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Profiling of lipophilic and phenolic phytochemicals of four cultivars from cherimoya (*Annona cherimola* Mill.)

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ABSTRACT

The lipophilic and phenolic extractives of the ripe mesocarp of four cherimoya cultivars ('Perry Vidal', 'Mateus I', 'Mateus III' and 'Funchal') from Madeira Island, were studied for the first time. The predominant lipophilic compounds are kaurene diterpenes (42.2–59.6%), fatty acids (18.0–35.6%) and sterols (9.6–23.7%). Kaur-16-en-19-oic acid is the major lipophilic component of all cultivars accounting between 554 and 1350 mg kg^{-1} of dry material.

The studied fruits also contain a high variety of flavan-3-ols, including galloylated and non-galloylated compounds. Five phenolic compounds were identified for the first time: catechin, (epi)catechin-(epi)gal locatechin, (epi)gallocatechin, (epi)afzelechin-(epi)catechin and procyanidin tetramer. 'Mateus I' and 'Mateus III' cultivars present the highest content of phenolic compounds (6299 and 9603 mg kg^{-1} of dry weight, respectively). These results support the use of this fruit as a rich source of health-promoting components, with the capacity to prevent or delay the progress of oxidative-stress related disorders.

1. Introduction

Annona, a plant genus from the family Annonaceae, comprises 119 species of which *Annona cherimola* Mill., *Annona muricata* L., *Annona squamosa* L., *Annona reticulata* L. and the interspecific hybrid *A. squamosa* L. × *A. cherimola* Mill. are of significant commercial importance (Pareek, Yahia, Pareek, & Kaushik, 2011; Pinto et al., 2005). Among these, *A. cherimola* Mill., commonly known as cherimoya, is the one with the strongest consumer demand (Pinto et al., 2005). Although native from South America and Antilles, *A. cherimola* is now cultivated in several tropical and subtropical areas around the world. Spain, Peru and Chile are the main producers of cherimoya while small production areas exist in California, Israel and Madeira Island, Portugal (Pinto et al., 2005).

The cherimoya shrub or small-tree is well adapted to the edaphoclimatic conditions of Madeira Island, Portugal, where the estimated production in 2014 was 1104 ton in an area of 115 ha (DRE, 2015), supporting the Portuguese demand for this fruit. After cherimoya introduction in the island in 1897 (Arun Jyothi, Venkatesh, Chakrapani, & Roja Rani, 2011), propagation by seeds prevailed and originated diverse vigorous plants from which cultivars were developed and improved (Nunes, 1997). Nowadays, there are several cherimoya cultivars, being 'Madeira', 'Perry Vidal', 'Mateus I' and 'Funchal' the most important, with a high potential to be commercialized in national and international markets. Fruits are heart-shaped, the skin is thin and delicate, differing in coloration at maturity being yellowish-green in "Funchal" and brownish-green in the others cultivars (Agripérola Cooperativa Agrícola CRL, 1998; Caldeira, Araujo, & Nunes, 1995).

Cherimoya fruits are mainly consumed fresh due to their pleasant taste and aroma but can also be used as semi-processed and processed products. This soft and nutritive fruit is known to be rich in minerals, vitamins, essential amino acids, volatile compounds and polysaccharides (Arun Jyothi et al., 2011; Cordeiro, Sousa, Freitas, & Gouveia, 2013; Egydio, Catarina, Floh, & Dos Santos, 2013; Ferreira, Perestrelo, & Câmara, 2009; Pareek et al., 2011). The presence of phenolic compounds in cherimoya has also been reported, namely flavanols and procyanidins (Barreca et al., 2011), although no information about the fruits origin (commercial or cultivars data) and the quantity of phenolic compounds was given. Furthermore, seeds, leaves and stems were also reported

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as important sources of essential oils, flavonoids, alkaloids, saponins, acetogenins and phytosterols, among others, with nutritional, pharmaceutical and industrial interest (Arun Jyothi et al., 2011; Chen, Chang, Pan, & Wu, 2001; Egydio & Santos, 2011; Pinto et al., 2005). Some of these compounds are bioactive as they display insecticidal, antimicrobial, antiparasitic, cytotoxic, antioxidant, antidepressant and anti-diabetic activities (Arun Jyothi et al., 2011; Barreca et al., 2011; Gupta-Elera, Garrett, Martinez, Robison, & O'Neill, 2011; Loizzo et al., 2012; Martínez-Vázquez et al., 2012).

However, there is limited and/or absent data regarding the characterization of cherimoya fruits pulp from 'Perry Vidal', 'Mateus I', 'Mateus III' and 'Funchal' cultivars. As far as we could ascertain, there is only one study reporting the volatile composition of cherimoya fruits from these cultivars (Ferreira et al., 2009).

The increasing recognition of cherimoya nutritional value highlights the importance of this fruit as a valuable supplement for diets, as well as for industrial applications. In addition to its nutritional value, the lipophilic and phenolic profiles of this fruit can be useful to determine its economic and health potential. Hence, and given that the phytochemicals or phytonutrients are dependent on the cultivar, geographic origin or climacteric conditions, the present study aims to evaluate the lipophilic and phenolic fractions composition of the ripe mesocarp of four cherimoya cultivars from Madeira Island, namely 'Perry Vidal', 'Mateus I', 'Mateus III' and 'Funchal', by gas chromatography–mass spectrometry (GC–MS) and ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-MS) analysis, respectively.

2. Material and methods

2.1. Chemicals

Dichloromethane (99% purity), *N,O*-bis(trimethylsilyl)trifluoroacetamide (99% purity), trimethylchlorosilane (99% purity), pyridine (99% purity), tetracosane (99% purity), stigmasterol (95% purity), octadecanoic acid (99% purity), nonadecan-1-ol (99% purity), gallic acid (purity higher than 97.5%) and Folin–Ciocalteu's phenol reagent were supplied by Sigma Chemical Co. (Madrid, Spain). Isorhamnetin (purity higher than 99%), luteolin (purity higher than 97%), formic acid (purity higher than 98%) and methanol (purity higher than 99.8%) were purchased from Fluka Chemie (Madrid, Spain). Sodium carbonate (99.9% purity) was supplied by Pronalab (Lisbon, Portugal). HPLC-grade methanol, water and acetonitrile were supplied by Fisher Scientific Chemicals (Loures, Portugal) and further filtered using a Solvent Filtration Apparatus 58061 from Supelco (Bellefonte, PA, USA).

2.2. Samples preparation and physicochemical parameters

Cherimoya (*Annona cherimola* Mill.) without evidence of physical or pathological injuries were selected from Centro de Fruticultura Subtropical do Funchal, Madeira Island, Portugal. Mature green fruits from 'Perry Vidal', 'Mateus I', 'Mateus III' and 'Funchal' cultivars were hand harvested in January 2015 (winter season) and then left to reach full ripeness at room temperature (20–23 °C). Ripe fruits (n = 6) were then peeled (peel was fully discarded), sliced and quick-frozen in liquid nitrogen. Frozen samples were lyophilized, milled to pass through a 40–60 mesh sieve and stored (humidity *ca.* 5%) in a freezer at −18 °C for further analyses.

Fruit firmness was determined after removing the skin on two opposite sides (n = 12) in the middle of each fruit using a pressure-testing instrument (Model FT 327) fitted with an 11.3 mm cylindrical plunger. The force required to penetrate into the flesh was expressed in Newtons (N). Fresh slices of each sample (n = 6) were used to measure fruit water content through a Gibertini–Eurotherm balance at 105 °C, as well as to determine the total soluble solids (TSS) or Brix percentage in a digital brix refractometer from ATAGO.

Data are reported as mean ± SD and analysed by one way analysis of variance (ANOVA) to determine differences between means at 5% confidence level. Statistical analyses were performed using SPSS v 23 for Windows.

2.3. Lipophilic and phenolic compounds extraction

Three grinded fruits (20 g) of each cultivar were Soxhlet extracted with dichloromethane during 6 h. The solvent was evaporated to dryness by low-pressure evaporation, the lipophilic extracts were weighted and the results were expressed in percent of dry material (% DW).

Subsequently, the solid residues from the dichloromethane extraction were suspended (m/v 1:100) in a methanol/water (MeOH:H_2O, 50:50 v/v) mixture, at room temperature for 24 h, under constant stirring. Then, the suspension was filtered, MeOH removed by low-pressure evaporation, and the phenolic extracts freeze-dried. The extraction yields were expressed in percent of dry material (% DW) (Santos et al., 2013).

2.4. GC–MS analysis

Before GC–MS analysis, two aliquots of each dried lipophilic extract (20 mg each) and an accurate amount of internal standard (tetracosane, 0.50 mg) were dissolved in 250 μL of pyridine. The compounds containing hydroxyl and carboxyl groups were converted into their trimethylsilyl (TMS) ethers and esters derivatives, respectively, by adding 250 μL of *N,O*-bis(trimethylsilyl)trifluoroacetamide and 50 μL of trimethylchlorosilane, standing the mixture at 70 °C for 30 min (Freire, Silvestre, Neto, & Cavaleiro, 2002). The derivatized extracts were analyzed by GC–MS on a Trace Gas Chromatograph 2000 Series, equipped with a Thermo Scientific DSQ II single-quadrupole mass spectrometer. The following conditions were used: electron impact energy 70 eV; collection rate: 1 scan s^{-1}; ion source temperature: 250 °C; *m/z* range: 33–700. The column used was a DB-1 J&W capillary column (30 m × 0.32 mm inner diameter, 0.25 μm film thickness) and helium was used as carrier gas (35 cm s^{-1}). The chromatographic conditions were as follows: initial temperature: 80 °C for 5 min; temperature gradient: 4 °C min^{-1}; final temperature: 260 °C; temperature gradient: 2 °C min^{-1}; final temperature: 285 °C for 8 min; injector temperature: 250 °C; transfer-line temperature: 290 °C; split ratio: 1:33.

To check the presence of lower volatility esterified structures, TMS derivatized samples were also analysed with a short DB-1 J&W capillary column (15 m × 0.32 mm inner diameter, 0.25 μm film thickness). The chromatographic conditions applied were as follows: initial temperature: 100 °C for 3 min; temperature gradient: 5 °C min^{-1}; final temperature: 340 °C for 12 min; injector temperature: 290 °C; transfer-line temperature: 290 °C; split ratio: 1:33.

Compounds were identified as TMS derivatives by comparing their mass spectra with the GC–MS spectral library (Wiley–NIST Mass Spectral Library 1999), with data from literature (AOCS Lipid Library, 2013; Oliveira et al., 2005), and, in some cases, by the injection of standards.

For semi-quantitative analysis, GC–MS was calibrated with pure reference compounds, representative of the major lipophilic extractive families (stigmasterol, octadecanoic acid and nonadecan-1-ol) relative to tetracosane. The respective response factors were calculated as an average of six GC–MS runs. For tocopherol and kaurene diterpenes the response factor of stigmasterol was used. Each aliquot was injected in triplicate. The presented