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Carbohydrates, volatile and phenolic compounds composition, and antioxidant activity of calabura (*Muntingia calabura* L.) fruit

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Catechin (PubChem CID: 73160)
Cyanidin-3-*O*-glucoside (PubChem CID: 441667)
Delphinidin-3-*O*-glucoside (PubChem CID: 443650)
Gallocatechin (PubChem CID: 65084)
Fructose (PubChem CID: 5984)
Gallic acid (PubChem CID: 370)
Glucose (PubChem CID: 79025)
Methyl salicylate (PubChem CID: 4133)
Quercetin (PubChem CID: 5280343)
β-Farnesene (PubChem CID: 5281517)

ABSTRACT

Soluble carbohydrates, volatile and phenolic compounds from calabura fruit as well as its antioxidant activity were assessed. The low amount of fermentable oligo-, di-, and monosaccharides and polyols (FODMAPs) and similar amount of glucose and fructose allow us to classify the calabura berry as low-FODMAPs. The terpenes β-Farnesene and dendrolasin identified by SPME-GC-MS were the major volatile components. UHPLC-MS/MS analysis revelled gallic acid (5325 μg/g dw) and cyanidin-3-*O*-glucoside (171 μg/g dw) as the main phenolic compounds, followed by gentisic acid, gallocatechin, caffeic acid and protocatechuic acid. In addition, gallic acid was found mainly in esterified (2883 μg/g dw) and insoluble-bound (2272 μg/g dw) forms. Free and glycosylated forms showed however the highest antioxidant activity due to occurrence of flavonoids (0.28–27 μg/g dw) in these fractions, such as catechin, gallocatechin, epigallocatechin, naringenin, and quercetin. These findings clearly suggest that calabura is a berry with low energy value and attractive colour and flavour that may contribute to the intake of several bioactive compounds with antioxidant activity. Furthermore, this berry have great potential for use in the food industry and as functional food.

1. Introduction

The species *Muntingia calabura* L. (see Fig. S1 in Supplementary material) belongs to Elaeocarpaceae family and it is the sole species within the genus *Muntingia*. This plant is native to Central America, but due to its good adaptation to soil and climate it has been widely grown in several countries of tropical climates, such as Brazil, China, India, Malaysia and Philippines (Mahmood et al., 2014). The calabura tree is also exotic and ornamental, and it grows fast even on poor soils. The first fruiting occurs one year after the planting and the fruiting occur throughout the year with peak from April to July.

Calabura is also a climacteric fruit that can ripen fully if they are harvested at completion of their growth period (Rahman, Solaiman, & Rahman, 2010). The fruit is small (average weight of 1.60 g) and red-coloured at mature stage, and shows a sweet and unique flavour, which attracts birds and also pleases the human taste. These ripe berries are very sweet due to high content of soluble solids (10.24°Brix) and low total titratable acidity (0.11 g citric acid per 100 g fruit, pH 5.64). Besides being very appreciated for its flavour and color, the fruit has been reported to contribute to the intake of carbohydrates (14.64%), proteins (2.64%), lipids (2.34%), fibers (1.75%) and minerals (1.28%), with low total energy value (Pereira, Tomé, Arruda, Fragiorge, & Ribeiro, 2016). In addition, it contains high content of soluble phenolic compounds, mainly phenolic acids and flavonoids (Lin et al., 2017; Rotta, Haminiuk, Maldaner, & Visentainer, 2017). Extracts rich in phenolic compounds from calabura fruits have been found to exhibit bioactivities such as

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antimicrobial activity against food borne pathogens (Sibi, Kaushik, Dhananjaya, Ravikumar, & Mallesha, 2013), antioxidant properties suppressing human LDL (Low Density Lipoprotein) oxidation (Lin, Chen, Chang, Chen, & Yang, 2017), and anti-inflammatory activity in carrageenan rat paw edema model (Gomathi, Anusuya, & Manian, 2013) and lipopolysaccharide-induced pro-inflammatory mediators in macrophages (Lin, Chang, et al., 2017).

The calabura fruits are eaten fresh and they are mostly consumed as homemade preparations, but these berries could have great potential for use in the food industry and as functional food due to its physicochemical properties and high content of antioxidant compounds (Lin, Chen, et al., 2017; Pereira et al., 2016). However, there is little information available in the literature about chemical composition of this berry; and the use of fruits in food science and industry depends mainly of its composition. Although there have been recent studies on the soluble phenolic compounds from calabura (Lin, Chang, et al., 2017; Lin, Chen, et al., 2017; Rotta et al., 2017), other fractions of phenolic compounds were not investigated, which can result in the underestimation of phenolic compounds content and consequently the actual biological properties of calabura fruits. In this approach, we evaluated the composition of volatile organic compounds, functional sugars and oligosaccharides, the individual anthocyanins content, and the soluble (free, esterified and glycosylated) and insoluble-bound forms of non-coloured phenolic compounds from calabura fruits. Colorimetric and fluorometric assays were also used to assess the phytochemicals and antioxidant activity of these berries. The aim of this study was therefore to investigate the functional components of calabura berry to help future studies about its potential functionality and uses in food industry.

2. Material and methods

2.1. Chemicals and reagents

Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), DPPH (2,2-diphenyl-1-picrylhydrazil), AAPH (2,2′-azobis(2-methylamidinopropane)-dihydrochloride), ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt), fluorescein sodium salt, sodium carbonate, and Folin–Ciocalteu reagent were purchased from Sigma–Aldrich (St. Louis, USA). Sugars (glucose, fructose and sucrose), polyols (xylitol, mannitol, and sorbitol), galactooligosaccharides (GOS; raffinose, stachyose, and verbascose), and maltooligosaccharides (MOS; maltotriose to maltoheptaose) were purchased from Sigma-Aldrich (St. Louis, USA), whereas the fructooligosaccharides (FOS; 1-kestose, nystose, and 1F-β-fructofuranosylnystose) were purchased from Wako (Wako Pure Chemicals Industries, Osaka, Japan). All phenolic compounds standards with purity ≥96% (gallic acid, gallocatechin, protocatechuic acid, epigallocatechin, catechin, chlorogenic acid, 4-hydroxybenzoic acid, epicatechin, caffeic acid, vanillic acid, gentisic acid, *p*-coumaric acid, sinapic acid, ferulic acid, rutin, quercetin, naringenin, cyanin, delphinidin-3-*O*-glucoside, cyaniding-3-*O*-glucoside, and methyl salicylate) were purchased from Sigma-Aldrich (St. Louis, USA). The saturated alkanes standard (C_7-C_{40}) was purchased from Supelco (Bellefonte, USA). Only HPLC grade solvents were used for preparing mobile phases. Ultrapure water (18 MΩ cm^{-1}) obtained from a Milli-Q water purification system (Millipore, Bedford, USA) was used.

2.2. Volatile constituents of calabura fruits by SPME and GC–MS

2.2.1. Harvest of the fruits

Ripe calabura fruits (red-coloured peel) were collected in July 2016 from five calabura trees located in Campinas-SP, Brazil. The fruits (10 fruits per tree in each day) were harvested at different date (once a week, totalling four days), and the analysis of volatile compounds from calabura fruits was carried out in triplicate on the same day of fruit harvest.

2.2.2. Solid phase microextraction (SPME)

SPME fiber (Supelco PDMS/DVB, 10 mm in length × 65 μm layer) was used. The fiber was activated according to the manufacturer's instructions. For each extraction, 5 g of the fruit homogenized and 15 mL of distilled water were placed in sterile flasks with a plastic cap with a PTFE septum. The sample flaks were put under magnetic stirring at 30 °C for 30 min. After this time, the SPME fiber was exposed to the headspace of the solution to adsorb the compounds. After 30 min, the SPME fiber was removed from the sample vial and introduced into the heated chromatograph injector (250 °C for 10 min) for desorption and analysis (Souza de Carvalho et al., 2014).

2.2.3. Gas chromatography-mass spectrometry (GC–MS)

The volatile compounds were analysed using an Agilent HP-7890 GC system coupled to a mass spectrometer HP-5975C (Agilent Technologies, Santa Clara, USA). Volatile components were separated using a capillary column (J&W Scientific HP-5MS, 30 m lenght × 0.25 mm i.d. × 0.25 μm of film thickness). The oven temperature programme was as follows: 40 °C (5 min), 40–120 °C at 5 °C/min, 120 °C (5 min), 120–180 °C at 3 °C/min, 180–240 °C at 10 °C/min, 240 °C (5 min). The carrier gas was helium at 1.0 mL/min. The analysis was performed in the splitless mode and temperatures of the injector and detector were set at 250 °C. The mass spectrometer transfer line was set at a temperature of 250 °C, impact energy of 70 + eV and the acquisition mass range of *m/z* 35–500. The identification of the volatile compounds was made by comparing their mass spectra with National Institute of Standards and Technology (NIST) mass spectral library (90% similarity) and linear retention index (LRI). The LRI of the identified compounds were calculated using a series of *n*-alkanes (C_7–C_{40}) injected in the same SPME-GC-MS conditions, and the LRI values were compared with those reported in the literature. The proportion of each compound was estimated dividing its mean area by the total area of the chromatogram, and expressed as percentage (Souza de Carvalho et al., 2014).

2.3. Plant material and sample preparation for carbohydrates, phenolic compounds and antioxidant analyses

Calabura fruits with full physiological maturity (red-coloured peel) were collected from June to July 2016. The collection of fruits was performed from five calabura trees located in Campinas-SP, Brazil. To avoid variation in the calabura composition, all fruits collected (1 kg) were mixed as a single lot. The fruits collected were immediately washed with distilled water to remove surface dirt, frozen at −20 °C, and thus all fruits frozen (1 kg) were freeze-dried. The average moisture content of the calabura fruit was 75.00 ± 1.6%. The freeze-dried material was powdered using a knife grinder and stored at freezer (−20 °C) until analysis.

2.4. Chromatographic analysis of sugars and oligosaccharides

Freeze-dried fruit was mixed with ultrapure water (1,10, w/v) with the aid of Ultra-Turrax Homogenizer at 11000 rpm for 30 s at room temperature. After the centrifugation (4000*g*, 5 min, 5 °C) the supernatant was filtered with a 0.22 μm filter and used for the analysis of sugars and oligosaccharides as described by Pereira, Arruda, Molina, and Pastore (2017), with some modifications.

High performance anion exchange chromatography coupled to pulsed amperometric detection (HPAEC-PAD) system model DIONEX ICS-5000 (Thermo Fisher Scientific, Waltham, USA) was used. The flow-rate was 1.0 mL/min, the column temperature was kept at 30 °C and the injection volume was 25 μL. Two different chromatographic column were used, one for the analysis of galactoligo-, mono- and disaccharides and polyols (Carbopac PA1 (250 × 4 mm, 10 μm particle size)), and the second one for the fructo- and maltooligosaccharides (CarboPac PA100 (250 × 4 mm, 8.5 μm particle size)). Isocratic mobile