Nutrición Hospitalaria

Nutr Hosp. 2015;32(5):2153-2161
ISSN 0212-1611 • CODEN NUHOEQ
S.V.R. 318

Original/*Investigación animal*

In vitro and *in vivo* antioxidant activity of Buriti fruit (*Mauritia flexuosa* L.f.)

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Abstract

Introduction: **studies have shown high concentration of monounsaturated fatty acids, carotenoids, polyphenols and ascorbic acid in Buriti fruit (*Mauritia flexuosa* L.f.). This study evaluated the *in vitro* and *in vivo* antioxidant activities of buriti fruit (*Mauritia flexuosa* L.f.).**

Methods: **the chemical composition and total phenolic and carotenoid contents of the buriti pulp and the feed rations were determined, and the *in vitro* antioxidant activity was analyzed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay. Wistar rats (21 days old) were randomly allocated (n=10) into a control groups and experimental groups (feed enriched with buriti pulp). After 60 days, the *in vivo* antioxidant activity was evaluated through the determination of the catalase activity and non-protein sulfhydryl (NPSH) groups in the liver and quantification of malondialdehyde (MDA) in the plasma and tissues.**

Results: **high contents of oleic fatty acids (73.3%), phenolic compounds (192 ± 0.3 mg/100 g) and carotenoids (23.9 ± 0.5 mg/100 g) as well as elevated *in vitro* antioxidant activity were found in the buriti pulp. The enriched diet had higher contents of phenols and carotenoids as well as higher antioxidant activity compared with the standard feed ($p < 0.05$). There were no differences between the groups regarding catalase activity in the liver and MDA concentrations in the plasma, liver and kidneys. The male rats of the experimental group had higher liver concentrations of NPSH compounds ($p < 0.05$).**

Conclusion: **these results may corroborate the claim that buriti fruit is an antioxidant functional food and support its utilization in a nutritionally balanced diet.**

(*Nutr Hosp*. 2015;32:2153-2161)

DOI:10.3305/nh.2015.32.5.9603

Key words: *Antioxidant activity. Bioactive compounds. Carotenoids. Dietary supplementation. Lipid peroxidation.*

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Recibido: 8-VII-2015.
Aceptado: 7-VIII-2015.

LA ACTIVIDAD ANTIOXIDANTE *IN VITRO* E *IN VIVO* DE LA FRUTA BURITÍ (*MAURITIA FLEXUOSA* L.F)

Resumen

Introducción: **estudios previos han demostrado que la fruta Burití (*Mauritia flexuosa* L.f). posee una alta concentración de ácidos grasos monoinsaturados, carotenoides, polifenoles y ácido ascórbico. Este estudio evaluó la actividad antioxidante *in vitro* e *in vivo* del Burití.**

Métodos: **fueron determinadas la composición química, el contenido de compuestos fenólicos y de carotenoides tanto de la pulpa del Burití como de las raciones de alimento. La actividad antioxidante *in vitro* fue analizada utilizando el ensayo del radical 2,2-difenil-1-picrilhidrazil (DPPH). Ratas Wistar (21 días de edad) fueron asignadas al azar (n = 10) en grupos controles y grupos experimentales (alimentación enriquecida con pulpa de Burití). Después de 60 días, la actividad antioxidante *in vivo* se evaluó mediante la actividad enzimática de la catalasa y grupos sulfhidrílo no proteico (NPSH) en el hígado, y se cuantificó el malondialdehído (MDA) en plasma y tejidos.**

Resultados: **la pulpa del Burití presentó alto contenido de ácido graso oleico (73,3%), compuestos fenólicos (192 ± 0,3 mg/100 g) y carotenoides (23,9 ± 0,5 mg/100 g), así como una elevada actividad antioxidante *in vitro*. La dieta enriquecida tenía mayor contenido de fenoles y carotenoides, y una mayor actividad antioxidante en comparación con la alimentación estándar ($p < 0,05$). No se observaron diferencias entre los grupos con respecto a la actividad de la catalasa en el hígado y las concentraciones de MDA en plasma, hígado y riñones. Las ratas macho del grupo experimental tuvieron concentraciones hepáticas más altas de NPSH ($p < 0,05$).**

Conclusión: **estos resultados pueden corroborar la hipótesis de que la fruta Burití es un alimento funcional antioxidante y su consumo es conveniente en una dieta nutricionalmente equilibrada.**

(*Nutr Hosp*. 2015;32:2153-2161)

DOI:10.3305/nh.2015.32.5.9603

Palabras clave: *Actividad antioxidante. Compuestos bioactivos. Carotenoides. Suplementos dietéticos. Peroxidación lipídica.*

Abbreviations

AOAC: Association of Official Analytical Chemists.
DPPH: 2,2-diphenyl-1-picrylhydrazyl.
DTNB: 5,5'-dithiobis (2-nitrobenzoic acid).
EDTA: Ethylenediaminetetraacetic acid.
FER: Food Efficiency Ratio.
GAE: Gallic acid equivalents.
GSH: Glutathione.
MDA: Malondialdehyde.
NF-kB: Nuclear transcription factor-kappa B.
NPSH: Non-protein sulfhydryl.
ROS: Reactive oxygen species.
SEM: Standard error of the mean.
SH: Sulfhydryl group.
TBARS: Thiobarbituric acid reactive substances.
TNB: Thionitrobenzoic.
UFPI: Federal University of Piaui.

Introduction

Studies in humans suggest that vegetables have health promoting properties and that a reduced consumption of these foods is related to an increased risk of developing cardiovascular disease[1] and cancer[2]. Accordingly, public awareness regarding the link between diet and health has aroused interest in the benefits of consuming fruit products and byproducts[3].

Among the bioactive compounds with functional properties in food, prebiotics, sulfur and nitrogen compounds, antioxidant vitamins and minerals, phenolic compounds, unsaturated fatty acids and dietary fiber are the most prominent[4]. Substances with antioxidant activity, including secondary plant compounds, have received considerable attention because these compounds may help to protect the human body against oxidative stress and thereby reduce the risk of a number of chronic degenerative disorders[3].

Oxidative stress results from an imbalance between the production of free radicals and the action of the antioxidant defense systems of the body, the main objective of which is to keep the oxidation process within physiological limits and thereby prevent an increase in oxidative damage that could culminate in cellular injury and toxicity[5].

Diet is an important factor in the regulation of oxidative stress. The effects of vitamin and mineral antioxidant supplements on oxidative stress are not yet conclusive, but positive effects on specific biomarkers, especially those related to the oxidation of the most prominent lipids, have been shown[5].

The Buritizeiro (*Mauritia flexuosa* L.f.) is a palm tree that is native to the Amazon rainforest as well as Northeast, Midwest and Central Brazil. Its fruit, the buriti, is widely consumed in the northeastern region of Brazil and has properties associated with protection against the development of chronic diseases[6]; however, there is little data in the available literature on the functional properties of this fruit in experimental studies.

Both the chemical composition and the antioxidant potential of the Buritizeiro fruit indicate that this fruit is rich in lipids, with a predominance of monounsaturated fatty acids, and has significant levels of carotenoids, polyphenols, total ascorbic acid and sulfuric amino acids[7].

Because the emergence of chronic diseases may be related to dietary habits, experimental studies investigating the effects of including regional foods with potential beneficial effects, such as buriti, in the diet are required to stimulate the consumption of these fruits as a part of a healthy diet. With this objective in mind, the following study evaluated the *in vitro* and *in vivo* antioxidant activity of buriti pulp.

Methods

Ingredients and preparation of feeds

Buriti pulp (5 Kg) was acquired from the local market in Teresina, PI, Brazil, and was added to standard rodent feed (Purina Labina) at a ratio of 10 g of dried pulp/100 g of the total weight of the feed. This supplemented feed was crushed and served daily for 60 days. The ratio of the fruit was chosen based on the study of Salgado, Mansi and Sousa[8], who used feed prepared with avocado, a fruit with a fatty acid profile similar to that found in buriti. The enriched feed was prepared weekly and stored under refrigeration in a closed, dark container until use.

Chemical analysis of buriti pulp, standard rodent feed and rodent feed enriched with buriti pulp

Nutritional composition and fatty acid determination

The moisture, protein, lipid and ash contents of the feed were determined according to the methodology described by the Association of Official Analytical Chemists (AOAC)[9]. To obtain the lipid fraction of the buriti pulp, an intermittent Soxhlet extractor (Merck, Darmstadt, Germany) with hexane as the solvent was used. After the transesterification reaction, the methyl esters of the fatty acids were identified by gas chromatography-mass spectrometry (SHIMADZU GC-17A, Shimadzu Corporation Inc., Kyoto, Japan)[10].

Phenolic compounds content

The quantification of phenolic compounds in the aqueous and methanolic extracts of the pulp and the feeds was performed according to the method of Swain and Hills[11], which was adapted by Lima[12] and based on the standard gallic acid curve (50 – 750

mg/L) obtained using Folin-Denis reagent. For this analysis, 0.5 mL samples of the extract were homogenized with 8 mL of distilled water, 0.5 mL of Folin-Denis reagent and 1 mL of an anhydrous sodium carbonate-saturated solution. The absorbance values were measured at 720 nm with a UV-VIS spectrophotometer (Biospectro SP-220, EQUIPAR Ltda., Curitiba, Brazil). All the determinations were performed in triplicate.

The methanol and aqueous extracts of the pulp and the feeds were prepared independently from defined quantities of the samples using the method of Sousa *et al*.[13], adapted to the quantities of the samples used. The samples were suspended in water or methanol (10% w/v) followed by vacuum filtration.

Determination of carotenoids

The carotenoid content of the buriti pulp was determined according to the method described by Rodriguez-Amaya[14]. The pulp was extracted and washed with acetone, and the acetonic extract was dissolved in petroleum ether and fractionated with distilled water. The carotenoid content in the ether phase was determined by measuring the absorbance at 450 nm with a UV-VIS spectrophotometer (Biospectro SP-220, EQUIPAR Ltda., Curitiba, Brazil) and using the specific absorption coefficient of β-carotene (2,592).

In vitro antioxidant activity

The *in vitro* antioxidant activity of the buriti pulp and the feeds was determined according to the method of Blois[15], which was adapted by Brand-Williams *et al*.[16] and Lima[12] and is based on the reduction of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical (Merck, Darmstadt, Germany). The tested samples (200 µL) were added to 2 mL of a 40 µg/mL DPPH methanol solution and allowed to react at room temperature. After 10 min and 30 min, the absorbance values were measured at 517 nm with a UV-VIS spectrophotometer (Biospectro SP-220, EQUIPAR Ltda., Curitiba, Brazil) and used to calculate the percentage of the antioxidant activity (AA%). Trolox, a synthetic antioxidant (Merck, Darmstadt, Germany), was used as a standard at concentrations of 15, 30, 45, 60 and 75 µM.

Animals

Weanling 21-day-old rats of both sexes (36 ± 0.66 g), from the laboratory of the Department of Physiology and Biophysics, Federal University of Piaui (UFPI), were randomly allocated into four groups (10 animals/group) and treated for 60 days with standard feed (Control-males and Control-females) or buriti pulp-enriched feed (Buriti-males and Buriti-females). The animals were kept in individual metabolic cages and received the standard or the buriti pulp-enriched diet and water *ad libitum* in a temperature-controlled room (25 ± 2ºC) with a photoperiod of 12 hours of light and 12 hours of darkness.

The daily body weights and feed intakes of the animals were recorded. A known amount of standard or buriti pulp-enriched feed was placed in the metabolic cage feeders, and the food intake was determined as the difference between this amount and what remained after 24 hours.

This research was approved by the Ethics Committee on Animal Experimentation of the UFPI under protocol ECAE/UFPI No. 057/11.

Euthanasia and collection of specimens for analysis

At the end of the 60-day experimental period, the animals from each group were euthanized with a lethal intraperitoneal dose of sodium thiopental (100 mg/kg) (Thiopentax, Cristália, SP, Brazil). A blood sample was collected from the caudal vena cava for the assessment of lipid peroxidation in the plasma. The liver was removed for antioxidant and lipid peroxidation analysis, and the kidneys were removed for the evaluation of lipid peroxidation. The samples were kept at -80ºC until processing.

In vivo antioxidant activity in hepatic tissue

The quantification of catalase activity was performed according to the method of Beutler[17], which follows the decomposition of hydrogen peroxide by monitoring the decrease in the absorbance at 230 nm with a UV-VIS spectrophotometer (Biospectro SP-220, EQUIPAR Ltda., Curitiba, Brazil). The non-protein sulfhydryl (NPSH) groups were quantified according to the methodology proposed by Sedlak and Lindsay[18]. The samples were homogenized in a refrigerated 0.02 M ethylenediaminetetraacetic acid, disodium salt (sodium EDTA) solution. The tissue proteins from the homogenate were precipitated with trichloroacetic acid solution (10%) and harvested by centrifugation (15 min, 3,000 rpm). An aliquot of the resulting supernatant was added to 0.4 M Tris/0.2 M EDTA, pH 8.9, and 0.01 M 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB; Sigma-Aldrich) diluted in methanol. The optical density of the thionitrobenzoic (TNB) ionic solution was evaluated against distilled water at 412 nm with a UV-VIS spectrophotometer (Biospectro SP-220, EQUIPAR Ltda., Curitiba, Brazil), and the concentration of the sulfhydryl groups was calculated by comparison with a standard calibration curve prepared with L-cysteine (Sigma-Aldrich). The NPSH group content was expressed as µM NPSH per weight (g) of wet tissue.

Lipid peroxidation in plasma and liver and kidney tissues

The evaluation of lipid peroxidation was performed by quantifying malondialdehyde (MDA) in the plasma as well as in the liver and kidney tissues. The MDA concentrations were determined by the production of thiobarbituric acid reactive substances (TBARS) according the method described by Ohkawa, Ohishi and Yagi[19].

The absorbance of the samples was determined with a UV-VIS spectrophotometer (Biospectro SP-220, EQUIPAR Ltda., Curitiba, Brazil) at 532, 510 and 560 nm to calculate a corrected absorbance as proposed by Pyles, Stejskal and Einzig[20], which minimizes the interference of heme pigments and hemoglobin.

Statistical analysis

The data were expressed as the means ± standard error of the mean (SEM). Normality was assessed using the D'Agostino and Pearson Omnibus normality test. Comparisons between groups were evaluated using unpaired "t" tests, and the significance level was set at $p < 0.05$.

Results

Table I presents the chemical compositions and the energy values of the buriti pulp, the standard feed and the feed enriched with buriti pulp. The buriti pulp proved to be a food with a high content of both carotenoids and lipids. The most abundant fatty acid in the buriti pulp was oleic acid (71.6%), followed by palmitic (16.9%), linoleic (9.9%) and stearic (1.6%) acids. Moreover, compared with the standard feed, the enriched feed presented a statistically higher caloric content (3%) and lipid content (31%) as well as a lower protein content (7.8%). The carotenoid content was approximately 3.31-fold higher in the enriched feed.

The amounts of phenolic compounds in the pulp as well as in the standard and enriched feeds are shown in table II. The pulp exhibited a high content of phenolic compounds, and the feeds used in the biological assay had statistically significant differences in the levels of phenolic compounds. The aqueous and methanol extracts of the enriched feed had significantly higher levels of phenolic compounds compared with the corresponding extracts of the standard feed.

Regarding the *in vitro* antioxidant activity as determined with the DPPH reduction method, the methanol extract of the buriti pulp had significantly higher ($p < 0.05$) antioxidant activity than the aqueous extract after both 10 and 30 min of reaction with the radical. A slight increase (5%) in the antioxidant activity of the aqueous extract from the feed enriched with buriti pulp was observed after only 10 min of reaction. There were no statistically significant differences between the antioxidant activities in the different aqueous extracts after 30 min (Table III).

During the 60-day monitoring period of the biological assay, the animals in the experimental group exhibited feed intakes and weights similar to those of the control group for the different periods of study (Fig. 1, Table IV). Regarding the Food Efficiency Ratio (FER), which represents the body weight gain per feed intake,[21] there were no significant differences between the groups (Fig. 2).

Buriti pulp intake did not affect the activity of catalase in the liver, but the levels of NPSH groups in the male rats from the experimental group were higher ($p < 0.05$) than those found in the animals from the respective control group, revealing a greater stimulation of antioxidant activity by including of this fruit in the diet. There were no statistically significant alterations in the concentrations of MDA in the plasma, kidneys or liver due to the consumption of feed enriched with buriti pulp (Table V).

Table I
Chemical composition, including macronutrients, moisture, ash, energy and carotenoids, of buriti pulp, standard feed and feed enriched with buriti pulp

Variable	*Buriti pulp*	*Feed*	
		Standard	*Enriched*
Moisture (%)	6.4 ± 0.1	6.2 ± 0.3	6.7 ± 0.2
Ash (g/100 g)	2.7 ± 0.0	10.5 ± 0.2	9.7 ± 0.3
Proteins (g/100 g)	9.0 ± 0.2	21.7 ± 0.3	20.0 ± 0.4[a]
Carbohydrates (g/100 g)	36.3 ± 0.4	57.1 ± 0.4	57.7 ± 0.5
Lipids (g/100 g)	45.7 ± 0.2	4.5 ± 0.3	5.9 ± 0.0[a]
Energy (Kcal/100 g)	592.0 ± 1.1	352.0 ± 1.2	363.0 ± 0.3[a]
Carotenoids (mg/100 g)	23.9 ± 0.5	1.6 ± 0.0	5.3 ± 0.4[a]

[a]Unpaired "t" test, $p < 0.05$ compared with the standard diet. The data are represented as the means ± SEM.

Table II
Phenolic compounds (gallic acid equivalents) in extracts of buriti pulp, standard feed and feed enriched with buriti pulp as measured in mg/100 g of wet sample.

Extract	Phenolic Compounds (mg/100 g)		
	Buriti pulp	Standard feed	Enriched feed
Methanolic	111.0 ± 5.2	26.6 ± 0.6	54.9 ± 0.3[a]
Aqueous	192.0 ± 0.3[a]	26.6 ± 0.9	65.9 ± 0.5[a]

[a]Unpaired "t" test, $p < 0.0001$ for the pulp compared with the methanol extract and for the feed compared with the same extracts from the standard feed. The data are represented as the means ± SEM.

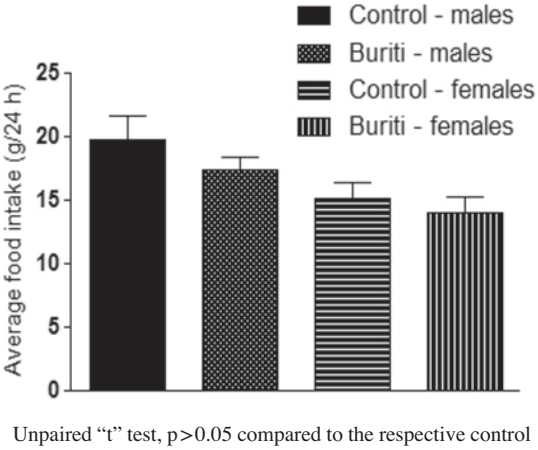
Table III
Trolox equivalent antioxidant activity (TEAC) in the extracts of buriti pulp, standard feed and feed enriched with buriti pulp, as measured by the reduction of DPPH quantified in µmol of Trolox/g of wet sample

Extract	Trolox equivalent antioxidant activity (µmol of trolox/g)					
	After 10 min of reaction			After 30 min of reaction		
	Buriti pulp	Standard feed	Enriched feed	Buriti pulp	Standard feed	Enriched feed
Methanolic	128.0 ± 3.1[a]	14.2 ± 015	14.5 ± 0.17	164.0 ± 2.7[a]	9.9 ± 0.6	10.5 ± 0.4
Aqueous	10.7 ± 0.4	9.9 ± 0.03	10.4 ± 0.03[a]	96.8 ± 8.7	9.0 ± 1.8	8.3 ± 0.4

[a]Unpaired "t" test, $p < 0.05$ compared with the same extract from the standard feed and the same reaction time. The data are represented as the means ± SEM.

Discussion

Edible native plants, such as araticum (*Annona crassiflora* Mart.), jatoba (*Hymenaea courbaril* L.), pequi (*Caryocar brasiliense*), mangaba (*Hancornia speciosa*), cagaita (*Eugenia dysenterica*) and buriti (*M. flexuosa* L.f.), are low-cost food sources with high nutritional value and are consumed by rural populations from Cerrado[22]. Moreover, the results from *in vitro* and *in vivo* studies aimed at elucidating the mechanisms by which non-nutrient bioactive compounds in plant foods contribute to maintaining health and reducing the risk of disease[23] have aroused the interest of the population in consuming plant-derived products and have created the possibility of using these plants as functional foods for special nutritional purposes[24].



Unpaired "t" test, $p > 0.05$ compared to the respective control group. Animals were in groups of 10. The data are represented as the means ± SEM.

Fig. 1.—Average daily food intake of the Rattus norvegicus groups fed standard feed (control) and feed enriched with buriti pulp (Buriti) for the 60-day experimental period.

Manhães[7] classifies buriti as a functional food because of the presence of bioactive substances, sulfur-containing amino acids, carotenoids, polyphenols, ascorbic acid and significant amounts of omega-9 fatty acids (oleic acid). The presence of these components imparts an antioxidant effect, and the food could potentially be consumed to reduce the risk of developing several diseases.

The buriti pulp used in this study had a high caloric value and high concentrations of lipids, carbohydrates and proteins. Carneiro and Carneiro[25], in a study on the chemical composition of buriti pulp from the state of Piaui, obtained lower values for protein (3.4%), carbohydrates (31.2%) and ash (1.6%) and higher caloric values (604 Kcal/100 g) and moisture (12.1%) and lipid (51.7%) levels compared with the data of the present study. Variations in the nutritional composition of the buriti pulp may be due to differences among the various regions of the country and harvesting conditions as well as the procurement, drying and storage of the fruit.

The profile of fatty acids present in the buriti pulp demonstrated that this fruit possesses high oleic acid content, followed by palmitic, stearic, and linoleic acids.

Table IV
Weight of the Rattus norvegicus groups fed standard feed (control) and a feed enriched with buriti pulp (Buriti) during the 60-day follow-up

Time (day)	*Body weight (g)*			
	Males		*Females*	
	Control	*Buriti*	*Control*	*Buriti*
1st	37.4 ± 3.2	34.2 ± 1.4	36.4 ± 2.7	36.0 ± 3.2
15th	109.0 ± 1.9	102.0 ± 7.2	97.7 ± 1.6	92.1 ± 1.8
30th	181.0 ± 3.7	167.0 ± 11.6	133.0 ± 0.4	132.0 ± 1.3
45th	247.0 ± 5.7	234.0 ± 14.3	161.0 ± 0.4	160.0 ± 1.3
60th	287.0 ± 6.9	276.0 ± 13.1	179.0 ± 0.7	177.0 ± 0.9

Unpaired "t" test, $p > 0.05$ compared with the respective control group. Animals were in groups of 10. The data are represented as the means ± SEM.

Manhães and Sabaa-Srur[26] described similar results, in which the presence of 73.3% oleic acid, 19.3% palmitic acid, 2.69% linoleic acid and 1.8% stearic acid was determined. Compared to other fruits, buriti presented a composition similar to that described for açai, the lipid content of which is predominantly composed of monounsaturated fatty acids (up to 61%) and polyunsaturated fatty acids (approximately 10.6%)[27].

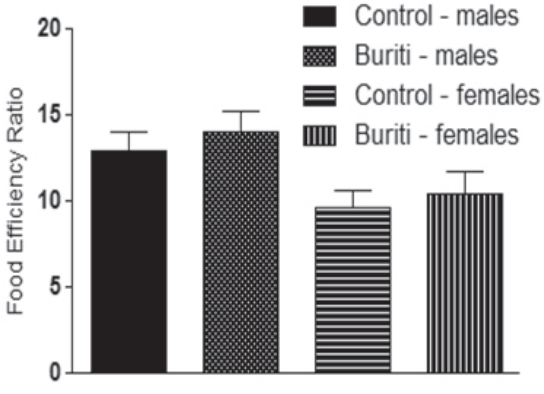
Regarding antioxidant compounds, the buriti pulp as well as the feed enriched with this fruit contained high concentrations of carotenoids and phenolic compounds when compared with the standard feed. The results for the quantification of carotenoids revealed that the pulp of the buriti has a content similar to that determined by Manhães and Sabaa-Srur[26], who obtained 23.3 mg/100 g of buriti pulp, which is less than the 51.4 mg/100 g of buriti pulp that was verified by Rosso and Mercadante[28] (via HPLC) and higher than the content in other Amazon fruits that are considered main sources of carotenoids, such as camu-camu (0.4 to 1.1 mg/100 g)[29] and acerola (0.4 to 1.9 mg/100 g)[30]. According to Rodriguez-Amaya, buriti has the highest carotenoid concentration of the food that has been analyzed in Brazil[31].

The total phenolic content of buriti pulp appears to be quite variable in multiple studies. Carneiro and Carneiro[25] found a content of phenolic compounds (expressed as gallic acid equivalents - GAE) of 18.2 ± 1.5 mg/100 g for the aqueous extract of buriti pulps. Moreover, Manhães and Sabaa-Srur[26] identified levels of polyphenols at 9.5 ± 3.1 mg of tannic acid/100 g. However, Koolen *et al*.[32] obtained 378 ± 3.1 mg GAE/100 g for the aqueous extract of the fruit, which is higher than that found in this study.

According to Tomás-Barberán and Espín[33], differences in the values for the concentrations of phenolic compounds may be influenced by factors such as the maturity, species, geographic origin, growth stage, harvest conditions and storage of the fruits.

In the evaluation of the *in vitro* antioxidant activity using the DPPH method, the methanol extract of the buriti pulp exhibited higher antioxidant activity than the aqueous extract. At 30 min, the antioxidant activity of the methanol extract of the pulp was higher than that of many wild fruits, including baguaçu (111.2 µmol/g) and jambolão (15 µmol/g) as well as the pulp of acerola (53.2 µmol/g), mango (12.9 µmol/g), cupuaçu (0.7 µmol/g) and passion fruit (0.9 µmol/g), which were all also evaluated by the DPPH method after 30 min[34]. These *in vitro* results should be carefully analyzed considering that DPPH is a non-physiological radical and that the body has several less stable oxidizing reactive species involving different redox systems.

In addition to the potential antioxidant effects of secondary compounds from plants, such as alkaloids, terpenoids and flavonoids, these compounds could present direct or indirect effects on the modulation of several inflammatory pathways in the body, including the activation of nuclear transcription factor-kappa B (NF-kB) and gene expression[35,36].



FER = body weight gain/ feed intake. Unpaired "t" test, $p > 0.05$ compared to the respective control group. Animals were in groups of 10. The data are represented as the means ± SEM.

Fig. 2.—Food Efficiency Ratio (FER) of the Rattus norvegicus groups fed standard feed (control) and feed enriched with buriti pulp (Buriti) for the 60-day experimental period.

Table V
Levels of catalase, non-protein sulfhydryl (NPSH) groups in liver tissue and malondialdehyde (MDA) in plasma, liver and kidneys of the Rattus norvegicus groups fed standard feed (control) and feed enriched with buriti pulp (Buriti) after the 60-day experimental period

Markers	*Males*		*Females*	
	Control	*Buriti*	*Control*	*Buriti*
Hepatic catalase (mmol/min g tissue)	56.9 ± 10.7	44.3 ± 13.9	59.7 ± 10.2	57.9 ± 10.7
Hepatic NPSH (μM/g tissue)	79.6 ± 19.3	136.0 ± 19.7[a]	110.0 ± 17.9	83.0 ± 18.7
MDA				
Plasma (nmol/nL)	1.7 ± 0.4	1.7 ± 0.2	1.9 ± 0.3	2.0 ± 0.4
Liver (nmol/g)	52.3 ± 3.8	48.4 ± 2.2	64.6 ± 6.7	57.9 ± 6.3
Kidney (nmol/g)	87.5 ± 5.5	91.1 ± 7.0	88.1 ± 4.3	103.0 ± 10.6

Regarding the role of diet in preventing oxidative stress, it is thought that antioxidant molecules are essential, but are not miraculous, because these compounds cannot prevent or cure diseases, especially when consumed in supplement form. However, these molecules can act together with the body's primary antioxidants, the superoxide dismutase, catalase and glutathione peroxidase enzymes, to maintain the levels of oxygen and nitrogen reactive species within physiological limits. For the body to regulate its requirement for antioxidant enzymes, the feed must contain adequate amounts of non-enzymatic antioxidants such as vitamins C, E and A[37].

In the present study, the intake of buriti pulp did not affect the concentrations of liver catalase but increased the levels of NPSH groups in the male rats from the experimental group, demonstrating a greater stimulation of antioxidant activity by including this fruit in the diet. Certain *in vivo* studies have demonstrated that the use of natural products was able to increase the levels of NPSH moieties and thereby prevent oxidative stress in male rats[38,39].

Glutathione (GSH) is the major NPSH group found in mammalian cells; GSH is a tripeptide found in high concentrations intracellularly that plays a central role in biotransformation, the elimination of xenobiotics and cellular protection against oxidative stress. Many GSH reactions involve the sulfhydryl group (SH), which is highly polarizable and, therefore, a good nucleophile for reaction with electrophilic chemical compounds[40].

Lipid peroxidation is a process resulting from the attack of reactive oxygen species (ROS) on unsaturated fatty acids in cell membranes. This mechanism leads to changes in membrane lipids, alters the membrane permeability and promotes the flow of cellular metabolites. The degree of lipid peroxidation is determined using the TBARS method[5,41]. This analytical method is used to detect MDA, an important marker of oxidative damage[41].

In the present study, no significant alterations in lipid peroxidation were demonstrated because there were no significant differences between the control and experimental groups in the concentrations of MDA in the plasma, kidneys and liver. This finding suggests that food enriched with fruit that is rich in unsaturated fatty acids resulted in no change in the lipid peroxidation.

Lima[12], in a study of the *in vivo* antioxidant activity of pequi (*Caryocar brasiliense* Camb.), a fruit that, similar to buriti, is rich in carotenoids and oleic acid, observed a reduction in the concentration of MDA in the liver tissues of animals treated with doses of 300 mg/kg of the fruit extract and of 100 mg/kg of free phenolic acids of the pulp for 30 days relative to the control groups.

Nevertheless, there is controversy concerning the antioxidant power of foods rich in bioactive compounds because certain factors in complex biological systems can reduce the effectiveness of these antioxidants[42]. A study designed to evaluate the antioxidant activity of fruta-do-lobo (*Solanum lycocarpum* St. Hill), a fruit from the Cerrado region that is rich in ascorbic acid and phenolic compounds, found that there was no inhibition of lipid peroxidation in the brains of rats when administered the extract of this fruit at doses of 450, 850 and 1,300 mg/kg for 18 days. The authors concluded that the antioxidants within the plant were unable to inhibit *in vivo* lipid peroxidation and also had no pro-oxidant action[43].

In healthy individuals, the regular intake of orange juice, a product with a high concentration of antioxidants, improved the antioxidant capacity in the blood but had no effect on lipid peroxidation[44].

The stimulation of the antioxidant GSH in the animals fed with enriched buriti pulp, without interfering with weight gain, food consumption or the redox state of the bodies of the young animals, demonstrates that the buriti fruit is a healthy food alternative for the general population. The discrete effect of the experimental diet on the NPSH levels in this study is most likely

a result of the use of a small proportion of buriti pulp in an *ad libitum* diet rather than treatment with an extract of whole pulp by gavage. Moreover, the fact that we did not investigate other markers of antioxidant activity, such as the activity of superoxide dismutase and glutathione peroxidase, constitutes a further limitation of the present study.

Further studies using different proportions of buriti pulp or with isolated nutrients that are part of its composition, such as β-carotene and oleic acid, are required to the assess the effects of these agents on the antioxidant activity in experimental models.

Conclusion

The consumption of a buriti-supplemented diet by young rats increased *in vitro* and *in vivo* antioxidant activities, while lipid peroxidation in the plasma, kidneys and liver was not affected. These results may corroborate the claim that buriti fruit is a functional food and support its utilization in a nutritionally balanced diet. However, further studies, including human clinical trials, are required to elucidate the role in maintaining health of the bioactive compounds present in buriti.

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