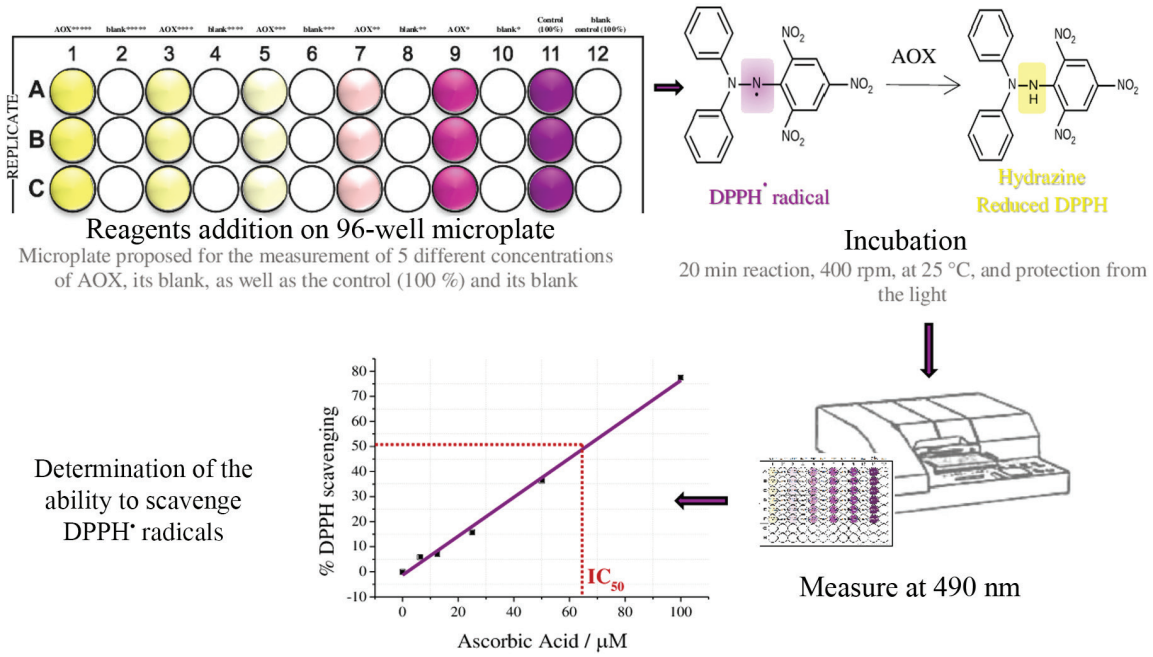

Figure 3. Miniaturized DPPH assay scheme.

and hydrophilic antioxidants of the lyophilized samples, the ABTS assay was applied in three extracts of different polarities: aqueous (PBS buffer), absolute ethanol and binary extraction (1:1, hydroethanolic). The $ABTS^{\bullet+}$ working solution was prepared by dilution to obtain to the control (100%) an absorbance around 0.7 units at 405 nm. The reaction mixture (AOX) was prepared by mixing 25 µL antioxidant (fruit extracts or standard antioxidant), and 225 µL $ABTS^{\bullet+}$ working solution. 250 µL solvent was used for the blank control (100%), and the solvent (225 µL)/antioxidant solution (25 µL) was used as blank. The decrease in absorbance after 6 min of reaction, at 25 °C, under stirring at 400 rpm and in the dark, was recorded at 405 nm. The ability to scavenge $ABTS^{\bullet+}$ radicals was calculated by IC_{50} (mg mL^{-1}) in ascorbic acid equivalents.

NBT assay

For the NBT assay, the method proposed by Cortina-Puig *et al.*[11] was applied with some modifications. All solutions were prepared in 50 mmol L^{-1} K-PBS (pH 7.5) containing EDTA (0.1 mmol L^{-1}) buffer due to the optimal enzyme conditions required. The control (100%) was prepared by mixing 175 µL K-PBS buffer, 25 µL 0.75 mmol L^{-1} HX, 25 µL 0.75 mmol L^{-1} NBT, followed by the incubation step (5 min, in the dark, 700 rpm, at 25 °C), and the addition of 25 µL 0.70 U mL^{-1} XOD to obtain after the final incubation step (15 min, in the dark, 700 rpm, at 25 °C) an absorbance around 0.28 ± 0.02 units at 560 nm. Fruit extracts (25 µL) were allowed to same reagents sequence addition but using 150 µL K-PBS buffer. The blank control and blank fruit were prepared in absence of XOD and adding 25 µL K-PBS buffer in order to obtain the final volume of 250 µL. The increase in absorbance after the final incubation step was recorded at 560 nm. The ability to scavenge $O_2^{\bullet-}$ radicals was calculated by IC_{50} (mg mL^{-1}) in ascorbic acid equivalents.

Results and Discussion

The antioxidant capacity results in IC_{50} for the different assays, in different solvents extracts, of the lyophilized red fruits are shown in Table 1. Ethanol and/or aqueous buffer were adopted as solvents in the samples antioxidants extractions after considering safety in handling, the human consumption in some food products such as beverages, wine, and liquors, as well as, the reference as a good solvent for antioxidant extraction.[14]

Strawberry pulp showed to have higher contents of lipophilic and hydrophilic antioxidants in view of the greater inhibition of the $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and $O_2^{\bullet-}$ radicals in all extracts, except in the ABTS assay for the ethanolic extract that showed similar results between the strawberry and cherry fruits. Comparing the IC_{50} value in the different solvent extractions in the ABTS method, it became evident that ethanol was not the better solvent for the extraction of the antioxidant compounds in the analyzed samples.

This study highlighted the advantages and limitations of the assays used. The ABTS method can be used in various solvents allowing the extraction of lipophilic

Table 1. The antioxidant capacity of red fruits, on dry basis, in IC_{50} of the different extracts expressed as mean ± standard deviation for n = 3

Sample	IC_{50} value / (mg mL^{-1})				
	$DPPH^{\bullet}$ ethanol	$O_2^{\bullet-}$ K-PBS	$ABTS^{\bullet+}$		
			Ethanol	ethanol:PBS	PBS
Strawberry (*Fragaria* spp.)	1.452 ± 0.014	0.123 ± 0.005	0.313 ± 0.002[a]	0.063 ± 0.003	0.072 ± 0.002
Cherry (*Prunus cerasus*)	10.962 ± 0.043	0.294 ± 0.004	0.311 ± 0.023[a]	0.134 ± 0.003	0.261 ± 0.004
Ascorbic acid	0.011	0.020	4.652×10^{-3}	1.652×10^{-3}	2.113×10^{-3}

Means followed by the same letter in the same columns do not differ significantly from each other by the Tukey's test at the 5% probability level; IC_{50}: concentrations of samples required to scavenge 50% of free radicals; $O_2^{\bullet-}$: superoxide radical; DPPH: 2,2-diphenyl-1-picrylhydrazyl; PBS: phosphate-buffered saline.

and hydrophilic antioxidants, and the better solvent system to be applied depends of the samples' antioxidant composition. The lower the IC_{50}, the higher is the fruit antioxidant potential, in this view it can be observed that the best inhibition rates have been obtained through extraction with the binary solvent mixture, followed by the PBS extraction. On the other hand, the DPPH radical showed to be more stable, probably due to the resonance effect in their chemical structure and only soluble in the ethanolic extract.[15] Superoxide anion radical ($O_2^{\bullet-}$) is one of the strongest ROS, which gets converted to other harmful ROS as well as free radicals such as hydrogen peroxide and hydroxyl radical in the cells.[16] In this study, the strawberry extracts showed the highest superoxide scavenging activity.

Table 2 compares the main spectrophotometric methods, taking into account not only its biochemical principle and operational conditions, but also its effectiveness.[7-10]

The different methods in the literature for the antioxidant capacity determination of biological systems involve different radicals/oxidant sources, consequently more than one chemical mechanism.[17] Additionally, the antioxidants chemical diversity allow different behavior to eliminate the unpaired condition of the free radical or to chelate metals. In view of this, no single assay accurately reflects the mechanism of action of all radical sources or all antioxidants in a complex system,[9] consequently more than one antioxidant capacity method must be used for comparing the mode of action of crude or pure compounds.[17,18]

In this work three methods, DPPH, ABTS and NBT, were chosen based upon different reaction mechanisms, NBT utilizing the SET mechanism to eliminate superoxide radicals which are oxidants present in all aerobic biological systems, while the other (DPPH and ABTS) using the ability to HAT and/or SET to neutralize the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ synthetic radicals.[7] In this view, NBT assay represents a more concise tool allowing investigators to assess a sample's antioxidant capacity against a specific, biologically relevant free radical, the superoxide radical ($O_2^{\bullet-}$), which is an oxygen-derived species that is potentially cytotoxic and causes damage to DNA, and therefore are related with a number of disorders such as Parkinson's disease and cancer.[19-22]

Table 3 summarizes a selection of research results obtained in the last four years using different extractions methodologies applied to strawberry and cherry fruits antioxidant capacity determination. The table includes

Table 2. Comparison of some spectrophotometric methods for antioxidant capacity scavenges regarding operation principle and advantages/disadvantages

Assay	Biochemical principle	Characteristic
DPPH	antioxidants neutralize $DPPH^{\bullet}$ radicals by SET and HAT; there is a decrease in absorbance over time, proportional to the antioxidant capacity	relatively stable radical; highly reproducible and precise; applied only in organic solvents; easy and fast
ABTS	antioxidants neutralize $ABTS^{\bullet+}$ radicals by SET and HAT; there is a decrease in absorbance over time, proportional to the antioxidant capacity	wide pH range; applied to hydrophilic and lipophilic antioxidants; long reaction time (> 6 min) could give incorrect results due to short assay; sensitive, easy and fast
NBT	antioxidants neutralize $O_2^{\bullet-}$ radicals by SET; there is a decrease in absorbance over time, proportional to the antioxidant capacity	applied only to hydrophilic antioxidants, and in physiological conditions (pH/salinity); highly reproducible, easy and fast

DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid; NBT: nitroblue tetrazolium; HAT: hydrogen atom transfer; SET: single electron transfer.

Table 3. Extraction conditions and results obtained from a selection of studies. Extraction conditions: solvent (volume %, the rest up to 100% is water unless indicated), solid-to-liquid ratio, temperature, extraction time

Extraction condition	Radical scavenging assay	Reference
Cherry (*Prunus cerasus*)		
Solvent extraction: ethanol (42.39%, acidified 1% formic acid), 1:15, 40 °C, 75 min	ABTS: 59.61 mM trolox mL^{-1}	23
Ultrasound assisted extraction: ethanol (40%), 1:15, 37 khz, 40 °C, 40 min	ABTS:105.87 mM trolox mL^{-1}	23
Strawberry (*Fragaria* spp.)		
Solvent extraction: absolute ethanol, 1:20, 60 °C, overnight	DPPH: (IC_{50}) 39.01 mg mL^{-1}	24
Ultrasound assisted extraction: ethanol:water:HCl (70:29:1), 1:10, 30 °C, 2 h	DPPH: 4250 μmol trolox equivalent g extract $weight^{-1}$	25
Solvent extraction: ethanol (95%), 1:4, at room temperature, 2 days	DPPH: (IC_{50}) 3.1 μg mL^{-1} ABTS: (IC_{50}) 9.9 μg mL^{-1}	16

ABTS: 2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid; DPPH: 2,2-diphenyl-1-picrylhydrazyl; IC_{50}: concentrations of samples required to scavenge 50% of free radicals.

the extraction conditions, results of radical scavenging assays, and the local sampling. A comparison between the results obtained from the antioxidant capacity and those reported in the literature is complex due to the samples and sampling conditions (climate, soil composition, varieties and cultivar), as well as different methods of extraction that significantly influence the results, radical final concentration used, and different ways in which the results are presented: different reference antioxidants are employed to express the results (usually gallic acid, trolox, ascorbic acid), numerous ways of expressing the results are used, such as percent inhibition of radical for a given concentration of the sample, mass equivalent of a reference antioxidant *per* gram of mass of the sample or extract, IC_{50}, among others. The standardization is longed for unifying quantities and units.

Despite the complexity, the strawberry antioxidant capacity was higher than those reported for Mandave *et al.*[24] for DPPH method, and lower than Basu and Maier[16] for DPPH and ABTS methods. In the other cases, unfortunately, no comparison can be made between our results and the literature used.

In this work, it was evidenced that the microtiter-adapted assays have allowed easy and fast analysis of numerous small samples at the same time. The plates' standardized dimensions make them ideal for automation and do not require special equipment. Moreover, their relatively small sample volumes, as well as the high densities, provide advantages in terms of reagent volume usage, cost and speed.

Conclusions

Because of the chemical heterogeneity of exogenous antioxidant compounds that may respond using more than one chemical mechanism to different sources of radicals or oxidants, there is still no universal method that can be employed. In view of this, there are different methods in the literature for the determination of the antioxidant capacity of biological systems and no assay accurately reflects the mechanism of action of all the oxidants sources or all the antioxidants in a complex system. This has led to a certain consensus of the researchers of the need to employ more than one method to determine the antioxidant capacity in order to compare the mode of action and obtain a more complete response.

DPPH, ABTS and NBT assays adapted on microtiter plates were applied to determinate the antioxidant capacity of two red fruits. The miniaturized assays have allowed rapid, inexpensive, and simultaneous analysis of the antioxidant potential of several red fruit samples. All three methods showed advantages and disadvantages inherent in the nature of the oxidizing source used and therefore of biological relevance, analytical performance (reproducibility, precision, sensitivity, response time) and solubility in solvents of different polarities. The choice of these methods should be made strictly according to the types of antioxidants to be tested.

This study is an important contribution for the food analysis area, since it will serve as a basis for the analyst to choose the best method to be used in evaluating the nutritional property of food sources.

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ESSENCIALIDADE E TOXICIDADE DE MANGANÊS E COBRE EM AMOSTRAS DE FRUTOS DA AMAZÔNIA BRASILEIRA

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Área

Alimentos

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Resumo

Cobre e manganês são microminerais essenciais ao organismo humano, sendo as frutas suas fontes naturais. Considerando a importância na nutrição humana, os teores de Cu e Mn foram quantificados simultaneamente por ICP-OES em nove frutos da Amazônia brasileira, e seus potenciais nutricionais avaliados. Os teores encontrados nos elementos sugerem que os frutos abiu, buriti e pajurá são fontes em Cu e monguba e uxi possuem alto teor; açaí e buriti possuem alto teor em Mn, e uxi é fonte natural nesse elemento. Os resultados representam novas informações sobre frutos da Amazônia brasileira, alguns ainda inexplorados, e sugerem sua inserção na dieta alimentar, por contribuírem com microminerais essenciais.

Palavras chaves

frutas amazônicas; microminerais; essencialidade

Introdução

Os minerais, originários sobretudo do solo e da água, são absorvidos pelas plantas e chegam ao homem através do consumo destes. No organismo humano formam os tecidos corpóreos, atuam na ativação e regulação enzimática, no controle de impulsos nervosos e em outras funções vitais (SMOLIN; GROSVENOR, 2007). Classificados como microminerais essenciais, as espécies químicas cobre e manganês são requeridas diariamente em pequenas quantidades, baseadas no consumo mínimo/máximo admitidos, a fim de prevenir deficiências que possam impedir o bom funcionamento do organismo, e evitando também a toxicidade, uma vez que o excesso e/ou deficiência desses minerais podem levar a desordens no sistema fisiológico e enfermidades (SHILS; OLSON; SHIKE, 1994). Estudos nutricionais demonstram que os níveis de ingestão diária recomendada (IDR) de cobre e manganês, para um homem adulto sadio, são de 0,9 mg e 2,3 mg, respectivamente (INSTITUTE OF MEDICINE, 2006). Os benefícios do cobre na nutrição humana incluem a manutenção da estrutura óssea, do sistema nervoso central e a absorção do ferro (VAITSMAN; DUTRA; AFONSO, 2001). A ingestão em quantidades insuficientes contribuem para o desenvolvimento de doenças cardiovasculares, infecções microbianas e anemias, enquanto seu excesso está relacionado à esclerose, asma, hipertensão, depressão, convulsões, necrose do fígado e problemas cardiorrespiratórios (PEDROZO, 2003; GOLDHABER, 2003). O manganês, por sua vez, atua no controle dos níveis de glicose, na proteção das células contra os radicais livres e nas atividades neuro-hormonais. Além disso, participa da formação e crescimento do feto e, provavelmente, combate o diabetes e a esquizofrenia (PEDROZO, 2003). O excesso deste elemento está relacionado às anormalidades nos pulmões e no cérebro (GOLDHABER,

2003). Diante desse contexto, e considerando que as frutas são fontes naturais em minerais, este trabalho visa avaliar o potencial nutricional de nove frutos nativos da Amazônia brasileira quanto à essencialidade e/ou toxicidade em cobre e manganês nos limites de tolerância fisiológicos.

Material e métodos

Os reagentes utilizados foram de grau de pureza analítica e a água utilizada foi deionizada. Os materiais utilizados foram previamente descontaminados em HNO_3 a 10% (v/v) por no mínimo 24 h. Nove frutos nativos da Amazônia brasileira foram adquiridos em seus ambientes de produção nos Estados de Roraima, Amazonas e Maranhão: abiu (*Pouteria caimito*), açaí (*Euterpe oleracea*), biribá (*Rhollinea orthopetala*), buriti (*Mauritia vinífera*), cupuaçu (*Theobroma grandiflorum*), inajá (*Maximiliana maripa*), monguba (*Pachira aquatica*), pajurá (*Couepia bracteosa*) e uxi (*Saccoglotis uchi*). Os frutos foram devidamente acondicionados, identificados e transportados ao laboratório. As amostras foram então lavadas em água corrente e em água deionizada, despolpadas, maceradas em gral de porcelana, homogeneizadas e refrigeradas a -20 °C. Foram tomadas porções de 0,2-0,5 g das amostras homogeneizadas, e adicionados sequencialmente 5,0 mL de HNO_3 concentrado, 2,0 mL de H_2O_2 a 30% (v/v) e 0,5 mL de ítrio (Y) a 100 mg L^{-1}, o qual foi usado como padrão interno. A mistura obtida foi homogeneizada e submetida a digestão em forno microondas (MARSX press 6.0), de acordo com o método estabelecido pela AOAC (2002). O digerido foi diluído com água deionizada para 25,0 mL e filtrado em papel de filtro quantitativo (28 µm) antes das análises dos teores dos minerais. As determinações dos elementos foram feitas por Espectrometria Ótica com Plasma Indutivamente Acoplado (ICP-OES) (Shimadzu, modelo 9820, com nebulizador concêntrico), sob 1,2 kW de potência, 10 L min^{-1} de argônio e comprimentos de onda de 327,396 nm e 383,826 nm, para o Cu e o Mn, respectivamente. As curvas analíticas foram definidas para 7 valores de concentração, obtidos a partir da diluição de soluções padrão a 1000 mg L^{-1} em 2 % (v/v) de HNO_3. Os critérios para avaliação da eficiência do método analítico foram baseados nas seguintes figuras de mérito: melhores respostas analíticas da razão sinal/ruído; precisão, baseada no desvio padrão relativo (RSD) para três determinações; sensibilidade, com base nos limites de detecção (LOD) e quantificação (LOQ) para dez determinações dos brancos (SKOOG, 2008), e exatidão, mediante ensaios de recuperação em dois níveis de concentrações.

Resultado e discussão

O método analítico mostrou-se preciso, com valores de RSD variando de 2,15 a 9,63 %, e de 2,42 a 8,45 %, para as determinações do Cu e Mn, respectivamente. As faixas lineares instrumentais foram de 0,03125 a 2,0 mg L^{-1} para o Cu e de 0,01875 a 1,2 mg L^{-1} para o Mn. Os valores de LOD e LOQ para o Cu foram de 4,0 10^{-4} e 17,0 10^{-4} mg L^{-1}, e para o Mn, de 7,12 10^{-6} e 1,2 10^{-5} mg L^{-1}, respectivamente. Índices de recuperação médios de 106,25 e 99,35 % foram obtidos para Cu e Mn, respectivamente. As concentrações médias de Cu e Mn (mg/100g de amostra) e seus respectivos RSD, bem como a classificação dos frutos como fonte nutritiva são apresentados na Tabela 1. A avaliação nutricional das amostras estudadas foi feita tendo como base o Relatório Técnico do Ministério da Saúde (BRASIL, 1998), o qual classifica um alimento como fonte em determinado mineral, quando 100 g deste apresentar de 15 a 29 % de sua IDR, e um alimento de alto teor mineral, quando este apresentar mais que 30 % da sua IDR. Avaliando a classificação mineral em relação a ingestão de cobre, observa-se que as polpas do *abiu*, *buriti* e *pajurá* podem ser classificadas como fontes, por atenderem 22,3 %, 17,7 %, 21,3 % e 15,6 % da IDR, respectivamente. As sementes de *monguba* e a polpa de *uxi* mostraram-se com alto teor em cobre, contribuindo com 83,0 e 35,7 % da IDR, respectivamente. Neste sentido, a inserção desses frutos na dieta seria bastante interessante, já que atenderia recomendações diárias e ao mesmo tempo evitaria danos à saúde causados por deficiência em cobre. Em relação ao manganês, a polpa de *uxi* pode ser classificada como fonte natural (29 % da IDR) e as polpas de *açaí* e *buriti* revelaram-se com alto teor nesse micromineral, excedendo 100 % da IDR. Apesar da grande quantidade de manganês fornecida através do consumo de 100 g das polpas de *açaí* e *buriti* estudadas, observa-se que os resultados das concentrações são inferiores ao nível máximo de ingestão tolerável (11 mg/dia). Além disso, deve-se considerar que apenas 3 a 5 % do manganês ingerido é efetivamente absorvido pelo organismo e disponibilizado para as funções metabólicas normais, uma vez que uma grande parcela é excretada pelas fezes via bile (INSTITUTE OF MEDICINE, 2006; MARTINS; LIMA, 2001). Logo, a efetiva inclusão dessas frutas na dieta alimentar traria diversos benefícios, como nos processos de formação dos ossos,

na função reprodutiva e no metabolismo de carboidratos e lipídeos (INSTITUTE OF MEDICINE, 2006). As concentrações obtidas para as polpas de *biribá*, *cupuaçu* e *inajá* podem contribuir para atingir a IDR em cobre e manganês através de complementações minerais de outros alimentos. Ainda que uma comparação entre resultados seja complexa, em virtude das concentrações dependerem de fatores inter-relacionadas como genética, solo, clima, estágio de maturidade da planta e biodisponibilidade (PEDROZO, 2003), comparando os valores obtidos com algumas referências da literatura, pode-se verificar que as concentrações de Cu para o *açaí* e Mn para o *abiu* mostraram certa concordância com os dados apresentados na TACO (2006), enquanto que os teores de Mn para o *açaí*, de Cu para o *abiu*, e de Cu e Mn para o *cupuaçu* e o *buriti* estão acima dos valores listados na TACO (2006) e também reportados por Manhães e Sabaa-Srur (2011). Por outro lado, os teores de Cu e Mn para o *biribá*, *pajurá* e *uxi* estão abaixo dos valores relatados em outros estudos (SMITH et al., 2013; BERTO et al., 2015). Não foram encontradas referências na literatura relacionadas aos teores desses minerais em polpas de *inajá*e sementes de *monguba*.

TABELA 1

FRUTAS	Cu	Mn	CLASSIFICAÇÃO
Abiu (*Pouteria caimito*)	0.20 (6.94)	0.08 (7.14)	Fonte em Cu
Açaí (*Euterpe oleracea*)	0,12 (5,79)	7,89 (3,56)	Alto teor em Mn
Biribá (*Rhollinea orthopetala*)	0.09 (5.33)	0.11 (5.15)	-
Buriti (*Mauritia vinifera*)	0,19 (2,15)	3,18 (4,10)	Fonte em Cu Alto Teor em Mn
Cupuaçu (*Theobroma grandiflorum*)	0.11 (5.65)	0.12 (5.58)	-
Inajá (*Maximiliana maripa*)	0,08 (9,10)	0,17 (7,04)	-
Monguba (*Pachira aquatica*)	0.75 (6.18)	0.20 (8.45)	Alto teor em Cu
Pajurá (*Couepia bracteosa*)	0.14 (9.36)	0.22 (2.42)	Fonte em Cu
Uxi (*Saccoglotis uchi*)	0.32 (7.46)	0.67 (2.80)	Alto teor em Cu Fonte em Mn

Teores de Cu e Mn nos frutos da Amazônia brasileira e sua classificação em relação à essencialidade mineral.

Conclusões

Os resultados aqui apresentados fornecem novas informações sobre microminerais em frutas nativas da região Amazônica, algumas inexploradas, sendo que tais informações serão úteis não só para a construção de uma base de dados de composição mineral desses alimentos, como também na sua popularização em outras regiões do Brasil e do mundo. A avaliação do potencial nutricional dos frutos selecionados permitiu classificá-los como sendo de elevado teor ou mesmo fonte em cobre e/ou manganês, destacando-se os frutos abiu, açaí, buriti, monguba, pajurá e uxi. De um modo geral, a inclusão dessas frutas na dieta alimentar brasileira é recomendada, uma vez que estas apresentaram concentrações de cobre e manganês em quantidades que podem contribuir para correções nutricionais e prevenir enfermidades. O conhecimento desta composição certamente auxiliará os consumidores na escolha dos alimentos, bem como contribuirá para a orientação nutricional por especialistas com princípios de desenvolvimento local e diversificação na alimentação.

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PERFIL PROTEÍCO DE FRUTOS NATIVOS DA AMAZÔNIA BRASILEIRA

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Área

Alimentos

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Resumo

As proteínas são nutrientes vitais ao organismo humano e, apesar das frutas não serem suas fontes reconhecidas, preocupações com a saúde humana têm incentivado a ingestão de proteínas de origem vegetal. Diante disso, determinou-se o teor de proteínas em 10 frutos nativos da Amazônia brasileira. Os resultados mostraram que as sementes de monguba possuem o maior teor proteico (2,44 %), seguido dos frutos uxi, buriti, biribá, pajurá, açaí, inajá, bacuri e abiu. De modo geral, as frutas estudadas possuem teores proteicos adequados, que possibilitam o seu uso na dieta humana e também na indústria alimentícia.

Palavras chaves

proteínas; frutas amazônicas; nutrientes

Introdução

As proteínas compreendem o grupo mais abundante de macromoléculas, encontradas dentro e fora das células e de importância vital aos seres vivos. Suas funções envolvem a catálise de reações químicas (enzimas), transporte de moléculas, transmissão de impulsos nervosos, proteção imunitária, entre outras (INSTITUTE OF MEDICINE, 2006). A ingestão diária recomendada (IDR) de proteínas para um adulto saudável é de 50 g (BRASIL, 2005). As principais fontes em proteína incluem carnes, cereais, ovos, leite e derivados, mas atualmente estes alimentos de origem animal ocupam destaque nas preocupações com a saúde humana devido aos altos níveis de gordura e a elevação do risco de doenças como o câncer (VILARTA *et al*., 2007; MCKEITH, 2016). Embora não sejam reconhecidas como fontes proteicas, muitas frutas são ricas em proteínas e fornecem ao organismo as chamadas proteínas completas, as quais são fontes em aminoácidos essenciais ao corpo (ACHKAR, 2013). Dessa forma a proteína de origem vegetal é uma alternativa da proteína animal para aplicações em alimentos, tendo em vista melhorar o valor nutricional de produtos alimentícios, suprir a ausência de alimentos à base de carne e aumentar a disponibilidade de proteínas (MOURE *et al*., 2006; PIRES *et al*., 2006). Diante desse contexto, e, considerando a importância do conhecimento da composição química, da valorização de alimentos locais, do estímulo a adequação da dieta alimentar baseada na diversificação e no desenvolvimento da economia local é que este trabalho visa determinar o conteúdo de proteína total em frutas nativas da Amazônia Brasileira.

Material e métodos

Dez frutos nativos da Amazônia brasileira foram coletados em seus ambientes de produção nos Estados de Roraima, Amazonas e Maranhão. As amostras foram lavadas em água corrente, seguida de água deionizada, despolpadas, homogeneizadas e o teor de proteínas determinado mediante a análise de nitrogênio pelo método

Kjeldahl da AOAC (CUNNIFF, 1998), o qual compreende três etapas: digestão, destilação e titulação. Na etapa da digestão o nitrogênio existente na amostra foi convertido em sal amoniacal. Para isto, 1 g das amostras foram tomados em tubos de digestão, adicionados 25 mL de H_2SO_4 a 0,05 mol L^{-1}, 6 g de mistura catalítica (TiO_2, $CuSO_4$ e K_2SO_4; 0,3:0,3:6) e a mistura submetida a digestão em bloco digestor. Em seguida, a amônia foi liberada do sal amoniacal por meio da destilação realizada com a adição, em ligeiro excesso, de NaOH a 30 % (m/v), sendo o destilado recebido em erlenmeyer previamente preenchido com indicador e uma solução ácida de volume e concentração conhecidos (25 mL de H_2SO_4 a 0,05 mol L^{-1}). Por fim, a quantidade de nitrogênio presente na amostra foi determinada através da titulação do excesso do H_2SO_4 a 0,05 mol L^{-1} com solução de NaOH a 0,1 mol L^{-1}, usando vermelho de metila como indicador. Considerando que o conteúdo de nitrogênio em proteínas presentes em alimentos vegetais é de aproximadamente 16%, empregou-se o fator 6,25 para converter a quantidade de nitrogênio em proteínas (Equação 1). % Proteínas(m/m)=(V.0,14.f)/P Equação (1) Em que: V é a diferença entre o volume em mL de ácido sulfúrico a 0,05 M e o de NaOH a 0,1 M gastos na titulação; P é a massa da amostra em g e f é o fator de conversão utilizado (6,25).

Resultado e discussão

As concentrações médias de proteínas (g/100g de amostra) obtidas para as amostras estudadas e seus respectivos desvios padrões, bem como os resultados obtidos por outros autores são apresentados na Tabela 1. As amêndoas do fruto monguba apresentaram os maiores teores proteicos (média de 2,44 %), representando 4,88 % da ingestão diária recomendada (IDR) (BRASIL, 2005). A polpa do fruto uxi apresentou o segundo maior teor em proteínas (2,17 %), seguida das polpas de buriti, biribá, pajurá, açaí, inajá, cupuaçu, bacuri e abiu. Aguiar (1995) analisou diversas amostras de frutas, hortaliças, carnes animais e outros alimentos provenientes de diferentes cidades do estado do Amazonas, a fim de elaborar uma Tabela de Composição Centesimal de Alimentos da Amazônia. Esta foi utilizada como comparativo para as amostras deste estudo. Da mesma maneira, neste trabalho destaca-se a publicação “Alimentos Regionais Brasileiros” (Brasil, 2015), que veio divulgar a imensa variedade de frutas brasileiras e apresentar suas composições químicas através de compilações de dados obtidos de trabalhos científicos diversos. Apesar de uma comparação dos valores obtidos com referências da literatura ser complexo, em virtude das concentrações dependerem de fatores inter- relacionadas como genética, solo, clima, estágio de maturidade da planta e biodisponibilidade (PEDROZO, 2003), observou-se que as concentrações obtidas para os frutos abiu, cupuaçu, inajá e para a semente de monguba tiveram valores inferiores a literatura (LOVE; PAULL, 2011; SILVA, 2014; MOTA; FRANÇA, 2007; AZEVEDO, 2008), enquanto que para as polpas dos frutos açaí, bacuri, biribá, buriti, pajurá e uxi houve certa concordância com os valores reportados por outros estudos (YUYAMA, *et al*., 2011; BERTO *et al*., 2015; MORTON, 1987; MANHÃES; SABAA-SRUR, 2011), conforme apresenta a Tabela 1. A inclusão de proteínas vegetais na dieta humana traz diversos benefícios a saúde em relação às animais, uma vez que são isentas em gorduras saturadas e aditivos químicos, auxiliam o transito intestinal, possuem uma elevada densidade nutritiva, um menor impacto ambiental em sua produção, entre outros (MOURE *et al*., 2006). Avaliando a ingestão de 200 g de polpa de buriti, uxi ou de sementes de monguba, observa-se que aproximadamente 10 % da IDR em proteínas para um adulto saudável são atendidos (50 g/dia). Considerando que a deficiência em proteínas afeta todos os órgãos do corpo e boa parte de seus sistemas, incluindo a função do cérebro e o sistema imunonlógico, recomenda-se a complementação da dieta alimentar com frutas por contribuir com quantidades de proteínas e outros nutrientes essenciais como vitaminas e minerais necessários para prevenir deficiências que possam impedir o bom funcionamento do organismo (INSTITUTE OF MEDICINE, 2006). Diante disso, recomenda-se a inserção das frutas estudadas na dieta alimentar por contribuírem com teores de proteínas que complementarão uma alimentação diversificada para um consumo equilibrado desse nutriente.

Tabela 1

FRUTAS	Teores de Proteína (g/100g)			
	Este estudo	Aguiar (1995)	Brasil (2015)	OUTROS ESTUDOS
Abiu	0,17 ± 0,00	-	0,8	0,8-2,1 (LOVE; PAULL, 2011)
Açaí	0,72 ± 0,05	3,60	0,8	0,59-1,03 (YUYAMA, *et al.*, 2011)
Bacuri	0,34 ± 0,00	-	1,9	0,29 (BERTO *et al.*, 2015)
Biribá	1,04 ± 0,10	-	0,6	1,90 (MORTON, 1987)
Buriti	2,14 ± 0,12	1,80	1,8	2,10 (MANHÃES; SABAA-SRUR, 2011)
Cupuaçu	0,40 ± 0,01	1,25	1,0	0,70-0,88 (SILVA, 2014)
Inajá	0,49 ± 0,01	-	-	3,14-4,78 (MOTA; FRANÇA, 2007)
Monguba	2,44 ± 0,13	-	-	14,01-14,85 (AZEVEDO, 2008)
Pajurá	0,93 ± 0,00	1,46	-	0,28 (BERTO *et al.*, 2015)
Uxi	2,17 ± 0,11	2,20	2,20	0,19 (BERTO *et al.*, 2015)

Comparação entre as faixas de concentrações de proteínas obtidas neste estudo e alguns teores encontrados na literatura.

Conclusões

A quantificação do teor de proteínas por meio do presente trabalho possibilitou divulgar uma variedade de frutas da Amazônia brasileira, muitas desapreciadas na alimentação, bem como valorizar a incorporação desses alimentos nas práticas alimentares, de forma a melhorar do padrão nutricional dos habitantes da região e até mesmo de outras regiões brasileiras. Os resultados apresentam dados úteis para a formação de uma estimativa de faixa de teor proteico nas frutas estudadas, considerando uma reunião de registros da literatura. O conhecimento desses valores certamente auxiliará os consumidores na escolha dos alimentos, bem como contribuirá para a orientação nutricional por especialistas com princípios de desenvolvimento local e diversificação na alimentação.

Agradecimentos

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DETECÇÃO DE MICROCISTINAS EM AMOSTRA DE ÁGUA NATURAL E POTÁVEL

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Área

Química Analítica

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Resumo

Este estudo objetivou desenvolver um biossensor amperométrico à base da enzima acetilcolinesterase ACHE (EE), para detecção indireta de microcistina-LR (MC-LR), baseada na ativação daquela pela presença desta. Foram testadas AChEs de varias fontes, livres e imobilizadas em sensores contendo como mediador eletroquímico tetracianoquinodimetano, TCNQ. Os testes de caracterização eletroquímica evidenciaram ser os biossensores precisos (CV médio intramedidas de 8,6 %) e sensíveis (LD e LQ de 0,27 e 0,91 µg L-1, respectivamente). O aumento da corrente após incubação do biossensor com a MC-LR, tanto para enzimas livres quanto para imobilizadas, mostraram que este poluente aquático é também um potente ativador da AChE, sendo possível sua detecção em níveis traços com o biossensor aqui descrito.

Palavras chaves

Microcistina-LR; Biossensores; Amperométricos

Introdução

As cianobactérias, também conhecidas como algas azuis, são bactérias Gram- negativas procariontes fotossintéticos que são encontradas em uma variedade de habitats, colonizando biótopos aquáticos e terrestres (MANKIEWICZ et al., 2003; BRIAND et al., 2003). Seu domínio sobre as demais espécies do ecossistema é uma indicação de que elas possuem algumas capacidades fisiológicas específicas que lhes permitem competir de forma muito eficiente (JAYARAJ, ANAND e RAO, 2006). Segundo MARTINS (2010), ambientes eutróficos, ou seja, rico em nutrientes, favorecem a proliferação e predominância de espécies de cianobactérias, que, por sua vez, podem produzir diversas toxinas e liberá-las no meio aquático, principalmente durante os fenômenos de florações (crescimento exuberante). Geralmente, estas florações têm grande impacto negativo nos corpos d'água alterando as características de qualidade, tais como odor e sabor, e de presença de toxinas específicas (BRASIL, 2000). A microcistina-LR é uma molécula, composta por sete aminoácidos, com peso molecular entre 0,9 a 1,0 kDaltons, classificadas como hepatotoxinas. Alguns pesquisadores da área têm mostrado que a microcistina-LR atua inibindo enzimas intracelulares denominadas fosfatases, que removem os grupamentos de fosfato das proteínas. Isso promove uma alteração na estrutura do esqueleto celular causando disfunção, ou seja, modifica a arquitetura e consequentemente a função das células do fígado. Os estudos chegam a admitir que a ação de doses sub- letais das hepatotoxinas provenientes de cianobactéria estaria associada ao desenvolvimento de câncer hepático. Esta suposição tem sido investigada na China onde existe cianobactéria nos mananciais de água fornecida a uma população que apresenta elevada frequência de dessa neoplasia (AZEVEDO, 2014). Vários métodos de detecção têm sido utilizados atualmente na detecção/quantificação de microcistinas, tais como a cromatografia líquida de alta eficiência acoplada à espectrometria de massas (HPLC/MS), os bioensaios envolvendo animais de

laboratórios, os testes de inibição enzimática (via colorimetria) e os testes imunológicos baseados em ELISA (do inglês, Enzyme Linked Immunosorbent Assay) (ETCHEGARAY E BUENO, 2010; LONG et al., 2009). Apesar de várias técnicas de análise para detecção de microcistinas tais como bioensaio, ELISA, HPLC e LC-MS já serem utilizadas, o desenvolvimento de biossensores abriu novas perspectivas, pois oferece uma detecção rápida e precisa, além de alta reprodutibilidade e sensibilidade (LEBOGANG, 2014; SINGH et al. ,2012). Destaca-se ainda a possibilidade desses aparatos virem a ser portáteis, além da miniaturização e rapidez de resposta (LEITE et al., 2013). O uso dos biossensores, na maioria dos casos, não necessita de técnicos ou especialistas, podendo, para alguns protótipos, dispensar o uso de reagentes. Podem ser confeccionados para uso contínuo, em linhas no processo, ou como descartáveis. Portanto, representam uma ferramenta promissora para suplementar as técnicas analíticas existentes (SILVA, 2011). Geralmente, um biossensor permite o uso de métodos “limpos” e de baixo custo, sem precisar de pré-tratamentos morosos e de grandes volumes de amostra. O seu uso, na maioria dos casos, não necessita de técnicos ou especialistas podendo, em alguns casos dispensar o uso de reagentes, e aparecendo tanto como aparelhos de uso contínuo ou como descartáveis (SALGADO; JACOBY; PAULA, 2015). Nas últimas décadas, estão sendo efetuados esforços pela comunidade científica objetivando o desenvolvimento de metodologias mais seguras, que melhorem a seletividade e a sensibilidade dos biossensores enzimáticos baseados no monitoramento do processo de inibição por analitos potencialmente tóxicos (MARQUES & YAMANAKA, 2008). Estudos recentes demonstraram que as microcistinas-LR podem aumentar a atividade das enzimas AChEs. O presente trabalho objetivou pesquisar possíveis interações de diferentes enzimas com a MC-LR, com foco no desenvolvimento de um biossensor para detectar esse contaminante a um preço mais acessível, sendo ainda o protótipo sensível, preciso e reprodutível.

Material e métodos

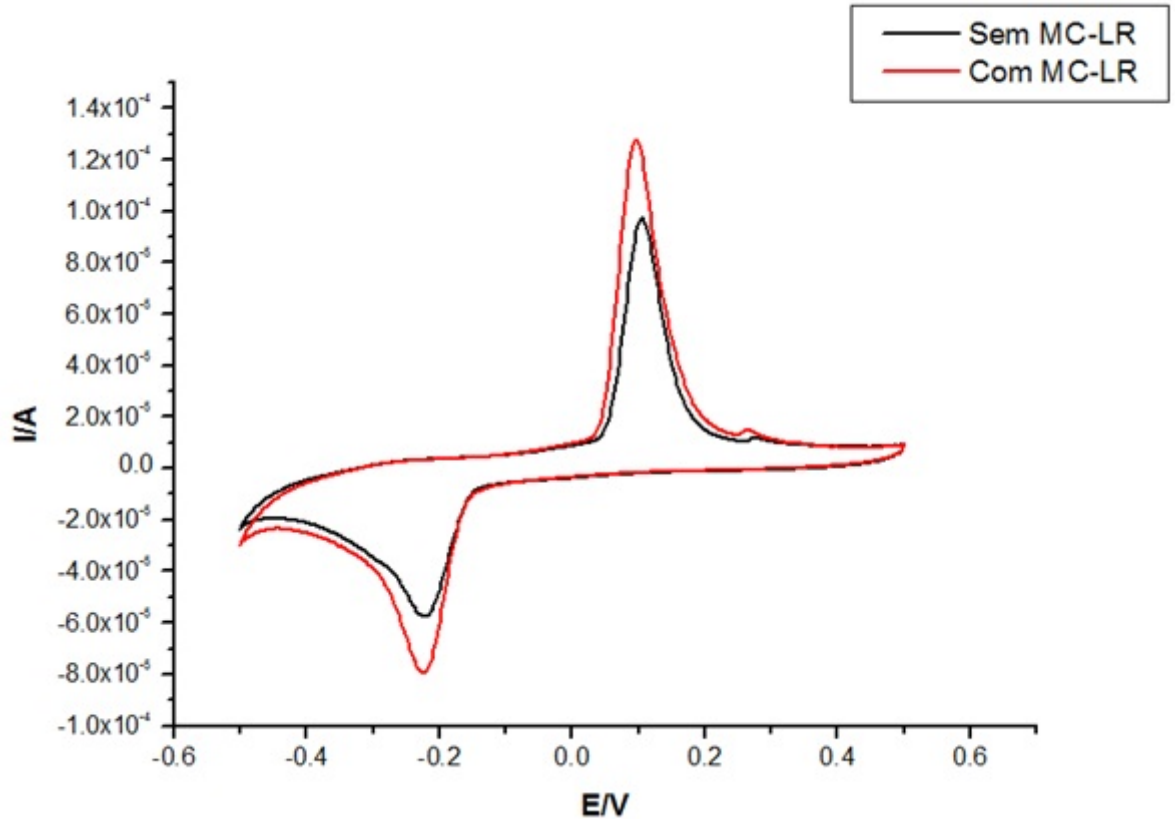
Foram preparadas pastas de grafite em pó contendo hidroxicetilcelulose, HEC, soroalbumina bovina, BSA, e glutaraldeído 5%, e incorporadas, a estas pastas, AChEs extraídas de: eritrócito bovino (EB), enguia elétrica (EE) e Drosophila melanogaster (enzimas geneticamente modificadas). A outra pasta foi incorporada a enzima butirilcolinesterase (BChE) obtida de soro humano. Uma porção de 2 mg de cada pasta sensivel foi depositada no eletrodo de trabalho de sensores serigrafados. Testes envolvendo voltametria de pulso diferencial (VPD), voltametria cíclica (VC) e leituras cronoamperométricas (CA) foram realizados e construídas curvas da ativação relativa percentual (AR %) em função da concentração da MC-LR. Algumas condições operacionais, tais como potencial de trabalho, mediador eletroquímico (foram testados tetracianoquinodimetano, TCNQ, e fitalocianina de cobalto, CoPC), pH do meio e concentração do substrato, foram otimizadas. O biossensor foi levado à refrigeração a 4°C durante 24h. Aferição do sinal da corrente: Foi inserido o biossensor em conector apropriado, que tornou possível o contato entre o potenciotasto (detector) e este. Inicialmente foi registrado o sinal obtido por meio da medida cronoamperométrica do sensor imerso em solução de substrato. Logo após, o biossensor foi lavado e seco e colocado em incubação em uma solução contendo a MC-LR, após a incubação, o eletrodo foi novamente lavado e seco, medindo-se, então, o sinal da atividade enzimática com o substrato. A percentagem de ativação foi determinada pelo aumento da corrente, sendo esta proporcional à concentração da microcistina conforme a equação: AR (%)= [I- Io /I)] x 100, onde AR =ativação relativa; Io = intensidade de corrente antes da ativação enzimática; I = intensidade de corrente após ativação enzimática

Resultado e discussão

Testes de VPD e VC revelaram que utilizando o mediador TCNQ, em pH 7,2, foi possível realizar as medidas de corrente a um potencial de trabalho relativamente baixo (100 mV) versus Ag/AgCl. A presença da MC-LR claramente ativou a enzima (Fig. 1), sendo que a melhor ativação foi obtida para a AChE- EE, estando esta imobilizada (Tab. 1). Os resultados de estabilidade do biossensor, coeficiente de variação CV= 8,32%, n=10, para ensaios com o mesmo sensor e CV= 9,33%, n=10 para ensaios com sensores diferentes, sendo valores bem precisos para esse tipo de protótipo, indicando que o biossensor é preciso e sensível. O biossensor desenvolvido mostrou-se sensível, apresentando limites de detecção e quantificação 0,27 µg L-1 e 0,91µg L-1 (Tab. 1). O Ministério da Saúde editou a portaria nº 518, de 25 de março de 2004 a qual estabeleceu o valor máximo de

microcistinas permitido na água de abastecimento público em 1µg. L-1, o limite de detecção encontrado é excelente para detecção de MC-LR uma vez que é menor do que valor máximo permitido pela legislação brasileira.

FIGURA 1



Voltamograma cíclico para AChE (EE) antes e após incubação com a MC-LR (mediador TCNQ).

TABELA 1

Método	Curva	R^2	Faixa de trabalho	LD	LQ
Enzima imobilizada	y= 9,77x + 5,55	0,9995	0,5 a 100 μgL^{-1}	0,5 μgL^{-1}	1.66 μgL^{-1}

Sensibilidade do Biossensor desenvolvido

Conclusões

De modo geral, o biossensor construído demonstra ser uma opção inovadora, já que opera por ativação enzimática na detecção de MC-LR. Além de ser de fácil construção e operação e de baixo custo, o biossensor é ainda preciso e sensível, sendo portanto adequado para monitoramentos ambientais da MC-LR em ambientes aquáticos suportamente contaminados. Esse trabalho representa, pois, uma grande contribuição para áreas ambiental e da saúde pública.

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Apoio

EXPERIMENTAÇÃO PROBLEMATIZADORA NO ENSINO DE QUÍMICA: UM LEVANTAMENTO ACERCA DOS ARTIGOS PUBLICADOS NA REVISTA QUÍMICA NOVA NA ESCOLA

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Área

Ensino de Química

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Resumo

Esta pesquisa objetivou analisar a experimentação problematizadora no ensino de Química, por meio de um levantamento acerca dos artigos publicados na Revista Química Nova na Escola. Assim, foi realizada análise dos trabalhos relacionados com à ação problematizadora e aqueles que tratam sobre o ensino de Química utilizando materiais de baixo custo para a realização de experimentos. Os método teve uma abordagem quantitativa e descritiva, por meio de consulta e análise dos arquivo da revista disponibilizado em sua página online. Esta pesquisa contribui para a construção de novas hipóteses sobre a ação problematizadora no ensino de Química, pois a partir desse estudo outras questões problemas podem ser levantadas, possibilitando uma maior exploração científica sobre a temática.

Palavras chaves

Ação problematizadora; Ensino de Química; Experimentação

Introdução

A sociedade atual tem defendido o processo de ensino-aprendizagem que visa a formação de cidadãos críticos, e nessa perspectiva o professor torna-se um mediador na construção do conhecimento. Com isso, é imprescindível que o currículo escolar seja voltado a projetos pedagógicos que torne os estudantes participativos e integrados na construção do conhecimento. Dessa forma, tendo em vista tais necessidades, é preciso propiciar aos estudantes a oportunidade para que façam uma nova leitura do conteúdo de Química e essa reconstrução do ensino pode ser enriquecida por meio da integração do cotidiano dos estudantes ao currículo escolar. Partindo desse entendimento, pesquisas no ensino de Química relacionadas com a utilização da ação problematizadora por meio dos pesquisadores que se dedicam a esta temática ao buscar iniciativas e ações para superar a forma de apresentação dos conteúdos químicos pautados na memorização de fórmulas e repasse sistemático dos conteúdos existentes na literatura convencional nas disciplinas de química, são de suma importância para o processo de ensino e aprendizagem. Deste modo, uma ação problematizadora do ensino de Química, baseada no levantamento de uma pergunta problematizadora, seguida do debate em torno da problemática levantada e a relação do conteúdo repassado pelo professor com o cotidiano dos estudantes. Essa interação professor-estudante deve ter uma perspectiva crítica, com o objetivo de permitir que o conhecimento seja entendido como

uma construção humana, que leve em consideração a relatividade, proporcionando com isso, a assimilação de uma nova ideia a partir do levantamento da questão problematizadora do conteúdo de Química. A partir dessa compreensão, o estudo objetivou analisar a experimentação problematizadora no ensino de Química, por meio de um levantamento dos artigos publicados na Revista Química Nova na Escola. Assim, foi realizado um levantamento acerca dos artigos relacionados com a ação problematizadora no ensino de Química, que foram publicados no período de 1995 a 2015, disponibilizados em formato PDF no portal da revista. Infere-se assim, a relevância desta pesquisa aos pois contribui para o aprofundamento do conhecimento acerca da ação problematizadora no ensino de Química, que permite, deste modo, adotar novas formas de abordagem para esta temática.

Material e métodos

Quanto à abordagem, esta pesquisa se enquadra em uma análise quantitativa, visto que o objetivo geral desse estudo é fazer um levantamento acerca dos artigos publicados no período de 1995 a 2015 na revista online Química Nova na Escola. Nesse sentido, a pesquisa quantitativa utiliza a coleta e análise de dados para responder às questões da pesquisa e testar hipóteses estabelecidas previamente, confiando na medição numérica, na contagem e, frequentemente, no uso de estatísticas para estabelecer com precisão a questão norteadora do estudo (SAMPIERI, 2006). Dessa forma, a pesquisa quantitativa se centra na objetividade influenciada pelo positivismo, considera que a realidade só pode ser compreendida com base na análise de dados ainda não quantificada, recolhidos com o auxílio de instrumentos padronizados e neutros (GERHARDT e SILVEIRA, 2009, p.34). Para alcançar o objetivo geral do estudo foi utilizada a pesquisa do tipo descritiva, pois este tipo de pesquisa tem como objetivo descrever os fatos e fenômenos de determinada realidade. Assim, “a pesquisa descritiva pretende medir ou coletar informações de maneira independente ou conjunta sobre os conceitos ou as variáveis a que se referem” (SAMPIERI, 2006). A pesquisa classifica-se como bibliográfica, pois a utilização dessa forma de pesquisa deve-se ao fato da necessidade de um embasamento teórico que é feito a partir do levantamento de referências teóricas já analisadas. Nesse sentido, a pesquisa bibliográfica é elaborada a partir de material já publicado, “constituído principalmente de livros, revistas, publicações em periódicos, artigos científicos, jornais, entre outros, com o objetivo de colocar o pesquisador em contato direto com todo material já escrito sobre o assunto da pesquisa” (GERHARDT e SILVEIRA, 2009, p.37). Logo, para melhor compreender o levantamento teórico feito, este foi estruturado com tópicos que discorreram sobre o Ensino de Química e Experimentação, Contextualização do Ensino de Química com o Cotidiano e a Ação Problematizadora no Ensino de Química. Portanto, “qualquer trabalho científico inicia-se com uma pesquisa bibliográfica, que permite ao pesquisador conhecer o que já se estudou sobre o assunto” (PRODANOV e FREITAS, 2013, p.56). Assim, é importante que o pesquisador verifique a veracidade dos dados obtidos, observando as possíveis incoerências ou contradições que as obras possam apresentar. Assim sendo, o estudo apresentado é de cunho bibliográfico, dada a necessidade de aprofundar o conhecimento a respeito do ensino de Química e fazer um levantamento acerca dos artigos que trabalham com a ação problematizadora, publicados no site da revista: qnesc.sbq.org.br, com intuito de contribuir de forma científica para a importância dos artigos publicados na revista para o meio acadêmico, professores e sociedade.

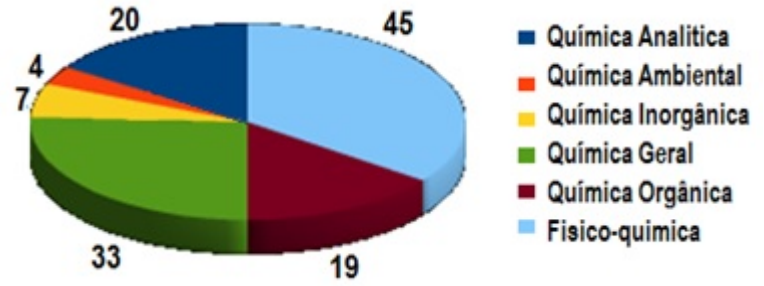
Resultado e discussão

Para este trabalho, foram selecionados 128 artigos científicos publicados entre os anos de 1995 a 2015 que passaram a ser objeto de análise. O critério de seleção foi a presença da descrição de experimentos no ensino de Ciências, em especial nas aulas de química, visando principalmente identificar quais deles abordam a ação problematizadora no seu desenvolvimento. Após a seleção, foi realizada uma leitura criteriosa de todos os artigos. Em seguida, eles foram divididos de duas formas: experimentos químicos desenvolvidos por meio da abordagem problematizadora e experimentos relatados sem essa ação. Ao fazer a leitura dos artigos na sua totalidade, verificou-se que apenas 34 retratam atividades experimentais desenvolvidas por meio da vertente problematizadora, o que corresponde ao percentual de 26,6%, e foram pautadas considerando o conhecimento prévio dos estudantes, e no ensino contextualizado, onde o objeto de investigação surgiu a partir das problemáticas presentes no dia a dia dos alunos. A partir da análise dos artigos, percebeu-se que a pedagogia

problematizadora baseada na metodologia proposta por Ferreira et al,(2008), adaptada a partir de Delizoicov, Angotti e Pernambuco (2002), denominada os Três Momentos Pedagógicos: Problematização Inicial, Organização e Aplicação do Conhecimento, estão presentes em grande parte das atividades experimentais dos 34 artigos selecionados. O autor Junior (2008) em seu artigo "Uma Abordagem Problematizadora para o Ensino de Interações Intermoleculares e Conceitos Afins", relata uma investigação baseada nestes três momentos, investigação dividida em levantamento das ideias prévias dos estudantes sobre o tema, problematização dessas ideias, apresentação e problematização de experimentos envolvendo interações moleculares, e enfim a avaliação, a qual foi realizada por meio de um questionário aberto contendo duas situações-problemas. A análise do questionário empregado foi positiva, tendo em vista que mais de 50% dos estudantes responderam corretamente às questões, o que indica que houve uma aprendizagem crítica, ou seja, os estudantes foram capazes de observar e registrar criticamente as modificações de um sistema, refletir, argumentar, apresentar explicações baseadas em suas anotações, bem como relatar isso tudo por escrito. Dessa forma, a ação problematizadora "naturalmente transforma o ato participativo em conhecimento dinâmico e importante para o estudante, que é reconhecido como sujeito pela sua capacidade de transformar e aprender junto, assim como o professor" (LEITE e SOARES, 2015, p.02). No decorrer da pesquisa, observou-se que a partir do ano de 2008, com uma maior possibilidade de publicação da revista, o crescimento de artigos científicos que indicam essa tendência é bem maior em relação aos anos anteriores. De outra parte, cumpre notar que foram identificados na análise 94 artigos, ou seja, 73,4%, que descreveram experimentos químicos que foram aplicados de forma tradicional, isto é, a metodologia utilizada nas aulas práticas consistiu-se na realização de procedimentos descritos em um roteiro elaborado, com explicações pré-formuladas, cabendo ao estudante a simples execução mecânica da experiência ou simples observação, o que o torna agente passivo no processo, sem uma participação ativa, principalmente nas discussões dos resultados. Por consequência, essa atividade perderá grande parte das potencialidades que a experimentação investigativa pode desenvolver. Em todos os artigos estudados foram identificados temas relacionados às seguintes áreas da Química: Química Analítica, Química Ambiental, Química Inorgânica, Química Geral, Química Orgânica e Físico-química. A partir dos dados apresentados no gráfico 1, verificou-se um maior número de artigos dirigidos à Físico-química (45), sendo que os assuntos mais empregados em dezesseis artigos correspondem ao ensino da Eletroquímica e Oxirredução. Acredita-se que a quantidade significativa de artigos publicados nesta área da química pode ser relacionada ao receio por partes de alguns educadores desta disciplina, que se utilizam de estratégias, como a atividade experimental para diminuir as dificuldades que os estudantes possuem em compreender a disciplina. Trabalhos relacionados à Química Geral (33), Química Analítica (20) e Química Orgânica (19) foram também encontrados em números significativos. Nos artigos correlatos à Química Geral observou-se que vários assuntos foram abordados, mas o tema com maior identificação foi Combustão. Esse conteúdo está presente em cinco trabalhos. O artigo publicado na primeira edição da revista "Repensando a Química", MALDANER et al (1995), é um exemplo de trabalho que aborda esta temática por meio do experimento "Combustão como transformação química", que centralizou-se no desenvolvimento do pensamento químico dos alunos da escola onde o experimento foi realizado. A Química Analítica, disciplina que analisa matérias e ajuda a compreender a sua composição, estrutura e quantidade, surge com 20 artigos. Acredita-se que a característica de estar presente nas atividades humanas, contribui grandemente para que a disciplina seja explorada nas aulas de química por meio da contextualização, conforme citam os autores Filho et al (2011) no artigo "Identificação de Ácido Salicílico em Produtos Dermatológicos". Logo em seguida vem a Química Orgânica, disciplina esta que estuda os compostos do carbono, com 19 artigos publicados. Por outro lado, os trabalhos que abordaram tópicos da Química Inorgânica (7) e Química Ambiental (4) foram os que menos tiveram publicações nestes vinte anos da revista. Os assuntos encontrados com maior frequência nas aulas de Química Inorgânica foram os Ácidos e Bases presentes nos artigos: Conceitos de Ácido e Base, FERREIRA (1996); Padronização de soluções ácida e básica, SUAREZ et al (2007); pH do Solo: Determinação com indicadores ácido-base no Ensino Médio, ANTUNES et al (2009). Analisando o gráfico 2, é possível identificar que a aprendizagem baseada em problemas está presente em grande parte na disciplina de Química Geral (17), correspondendo a mais de 50% dos trabalhos, seguida da Físico-química (10). Estes dados positivos que a Química Geral apresenta se deve ao fato de esta área possuir conteúdos mais acessíveis para a geração de questões problemas, característica esta primordial presente na teoria da aprendizagem por meio da problematização. Como exemplo, podemos citar o trabalho A Estratégia "Laboratório Aberto" (SUART et al, 2010), onde é proposto aos alunos uma atividade investigativa com o objetivo de desenvolver o conceito de temperatura de ebulição por meio da resolução de problemas. A partir desta

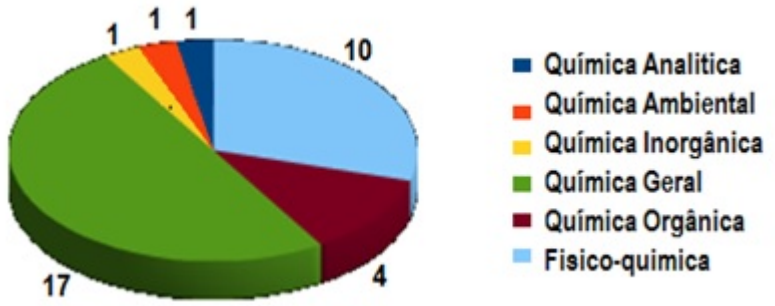
metodologia utilizada pelo professor, os estudantes puderam elaborar hipóteses, coletar e analisar dados, emitir conclusões que foram discutidas posteriormente com a turma. A ausência de laboratórios equipados e de espaços apropriados são considerados por alguns professores uma limitação para a realização de aulas experimentais em grande parte das unidades educacionais do Brasil, além disso, o custo financeiro significativo para a aquisição de materiais e reagentes contribuem para que isso aconteça. Mas, o uso de materiais usuais pode diminuir relativamente tais custos, ampliando a possibilidade de aplicação de aulas práticas nas escolas. É importante salientar que, para propor uma atividade contextualizada com a realidade dos alunos, o professor deve considerar a importância de colocá- los à frente de situações-problemas, o que propicia a construção do próprio conhecimento. Desta forma, os estudantes são problematizados e motivados a explorar e desenvolverem estudos sobre os temas presentes no dia a dia, exercitam ideias abrangentes, produzindo pensamentos coerentes com a forma química de entender e relacionar com a realidade.

Gráfico 1



Quantidade de trabalhos relacionados a cada área de ensino de Química.

Gráfico 2



Artigos por área de estudo da Química publicados na revista Química Nova na Escola que abordam a problematização

Conclusões

Diante dos resultados, nota-se que apenas 34 artigos publicados nestes vinte anos de existência da Revista Química Nova na Escola, evidenciam a prática problematizadora com o levantamento prévio das ideias dos alunos sobre o tema, realização de experimentos e discussão problematizadora. Logo, foi possível verificar que essa sequência de atividades contribuiu positivamente para os resultados do processo de ensino-aprendizagem, promovendo a participação ativa e apropriação do conhecimento científico pelos alunos de forma crítica e reflexiva. A partir dos estudos correlatos, cumpre notar que os conteúdos pertinentes à Química Geral predominam nos artigos da Revista Química Nova na Escola que apresentam a metodologia da problematização. Acredita-se que isso se deve ao fato de a mesma possuir assuntos mais acessíveis para a criação de problemas reais que permitam a contextualização. Desta forma, para que os estudantes se tornem participantes do processo de ensino-aprendizagem dos conteúdos da disciplina de Química a partir da utilização da metodologia problematizadora, é necessário que os professores incluam no seu planejamento pedagógico atividades como: discussões estruturadas que instiguem o levantamento da pergunta problematizadora, debates, estudo de caso e etc. Em suma, é importante destacar a relevância da revista científica Química Nova na Escola, pois desde a sua criação, as pesquisas voltadas ao ensino de Química se ampliaram, houve uma propagação dos conteúdos

relacionados à ação problematizadora e com isso, os artigos publicados na revista têm contribuído na prática pedagógica dos professores de Química. Portanto, a inserção da ação problematizadora no ensino de Química, significa munir-se de ferramentas que contribuem para colocar em prática o conhecimento construído, implicando a problematização da realidade e a reconstrução do processo de ensino-aprendizagem dos conteúdos curriculares do ensino de Química.

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IMPACTO DAS ATIVIDADES HUMANAS EM RELAÇÃO À PRESENÇA DE METAIS PESADOS EM AMBIENTES AQUÁTICOS - PROPOSTA DE ESTUDO COM O RIO PACIÊNCIA EM SÃO LUÍS - MA

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Resumo

A contaminação por metais pesados desperta muita atenção pois essas substâncias têm alta persistência, toxicidade e bioacumulação no ambiente e organismos. A presente revisão objetivou realizar um levantamento dos estudos já publicados no Brasil, evidenciando a presença de metais pesados em ambientes aquáticos. Além disso, foi proposto um estudo no Rio Paciência, importante ecossistema aquático maranhense que vem sendo degradado com o crescente desenvolvimento da região urbana de São Luís. A análise da literatura científica foi feita em revistas, dissertações e periodicos cientificos. Os resultado reforçaram as premissas iniciais, em relação à toxicidade e mobilidade desses poluentes nos compartimentos ambientais, bem como a necessidade de se realizar o referido estudo no rio Paciência.

Palavras chaves

metais pesados; ambientes aquáticos; rio paciência

Introdução

Um dos principais problemas que o Brasil enfrenta, no tocante a preservação e ao manejo dos recursos hídricos continentais e costeiros, diz respeito a contaminação por efluentes domésticos. (ROCHA; ROSA; CARDOSO, 2009). O grande aumento na quantidade de substâncias que estão sendo constantemente liberadas no ambiente, levou ao esgotamento da capacidade dos sistemas de transformar ou eliminar o excesso, ocasionando em alteração do equilíbrio ambiental (SISINNO; OLIVEIRA-FILHO, 2013). Uma das classes de substâncias que mais impactam o meio ambiente é a dos metais. Estes tem recebido atenção especial nas últimas décadas por serem conservativos e não biodegradáveis (MASUTTI, 2004). Os metais pesados compreendem os elementos que possuem densidade superior a 5 g/cm³ e muitos deles são essenciais para o organismo humano, como manganês, cobalto, cobre, molibdênio, vanádio, estrôncio e zinco, ao passo que outros como chumbo, cádmio e mercúrio não possuem função nenhuma no organismo e podem causar doenças graves (NUNES et. al., 2009). Muitas são as formas pelas quais esses elementos podem afetar negativamente os sistemas biológicos sendo algumas delas a inibição de enzimas biologicamente críticas, danos oxidativos e mimetismo (ligação a locais fisiológicos normalmente ocupados por metais essenciais) (KLAASEN; WATKINS III, 2012). O Rio Paciência, importante curso d'água presente em São Luís no estado do Maranhão, Brasil, constitui-se em um dos principais mananciais da Ilha, tendo importante contribuição no sistema de abastecimento da capital. Atualmente, recebe cargas acentuadas de efluentes domésticos ao longo do seu curso, comprometendo a qualidade de suas águas e inviabilizando o potencial de usos múltiplos (CASTRO, 2001).

Material e métodos

Foi realizado um levantamento bibliográfico sobre o histórico da contaminação de ambientes aquáticos por metais pesados, bem como sobre os principais impactos dessas substâncias para saúde humana. Em seguida efetuou- se um levantamento sobre o impacto dessas substancias sobre variados corpos de águas superficiais do Brasil. As

fontes de poluição e seus impactos ao ambiente foram comparados e discutidos.

Resultado e discussão

Historicamente, o desenvolvimento das civilizações ocorreu ao redor de rios por causa da facilidade de obtenção de água doce e como via de transporte. Desde então, as águas dos rios vêm sendo afetadas pelas atividades humanas como a agricultura e o despejo de efluentes industriais e domésticos (CAMPOS, 2010). Tais ações causam impactos negativos ao ecossistema e consequentemente a saúde dos que dele dependem. Em estudos realizados no Vale do Rio dos Sinos, RS (ROBAINA et.al.,2002) constataram que os processos de urbanização e industrialização estão associados com alta concentração de metais pesados. Os metais Cr, Cu e Zn apresentam risco de muito alto a extremo em algumas drenagens da área. (RANGEL et.al., 2013) dizem que o processo de urbanização na bacia do Rio Paciência iniciou-se nas décadas de 70 e 80, tornando-se mais evidente devido à criação de diversos conjuntos habitacionais. Este processo tem dado ênfase a uma forte concentração do setor terciário e de serviços, em que acabam prejudicando a bacia jogando lixo nos igarapés e desmatando. Em consequência desses usos, a bacia apresenta uma grande fragilidade ambiental com riscos de contaminação por metais pesados. Sabe-se que o rio Paciência desempenha um importante papel na economia local, através da irrigação da horticultura e floricultura, além ter sido fonte de lazer nos finais de semana em alguns trechos do seu curso, o que justifica a importância do desenvolvimento de estudos quanto ao seu grau de contaminação. Segundo o ofício 50 feito em 2012 pelo Ministério da saúde, a exposição humana a contaminantes químicos como o chumbo, mercúrio e cádmio, pode causar efeitos tóxicos, mesmo quando ingeridos em baixas concentrações, devido à sua capacidade de bioacumulação na cadeia trófica.

Conclusões

Os dados apresentados mostram o quanto o dejeto de efluentes podem ser nocivos aos ecossistemas. Dessa forma, é crucial que sejam realizados estudos a fim de avaliar o grau de contaminação de águas superficiais. Neste contexto, se enquadra o Rio Paciência, que vem sendo degradado com o crescente desenvolvimento da região metropolitana de São Luís. A presente revisão teve como objetivo propor a avalição da contaminação por metais pesados sobre este rio o qual não possui muitos estudos acerca dessa variável sendo que as atividades em torno de seu curso sugerem tal contaminação.

Agradecimentos

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14 SIMPEQUI
Simpósio Brasileiro de Educação Química
Base Curricular Nacional para a Química na Educação Básica

A CONCEPÇÃO DO CONCEITO DO PRINCÍPIO LE CHATELIER DE ESTUDANTES EM UMA ESCOLA PÚBLICA NO MUNICÍPIO DE RORAINÓPOLIS-RR AO ANALISAR SEUS MODELOS MENTAIS

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Resumo

Por meio do processo de observação, análise crítica, experimentação ou decisão é possível construir e reconstruir o conhecimento, tais processos estão relacionados ao conceito de Modelos Mentais, que nos quais justificam o desenvolvimento do raciocínio humano. Esses modelos mentais se tratam de uma teoria psicológica baseada nos estudos de Philip Johnson-Laird, formalizada em 1983. Assim, o presente trabalho tem como enfoque a utilização desta teoria em sala de aula por meio da metodologia que abrange o Princípio de Lê Chatelier, envolvendo a resolução de textos situações-problemas nas séries matutinas de 2° ano do Ensino Médio, situadas na Escola Estadual José de Alencar, município de Rorainópolis-RR.

Palavras chaves

Modelos Mentais; Princípio de Le Chatelier; Situações-Problemas

Introdução

A produção do conhecimento humano já se tornou prática social indispensável ao desenvolvimento geral da sociedade. A todo instante o indivíduo está em momento de aprendizagem, aonde pode ocorrer de forma consciente ou inconsciente. Deste modo, aprender de forma consciente é levar o indivíduo a refletir sobre tal assunto, que pode ser em um ambiente escolar, por exemplo, onde o professor explica o conteúdo ao aluno, com o objetivo que este adquira conhecimento de forma induzida, sabendo que, o estudante vai à escola com o intuito de obter conhecimento e desenvolver habilidades. Aprender de forma inconsciente é a maneira mais remota de aprendizagem que existe, onde o sujeito aprende com as vivências no seu dia a dia, superando desafios, enfrentando obstáculos, confrontando problemas, ou simplesmente em seu cotidiano. Todas são maneiras de obter conhecimento e criar modelos mentais sem instrução prévia e de forma implícita. Assim, o sujeito apenas será capaz de refletir, questionar, resolver problemas e compreender qualquer assunto, se este tiver um modelo de determinado fenômeno em sua mente. Entendendo o fenômeno, o indivíduo terá condições de

obter resultados e saberá como controlá-lo ou alterá-lo, ou ainda, relacioná-lo com outros fenômenos (JOHNSON-LAIRD, 1983). Logo, em sala de aula, o estudante somente conseguirá fazer comentários sobre determinado conteúdo, se ele já obtiver alguma compreensão daquilo que está sendo explanado, e para compreender qualquer assunto é necessário que o estudante tenha um modelo funcional dele, caso contrário, não terá bases cognitivas para que seja possível fazer suas analogias. Em se tratando de modelos mentais, é extremamente necessário compreender a relevância e a cooperação das estruturas mentais no processo de ensino aprendizagem, que são representados pelos modelos mentais já estruturados, deste modo os conteúdos devem ter significado para o estudante, fazendo-se necessária a modificação e a contextualização do eixo temático. Todavia, na prática educacional, essa tarefa se torna muito complicada, mesmo quando o professor se dispõe aos novos planejamentos, devido à dificuldade de encontrar uma metodologia para se trabalhar com o modelo mental já estruturado do estudante dentro de sala de aula de maneira cômoda. Desta forma, este trabalho tem como finalidade apresentar um método alternativo, uma vez que os resultados reagiram de acordo com os objetivos, para se desenvolver de maneira adequada, respeitando o tempo do professor em sala de aula e a praticidade de planejar, executar e avaliar os seus estudantes por meio de Textos Situações-Problemas. Os Textos Situações-Problemas se desenvolvem a partir de hipóteses e evidências, com intuito de obter uma conclusão coerente, na qual o estudante será responsável por toda a evolução e resolução da pesquisa. Logo, o professor exercerá função de orientador, permitindo aos seus estudante o contato com os diferentes tipos de conhecimento e ajudando-os na organização, na avaliação e na sua utilização em diferentes contextos. Por conseguinte, esse método tem o desígnio de colocar os estudantes em contato com os problemas reais, com o propósito de estimular o desenvolvimento do pensamento crítico, a habilidade na resolução de problemas e a aprendizagem de conceitos da disciplina em questão, possuindo a característica de enfatizar o aprendizado autodirigido. A pesquisa tem como problemática a questão de como os textos situações- problemas influenciam nos modelos mentais dos estudantes, auxiliando na resolução de problemas e na construção conceitual do Princípio de Le Chatelier. Desta maneira, o objetivo da pesquisa é verificar a concepção do Princípio de Le Chatelier dos estudantes de 2° ano de Ensino Médio da Escola Estadual José de Alencar por meio de seus modelos mentais, partindo da utilização de textos situações-problemas. Portanto, para desenvolver a pesquisa adequadamente, é importante analisar o desenvolvimento dos estudantes em sala de aula, identificando, suas possíveis dificuldades de compreensão e interpretação conceitual, e intervir em suas dificuldades de aprendizagem por meio de textos situações-problemas.

Material e métodos

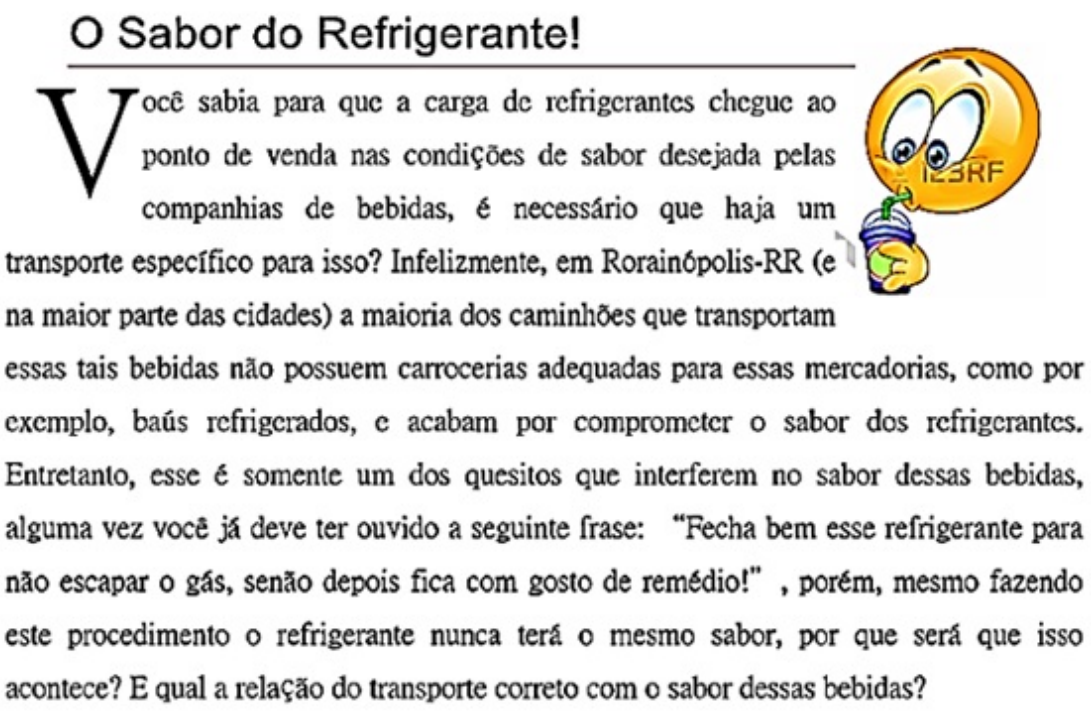
O campo de abrangência se constitui em todas as turmas matutinas de 2° ano do ensino médio regular, da Escola Estadual José de Alencar, situada no município de Rorainópolis, sul do Estado de Roraima. Assim, a amostra é composta de três turmas, onde 2° ano A apresenta vinte e três estudantes, 2° ano B compreende vinte e um estudantes, e 2° ano C inclui vinte estudantes, totalizando uma amostra de sessenta e quatro indivíduos. Trata-se de uma pesquisa com classificação exploratória e de natureza qualitativa. Para Zikmund (2000), “a pesquisa exploratória é empregada quando se tem o propósito de diagnosticar situações, explorar alternativas ou descobrir novas ideais, permitindo haver explicações alternativas para tal fato”. Deste modo, o objetivo da pesquisa é analisar o desenvolvimento dos estudantes, diagnosticando a situação de suas possíveis dificuldades de compreensão e interpretação conceitual, para que, posteriormente, seja explorada a alternativa de uma metodologia diferenciada, com base em resolução de problemas, por meio de textos situações-problemas, verificando sua eficácia ou ineficácia. A necessidade da abordagem qualitativa é devida suas particularidades que possibilita uma espécie de representatividade do grupo maior dos sujeitos que participarão no estudo, que, no caso, não é preocupação da amostra a quantificação da dos resultados, ao contrário, decide intencionalmente, considerando uma série de condições (TRIVIÑOS, 1987). Uma das técnicas de coleta de dados utilizada nesta pesquisa foi a observação, que segundo Marconi & Lakatos (1996), este instrumento é apropriado quando a finalidade é identificar e obter provas a respeito de objetivos sobre os quais os indivíduos não têm consciência, mas que orientam seu comportamento. Assim, utilizou-se, especificamente, a observação sistemática, pois se tratou de uma observação estruturada, esquematizada e controlada, compreendendo seu objetivo de estudo, a fim de eliminar falhas e distorções que poderiam ocasionar incoerência em tal pesquisa. Outra técnica de coleta de dados utilizada nesta pesquisa foi aplicação de questionários, que segundo Cervo & Bervian (2002), esta ferramenta refere-se a um meio de obter respostas às questões por uma fórmula que o próprio informante preenche. O uso dos questionários possibilitou o alcance de um maior número de estudantes, onde todos puderam participar de maneira anônima, sendo que a padronização das questões permitiu uma interpretação mais ordenada e objetiva, facilitando a comparação das respostas em alguns casos, onde eram necessárias para obter informações específicas ao conteúdo.

Resultado e discussão

Foi feita análise dos perfis iniciais dos estudantes segundo seus entendimentos sobre o conteúdo de Equilíbrio Químico, por meio de questões relevantes para o início da pesquisa. Tais estudantes encontravam-se no início do terceiro bimestre, onde, até o momento, tiveram a proximidade com o conteúdo em sala de aula por meio de resumo solicitado pelo professor titular das turmas. Assim, essa avaliação inicial tem por intento sondar o conhecimento do estudante, a fim de ter subsídios que fundamentem, futuramente, o caminho a ser desenvolvido em sala de aula pelo professor estagiário durante o processo de avaliação desses alunos. Segundo Luckesi (2002), a avaliação envolve um ato que excede o alcance da configuração do objeto, exigindo decisão do que fazer com ele, direcionando o objeto numa trilha dinâmica da ação. É confundida comumente com a verificação, que na qual esta é uma ação que "petrifica" o objeto. Com base nessa argumentação, fez-se a análise de dados, onde sessenta e quatro estudantes responderam a quatro questões contidas no questionário de avaliação inicial. Em primeiro interesse, averiguaram-se quais sentenças caracterizavam um sistema em Equilíbrio Químico, por meio de sublinhamentos feito pelos estudantes. É possível alegar que os estudantes não souberam indicar somente as sentenças que caracterizam um sistema em equilíbrio químico, onde houve uma série de características, que entre elas estão algumas sentenças corretas e outras sentenças que não se aplicam ao sentido de equilíbrio químico, podendo perceber uma confusão de raciocínio entre as propriedades escolhidas por eles. A segunda questão do questionário fundamentava a construção do conceito do princípio de Le Chatelier, em que os estudantes poderiam utilizar palavras mencionadas na própria questão, a fim de facilitar a elaboração do conceito objetivado. No entanto, 89% dos estudantes não souberam construir o conceito do princípio de Le Chatelier, mesmo com a utilização das palavras mencionadas na questão, onde muitos afirmam que não conseguiram fazer tal construção por nunca ouvirem falar em Le Chatelier, mesmo que em seus cadernos, quando feito os resumos à pedido do professor titular das turmas, estaria presente todo o conteúdo envolvendo o princípio de Le Chatelier. Na terceira questão, cabiam aos estudantes responder se a reação química genérica tratava-se de uma reação em equilíbrio químico, justificando de forma lógica, entretanto, cerca de 84%, não soube justificar a questão baseada na reação genérica, tendo várias justificativas como "não sei o que significa isso" e "nunca vi esse tipo de reação". Porventura, os estudantes não apresentavam condições de assimilar a reação química genérica com o conceito de equilíbrio químico, supostamente por possuírem um modelo mental ainda não estruturado. A última questão do questionário de avaliação inicial constituiu na forma de múltipla escolha, onde competia aos estudantes apontar as situações que favoreciam a formação do ozônio, porém muitos não obtiveram êxito ao apontar as situações que favorecem a formação do ozônio, havendo grande incoerência em suas respostas. Provavelmente, os estudantes não continham embasamentos suficientes sobre os fatores que afetam o equilíbrio químico para ter condições de analisar a reação química fornecida na questão. Após a avaliação inicial, foram aplicados três diferentes textos situações- problemas envolvendo o princípio de Le Chatelier, que nos quais consiste o destaque de temas voltado para a realidade dos estudantes, a fim de ampliar o conteúdo em suas inserções sociais, despertando o seu interesse, uma vez que se trata de textos que consideram os interesses de sua vivência diária, como mostram as figuras abaixo. Cada turma matutina de 2° ano de ensino médio foi divida em três grupos, onde cada grupo recebeu um texto situação-problema que, após um prazo de vinte e cinco dias (período suficiente para que os estudantes fizessem pesquisas e desenvolvessem o trabalho), deveria apresentar hipóteses que comprovassem suas explicações perante a turma. Por fim, as hipóteses seriam analisadas e avaliadas pelo professor titular das turmas. A resolução de problemas através de textos que envolviam o princípio de Le Chatelier foi desenvolvida em sala de aula através das seguintes fases utilizadas por Gagné (1976): I. Fase inicial: - proposição do problema. -compreensão do problema. II. Fase intermediária: - construção da solução/hipótese. - testagem da solução/hipótese. III. Fase final: - aceitação ou rejeição da solução/hipótese. A finalidade da utilização desta metodologia diferenciada é que haja um estímulo no cognitivo do estudante, fazendo com que ele aprenda a questionar o seu próprio pensamento, desenvolvimento, consequentemente, dos seus modelos mentais, possibilitando à sua própria capacidade o ato de construir e recriar mentalmente suas representações do mundo exterior (JOHNSON-LAIRD, 1983). Após um intervalo de, aproximadamente, quatro meses, contados a partir das apresentações e defesas das hipóteses dos textos situações-problemas em sala de aula, houve a aplicação de questionários de avaliação final, a fim de verificar e apontar se houve progresso em longo prazo no desenvolvimento conceitual dos estudantes em relação ao princípio de Le Chatelier. Com quatro perguntas abertas, o questionário final verificou questões que envolviam Equilíbrio Químico, mas especificamente o princípio de Le Chatelier, nos quais foram trabalhados em sala de aula por meio da metodologia de textos-situações problemas. A primeira pergunta tem como objetivo a construção do conceito de Equilíbrio Químico. Pode-se dizer que os estudantes obtiveram um avanço significativo na primeira pergunta, mesmo ainda tendo cerca de

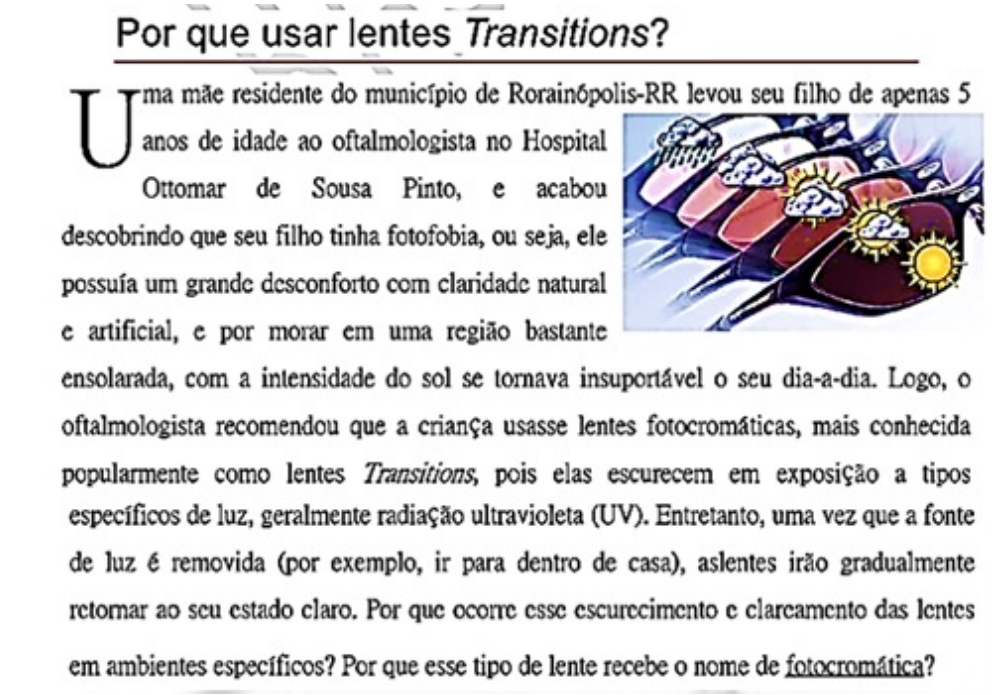
39% de estudantes que participaram da metodologia aplicada em sala de aula, não saber responder com coerência a pergunta avaliativa. A segunda questão induzia o estudante a construir um conceito lógico sobre o princípio de Le Chatelier, com o intuito de avaliar o que haveria em seu modelo mental sobre o determinado conteúdo, onde a análise mostrou que houve progresso nas respostas pertencentes à segunda questão avaliativa, sendo que 69% dos estudantes responderam coerentemente. A terceira pergunta faz referência aos fatores que afetam um sistema em Equilíbrio Químico, uma vez que a questão envolviam respostas relacionadas aos textos situações-problemas apresentadas pelos estudantes, onde para resolver tais problemas contidos nos textos, primeiramente, deveriam apontar os fatores que afetavam tais sistemas. Com isso, todos os estudantes conseguiram responder à esta questão corretamente. A última questão se tratava de um "desafio", onde os estudantes deveriam escolher e explicar, resumidamente, sobre um fator que perturbava um sistema em equilíbrio, que por meio de análise de dados, percebeu-se que a maior parte dos estudantes escolheu o fator temperatura para poder explicar a maneira de como ela afeta um sistema em equilíbrio químico, cerca de 52%, podendo afirmar que eles assimilaram com mais facilidade os conceitos que envolviam este fator. Deste modo, houve um progresso notório no desenvolvimento conceitual dos estudantes, sendo que, apesar de um intervalo de, aproximadamente, quatro meses, estes conseguiram resolver questões envolvendo o conceito de equilíbrio químico, mais numerosamente, questões do princípio de Le Chatelier. Entretanto, apesar desse progresso, ainda existem estudantes que não obtiveram êxito após participaram da metodologia de resoluções de textos situações-problemas e, por não ter tido condições de responder as questões de avalição final, apresentaram-se justificativas como: "simplesmente não me lembro"; "tem que revisar o conteúdo pra eu recordar"; "não estudei direito" e "não aprendi nada".

Figura 1



Texto situação-problema sobre o sabor do refrigerante

Figura-2



Por que usar lentes *Transitions*?

Uma mãe residente do município de Rorainópolis-RR levou seu filho de apenas 5 anos de idade ao oftalmologista no Hospital Ottomar de Sousa Pinto, e acabou descobrindo que seu filho tinha fotofobia, ou seja, ele possuía um grande desconforto com claridade natural e artificial, e por morar em uma região bastante ensolarada, com a intensidade do sol se tornava insuportável o seu dia-a-dia. Logo, o oftalmologista recomendou que a criança usasse lentes fotocromáticas, mais conhecida popularmente como lentes *Transitions*, pois elas escurecem em exposição a tipos específicos de luz, geralmente radiação ultravioleta (UV). Entretanto, uma vez que a fonte de luz é removida (por exemplo, ir para dentro de casa), aslentes irão gradualmente retornar ao seu estado claro. Por que ocorre esse escurecimento e clareamento das lentes em ambientes específicos? Por que esse tipo de lente recebe o nome de fotocromática?

Texto situação-problema sobre as lentes Transitions.

Conclusões

Ao se determinar os percentuais de respostas dos estudantes nos questionários iniciais, obteve-se um indicativo de que a grande maioria não domina a parte conceitual do estudo de Equilíbrio Químico, mais precisamente, o princípio de Le Chatelier, onde muitos não responderam coerentemente às perguntas de conhecimento básico sobre o conteúdo. Vários podem ser os motivos em relação à dificuldade que os estudantes possuem em formar conceitos como, por exemplo, uns dos fatores que dificultam a construção do conhecimento, é o tratamento do conteúdo de maneira superficial, tanto pelo professor quanto pelo livro didático, tempo disponibilizado ao tratamento do conteúdo, aulas extremamente teóricas, portanto cansativas, e outros. Sabe-se que, por meio das repostas provenientes do questionário inicial, os estudantes possuíam um modelo mental sobre o conteúdo abordado, frágil, mal estruturado e incompleto, que resulta em um processo de aprendizagem irrelevante, onde este não terá bagagens suficientes para resolver questões sobre o tema, pois seu modelo mental não permitirá por falta de conhecimento assimilado. A intenção da pesquisa foi buscar caminhos que tentasse solucionar o problema questionado, concebendo ao estudante ser o centro do processo de sua aprendizagem, buscando ser ativos, construindo seu conhecimento por meio de um diálogo e pesquisa. Consequentemente, a metodologia de textos situações-problemas se mostrou eficaz, correspondendo aos objetivos desta pesquisa, sendo que esta possibilitou uma interação maior entre os elementos que constituem uma sala de aula, cometendo ao estudante que seja o sujeito de sua própria aprendizagem, e com isso estruturar seu modelo mental sobre o conteúdo abordado nesse período.

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ANTIOXIDANT CAPACITY OF NATIVES AMAZONIAN FRUITS BY NITROTETRAZOLIUM BLUE CHLORIDE (NBT) METHOD

Área

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Alimentos

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Resumo

The increase of oxidative stress drives the search for foods that can prevent or minimize oxidative damage. The antioxidant capacity of four native fruits of the Amazon region was evaluated by NBT method. All the fruits showed antioxidant activity, and cupuaçu and bacuri showed similar statistically by F test and t Student unpaired ($p < 0.05$). The inhibition percentage of radical $O_2^{\bullet-}$ was greater for acerola (96.84%) followed by the açaí, murici, cupuaçu/bacuri. This study provided new data on the antioxidant activity of native fruits of the Amazon region, as well as new options for assessing antioxidant capacity of complex matrices. Based on this study, it can be said that these fruits are suitable for use in the food and cosmetics industries as well as in pharmaceutical compositions.

Palavras chaves

Amazonian; antioxidant potential; fruits

Introdução

Amazon Region is formed by a complex mosaic of endemic areas with rich diversity of fruit species which are distributed in accordance with their biota specificities [1]. Recognized sources of nutrients, fruits comprise important foods nutritionally in the human diet and have in recent years received increased attention due to epidemiological evidence that shows that the regular consumption of vegetables reduces mortality and morbidity due to some chronic diseases [2, 3]. The protective effect exerted by these foods has been attributed to high levels of phytochemicals with antioxidant properties that contribute to the prevention of various diseases [4]. Antioxidants are natural or synthetic substances that prevent or delay the oxidative damage by scavenging the free radicals, even in small concentrations, being formed by enzymes, vitamins, minerals, phenolic compounds, flavonoids and protein [5, 6]. Although some antioxidant molecules have biological origin, the daily intake of antioxidants through diet is necessary in view of its constant physiological consumption for maintaining the balance of free radicals. Once in healthy individuals the production of free radicals is balanced by the antioxidative defense system, the imbalance in favor of free radicals generates the oxidative stress that may be a contributory factor to the pathogenesis such as neurodegenerative disorders, Alzheimer's diseases, Parkinson's diseases, cancer, cardiovascular diseases, atherosclerosis, cataracts, cognitive dysfunction and inflammations [7-10]. Free radicals are highly unstable molecules oxidation with available electrons that can be generated in vivo during metabolic processes and/or by exogenous sources, as some carcinogenic compounds and ionizant reactions. The Reactive Oxygen Species (ROS) are the most damage class of free radicals in biological systems, and include the superoxide anion ($O_2^{\bullet-}$), singlet oxygen (O_21), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), hydroxyl radical ($OH^{\bullet}$), peroxynitrite (ONOO-), peroxyl ($ROO^{\bullet}$), alkoxyl radicals ($RO^{\bullet}$) and others [11]. Thus, it is important to know the antioxidant content and their efficacy in fruits, for preservation or protection against oxidative damage, as well as for future cosmetic, therapeutic, medical and food technology applications. There are many spectrophotometric methods for measuring the antioxidants potential in vegetables. In general, are simple and rapid assays and needs only a UV-vis spectrophotometer. But the most measure the ability to inhibit a commercial free radical, that is, a free radical with chemical structures other than free radicals produced in biological systems. In this view, the NBT method shows advantages, once evaluate the superoxide scavenging capacity of the antioxidants in the sample, in physiological conditions. Established by Cortina Puig et al. (2009), in the NBT method, $O_2^{\bullet-}$ radicals and acid uric are generate in vitro by the HX/XOD system. The $O_2^{\bullet-}$ radicals reduce the NBT reagent (yellow color) into formazan (purple color), which is measure spectrophotometrically at 560 nm (Figure 1). The presence of radical scavengers (the antioxidant sample) generates inhibition (competitive) in the formation of formazan leading to the decrease of its production rate. Considering the unexplored antioxidant potential of fruits native to the Amazon region, this paper determined the antioxidant capacity by colorimetric assay for four Amazonian fruits, popularly known as açaí (Euterpe oleracea), bacuri (Platonia insignis), cupuaçu (Theobroma grandiflorum) and murici (Byrsonima dealbata). For this, the production of oxygen radicals from hypoxanthine system (HX) / xanthine oxidase (XOD) was carried out in the presence of nitrotetrazolium blue chloride (NBT), allowing the formation of a product with a linear purple color and stable which is measured spectrophotometrically at 560 nm [12]. Before the unexplored antioxidant potential of fruits native to the Amazon region, this paper determined the antioxidant capacity by colorimetric assay for four Amazonian fruits, popularly known as açaí (Euterpe oleracea), bacuri (Platonia insignis), cupuaçu (Theobroma grandiflorum) and murici (Byrsonima dealbata). For this, the production of oxygen radicals from hypoxanthine system (HX) / xanthine oxidase (XOD) was carried out in the presence of nitrotetrazolium blue chloride (NBT), allowing the formation of a product with a linear purple color and stable which is measured spectrophotometrically at 560 nm [12].

Material e métodos

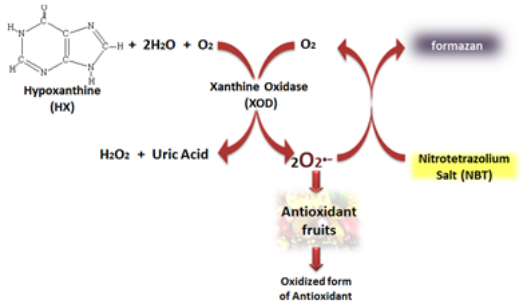
CHEMICAL REAGENTS The reagents used were NBT (N6876), HX (H9377), L-ascorbic acid (A5960), XOD from bovine milk (X4376). All reagents were of analytical grade and all solutions were prepared using Milli-Q water. INSTRUMENTATION Colorimetric measurements were performed with a Beckman DU520 UV–Vis Spectrophotometer (Beckman Coulter France, S.A., Roissy CDG, France). PREPARATION OF THE ANTIOXIDANT SAMPLES Four native amazonic fruits were included in this study: açaí (Euterpe oleracea), bacuri (Platonia insignis), cupuaçu (Theobroma grandiflorum) and murici (Byrsonima dealbata). The fruits were obtained from São Luís, Maranhão, Brazil in Ceasa market. Acerola was also evaluated in this study in order to compare their results with other fruit, in view because it is a known high antioxidant potential [13, 14]. The samples were washed, pulped and mixed with the aid of a mixer of stainless steel. Approximately, and triplicate samples of 200 g were taken, filtered in vacuum Buchner funnel with qualitative filter paper, followed by a new filtration with a quantitative filter paper (1.2 mM). The volume of 500 uL of the obtained extract was diluted to 1 mL in 50 mM Phosphate-Buffered Potassium (K-PB) pH 7.5 with 0.1 mM EDTA.

From this solution, sequential dilutions were performed using 50 mM Phosphate-Buffered Potassium (K-PB) pH 7.5 with 0.1 mM EDTA. MEASUREMENT OF THE SUPEROXIDE SCAVENGING CAPACITY USING THE NBT METHOD A reaction mixture was prepared with 50 mM K-PB pH 7.5 containing EDTA (0.1 mM), 25 µM HX, 50 µM NBT, the antioxidant extract (distilled water for the blank) and 0.2 U mL−1 XOD, which was added last. The increase in absorbance for 3 min was recorded at 560 nm. Stock solutions of NBT, HX and XOD were prepared in 50 mM K-PB pH 7.5 containing EDTA (0.1 mM). The % radical scavenging activity (RSA) of the plant extracts was calculated using the following formula: RSA % = 100 x [(Abs. control - Abs. sample)/Abs control] Where, Abs. control is the absorbance of formazan without the sample; Abs. sample is the absorbance of formazan with the sample.

Resultado e discussão

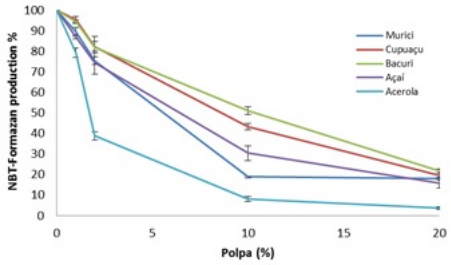
Results of the antioxidant activity of fruits under study expressed in function de formazan production rate for different title mass (% m/v) of analyzed fruits and standard deviation for each analysis are shown in the Figure 2. There was observed that all the fruits showed antioxidant activity. The inhibition of $O_2^{\bullet-}$ radicals generated by the antioxidant action of the studied pulps are revealed by the small NBT amount reduced to formazan. Evaluating the closeness of the results obtained for bacuri and cupuaçu samples, F and unpaired t tests were applied using 95 % confidence limits to see if they show significant differences between their accuracy and absorbance averages, respectively. Table 1 shows the F and unpaired t values calculated. The results of the F and unpaired t tests showed that there aren't significant differences between the precision and the percentage of formazan production obtained in samples of cupuaçu and bacuri, for all dilutions, at 95 % confidence, revealing antioxidant activities similar to those plant species. The results shows the superoxide RSA was biggest in acerola (96.39 %), and lower for the following fruits by açaí (84.21 %), murici (83.91 %), cupuaçu (80.54 %) and bacuri (78.34 %).

Figure 1



Reactions involved in the measurement of the superoxide scavenging capacity using the NBT chromogenic reagent.

Figure 2



Representation of the formazan production rate by different concentrations of pulp fruits.

Table 1 - Values of F and t calculated for Bacuri and Cupuacu.

% Pulp	1	2	10	20
Test F	2.33	3.26	14.28	2.33
Test *t*	0.65	0.05	0.79	1.27

F critical at 95 % confidence for 2 degrees of freedom: 19.0; t critical at 95 % confidence for 4 degrees of freedom: 2.78.

Conclusões

The NBT method employed shown an alternative for the determination of antioxidant capacity in fruits, being promising for application in other matrices, in the same conditions. The addition of fruits pulp samples caused the decrease in absorbance signal, allowing the quantification of their antioxidant capacity. The results reveal that all the fruit studies showed antioxidant activity. The acerola has the highest antioxidant activity, followed by açaí, murici and cupuaçu/bacuri. Cupuaçu and bacuri have similar antioxidant activity for all dilutions made, with 95 % confidence level. Overall, this study provides data on the antioxidant activity of native fruits of the Amazon Region.

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DETERMINAÇÃO DOS ELEMENTOS MINERAIS CA, CU, FE, MG, MN, NA E ZN EM TRÊS VARIEDADES DE PIMENTÕES COMERCIALIZADOS NA CIDADE DE SÃO LUÍS-MA

Área

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Resumo

Foram analisadas amostras de pimentão verde, pimentão vermelho e pimentão amarelo, comercializados em supermercados localizados na cidade de São Luís-MA, quanto à presença de macro e micro minerais essenciais ao organismo. Foram analisados quantitativamente cálcio, cobre, ferro, magnésio, manganês, sódio e zinco, empregando Espectrometria Ótica com Plasma Indutivamente Acoplado (ICP- OES). Foram encontrados teores dos minerais, em mg/100g, nas seguintes faixas: 7,29-10,25 para Ca, 0,05-0,13 para Cu, 0,40-0,88 para Fe, 11,83-14,07 para Mg, 0,3-0,11 para Mn, 1,34-2,92 para Na e 0,68-0,91 para Zn. Entre as hortaliças estudadas, o pimentão amarelo apresentou os melhores resultados para Cu e Zn, correspondendo a 13,33% e 12,86%, respectivamente, aos índices diários recomendados (IDR).

Palavras chaves

Pimentões ; teores de minerais; São Luís-MA

Introdução

Consideradas alimentos reguladores, as hortaliças são fundamentais para fazer o corpo humano funcionar de maneira adequada e harmônica, por apresentarem uma densidade energética baixa e serem ricas em micronutrientes, fibras e outros elementos fundamentais (HORTALIÇAS EM REVISTA, 2012). Algumas hortaliças fazem parte da mesa do consumidor diariamente, a exemplo disso tem-se o pimentão (Capsicum annuum L.), que está entre as hortaliças mais cultivadas no Brasil e de maior importância econômica e social (HENZ et al., 2007), por conta de sua forte participação na culinária doméstica e empresarial e na geração de emprego e renda para muitas famílias (ANUÁRIO BRASILEIRO DE HORTALIÇAS, 2012). Os elementos minerais são originários do solo, que são absorvidos pelas plantas, tornando o consumo de frutas e hortaliças um dos seus maiores vinculadores. Sabe-se que o corpo humano é composto por 4% a 5% de minerais, os quais possuem funções essenciais à saúde, como controlar impulsos nervosos, auxiliar na atividade muscular e no balanço ácido-base, além de agir como constituintes estruturais de tecidos corpóreos e como ativadores/reguladores de enzimas (TAIZ e ZEIGER, 2009). Porém, como o organismo não pode produzi- los, deve-se utilizar fontes externas, como os alimentos, para assegurar uma ingestão adequada. Os minerais Ca, Cu, Fe, Mg, Mn, Na e Zn são considerados nutrientes essenciais de grande importância na nutrição humana, pois apresentam um papel importante em várias vias metabólicas. Dessa forma, o presente estudo teve o objetivo de investigar o potencial nutricional de pimentões amarelos, verdes e vermelhos comercializados em supermercados da cidade de São Luís-MA, em relação a presença desses minerais.

Material e métodos

Os reagentes utilizados foram de grau de pureza analítica e a água utilizada foi deionizada. Os materiais utilizados foram previamente descontaminados em HNO_3 a 10% (v/v) por no mínimo 24 h. As três variedades de pimentões (amarelo, vermelho, verde) foram adquiridas em supermercados localizados na região metropolitana de São Luís-MA, tendo sido devidamente acondicionadas, identificadas e transportadas ao laboratório. A porções de 0,2-0,5 g das amostras foram adicionados, sequencialmente, 5,0 mL de HNO_3 concentrado, 2,0 mL de H_2O_2 a 30% (v/v) e 0,5 mL de ítrio (Y) a 100 mg L^{-1}, este último usado como padrão interno. A mistura foi submetida à digestão em forno microondas (MARSX press 6.0), de acordo com o método estabelecido pela AOAC (2002). O digerido foi diluído com água deionizada para 25,0 mL e filtrado em papel de filtro quantitativo (28 µm), antes das análises dos teores dos minerais. As determinações dos elementos foram feitas por Espectrometria Ótica com Plasma Indutivamente Acoplado (ICP-OES) (Shimadzu, modelo 9820, com nebulizador concêntrico), sob 1,2 kW de potência, 10 L min^{-1} de argônio e os seguintes comprimentos de onda: 616.217nm (Ca), 327.396nm (Cu), 259.940nm (Fe), 383.826nm (Mg), 257.610nm (Mn), 589.592nm (Na) e 213.856nm (Zn). As curvas analíticas foram definidas para 4 valores de concentração, obtidos a partir da diluição de soluções padrão a 1000 mg L^{-1} em HNO_3 a 2 % (v/v). Os critérios para avaliação da eficiência do método analítico foram baseados nas seguintes figuras de mérito: melhores respostas analíticas da razão sinal/ruído; precisão, baseada no desvio padrão relativo (RSD) para três determinações, e sensibilidade, com base nos limites de detecção (LOD) e quantificação (LOQ) para dez determinações dos brancos (SKOOG, 2008).

Resultado e discussão

As concentrações médias dos minerais investigados (mg/100g de amostra) e seus respectivos RSD, são apresentados na Tabela 1, onde observa-se que o método analítico mostrou-se preciso, com valores de RSD variando de 0,2 a 8,77 %, para as determinações. A avaliação nutricional das amostras estudadas foi feita tendo como base o Relatório Técnico do Ministério da Saúde (BRASIL, 1998), o qual classifica um alimento como fonte em determinado mineral, quando 100 g deste apresentar 15 % de sua IDR, esses valores são mostrados na Tabela 2. Avaliando a classificação mineral em relação a ingestão dos elementos estudados, observa-se que as hortaliças forneceram teores dos minerais inferiores a IDR, no entanto, os resultados obtidos mostram que algumas das amostras podem, perfeitamente, ser usadas na elaboração de um complemento alimentar. Assim como o pimentão amarelo que apresentou 13,33% da IDR para o Cu e 12,86% para o Zn, sendo muito importante pois o Cu é essencial como constituinte de algumas metaloenzimas requeridas na síntese da hemoglobina e na catálise de oxidação metabólica e o Zn também faz parte da composição de várias enzimas envolvidas em funções fisiológicas (Onianwa, P. C.; Adeyemo, A. O.;

Idowu, O. E., 2001). Neste estudo, foram encontrados teores de sódio para os pimentões amarelo, verde e vermelho iguais a 1,36; 1,37; e 2,86 mg 100g-1 respectivamente, além disso, a ingestão de 100 g das partes comestíveis dos pimentões estudados fornecem teores de Na inferiores a 15 % da IDR, sendo estes resultados satisfatórios já que o Ministério da Saúde tem coordenado estratégias nacionais com vistas à redução do consumo de sódio, através de ações articuladas a planos setoriais como o Plano Nacional de Saúde 2012–2015 (NILSON et al., 2012).

Tabela 1- Teores de elementos minerais (mg 100g-1) nas amostras

Elemento mineral	Hortaliças Estudadas CONCENTRAÇÃO mg $100g^{-1}$ (RSD)		
	Pimentão amarelo	Pimentão verde	Pimentão vermelho
Ca	7,80 ^{b}(4,14)	7,29 ^{b}(0,02)	9,87^{a} (3,85)
Cu	0,12^{a} (6,48)	0,05^{c} (3,40)	0,08^{b} (3,91)
Fe	0,84^{a}(4,22)	0,36^{c}(6,48)	0,71^{b}(1,50)
Mg	13,88^{a}(1,84)	13,07^{b}(1,97)	11,94^{c}(0,96)
Mn	0,11^{a}(3,22)	0,11^{a}(0,42)	0,03^{b}(2,52)
Na	1,36^{b}(8,77)	1,37^{b}(1,91)	2,84^{a}(1,94)
Zn	0,90^{a}(1,40)	0,76^{a}(2,91)	0,83^{a}(3,33)
As médias seguidas da mesma letra não diferem estatisticamente entre si pelo teste t de Student em um nivelo de 95% de probabilidade.			

Tabela 2 - Valores da IDR em minerais

%IDR ATENDIDO PELOS PIMENTÕES			
Elemento (IDR (mg dia^{-1}))	Amarelo	Verde	Vermelho
Ca (1000) ^{a}	0,78	0,73	0,99
Cu (0,9) ^{a}	13,33	5,56	8,89
Fe (14) ^{a}	6,00	2,70	5,07
Mg (260) ^{a}	5,34	5,03	4,59
Mn (2,3) ^{a}	4,78	5,22	1,30
Na (1500) ^{b}	0,09	0,09	0,19
Zn (7) ^{a}	12,86	10,00	11,86
^{a}BRASIL, 2005; ^{b}INSTITUTE OF MEDICINE, 2006.			

Conclusões

O desenvolvimento do trabalho foi importante para contribuir com novas informações sobre micro e macro minerais em amostras de três variedades de pimentões, ampliando a literatura e apresentando dados regionais. Entes as hortaliças estudadas, o pimentão amarelo apresentou os melhores resultados para Cu e Zn, em faixas de 0,05-0,13 mg/100g para Cu e 0,68-0,91 mg/100g para Zn, correspondendo a 13,33% e 12,86%, respectivamente, aos valores de IDR, sendo portanto uma boa contribuição para alimentação de quem os consome.

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ANTIOXIDANT ACTIVITY ASSESSMENT OF EXOTIC FRUITS BY DIFFERENT ANALYTICAL METHODS

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For all living aerobic organisms, the molecular oxygen (O_2) is required for the cellular respiration which is reduced to water with the inevitable formation of Reactive Oxygen Species (ROS). The recent growth in the knowledge of ROS demonstrates that a balance between ROS and antioxidants is necessary to maintain the normal physiological function. Nevertheless, if the ROS overwhelm the body's ability to regulate them, a condition known as oxidative stress ensues, involving in oxidative injury in cellular components and consequently the development of a number of diseases. Antioxidants are well-known for their ability to scavenge ROS and this provided protection stimulates search by natural antioxidants that can prevent or minimize the oxidative damage. In this view, the Brazilian flora presents a vast biodiversity with great chemical and pharmacological potentialities unexplored.

The main objective of this work is to identify new potential natural sources of antioxidants. For this, the antioxidant capacity based on the superoxide radical ($O_2^{\bullet-}$) scavenging ability was determined by a spectrophotometric bio-assay and an amperometric biosensor. In both cases, the superoxide radicals were generated in vitro during the catalytic oxidation of hypoxanthine by Xanthine OxiDase (XOD). The miniaturized bio-assay allows a rapid and reasonably accurate measurement of fruit extract. The design of the biosensor is based on the measurement of antioxidant oxidation at E=0V vs. Ag/AgCl with PEDOT modified screen printed electrode. Hypoxanthine addition triggers the $O_2^{\bullet-}$ production which is scavenged by the antioxidant, inducing the decrease of the measured current due to the antioxidant concentration reduction. Thus, this decrease can be correlated to the antioxidant capacity.

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Title:

Amperometric biosensor for the evaluation of antioxidant capacity in fruits matrices

Authors & affiliations:

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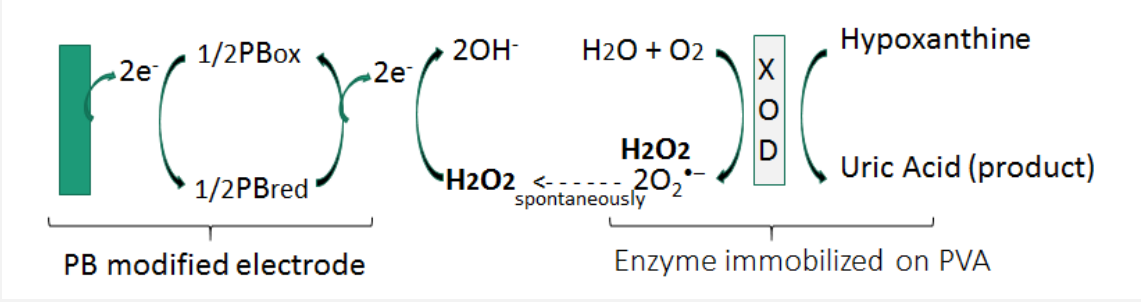
In recent years, there is a growing interest to obtain the biologically active compounds from natural sources due to the protection they provide against cardiovascular diseases and certain cancers. The protective effect is mainly attributed to the presence of constituents with antioxidant properties. This work describes the design of an amperometric biosensor to determinate the antioxidant capacity of relatively less explored native fruits of the Amazon region with aim to identify new potential natural sources of antioxidants. For this, a conventional system of three screen-printed electrodes was used as transducer platform based on a Prussian Blue (PB) modified working electrode, a pseudo reference silver electrode and a counter electrode. The xanthine oxidase (XOD) was immobilized onto the PB modified electrode surface by crosslinking with polyvinyl alcohol (PVA). The detection principle is based on the measurement of the H_2O_2 generated as a final product of the enzymatic reaction between the hypoxanthine (HP) and the XOD or by spontaneously dismutation of the $O_2^{\bullet-}$ radicals. The enzymatic generated H_2O_2 is reduced on the electrode surface (E~0V vs. Ag/AgCl) and the measured current is proportional to its concentration. As the antioxidants react with $O_2^{\bullet-}$ radicals and/or H_2O_2, their addition induces a decrease in the negative current, allowing the evaluation of the antioxidant capacity. The interest of this biosensor is to use real free radical oxygen species and not artificial radical such as ABTS or DPPH.



The applicability of this biosensor is demonstrated by *in vitro* analysis of standards antioxidants for later application in environmental matrices as Amazon fruits.

Acknowledgments: Scholarship from CAPES/Doctoral Sandwich Program Abroad/Case nº 88881.133624/2016-01.

APLICABILIDADE DA PCA E HCA NA DISCRIMINAÇÃO DE FRUTOS ARECACEAE

Área

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Química Analítica

Autores

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Resumo

A composição bromatológica e mineral, a capacidade antioxidante e o conteúdo em compostos bioativos de 3 frutos da família Arecaceae, nativos da região Amazônica, foram previamente avaliados. Análises quimiométricas de componente principais (PCA) e de agrupamento hierárquico (HCA) foram aplicadas possibilitando a discriminação entre as amostras pretencentes à mesma família botânica.

Palavras chaves

Arecaceae; avaliação nutricional; quimiometria

Introdução

Constituída por 283 espécies no Brasil, sendo 147 destes presentes no bioma Amazônia, a família Arecaceae é genericamente conhecida como palmeiras (Leitman et al. 2016). Fontes reconhecidas de nutrientes, os frutos de palmeiras compreendem alimentos nutricionalmente importantes, devido ao seu efeito protetor atribuído à presença de constituintes, como minerais e altos níveis de fitoquímicos com propriedades antioxidantes (NUNES et al., 2011; KAHL et al., 2012; LIU, 2013; KOZLOWSKA & SZOTASK-WEGIEREK, 2014; WANG et al. 2013). Nesse sentido, foram avaliados a composição bromatológica, o teor de minerais, a presença de compostos bioativos e a capacidade antioxidante de três frutos da família *Arecaceae* nativos de Amazônia: açaí (*Euterpe oleracea*), buriti (*Mauritia flexuosa*) e inajá (*Maximiliana maripa*). A fim de analisar os dados experimentais obtidos e possibilitar a discriminação composicional das amostras e a elucidação de seus comportamentos químicos, as técnicas de reconhecimento de padrões não supervisionadas, como PCA (análise de componentes principais) e HCA (análise agrupamento hierárquico), foram aplicadas aos resultados obtidos.

Material e métodos

O programa PAST foi usado para executar as análises por PCA e a HCA. As análises químicas foram realizadas na porção convencionalmente comestível dos frutos de açaí, buriti e inajá, em triplicata, para a construção da matriz de dados 9x17 (nove amostras e dezessete variáveis), sendo as variáveis compostas por 7 parâmetros bromatólogicos (umidade-MC, cinzas-TA, pH, lípidos-TL, proteínas- CP, acidez-ACIDEZ e acidez em ácido cítrico-CA), 7 elementos minerais (Ca, Cu, Fe, Mg, Mn, Na e Zn), 2 compostos bioativos (compostos fenólicos-PHENOLIC e vitamina C-VIT-C) e a capacidade antioxidante (ANT-CAP) contra radicais superóxido de importancia fisiológica Os dados foram previamente auto-escalonados, antes de serem submetidos às análises por PCA e HCA, pois havia grande variação de respostas das diversas variáveis, já que estas diferiam em ordem de grandeza, atribuindo assim o mesmo peso a todas as variáveis. A distância euclidiana foi utilizada para obter o dendrograma HCA.

Resultado e discussão

A Figura 1 mostra os resultados da análise multivariada da composição química com o diagrama de dispersão da PCA. Os componentes PC1 e PC2 descrevem 100% da variação total dos dados e alguns fornecem informações discriminatórias das amostras. O primeiro componente principal (PC1) descreve 68,46% da variação total e o segundo (PC2), 31,54%. Analisando os scores através do PC1, é possível observar a discriminação de dois grandes grupos: de um lado, as amostras de buriti e, de outro, as amostras de inajá e açaí. Em relação aos sobrepostos, observa-se forte influência das variáveis nas amostras. O comportamento distintivo apresentado pelas amostras de buriti, que descreve uma discriminação composicional em relação às amostras de inajá e açaí, foi ocasionado principalmente pelos maiores teores em TL, CP, CA, Ca, Mg, Cu e Fe. Por outro lado, as amostras de açaí e inajá apresentam os maiores teores em Na, Zn, MC, Vitamina C e maior valor de pH. Os resultados obtidos pela PCA foram confirmados pelo dendrograma obtido pela HCA (Figura 2). É possível observar a formação de três clusters, um para cada fruto, nos quais os agrupamentos de inajá e açaí apresentam maiores semelhanças.

Figura 1

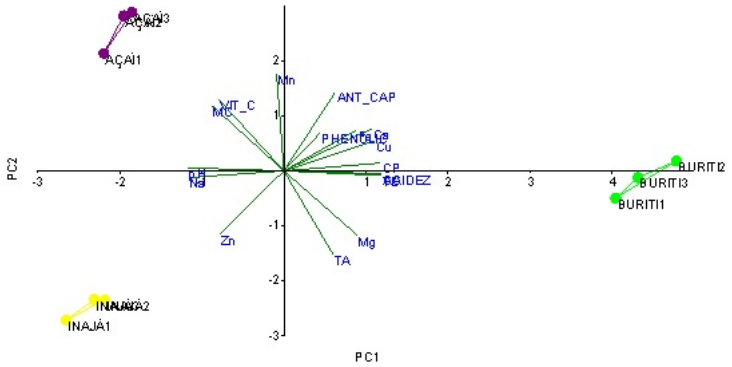
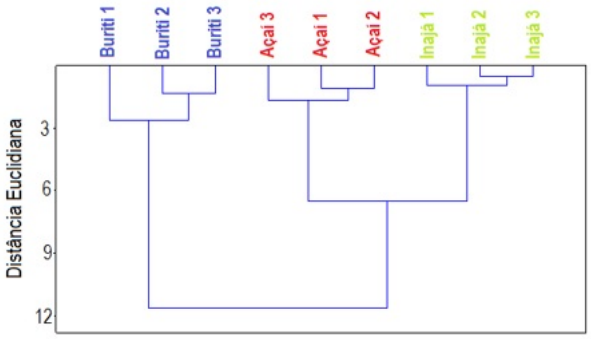


Diagrama de dispersão da PCA da composição química em frutos de Arecaceae.

Figura 2



Dendrograma representando as relações de similaridade da composição química de frutos de Arecaceae usando Ward's como método de ligação e a distância

Conclusões

A análise multivariada da composição química obtida pela PCA mostrou um comportamento distintivo nas amostras de buriti, causado pela discriminação composicional, especialmente pelo maior conteúdo na maioria dos nutrientes. A HCA confirmou a formação de três agrupamentos, um para cada fruto, sendo que o dos frutos inajá e açaí apresentou maiores semelhanças composicionais. A aplicação da análise exploratória de dados (HCA e PCA) nos frutos de mesma família botânica permitiu a obtenção de informações rápidas e eficientes sobre a similaridade entre as amostras pela visualização gráfica. O presente trabalho trouxe uma contribuição acerca de espécies de frutos considerados muito importantes para a região amazônica, tanto do ponto de vista nutricional quanto cultural, já que fazem parte do "saber fazer", podendo ser consumidos in natura, mas também utilizados na elaboração de alimentos regionais, tais como sucos, doces, sorvetes, compotas, entre outros.

Agradecimentos

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AVALIAÇÃO DA PRESENÇA DO MICROMINERAL LÍTIO NO AÇAÍ PROVENIENTE DE BOA VISTA, RORAIMA, EM RELAÇÃO À TOXICIDADE E À ESSENCIALIDADE NUTRICIONAL

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Área

Alimentos

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Resumo

A relação entre a deficiência de lítio endógeno e o desenvolvimento de psicopatologias humanas graves tem sido reportadas nas últimas décadas. Diante da importância em investigar o teor de lítio em alimentos naturais, este trabalho objetivou determinar este mineral, por ICP-OES, em amostras de açaí. Os resultados revelam que o fruto possui um alto teor desse micronutriente, o que tem favorecido nutricionalmente, não só a população do Estado de RR, mas tambpém toda a região amazônica.

Palavras chaves

lítio; açaí; ICP-OES

Introdução

O lítio é um metal alcalino de ocorrência natural, que os organismos vivos ingerem a partir de fontes alimentares, especiamente grãos e vegetais, e pelo consumo d'água, estando por isso presente em traços no corpo humano (DEMLING et al., 2001). Sua presença nos frutos varia conforme a origem, e depende de fatores naturais, como qualidade do solo, clima, espécie do fruto, entre outros (ANKER, SCHAFER e AMHOLD, 2003). Doenças relacionadas à deficiência de lítio não estão ainda bem elucidadas e os mecanismos bioquímicos de ação são complexos e inter-relacionados às funções de várias enzimas, hormônios e vitaminas, bem como com a fatores de crescimento e transformadores (SCHRAUZER, 2002). A quase ausência de lítio na água de abastecimento foi associada ao aumento das taxas de suicídios, homicídios e taxas de prisão por uso de drogas e outros crimes por Schrauzer e Shrestha (1990). Além disso, o uso do lítio como medicamento no controle de algumas doenças mentais e estados emocionais caracterizados por grandes e frequentes alterações de humor, incluindo transtornos depressivos maníacos, tem sido uma prática corriqueira na área psiquiátrica (DEMLING et al., 2001). Diante desse contexto, e considerando que as frutas são fontes naturais em minerais, este trabalho avaliou o potencial nutricional da polpa de açaí quanto à essencialidade e/ou toxicidade em lítio nos limites de tolerância fisiológicos.

Material e métodos

Os reagentes utilizados foram de grau de pureza analítica e a água utilizada foi deionizada. Os materiais utilizados foram previamente descontaminados em HNO_3 a 10% (v/v) por no mínimo 24h. Frutos de açaí foram adquiridos em seus ambientes de produção em Boa Vista, Roraima. Foram tomadas porções de 0,2-0,5 g das amostras homogeneizadas, e adicionados sequencialmente 5,0 mL de HNO_3 concentrado, 2,0 mL de H_2O_2 a 30% (v/v) e 0,5 mL de ítrio (Y) a 100 $mg.L^{-1}$,o qual foi usado como padrão interno. A mistura obtida foi homogeneizada e submetida à digestão em forno microondas (MARSX press 6.0), de acordo com o método estabelecido pela AOAC (2002). O material digerido foi diluído com água deionizada para 25,0 mL e filtrado em papel de filtro quantitativo (28 µm),antes das análises dos teores do mineral. As determinações do teor de lítio foram feitas por Espectrometria Ótica com Plasma Indutivamente Acoplado (ICP-OES) (Shimadzu, modelo 9820, com nebulizador concêntrico), sob 1,2 kW de potência, 10 $L.min^{-1}$ de argônio e comprimentos de onda de 610,364 nm. A curva analítica foi construída com 7 valores de concentração, mediante diluição de uma solução padrão de Li a 1000 $mg.L^{-1}$ em 2 % (v/v) de HNO_3.

Resultado e discussão

Eficiência da metodologia analítica O método empregado para a determinação do teor de Li nas amostras de açaí mostrou-se preciso, com valores de RSD de 5,37%. A faixa linear instrumental, obtida mediante injeção das soluções padrão, foi de 0,01875 a 20,0 $mg.L^{-1}$. Os valores de limite de detecção (LOD) e de limite de quantificação (LOQ) foram de 0,1 e 0,4 $\mu g.L^{-1}$, respectivamente, acima do limite do instrumento para o elemento, porém dentro de um elevado nível de sensibilidade, considerando a linearidade. *Avaliação da presença de Li no fruto* A concentração média de lítio encontrada nas amostras de açaí foi de 15,34 $mg.100g^{-1}$ de amostra fresca. Fazendo-se uma comparação com outros frutos também muito consumidos na região, esse teor médio foi superior ao encontrado no fruto abricó do norte (9,5 $mg.100g^{-1}$), também amazônico, e inferior ao teor médio de Li encontrado na maçã (35 $mg.100g^{-1}$), uma fruta não autóctone, mas que fora paulatinamente introduzida na alimentação, sendo igualmente apreciada pelos amazonenses e nortistas (VAITSMAN, VAITSMAN, e AZEVEDO, 1991). A exigência diária mínima de lítio têm sido motivo de divergências. Apesar de Schrauzer (2002) defender ser 1 $mg.dia^{-1}$, Marshall (2015) estima uma necessidade de quantidades maiores, por considerar que existem diferenças individuais que podem exigir ingestão maior para uma saúde ideal, levando em conta ainda a perda de energia diária, que é diferente para cada indivíduo. Além disso, a EPA estimou que uma ingestão dietética de lítio nos EUA entre 0,6 a 3,1 $mg.dia^{-1}$ (NATIONAL INSTITUTES OF HEALTH, 1985). Nos Andes, ao norte da Argentina, o consumo estimado é de 2 a 30 $mg.dia^{-1}$(CONCHA et al., 2010). Em consumidores franceses, estimou-se um consumo médio díario de 11 µg para lítio (NOËL et al., 2003). Embora exista divergência na ingestão diária recomendada (IDR), a avaliação nutricional da polpa de açaí permite classificar o fruto com sendo um alimento de alto teor em lítio, tendo como base o Relatório Técnico do Ministério da Saúde (BRASIL, 1998), o qual classifica um alimento como fonte em determinado mineral, quando 100 g deste apresentar de 15 a 29 % de sua IDR, e um alimento de alto teor mineral, quando este apresentar mais que 30 % da sua IDR. É importante salientar que mecanismos muito diferentes governam altas e baixas doses de lítio. Somente em doses muito altas, cerca de 50 a 300 vezes maior do que a ingestão dietética natural de alimentos

e água, o lítio atua como uma droga (NOËL et al., 2003). Neste sentido, não são ainda conhecidos os valores basais para o lítio na região em que as amostras de açaí foram produzidas. Assim, os valores reportados no presente estudo representam novos dados referentes ao potencial nutricional do fruto.

Conclusões

Os resultados aqui apresentados fornecem novas informações sobre o conteúdo de lítio presente na polpa do açaí estudada. Apesar de não serem conhecidos os valores basais para o lítio na região norte brasileira, as informações aqui apresentadas são importantes para profissionais da química, geoquímica, medicina, nutrição, biologia e biotecnologia.

Agradecimentos

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DETERMINAÇÃO DE COMPOSTOS FENÓLICOS EM TRÊS FRUTOS DA PRÉ-AMAZÔNIA MARANHENSE

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Área

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Resumo

Considerando a imensa diversidade de frutos tropicais do Brasil e as escassas informações sobre suas composições químicas, este trabalho teve como objetivo avaliar três espécies: abricó (Mammea americana), ingá (Inga edulis Mart) e murta (Eugenia punicifolia), por meio da determinação do teor de compostos fenólicos. Em todos os frutos foram detectadas concentrações significativas de fenóis totais, sendo que a murta apresentou o maior potencial (734.08 ± 26.31) seguida do ingá (660.49 ± 11.09) e abricó (223.87 ± 13.75). Dados inéditos foram apresentados neste estudo para a murta. De modo geral, os resultados fornecem informações da composição química de frutos tropicais pouco conhecidos que poderão ser explorados tanto na indústria de alimentos quanto de cosméticos e/ou farmacêutica.

Palavras chaves

Compostos fenólicos ; frutos tropicais; Pré-Amazônia Maranhense

Introdução

O Maranhão representa uma área de transição entre o Nordeste e a região amazônica. Dessa forma, reúne uma diversificação ecológica com flora rica e variada com valioso potencial genético de espécies nativas produtoras de frutos. Entretanto, dados sobre a realidade social, econômica e o quadro de precariedade da saúde e da nutrição registrado na região contrastam com a sua riqueza em recursos biológicos (MARTINS; OLIVEIRA, 2011). Em relação aos compostos fenólicos, sabe-se que estes são metabólitos secundários de plantas que exercem função de fotoproteção, defesa contra microorganismos e insetos, além de serem responsáveis pela pigmentação e por algumas características organolépticas dos alimentos (HORST; LAJOLO, 2014; ALVES, 2010). Vários estudos epidemiológicos indicam que a alta ingestão de produtos vegetais está associada com uma redução no risco de uma variedade de doenças crônicas como aterosclerose e câncer, efeitos que são atribuídos, entre outras substancias, aos compostos fenólicos, especialmente os flavonóides, e os carotenoides (CARDOSO et al.; 2010). Diante disso, a caracterização de frutos em relação à compostos fenólicos é de grande relevância por se referir a novos dados de constituintes considerados importantes à saúde humana. Considerando a importância destes alimentos aliado a potencialidade de algumas espécies ainda pouco exploradas, esse trabalho objetivou realizar esta caracterização em três frutos tropicais ainda pouco conhecidos, coletados no estado do Maranhão: abricó (Mammea americana), ingá (Inga edulis Mart) e murta (Eugenia punicifolia).

Material e métodos

Amostra e amostragem Foi contemplada, nesse estudo, os frutos: abricó (Mammea americana), ingá (Inga edulis Mart) e murta (Eugenia punicifolia), adquiridos in natura no município de Paço do Lumiar (MA). As amostras foram devidamente acondicionadas em sacos plásticos, rotuladas, armazenadas e transportadas ao laboratório. No laboratório as amostras foram lavadas com a finalidade de remover partículas do solo, poeiras e outros resíduos e armazenadas a - 20 °C (freezer) até o momento da análise. Determinação de compostos fenólicos O teor total de fenol foi determinado de acordo com Pueyo & Calvo (2009) e Berker et al. (2010), com algumas modificações e adaptadas ao leitor de microplacas. Foram adicionados, nos poços das microplacas, 25 µL de extrato etanólico de polpa, 157,5 µL de água desionizada, 5 µL de HCl (1 mol.L-1) 37,5 µL K3 [Fe (CN) 6] (1% m / v), 12,5 de dodecil sulfato de sio (1% v / v) e 12,5 de FeCl3.6H2O (0,2% m / v). A leitura da absorvância foi realizada após 30 minutos a 750 nm utilizando um leitor de ELISA (Biotek). A curva de calibração foi obtida utilizando soluções padrões de ácido gálico em diferentes concentrações (R^2 = 0,9947). Os resultados foram expressos em equivalentes de ácido gálico em miligramas por 100 g de polpa (EAG mg.100 g-1).

Resultado e discussão

Os resultados para as concentrações obtidas de compostos fenólicos nos frutos estudados foram submetidos à análise de variância (ANOVA), seguida da aplicação do teste Tukey (5 % de significância), a fim de verificar a existência de diferenças significativas entre as concentrações. Os resultados das concentrações, em EAG mg.100g-1, com seus respectivos desvios-padrão são apresentados na Tabela 1. Entre as frutas estudadas, murta apresentou o maior teor de compostos fenólicos, seguido de ingá e abricó. Comparando os resultados obtidos com os dados da literatura, é possível observar que os teores de compostos fenólicos obtidos são superiores ao intervalo relatado por Braga et al. (2010) (23,11-27,71 mg EAG.100g-1) e Vasconcelos (2015) (132 mg EAG.100g-1) para a polpa fresca do abricó. Os teores obtidos neste estudo para polpa de ingá são superiores aos descritos por Souza et al. (2008) (230-250 mg GAE.100g-1, base seca) e menor que Pompeu et al. (2012) (980 mg EAG.100g-1, base seca). Nenhum registro de compostos fenólicos foi encontrado para murta. De modo geral os frutos contemplados podem ser considerados fontes promissoras de compostos fenólicos, estando atreladas a eles, elevadas propriedades antioxidantes, as quais são potenciais na aplicação nas indústrias farmacêutica, cosmética e de alimentos.

Tabela 1 – Concentrações dos compostos fenólicos para os frutos estuda

Frutos	Compostos fenólicos EAG mg 100 g^{-1}
Abricó	223.87 ± 13.75
Ingá	660.49 ± 11.09
Murta	734.08 ± 26.31

Médias seguidas da mesma letra, nas colunas, não diferem significativamente entre si, pelo teste de Tukey em um nível de 5% de probabilidade.

Conclusões

Todos os frutos apresentaram concentrações significativas de compostos fenólicos, sendo que a polpa de murta apresentou o maior conteúdo. Os resultados dos frutos foram satisfatórios e revelaram que eles são fontes promissoras para exploração industrial. Este estudo trouxe novas informações sobre composições químicas úteis para os setores da biotecnologia e que certamente auxiliará os consumidores na escolha dos alimentos, bem como contribuirá para a orientação nutricional por especialistas com princípios de desenvolvimento local e diversificação na alimentação.

Agradecimentos

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DETERMINAÇÃO DE ELEMENTOS TRAÇOS NA ÁGUA E NO SEDIMENTO DO BAIXO CURSO DA BACIA DO ITAPECURU-MA, BRASIL

Área

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Ambiental

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Resumo

O presente trabalho objetivou avaliar parâmetros físico-químicos nas águas e de elementos metálicos (Al, Cd, Cr, Fe, Mn, Pb, Se e Zn) na água e no sedimento no baixo curso do rio Itapecuru, em função de suas variações espaciais (três cidades Rosário, Santa Rita e Itapecuru Mirim) e temporais (estações seca e chuvosa). Em relação aos limites estabelecidos pela legislação brasileira, os resultados das análises físico-químicas da água para o período chuvoso a qualificam para o consumo humano, enquanto que para a estação seca observam-se inadequações nos parâmetros turbidez, oxigênio dissolvido, sólidos totais dissolvidos e salinidade. De modo geral, os resultados evidenciam um efeito sazonal significativo ($p < 0,05$) nas amostras de água e de sedimento.

Palavras chaves

Rio Itapecuru; Poluição; Elemento traço

Introdução

Rico em bacias hidrográficas de grandes dimensões, o Estado do Maranhão diferencia-se pela estabilidade e volume expressivos de água durante todo o ano. Dentre as bacias hidrográficas, a do rio Itapecuru merece destaque, em razão de ser a segunda maior bacia genuinamente maranhense, com 52.972 krn2 de extensão, correspondendo a 16% da superfície do estado (BARROS, FRAGA e BIRINDELLI, 2011). O rio Itapecuru e seus afluentes atravessam 55 cidades e, assim, vem contribuindo historicamente para o desenvolvimento destas, desempenhando papel relevante no povoamento, na expansão da produção agrícola por meio de importantes rotas de navegação para o interior do estado, bem como no progresso de usinas e indústrias de beneficiamento. Mas, apesar de sua inegável importância, em toda a sua extensão da bacia é possível observar diversas evidências de degradação ambiental, tais como descargas de efluentes domésticos e industriais, lixiviação de pesticidas de áreas agrícolas, degradação do solo, destruição da mata ciliar, escassez de diversidade de peixes e remoção ilegal da areia. A combinação desses fatores adversos têm sido responsável pela perda de 73% do seu volume (COSTA, et al., 2015). O contínuos dano às potencialidades naturais da bacia evidenciam a necessidade de estudos ambientais da qualidade da água e dos sedimentos, de modo a se identificar fontes de poluição e, a partir daí, estabelecer medidas concretas para sua recuperação. A composição físico-química de um corpo d'água é um indicador da sua qualidade, uma vez que reflete o ambiente natural, bem como revela a introdução de substâncias químicas, quando valores superiores aos estabelecidos pela legislação são atingidos (GUPTA, PANDEY e HUSSAIN, 2017). De modo semelhante, a ocorrência de elevadas concentrações de espécies inorgânicas, tais como íons metálicos nos sedimentos, pode ser uma boa indicação de poluição antropogênica (KUMAR, et al., 2016). A contaminação por esses elementos no ambiente aquático, em especial os metais pesados, é preocupante, em razão da abundância, persistência e ecotoxicidade desses poluentes (ISLAM, et al., 2015; AHMED, et al., 2015). Como os sedimentos integram os contaminantes ao longo do tempo e estão em constante fluxo sobrejacente com a coluna d'água, a análise dessas espécies químicas permite o conhecimento da sua distribuição e comportamento nos sedimentos costeiros, fornecendo ainda um registro da história espacial e temporal da poluição em uma determinada região ou ecossistema (LI, et al., 2018). O presente estudo teve por objetivo avaliar os parâmetros físico-químicos da água, no baixo curso do rio Itapecuru bem como analisar a distribuição de algumas espécies metálicas na coluna d'água e no sedimento, nas duas estações do ano e em diferentes cidades. O foco dessa avaliação foi um pré- diagnóstico da situação hidrografia do rio, urbanização, empresas industriais e demais atividades antropogênicas.

Material e métodos

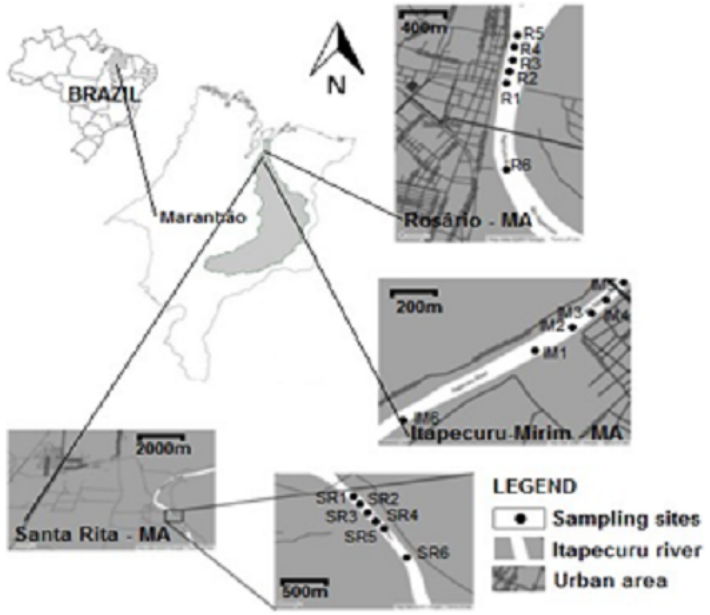
As amostras da água e sedimentos foram coletadas nas cidades de Santa Rita, Rosário e Itapecuru-Mirim (Figura 1), nos meses de setembro a novembro de 2016 (estação seca) e em abril de 2017 (estação chuvosa). Foram definidos seis pontos amostrais para cada cidade, considerando, na seleção destes, algumas características de área, como proximidade de atividades humanas como o desmatamento, perda de solo, erosão e despejo de dejetos domiciliares e industriais (Santos, et al., 2015; Feitosa e Almeida, 2012). Para a determinação das espécies metálicas, foi realizado o preparo das amostras, onde uma porção de 5 mg do sedimento foi transferida para o vaso de polipropileno que, que acompanha o aparelho (MARS X-Press), e a este adicionado um volume de 10 mL de HNO_3 concentrado. Em seguida, a amostra foi submetida à digestão no forno micro-ondas, conforme programa contido no Workstation do equipamento,baseado no método EPA 3015a (US-EPA, 2007). Após digestão, a mistura resultante foi diluída para 50,0 mL com uma solução de HNO_3 a 2 %(v/v), depois filtrada através de papel filtro quantitativo (28 µm) diretamente para frascos de polietileno, em seguida analisada por ICP- OES. Imediatamente antes das leituras, a solução foi diluída na proporção 1:10, devido à elevada concentração dos elementos, que a princípio fornecia medidas por ICP-OES acima das faixas lineares. Com relação às amostras de água, não houve necessidade da etapa de digestão ácida; um volume de 100 mL de cada amostra foi submetido à filtração a vácuo, seguida de adição de 2mL de HNO_3 concentrado e conservação a -10°C, até o momento da análise por ICP- OES. Amostras em branco, consistindo de água deionizada acidificada do mesmo jeito, foram preparadas para cada análise, tendo sido esta realizada em triplicata. Após testes da resposta espectrofotométrica baseados na avaliação da razão sinal/ruído, como maior sensibilidade de cada elemento, foram estabelecidas, otimizadas a saber: nebulizador: do tipo pneumático de tubo concêntrico; software: ICPE - solutionLauncher; gerador de radiofrequência a 1,2 KW; Vazões do Argônio: 0,6 L min^{-1}e 10 L min^{-1} para os cilindros auxiliar e principal, respectivamente; fluxo do gás de arraste: 0,7 L min^{-1}; velocidade de rotação da bomba peristáltica: 40 rpm; correção de fundo: 2 pontos; visão axial com os seguintes valores de comprimento de onda (nm): Al = 167,081; Cd = 226,502; Cr = 267,716; Fe = 239,562; Pb = 220,353; Se = 196,090, e Zn = 213,856. De modo a caracterizar a eficiência do método como um todo, desde o preparo das amostras até a análise por ICP- OES, foram adotadas as seguintes figuras de

mérito: precisão, determinada por meio dos cálculos dos desvios padrão relativos (RSD); sensibilidade, através da determinação dos limites de detecção (LDs) e de quantificação (LQs), e linearidade. Todos os cálculos foram feitos a partir da curva analítica de cada elemento, construída com no mínimo sete pontos (Vanini, et al., 2015).

Resultado e discussão

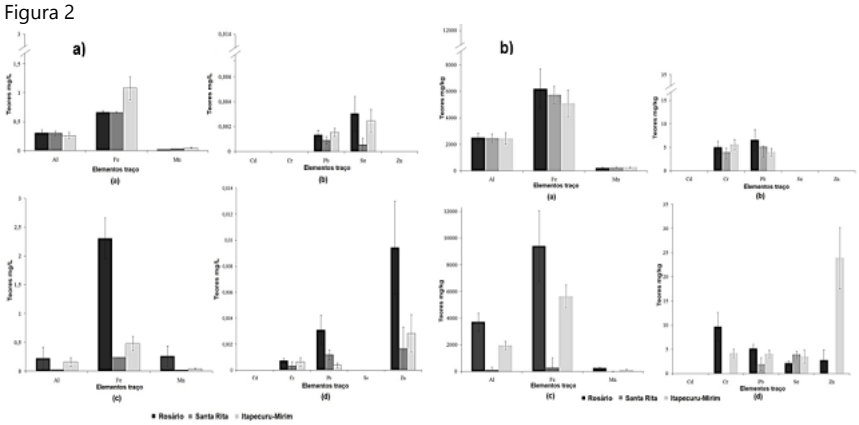
Os resultados das análises físico-químicas das amostras de águas superficiais, bem como os valores estabelecidos pela legislação como limites máximos a resolução 357/2005 do Conselho Nacional do Meio Ambiente (CONAMA, 2005). As temperaturas da água estiveram na faixa de 29,9 a 32,2°C na estação seca e 28,34 a 31,76°C no período chuvosa, não tendo sido verificadas diferenças significativas entre os resultados ($p > 0,05$). As maiores temperaturas durante o período de estiagem (estação seca) coincidiram com o período de menor pluviosidade e vazão dos respectivos pontos de coleta. Em relação ao pH, todos os valores estiveram dentro da faixa ideal (6,0 - 9,0) (CONAMA, 2005), exceto para o ponto R1 na estação seca, que apresentou pH 5,4. O potencial de oxidação/redução (POR) foram obtidos para os pontos na cidade de Rosário (177 – 183 mV), enquanto que durante a estação chuvosa, foi possível notar certa concordância entre os resultados em todas as localidades. Nas cidades de Santa Rita e Itapecuru Mirim, os resultados de turbidez (TU), em ambas as estações, atenderam aos valores máximos permitidos (VMP) para águas doce (>100 NTU); porém, para a cidade de rosário, valores de turbidez muito elevada (102 -165 NTU) foi verificado, no período seco, com exceção do ponto R1. Os valores de oxigênio dissolvido (OD) encontrados na estação chuvosa variaram de 4,87 a 7,98 mg.L- 1, tendo sido superiores àqueles obtidos na estação seca (3,34 - 6,67 mg.L- 1). Tal diferença, confirmada estatisticamente ($p < 0,05$), pode ser atribuída, entre outros fatores, ao fato de que a solubilidade dos gases em água diminui com a elevação da temperatura (Canpana, et al.,1996). Comparando-se o ponto 6 aos demais pontos coletado, durante a estação seca, observa-se uma diminuição do nível de OD nas cidades de Santa Rita e Itapecuru-Mirim, o que pode estar associado ao despejo de origem orgânica, como efluentes domésticos vindos das comunidades ribeirinhas localizadas nas proximidades. Confrontando os resultados de OD obtidos na estação chuvosa com os valor de referência (OD > 5 mg.L-1)(Conama, 2005), observa-se que, com exceção do ponto amostral IM6, todos os pontos mostraram possuir água adequada de qualidade. Na estação seca, a maioria dos pontos revelou valores de OD próximos ou um pouco inferiores a esse limite, principalmente na cidade de Rosário. O parâmetro sólidos totais dissolvidos (STD) apresentou valores superiores ao valor de referência (STD ≤ 0,5) (CONAMA, 2005), na estação seca. Nessas áreas, a lixiviação agrícola, a presença de rejeitos da pecuária, a contaminação do solo, com conseqüente poluição da água pelo uso de fertilizantes são fatores, além das fontes pontuais de descarga de águas poluídas (Parron, Muniz e Pereira, 2011), são fatores que resultam na elevada quantidade de íons dissolvidos na água. Durante a estação seca, a maioria dos pontos avaliados na cidade de Rosário não atendeu aos valores de referência em relação aos parâmetros TU, OD, STD e SAL, bem como alguns pontos das cidades de Santa Rita e Itapecuru-Mirim, em relação ao parâmetro OD. Por outro lado, durante a estação chuvosa, todos os parâmetros físico- químicos em todas as cidades encontram-se dentro da faixa admitida, exceto OD no ponto IM6, como já mencionado. A média das concentrações dos 8 elementos traços em águas superficiais, de acordo com a estação (Seca/chuvosa), são mostrados na Figura 2a (a – d). Foram detectados teores dos elementos alumínio (Al) e ferro (Fe) acima dos valores máximos permitidos (VMPs) (CONAMA, 2005), em alguns pontos das cidades de Rosário e Itapecuru-Mirim, durante a estação seca. Isso revelou os impactos provocados não só pelos despejos industriais, provavelmente provenientes das siderurgias, mas também pelos lixões localizados nas proximidades dos pontos amostrais, sendo que estes últimos vêm ocasionando contínua contaminação dos solos e consequentemente das águas do rio. Isso ficou evidente em decorrência da discrepância dos teores desses elementos observados nos demais pontos amostrados na mesma área. De toda forma, as altas concentrações de Al podem estar influenciando negativamente a vida aquática nessas localidades. Vale mencionar que já foram observadas alterações fisiológicas em peixes causadas pelo Al, além de desregulações hematológicas, metabólicas, respiratórias e do sistema nervoso (Sivakumar, khatiwada e sivasubramanian., 2012; Meyer- Baron, et al., 2007). Em humanos, estudos associam a presença do Al à ocorrência de casos de autismo (Mold, et al., 2018) e mal de Alzheimer (Mirza, et al., 2017; Stephens e Jolliff, 2015). Hoppe, et al. (2015) afirmam ainda que a presença do íon $Al3+$ no meio aquoso influencia a especiação de outros metais, como observado nos ensaios ecotoxicológicos empregando Daphynia magna; neste mesmo estudo, foi observado ainda que a presença do Al favorece a biodisponibilidade de Cu, o que pode representar um aumento da biossorção desse metal tóxico. Foram detectadas concentrações de Fe próximas de 3,0 mg.L-1 em algumas amostras de água na cidade de Rosário (R3 e R6), no período seco. Estudos recentes, envolvendo ensaios ecotoxicológicos, mostraram que a espécie de peixe Daniorerio sofreu bioacumulação na faixa de 1,25 a 1,32 mg.g-1 (Zhang, et al., 2015). No tocante ao Mn, as concentrações detectadas na água estiveram abaixo dos VMPs na maioria das cidades, tanto no período úmido quanto no seco. A exceção foi verificada também na cidade de Rosário, durante a estação seca, com teores de Mn iguais ou superiores aos valores limites. Todos os pontos amostrados apresentaram concentrações das espécies metálicas Cd, Cr, Pb, Se e Zn abaixo dos valores de referências. As concentrações médias de Al e Se apresentaram-se mais elevadas durante o período seco nas cidades de Santa Rita e Itapecuru-Mirim. Por outro lado, maiores concentrações de Zn, em todos os pontos amostrados, foram obtidas durante a estação chuvosa, assim como Fe e Mn em Rosário, e Cr e Pb em Rosário e Santa Rita. As concentrações desses microelementos diferiram estatisticamente ($p < 0,05$) nos dois períodos sazonais. De modo geral, concentrações de Al e Fe acima do VMP foram obtidas nas amostras de água avaliadas durante a estação seca nas cidades de Rosário e Itapecuru-Mirim, e, durante a estação chuvosa, em todas as cidades. Apenas um ponto de coleta (R5) mostrou teores de Mn acima do VMP durante a estação seca. A hidrogeomorfologia, aliada às atividades antropogênicas, como presença de lixões, esgotos e usinas metalúrgicas nas proximidades das margens do rio, no referido ponto, podem estar contribuindo para estes resultados. Os teores de quatro espécies metálicas (Cd, Cr, Pb e Zn), encontrados na amostras de sedimentos, foram comparados aos valores de referência propostos pelo Conama (2012), a qual baseia-se nos limites estabelecidos pelo Conselho Canadense do Ministério do Meio Ambiente (CCME) (Rezende, et al., 2011). Para os demais elementos, não existem valores de referência para sedimentos, e observou-se ainda ausência de legislação específica de solos e sedimento para o Estado do Maranhão. Verificou-se que todas as amostras analisadas apresentaram concentrações dos elementos Cd, Cr, Pb e Zn menores que os VMPs estabelecidos por essa norma. Da mesma forma que nas águas, os elementos Al e Fe apresentaram-se em altas concentrações nos sedimentos, na cidade de Rosário. As espécies metálicas tóxicas Cr e Pb também foram encontradas em maior quantidade nos sedimentos coletados em Rosário ($p < 0,05$), o que torna evidente a influência das atividades antropogênicas nessas áreas que, de todas as analisadas, apresentaram maior densidade populacional. Apesar das concentrações de Mn, nas amostras de água (Fig. 2a), terem sido relativamente baixas, no sedimento estas foram consideradas altas (Fig. 2 b). Esses resultados revelam uma situação preocupante, pois a presença desses micropoluentes pode estar comprometendo seriamente a biodiversidade aquática.

Figura 1



Localização da área de estudo e dos pontos de coleta das amostras de água.

Figura 2



Média das concentrações dos 8 elementos traços em águas superficiais, de acordo com a estação (Seca/chuvosa)(a) e sedimentos (b).

Conclusões

No presente estudo, foi avaliada a qualidade da água do baixo curso do rio Itapecuru, em relação aos parâmetros convencionais de qualidade e aos teores de diversas espécies de macro e microminerais, nas estações seca e chuvosa. Embora a maioria dos resultados dos parâmetros físico-químicos indicou uma qualidade da água relativamente boa, os teores de espécies minerais, sobretudo de alguns metais pesados, por sua vez revelam um quadro de contaminação e suscitam estudos mais aprofundados. Apesar da elevada importância da bacia do rio Itapecuru, não só para a manutenção da vida, mas também por servir de fonte de captação hídrica para milhares de famílias maranhenses que utilizam suas águas para diversas atividades, comprovou-se, pelas análises químicas, que esse enorme corpo d'água vem sendo atingindo negativamente pela deficiência na gestão pública com relação ao descarte de resíduos sólidos e líquidos contaminados. Tais efluentes são provenientes de várias fontes, tais como esgotos domésticos, efluentes industriais e agrícolas, resíduos de siderurgias e chorume escoado dos lixões localizados no entorno. Levando em consideração a escassez de trabalhos desse tipo para o Estado do Maranhão, especialmente para o Rio Itapecuru, o presente estudo evidenciou ainda a necessidade de uma análise mais detalhada, baseada na avaliação dos níveis de contaminação por esses elementos em toda a extensão da bacia do rio Itapecuru, não só na água e nos sedimentos, mas também no solo, na fauna aquática, e também nos efluentes provenientes de empreendimentos eatividades poluidoras. Contudo, ainda que sejam preliminares, os resultados aqui apresentados permitem concluir que existe um caso preocupante de contaminação por elementos traço prejudiciais não só à biota aquática, como também à saúde humana. Nessa perspectiva, esse estudo evidencia um início promissor de uma linha de pesquisa que, apesar de ser bastante explorada em todo o mundo, ainda é escassa para o Estado do Maranhão.

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DETERMINAÇÃO DO POTENCIAL ANTIOXIDANTE O ABRICÓ (MAMMEA AMERICANA) EMPREGANDO O MÉTODO NBT

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Área

Alimentos

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Resumo

O aumento do estresse oxidativo leva à busca de alimentos que possam prevenir ou minimizar o dano oxidativo. A capacidade antioxidante do abricó (*Mammea americana*) foi avaliada pelo método NBT. Foi feita uma comparação com a acerola, que é uma reconhecida fonte em antioxidantes. A porcentagem de inibição do radical $O2^{\bullet -}$ do abricó foi de 88,17% estando bem próxima à da acerola (96,84%). Este estudo forneceu novos dados sobre a atividade antioxidante de um fruto pouco explorado, bem como uma nova alternativa analítica para se avaliar tal propriedade em matrizes complexas. Com base no presente estudo poder-se-ia considerar o abricó uma fruta adequada para uso nas indústrias de alimentos e cosméticos, bem como em composições farmacêuticas.

Palavras chaves

Mammea americana; atividade antioxidante ; NBT

Introdução

Os radicais livres possuem diferentes papéis no organismo e encontram-se envolvidos em uma série de reações químicas e biológicas importantes. Entretanto, seu excesso apresenta efeitos deletérios, tais como danos ao DNA e proteínas provocando alterações na estrutura e funções celulares e, dessa forma, se encontram envolvidos em diversas patologias (ALVES et al., 2010). Tais espécies químicas são bastante reativas e instáveis, possuindo vida curta. A formação destas moléculas ocorre naturalmente no organismo de todos os seres vivos, devido à exposição ao oxigênio molecular; no entanto, a produção aumentada das espécies de oxigênio reativo podem conduzir ao chamado estresse oxidativo (VASCONCELOS et al., 2014), dificultando a manutenção de muitas funções fisiológicas. A produção destas substancias pode ser controlada por diversos compostos antioxidantes, presentes em diversos alimentos como os frutos, e isso tem sido muito explorado pelas indústrias de alimentos e farmacêuticas. Numa dieta saudável, as frutas desempenham papel de grande destaque pela saúde que nos proporcionam, traduzida em aumento da expectativa de vida, vitalidade e prevenção de inúmeras doenças devendo estar presentes diariamente nas refeições (LORENZI et al., 2006). Apesar da diversidade em frutos tropicais, e da exploração reconhecida para algumas espécies, ainda são necessárias pesquisas para a completa elucidação dos seus constituintes químicos. Dentro deste contexto, o desenvolvimento de pesquisas visando ao estabelecimento de estratégias para a aquisição de novas informações sobre a composição química de frutos tropicais, tais como o abricó, é pertinente, pois possibilitará conhecer os seus estágios atuais de produção, consumo e a influência sofrida por estas espécies vegetais nos processos ambientais.

Material e métodos

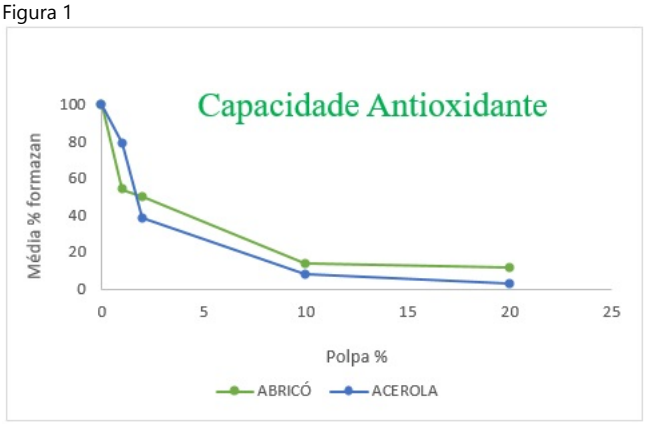
Os reagentes utilizados foram: Nitrotetrazolium blue chloride (NBT, ref. N6876); hipoxantina (HX, ref. H9377), ácido L-ascórbico (ref. A5960), enzima xantonoxidade extraída de leite bovino (XOD, ref. X4376), todos da Sigma. As soluções foram preparadas usando água Milli-Q. O fruto utilizado no estudo, abricó (Mammea americana), foi obtido em Paço do Lumiar, Maranhão, Brasil. Também foi avaliada a acerola, para fins de comparação, por conta de seu reconhecido potencial antioxidante. Inicialmente, as amostras foram lavadas, despolpadas e misturadas com o auxílio de um misturador de aço inoxidável. Em seguida, foram filtradas a vácuo em funil de Buchner com papel de filtro qualitativo, seguido por uma nova filtração com papel de filtro quantitativo (1,2 µm). Um volume de 500 uL do extracto obtido foi diluído para 1 mL com solução de fosfato de potássio (K-PB) 50 mM contendo EDTA 0,1 mM, pH 7,5.. A partir desta solução, foram efetuadas diluições sequenciais utilizando a mesma solução tampão. Feito isso, uma mistura reacional foi preparada com 175 µL de solução de K-PB 50 mM contendo EDTA (0,1 mM), pH 7,5, 25 µL de solução de HX 25 µM, 25 µL de solução de NBT 50 µM, 25 µL do extrato antioxidante (água destilada para o branco) e 25 µL da solução da enzima XOD com uma atividade específica de 0,2 U mL^{-1}, sendo esta última adicionada sempre por último. Em seguida, foram realizadas medições colorimétricas com um espectrofotômetro UV- Vis Beckman DU520 (Beckman Coulter France, S. A., Roissy CDG, France). O aumento da absorbância durante 3 min foi registado a 560 nm. A porcentagem de atividade sequestradora de radicais livres (RSA) dos extratos vegetais foi calculada usando a seguinte fórmula: % RSA = 100 x [(controle abs. - amostra abs.) / Controle abs] Onde, Abs. controle é a absorbância do formazan sem a amostra; Abs. amostra é a absorbância de formazan com a amostra. O experimento teve delineamento inteiramente casualizado e os dados gerados foram analisados estatisticamente, por comparação empregando o teste t de Student.

Resultado e discussão

A atividade antioxidante dos frutos abricó e da acerola é expressa pela taxa de produção de formazan para diferentes massas de títulos (% m / v), e os resultados são mostrados na Fig. 1. A inibição dos radicais $O2^{\bullet -}$ gerados pela ação antioxidante das polpas é revelada pela pequena quantidade de NBT reduzida a formazan. Aqui, observou-se que o abricó apresentou boa atividade antioxidante. Avaliando a proximidade dos resultados obtidos para o abricó e a acerola, testes F e t não pareados foram aplicados usando limites de confiança de 95% para ver se eles mostram diferenças significativas entre a precisão e as médias de absorbância, respectivamente. A Tab. 1 mostra os valores de F e t calculados. Os resultados dos testes t e F mostraram que houve diferenças significativas entre os valores de produção de formazan (%) obtidos para as amostras estudadas para todas as diluições, com 95% de confiança. Os resultados mostraram que o superóxido RSA na acerola foi de 96,39% e 88,17% para o abricó. Os resultados não foram estatisticamente semelhantes; no entanto, os frutos

são de grande importância, do ponto de vista de capacidade antioxidante, com destaque especial ao abricó que ainda é um fruto pouco conhecido e explorado.

Figura 1



Representação da produção média de NBT-formazan em concentrações decrescentes dos frutos amazônicos estudados

Tabela 1

%POLPA	1	2	10	20
Teste t	6,42	3,77	4,86	6,74
Teste F	0,21	0,08	0,62	0,05

F crítico com 95% de confiança para 2 graus de liberdade: 19,0; t crítico com 95% de confiança para 2 graus de liberdade: 2.78

valores de F e t calculados

Conclusões

O método NBT empregado mostrou ser uma alternativa analítica simples, rápida e relativamente barata para a determinação da capacidade antioxidante de frutos. A adição de amostras de frutas causou a diminuição do sinal de absorbância, permitindo a quantificação de sua capacidade antioxidante. Os resultados revelaram que os frutos estudados apresentam elevada atividade antioxidante. A acerola possui potencial antioxidante um pouco maior; no entanto, o abricó também apresentou um bom resultado. De modo geral os frutos contemplados neste estudo podem ser considerados excelentes fontes de antioxidantes, tendo grande valor para aplicação nas indústrias farmacêutica e de alimentos.

Agradecimentos

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DETERMINAÇÃO DO POTENCIAL MICROMINERAL DE MURTA (EUGENIA PUNICIFOLIA)

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Área

Alimentos

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Resumo

Foram analisadas amostras de Murta (Eugenia punicifolia) coletada no município de Paço do Lumiar- MA, quanto à presença de microminerais essenciais ao organismo. Analisou-se quantitativamente cobre, ferro, manganês e zinco, empregando Espectrometria Ótica com Plasma Indutivamente Acoplado (ICP- OES). Foram encontrados os seguintes teores dos minerais, em mg/100g: 0,16 para Cu; 0,75 para Fe; 0,42 para Mn e 0,07 para Zn. A metodologia analítica utilizada foi precisa com coeficientes de variação (CV) menores que 10 %. O fruto mostrou ser fonte em Cu e Mn correspondendo a 17,78% e 18,26%, respectivamente, aos índices diários recomendados (IDR). Os valores apresentados fornecem novas informações sendo úteis para a construção de uma base de dados de composição mineral e aplicação industrial.

Palavras chaves

Murta; ICP-OES; microminerais

Introdução

Numa dieta saudável, as frutas desempenham papel de grande destaque pela saúde que nos proporcionam, traduzida em aumento da expectativa de vida, vitalidade e prevenção de inúmeras doenças por serem ricas em uma vasta gama de vitaminas, minerais e fibras (LORENZI et al., 2006), devendo estar presentes diariamente nas refeições. Somado a isso, estudos revelam que plantas alimentícias não-convencionais são mais ricas nutricionalmente do que plantas domesticadas (KINUPP; BARROS, 2008). No Brasil, diversas espécies não tradicionais vêm sendo utilizadas pelas populações locais, por apresentarem grande potencial para exploração no mercado de consumo in natura e/ou na industrialização. No entanto, tais espécies necessitam ser preservadas, cultivadas racionalmente e caracterizadas através do estudo de suas propriedades, visando sua utilização no mercado de alimentos funcionais (RUFINO, 2008). Considerando a importância dos frutos tropicais aliada à potencialidade de algumas espécies ainda pouco exploradas, esse trabalho objetivou realizar a determinação de microminerais (cobre, ferro, manganês e zinco) em uma fruta tropical ainda pouco conhecida, coletada no estado do Maranhão, murta (*Eugenia punicifolia*). A murta (*Eugenia punicifolia*) é uma planta arbustiva de até 3 m de altura, sendo nativa e endêmica do Brasil, distribuída em todos os ecossistemas brasileiro. Suas flores e frutos são encontrados durante o ano inteiro, sendo estes, pequenos e arredondados de cor vermelho-alaranjado quando maduro, contendo 1 a 2 sementes. Em geral, seus frutos são consumidos in natura. Não obstante sua utilização para fins alimentício e terapeutico, a murta é pouco conhecida pelos brasileiros não sendo reconhecida ainda como uma espécie de importância para exploração econômica. Dados sobre a composição mineral são escassos. Diante disso, a caracterização deste fruto em relação à presença de minerais, é importante, não só por se referir a novos dados de constituintes considerados importantes à saúde humana, mas também por estar relacionado à saúde e produtividade do vegetal. O conhecimento da composição de frutas regionais é fundamental para incentivar a comercialização nacional e internacional; para a rotulagem nutricional a fim de auxiliar consumidores na escolha dos alimentos; para a orientação da educação nutricional por especialistas baseada em princípios de desenvolvimento local e diversificação da alimentação, assim como, para a avaliação e adequação da ingestão de nutrientes de indivíduos ou populações.

Material e métodos

Amostra e amostragem Foi contemplada, nesse estudo, o fruto murta (Eugenia punicifolia), adquirida in natura no município de Paço do Lumiar (MA), sendo S 02˚29'39.7" W044˚08'41.4" as coordenadas do ambiente de coleta . As amostras foram devidamente acondicionadas em sacos plásticos, rotuladas, armazenadas e transportadas ao laboratório, onde foram lavadas para remoção das partículas do solo, poeiras e outros resíduos e, em seguida, armazenadas a -20 °C até o momento da análise. *Determinação dos microminerais* O procedimento de digestão baseou-se no método AOAC (2002), utilizando 0,5 ml de ítrio (100 $mg.L^{-1}$) como padrão interno, em forno de microondas fechado (MARSX press 6.0). A solução resultante foi diluída com água desionizada para 25,0 ml em um balão volumétrico antes de ser analisada por espectrômetro de emissão óptica de plasma indutivamente acoplado (ICP-OES). Todas as análises foram realizadas em triplicata. Concentrações de quatro minerais (Cu, Fe, Mn e Zn) foram determinadas. As medições para determinação simultânea foram realizadas com um ICP OES (Shimadzu, modelo 9820), equipado com nebulizador concêntrico e permitindo a escolha da configuração entre o modo radial ou axial em uma unidade integrada. As condições operacionais estão resumidas na Tabela 1.

Resultado e discussão

Os resultados para as espécies minerais foram submetidos à análise de variância (ANOVA), seguida da aplicação do teste Tukey (5 % de significância), a fim de verificar a existência de diferenças significativas entre as concentrações. A avaliação nutricional das amostras estudadas foi feita tendo como referência o Relatório Técnico do Ministério da Saúde (BRASIL, 1998). Os resultados das concentrações, em $mg.100g^{-1}$, com seus respectivos CVs (%), bem como os LDs e LQs do método analítico utilizado são apresentados na Tabela 2. Avaliando os resultados das concentrações (Tabela 1), observa-se que os valores dos coeficientes de variaçã0 (CV%) estão abaixo de 8,99 %, indicando a precisão das determinações. A ingestão de 100 g das partes comestíveis da murta fornece 5,36; 1,0; 18,26 e 17,78 % de Fe Zn, Mn e Cu, respectivamente. Sendo estas duas últimas classificadas como fonte dos elementos por apresentarem teor acima de 15% da IDR. Do ponto de vista nutricional isso é muito importante, pois o Cu é essencial como constituinte de algumas metaloenzimas requeridas na síntese

da hemoglobina e na catálise de oxidação metabólica e o Mn previne sintomas que vão desde atraso no crescimento até ataxia (Onianwa; Adeyemo; Idowu, 2001; ERIKSON et al., 2005). Estudos indicam que o Zn faz parte da composição de várias enzimas envolvidas em funções fisiológicas, como síntese de proteínas e metabolismo energético. Já o Fe é responsável pela síntese das células vermelhas do sangue e também pelo transporte de oxigênio para todas as células do corpo (MAPA, 2010). Contudo, o fruto aqui investigado apresentou-se abaixo da IDR, o que torna necessária uma complementação alimentar para que o índice diário recomendando seja atingido. Não foram encontrados, na literatura, registros da concentração de minerais para murta. Vale ressaltar que o presente trabalho é o primeiro a apresentar dados destes parâmetros para esta fruta. Entretanto, ainda que uma comparação entre resultados seja complexa, em virtude das concentrações dependerem de fatores inter-relacionados como genética, solo, clima, estágio de maturidade da planta e biodisponibilidade (PEDROZO, 2003), fazendo-se uma comparação com os resultados divulgados pelo IBGE (2009) para os frutos pitanga (*Eugenia uniflora*) e araçá-açu (*Eugenia stipitata*), pertencentes ao mesmo gênero da murta, observa-se que a murta apresentou concentrações de Cu, Mn e Fe superiores aos relatados para pitanga, assim como as concentrações de Fe e Mn em araçá-açu estiveram inferiores as da murta. No entanto quando feita uma comparação entre as concentrações Zn do araçá-açu e da pitanga com as aqui relatadas, observa-se que os valores para murta foram inferiores.

Tabela 1

Parameter	Value
Radio Frequency Power	1.2 kW
Plasma argon flow rate	10 L min^{-1}
Auxiliary argon flow rate	0.6 L min^{-1}
Carrier Gas	0.7 L min^{-1}
Exposure time	30 s
Solvent Rinse Time	30 s
Peristaltic Pump Rotation Speed	20-60 rpm
View Direction	Axial for Cu, Fe, Mn and Zn
Nebulizer	Concentric
Emission lines (λ nm)	Cu (327.396); Fe (259.940); Mn (257.610); Zn (213.856).

Condições operacionais do método ICP-OES utilizado durante as análises

Tabela 2

	MINERAIS			
	Cu	Fe	Mn	Zn
LD(mg/L)	4,0 10-4	5,0 10-4	7,12 10-6	3,0 10-4
LQ (mg/L)	1,7 10-3	2,9 10-3	1,2 10-5	1,7 10-3
Fruto	CONCENTRAÇÃO (mg.100g^{-1})			
Murta	0,16 (3,16)	0,75 (8,99)	0,42 (1,91)	0,07 (8,48)
Valores entre parênteses: coeficientes de variação (CV%).				

Composição mineral dos frutos estudados

Conclusões

Com a avaliação da composição mineral do fruto, a murta se mostrou como fonte potencial dos minerais Cu e Mn, apresentando valores superiores a 15% do índice diário recomendado (IDR), 18,26% e 17,78%, respectivamente. Este estudo é um dos primeiros a fornecer uma avaliação detalhada das composições nutricionais da Eugenia punicifolia, ainda pouco explorada, utilizando métodos de ICP-OES. Trouxe ainda novas informações sobre composição química úteis que contribuirá para a orientação nutricional por especialistas com princípios de desenvolvimento local e diversificação na alimentação.

Agradecimentos

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ESTUDO DO NÍVEL PROTEICO, LIPDICO E AVALIAÇÃO DO PEREFIL ENERGÉTICO EM TRÊS FRUTOS DA PRÉ-AMAZÔNIA MARANHENSE

Área

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Resumo

Considerando a imensa diversidade de frutos tropicais do Brasil e as escassas informações sobre suas composições químicas, este trabalho teve como objetivo avaliar três espécies: abricó (Mammea americana), ingá (Inga edulis Mart) e murta (Eugenia punicifolia), por meio da determinação de proteínas e lipídios, assim como análise do perfil energético. As amostras avaliadas apresentaram um alto valor energético superiores a 50,8 Kcal 100g-1 e o maior teor em lipídeo foi obtido para a polpa de abricó (1,432 %), enquanto que, o ingá apresentou o maior teor proteico (1,52 %). De modo geral, os resultados fornecem informações da composição química de frutos tropicais pouco conhecidos que poderão ser explorados tanto na indústria de alimentos quanto de cosméticos e/ou farmacêutica.

Palavras chaves

Bromatologia; frutos tropicais ; Pré-Amazônia Maranhense

Introdução

O Maranhão representa uma área de transição entre o Nordeste e a região amazônica. Dessa forma, reúne uma diversificação ecológica com flora rica e variada com valioso potencial genético de espécies nativas produtoras de frutos. Entretanto, dados sobre a realidade social, econômica e o quadro de precariedade da saúde e da nutrição registrado na região contrastam com a sua riqueza em recursos biológicos (MARTINS; OLIVEIRA, 2011). Numa dieta saudável, as frutas desempenham papel de grande destaque pela saúde que nos proporcionam, traduzida em aumento da expectativa de vida, vitalidade e prevenção de inúmeras doenças por serem ricas em uma vasta gama de vitaminas, minerais e fibras (LORENZI et al., 2006), devendo estar presentes diariamente nas refeições. Em caso de comercialização, bem como, para a orientação da educação alimentar por especialistas o conhecimento da composição de frutas é fundamental para a rotulagem nutricional a fim de auxiliar consumidores na escolha dos alimentos. Portanto, esse trabalho objetivou realizar a determinação de proteínas e lipídios, assim como análise do perfil energético de três frutos ainda pouco conhecidos, coletados no Estado do Maranhão: abricó (Mammea americana), ingá (Inga edulis Mart) e murta (Eugenia punicifolia).

Material e métodos

Foi contemplada, nesse estudo, os frutos: abricó (Mammea americana), ingá (Inga edulis Mart) e murta (Eugenia punicifolia), adquiridos in natura no município de Paço do Lumiar (MA). O teor de proteína, lipídios e valor da energia total foi realizada de acordo com a Associação de Métodos Analíticos (métodos AOAC), com adaptações. O teor de proteínas foi determinado mediante a análise de nitrogênio pelo método Kjeldahl, que compreende três etapas: digestão, destilação e titulação. Na etapa da digestão 0,1 g das amostras foram tomados em tubos de digestão, adicionados 25 mL de H_2SO_4 a 0,05 mol L-1, e a mistura (TiO_2 e K_2SO_4; 1:2.) e submetida a digestão. Depois foi feita uma destilação com adição de NaOH a 40 % (m/v). Por fim, a quantidade de nitrogênio presente na amostra foi determinada pela titulação do H_2SO_4 a 0,05 mol L-1 com NaOH a 0,1 mol L-1, usando vermelho de metila como indicador. Para análise de lipídios foi feita extração direta em Soxhlet empregando como solvente hexano. 3 g da amostra foi tomada e transferida ao aparelho extrato, acoplou-se ao balão de fundo chato previamente tarado a 105 °C, adicionou-se solvente em quantidade suficiente para um Soxhlet e meio, adaptou-se ao refrigerador e aqueceu-se mantendo em extração continua por cerca de 6h. Destilou-se o hexano e aqueceu-se o balão com o resíduo extraído em estufa a 105°C por 1h. O teor de lipídeos foi obtido pela razão entre a massa de lipídeos e de amostra, multiplicada por 100. Os carboidratos totais foram determinados pela seguinte equação: [carboidratos totais = 100 - (umidade + cinzas totais + proteínas + lipídeos)] e o valor da energia total, em Kcal/100 g de amostra, foi estimado considerando o calor de combustão e a disgestibilidade a partir dos teores de proteínas, lipídios e carboidratos, utilizando o coeficiente de conversão de 9 Kcal por grama de lipídeos e 4 Kcal/g de proteínas e carboidratos.

Resultado e discussão

Os resultados obtidos para os frutos contemplados neste estudo são apresentados na Tabela 1, onde observa-se elevada precisão dos resultados, com coeficientes de variação inferiores a 10 %. Os resultados de cada parâmetro foram submetidos à análise de variância (ANOVA, $\alpha = 0,05$) seguidas do teste Tukey (5 % de significância), a fim de verificar a existência de diferenças significativas entre as concentrações obtidas. Segundo Moure et al., (2006), embora não sejam reconhecidas como fontes proteicas, a inclusão de proteínas vegetais na dieta humana traz diversos benefícios a saúde em relação aos animais, uma vez que são isentas em gorduras saturadas e aditivos químicos. Os frutos aqui estudados apresentaram os seguintes valores proteicos: 0,44%; 1,52% e 1,17% para o abricó, ingá e murta respectivamente. Uma comparação feita com a literatura mostrou que o fruto ingá apresentou teor de proteína superior aos teores reportados por Rueda (2012) e inferior aos reportados por Caramori, Souza e Fernandes. (2008). Os lipídios desempenham importantes funções no organismo dos seres vivos, sendo os principais depósitos de energia. Na indústria alimentícia, fornecem aroma, sabor e palatabilidade aos alimentos (FOOD INGREDIENTES BRASIL, 2016). O fruto que mostrou o maior conteúdo lipídico foi o abricó (1,432). O conteúdo energético está diretamente relacionado aos teores de lipídeos

nas amostras (R2= 0,99). As amostras avaliadas possuem um alto valor energético superiores a 50,8 Kcal 100g-1, podendo assim serem indicadas em dietas hipercalóricas.

Tabela 2 – Caracterização físico-química dos frutos estudados

Matriz	Proteína % (RSD)	Lipídeos % (RSD)	Carboidratos	Energia Kcal/100g
Abricó	0,44^{c} (6,35)	1,432^{a} (7,92)	20,05	62,05
Ingá	1,52^{b} (6,84)	0,72^{b} (8,49)	12,01	56,25
Murta	1,17^{a} (8,98)	0,03^{c} (9,43)	11,12	50,80

Médias seguidas da mesma letra, nas colunas, não diferem significativamente entre si, pelo teste de Tukey ao nível de 5% de probabilidade.

Conclusões

Os resultados dos frutos foram satisfatórios revelando-se fontes promissoras para exploração industrial. As amostras avaliadas possuem um alto valor energético superiores a 50,8 Kcal 100g-1 e o maior teor em lipídeo foi obtido para a polpa de abricó (1,432 %), já o ingá apresentou o maior teor proteico (1,52 %). Este estudo trouxe novas informações sobre composições químicas que auxiliará os consumidores na escolha dos alimentos e contribuirá para a orientação nutricional com princípios de desenvolvimento local e diversificação na alimentação.

Agradecimentos

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Sakhalin and the Czech knotweed is limited. Hence, the characterization of these two taxons represent the new challenge due to their altered biological and chemical activity.

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KN.2.4. FROM PRO-INFLAMMATORY MOLECULES TO THE BRAIN'S RESTING-STATE CONNECTIVITY. THE FUTURE OF CLINICAL DIAGNOSIS OF DEPRESSION

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Although scientists have learned a lot about the brain over the last few decades, the approach to treating mental illness has not kept pace with these findings. Without knowing the causes of the mental illness they were treating, the psychiatrists focused on the subjective symptoms and this approach led them the wrong way. In short, patients have been given names for syndromes or disorders that are not really known to be real entities or to what extent such an entity is different from another. The aim of this paper is to summarize some of the findings from neuroscience which could help finding the mechanisms behind depressive disorders. In the last two decades have been identified biomarkers that can predict response to antidepressant medications, such as hemolytic-encephalopathies barrier function, hormonal dysfunctions, and mechanisms of plasticity. Also, immune analysis allows an early screening of people presenting an increased risk for developing affective disorders, or are in an early stage of these. In addition, imaging and anatomical studies have found alterations in both the structure and function in regions that belong to the some of the brain's networks, thus suggesting a basis for the cognitive deficits associated with depression.

O.2.1. COMBINED EXPERIMENTAL APPROACHES TO ASSESS THE ANTIOXIDANT ACTIVITY IN FOOD

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Several epidemiological studies have suggested that antioxidant compounds may have a modulating role on risk factors for chronic diseases. These positive effects have been partially associated with antioxidant molecules, including vitamin C and flavonoids, which are present in significant amount in food and beverages (e.g., wine, juices, tea). These compounds can counteract the development of free radicals, involved in several deleterious effects on many biological targets. Considering that the *in vitro* evaluation of antioxidant activity is highly useful to establish a correlation with possible *in vivo* effects, increasing interest has been raised for fast analytical/biological methods.

The aim of the study was to compare the antioxidant properties of some food and beverage, measured by different analytical approaches.

Grape juices, wines and food supplements containing acerola and melatonin were firstly tested for their antioxidant activity with a novel method based on an electrochemical biosensor. The method was then compared with spectrophotometric (DPPH and ABTS assays) and chromatographic (High Performance Thin Layer Chromatography) assays.

Although with some differences, the results obtained with all tests used showed a similar trend: red wines and food supplements containing acerola showed the highest antioxidant activity. An emerging point was the interference of food matrix, which could be responsible for the differences observed intra- and inter-methods.

The use of biosensor integrated with other assays seems to offer a reliable body of data to evaluate the antioxidant activity of food and foodstuffs, reflecting at least partially the *in vivo* protecting potency.

O.2.2. UNDERSTANDING THE POTENTIAL OF ANTIOXIDANTS: HOW EFFICIENT? WHICH METHODS AND APPLICATIONS?

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The antioxidant potential of exogenous natural sources has received great attention due to the increased oxidative stress that has been identified as the cause of the development and progression of various diseases. Similarly, the number of methods and variations for measuring antioxidants in plants has increased considerably. In view of this, the applications and limitations of the main methods, as well as, the potential end- uses of the antioxidants are discussed. Spectrometry, electrochemical and chromatography analytical methods have been used for measuring the antioxidant potential, these assays differ in the mechanism of generation of different radical species and/or target molecules and in the way end products are measured. The uses of antioxidants in pharmacological, medicinal, and therapeutic applications have been intensively reported in the preventing and treatment of several diseases, especially degenerative disorders such as cancer. The use of preservative antioxidants has immense industrial applications such as food additives to increase the oxidative stability, cosmetics to prevent rancidity and aging, rubber and plastic to prevent the

degradation and reducing the wastage of raw materials, fuels and lubricants to prevent the oxidation and damage the engines. Due to the antioxidants behaviour that may respond in a different manner to different radical or oxidant sources, the measurement of antioxidants is not a simple process and there is no yet unique simple universal method that can be measured accurately and quantitatively. However, the standardization is longed for unifying quantities and units. Plants with high antioxidant capacity represent an interesting potential for many applications.

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O.2.3. FUNCTIONAL FOOD IN IRRITABLE BOWEL SYNDROM

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Irritable bowel syndrome (IBS) is one of the most common functional gastrointestinal diseases that affects in our geographical area round. 14% of the people. Management of this disease is difficult and requires a good doctor-patient communication. The therapeutic decision is difficult and need to consider the intricate etiology of the syndrome. Both the pharmacological and non-pharmacological treatment will be take into discussion. A large number of patients fail to respond to pharmacological therapy. Some diets improve symptoms of IBS, but data supporting their use are limited. Some specific dietary intervention were tested in IBS: dietary fiber supplementation, elimination diets, very low carbohydrate diet, no-gluten diet, Low Fermentable Oligosaccharides, Disaccharides, Monosaccharides, and Polyol Diet (FODMAPs).

There is a growing body of evidence to support the use of a low FODMAP diet in IBS patients. Diet is safe on short-term, but some issues are waiting for the answerer: the type of food, quantity of food, the additive effect of various food, safety and long term efficacy. Low FODMAPs diet reduces IBS symptoms. These carbohydrates are represented by fructose and lactose (apples, pears, watermelon, fruit juices, dried fruit, milk and derivatives), polyols used to produce low calories food, galactan and fructans (wheat, onion, garlic, cabbage, soybeans, broccoli).

The current researches will try to identify fecal bacterial profile of patients who responded to dietary intervention in IBS.

O.2.4. AMPEROMETRIC BIOSENSOR BASED ON GRAPHENE/FERROCENE CARBOXYLIC ACID/L-AMINO ACID OXIDASE NANOCOMPOSITE FOR THE DETECTION OF L-ALANINE

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Detection of optical-active amino acids has received special attention due to its significant impacts on chemical, biological, and pharmaceutical sciences. Among the different analytical techniques, electrochemical biosensors based on enzyme-nanocomposite are attractive due to its simple construction, rapidity, and very good sensitivity.

In this study, a novel amperometric biosensor for the detection of L-alanine in pharmaceutical samples was developed. The sensing material is based on covalent immobilized L-amino acid oxidase onto ferrocene carboxylic acid functionalized graphene thin film.

L-amino acid oxidase enzyme immobilization was carried out by cross-linking with glutaraldehyde. Ferrocene carboxylic acid was used as redox probe due to its electroactivity. The molecular architecture and interactions among components of sensing layer was determined by using FTIR technique. The morphology of sensing layer was studied by scanning electron microscopy.

After the biosensor testing towards L-alanine and D-alanine respectively, a larger current response was obtained from L-alanine. The optimizations of supporting electrolyte properties (pH and temperature) and of detection technique parameters (applied potential, stirring rate) were carried out. The biosensor presented an optimal response when a potential of -0.5 V was applied in phosphate buffer solution of pH 8.0. The linear range of the biosensor under the optimum working conditions was from 1.0×10^{-8} to 1.0×10^{-4} M with a lower detection limit of 4.2×10^{-9} M (S/N =3). The interfering effects were studied by standard addition method obtaining an excellent average recovery of 100.5%.

The biosensor was validated by quantification of L-alanine in pharmaceutical sample, when excellent results were achieved.

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