Scientia Horticulturae 227 (2018) 10–21

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Research Paper

Bioactive compounds and antioxidant activity of buriti fruits, during the postharvest, harvested at different ripening stages

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ARTICLE INFO

Keywords:
Mauritia flexuosa L.f.
Functional
Amazon
Shelf file

ABSTRACT

The buriti (*Mauritia flexuosa* L.f.) is a highly nutritious fruit with a yellowish-orange pulp and a bittersweet taste. Its endocarp is surrounded by a spongy material made of starch and oil, while the fruit skin is hard, consisted of small reddish-brown scales. This work aimed to characterize the bioactive compounds and the antioxidant activity of buriti fruits, during the postharvest time, harvested at different ripening stages. The fruits were harvested in a transitional environment savannah/forest at private properties in the city of Boa Vista, Roraima, Brazil. Fruits were analyzed for five consecutive years (2012–2016), at three different ripening stages: immature (with up to 75% green color between the scales), mature (with up to 50% green color between the scales) and ripened (with up to 25% green color between the scales). The selected fruits were standardized on the basis of their appearance, such as color of the epidermis and phytosanitary conditions, and later sanitized. Different fruit variables were analyzed every 2 days during a 10-day postharvest period including loss of fresh mass, pH, total acidity, soluble solids, total pectins, pectin methyl esterase and polygalacturonase enzyme activity, respiratory behavior (CO_2 and ethylene), and functional activity such as phenolic compounds, carotenoids and antioxidant activity (ORAC and DPPH). High antioxidant activity, in both methods, was observed in buriti fruits at different ripening stages during the postharvest storage period, characterized by high phenolic and carotenoid compounds. Fruits harvested at mature and immature stages showed a longer postharvest shelf life. It was observed a climacteric behavior of the buriti fruits here analyzed, that showed an increase in the respiration rate and ethylene production, indicated by the peak of CO_2 and ethylene production. Similar changes were observed in the fruit pH, total acidity and soluble solids, as well as in the activity of peptic enzymes whereas a decrease was observed in the total pectin contents. The fruits showed reasonable amount of phenolic compounds, carotenoids and antioxidant activity, thus confirming the functional potential of these fruits.

1. Introduction

Buriti (*Mauritia flexuosa* L.F.) is considered to be the most abundant palm tree of Brazil. This specie grows naturally in the Amazonian biome and present great socioeconomic potential. Buriti fruit is a good source of vitamins and minerals and are widely used for commercial purposes, such as in the production of non-food by-products, serving as raw material for the construction of houses, beverages manufacturing and direct use as food (Cunha et al., 2012).

Buriti not only contribute as a fauna preservation strategy by serving as food source to many birds and mammal species but is also a good indicator of poorly drained or water logged soil since it grows only near swamps, water springs and wells (Vieira et al., 2010).

The pulp of buriti fruit contains high amounts of carotenoids, polyphenols and ascorbic acid and has the potential of preventing oxidative stress diseases (Koolen et al., 2013). Its lipidic fraction is basically composed of tocopherol and oils with predominance of oleic and palmitic fatty acids, which help in the prevention of cardiovascular diseases (Barreto et al., 2009; Manhães and Sabaa-Srur, 2011). Although very little have been reported on the functional activities of buriti fruit, it has showed high photoprotection potential due to the presence of carotenoids with its antibacterial and healing property (Batista et al., 2012).

Based on the significant importance of buriti fruit, its chemical properties, pulp consumption and wide distribution in the Amazon (Canuto et al., 2010; Castro et al., 2014; Milanez et al., 2016), this work aimed to characterize the physicochemical and functional properties of fruits harvested at different stages of ripening, aiming to support its

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http://dx.doi.org/10.1016/j.scienta.2017.08.045
Received 13 December 2016; Received in revised form 25 August 2017; Accepted 29 August 2017

technological development.

2. Material and methods

2.1. Method of harvest

Buriti fruits (*Mauritia flexuosa* L.f.) were harvested during five consecutive years (2012–2016) from May to October in each season, in private farm located at transition savannah/forest area in the municipality of Amajari, Brazil, RR.

Initially, selected buriti palm trees were identified considering fruit bunches in full development, whereas fruits were harvested at an interval of 60-days during each year of evaluation. Fruit harvest at different ripening stages included (Amarante and Megguer, 2008): immature (with up to 75% green color between the scales), mature (with up to 50% green color between the scales) and ripened (with up to 25% green color between the scales).

After harvest, the fruits were transported in small plastic containers, without any cover or temperature control (time: less than 2 h), to the Food Technology Laboratory, Federal University of Roraima, Brazil. Fruits were selected and standardized based on their appearance (skin color and health condition). Later, they were sanitized with 30 ppm hypochlorite for 10 min, air dried at room temperature (24 ± 3 °C and RH 80 ± 5%) and placed in open 0.010 mm thick (TpO_2 of 11,234 $cm^3\ m^{-2}\ day^{-1}$, 25 °C and 1 atm; $TpCO_2$ of 36,705 $cm^3\ m^{-2}\ day^{-1}$, 25 °C and 1 atm; area of permeability of 805 cm^2) transparent plastic bags (PEBD).

2.2. Analyses performed

The analyses were divided into two categories (destructive and non-destructive non-destructive), as follows:

2.2.1. Destructive analysis

since the pulp was used for the analysis therefore in each phase of the analysis, the pulp was extracted by separating it from the peel and seed. After that, it was blended in order to obtain a homogenous mass, which was then used for the evaluations of all destructive analysis in triplicate. The pH was measured using a potentiometer with a glass electrode. The total acidity (TA) was calculated by titration of the filtrate (1:5 dilution) with 0.1 N NaOH, and the results were expressed in mg of citric acid.100 g^{-1} (IAL, 2008). The soluble solids contents (SS) were evaluated according to the methodology of IAL (2008) using a refractometer (model RT-30 ATC) whereas the results were expressed in °Brix.

For analysis of total and soluble pectin, McCready and McCoomb (1952) extraction technique was being used while calculated using Bitter and Muir technique (1962), i.e. by reaction with carbazole. The levels of total and soluble pectin were presented in percentage of galacturonic acid.100 g^{-1} of the pulp.

The activity of the enzyme pectin methyl esterase (PME) was determined using a method described by Jen and Robinson (1984), and the results are expressed in μmol of NaOH $g^{-1}\ min^{-1}$. The activity of the enzyme polygalacturonase (PG) was measured using Pressey and Avants (1973) approach and the results are presented as enzymatic activity unit (EAU) $g^{-1}\ min^{-1}$.

For the follow destructive analysis, it was used lyophilized fruits. In the same way, two types of extractors (water and ethanol) were used for the analysis of total phenolics and antioxidant activity (ORAC and DPPH).

Total phenolics were calculated according to the methodology described by Wettasinghe and Shahidi (1999). A spectrophotometer was used for evaluation using the Folin-Ciocalteau reagent (Merck) and the gallic acid standard curve. The results, in dry basis, were expressed as GAE mg.100 g^{-1} (gallic acid equivalent).

For the total carotenoid content determination of each evaluation period, 0.2 g of freeze dried pulp samples were taken in a test tube covered with aluminum foil and added with 10 mL of hexane-acetone (6:4) extraction solution. The extracts were than stirred in tubes shaker for 1 min and left stagnant for 9 min after that to settle down, and filtered through cotton and straightaway tested (in triplicates) in a spectrophotometer at 450 nm wavelength. ß-carotene was used as standard to construct the calibration curve. The results, in dry basis, were expressed in μg of ß-carotene per 100 g of sample (AOAC, 2010).

The antioxidant activity was determined using ORAC (Oxygen Radical Absorbance Capacity) method, according to the methodology of Ou et al. (2001) adapted by Huang et al. (2002), in microplates with fluorescein. The analyses were performed using 96-well microplates (Synergy HT Multi-Mode Reader, Biotek Industries, USA). A sample of 25 μL was mixed with 150 mL of fluorescein (55.5 nm) and incubated for 15 min at 37 °C in nanomicroplates before the automatic injection of 25 μL AAPH solution (155 mM). The reading of fluorescence was observed for at least 50 min (excitement = 485 nm; emission = 520 nm). Trolox solutions were prepared (8, 16, 24, 32 and 40 M) for the calibration curve. All solutions were diluted in phosphate buffer (75 mM, pH 7.4). Samples were analyzed in three dilutions and taking its mean as the final ORAC value as recommended by Huang et al. (2002). The quantification of antioxidant activity was based on calculating the area under the fluorescence curve as proposed by Prior et al. (2005). The results, in dry basis, were expressed in m mol Trolox Equivalent (TE) per 100 g of the sample.

The antioxidant activity (radical 1,1-difenil-2-picrilhidrazila) − DPPH was determined according to Brand-Williams et al. (1995) methodology with some modifications. The analyses were performed in 96-well microplates (Synergy HT Multi-Mode Reader, Biotex Industries, USA) and the decrease in the absorbance was monitored at 517 nm every 5-min interval until the reaction stabilized. The values were determined by adding 40 μL of DPPH solution to each well of 250 μL microplate and methanol into the control, or the same volume for standard solutions (BHA, BHT, ascorbic acid, chlorogenic acid and quercetin) in the sample extracts. The remaining DPPH at the end of the reaction was calculated as the DPPH radical activity by using a Trolox standard curve. The DPPH antioxidant method, in dry basis, was expressed in m mol Trolox Equivalent (TE) per 100 g of sample.

2.2.2. Non-destructive analysis

For measurements of respiratory behavior and ethylene production, approximately 1 kg of fruits were placed in airtight containers, each one with a capacity of 1 L, for 1 h at 22 ± 1 °C. After this period, 5 mL of gas was collected from each container with the help of a hypodermic syringe, for ethylene and CO_2 dosing. The ethylene concentration was measured by gas chromatography using Varian® brand, Model 3300, equipped with stainless 1/8" column, prepared with Porapak® N and flame ionization detector. The CO_2 concentration was measured in a Shimadzu® CR 950 chromatograph, equipped with a thermal conductivity detection system. Standard solution of 100 ppm and 5% were used for ethylene and CO_2, respectively. The results were expressed in mL of CO_2 kg-1 h-1 and μL of ethylene kg-1 h-1 (AOAC, 2010).

The weight loss (%) was calculated as a percentage of weight of the fruit at the beginning and at the end of 10 days of storage period (24 ± 3 °C and 75 ± R.H.). The difference as a percentage from the original weight was calculated: weight loss% = ([initial weight − final weight]/initial weight) × 100 (Youssef and Roberto, 2014).

2.3. Statistical analysis

It is important to mention that this work presents averages for the five seasons performed, where supposedly has no detected any statistical significant variations between the years. In this sense, all the data from all the five seasons performed were submitted to an analysis of variance (ANOVA) to test the difference between the results and the means were compared by the Tukey test at 5% statistical probability