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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

results are the average of the concordant values obtained for the six aliquots (less than 5% variation between injections of the same aliquot and between aliquots of the same cherimoya variety extracts).

2.5. UHPLC procedure

The UHPLC system consisted of a variable loop Accela autosampler (200 vial capacity set at 15 °C), an Accela 600 LC pump and an Accela 80 Hz PDA detector (Thermo Fisher Scientific, San Jose, CA, USA). The separation of the compounds was carried out with a gradient elution program at a flow rate of 0.5 mL min^{-1}, at 45 °C, by using a Kinetex™ C_{18} (50 mm × 2.1 mm × 1.7 μm) column supplied by Phenomenex (Thermo Fisher Scientific, San Jose, CA, USA). The injection volume in the UHPLC system was 6 μL and the mobile phase consisted in water:acetonitrile (99:1, v/v) (A) and acetonitrile (B), both with 0.1% of formic acid. The following linear gradient was applied: 0–4 min: 2%B, 4–7.5 min: 2–4.5%B, 7.5–25.5 min: 4.5–11%B, 25.5–26 min: 11–12.8%B, 26–26.5 min: 12.8–30%B, 26.5–32 min: 30–39%B, 32–35 min: 39–47.5%B, 35–39 min: 47.5–65%B, 39–44 min: 65–100%B, 44–48 min: 100-0%B, followed by re-equilibration of the column for 4 min before the next run. Double online detection was carried out in the diode array detector, at 280 and 340 nm, and UV spectra in a range of 200–600 nm were also recorded. Before the injection, each MeOH:H_2O extract was dissolved in MeOH/H_2O (50:50, v/v) HPLC grade, to obtain a final extract concentration of 30 mg mL^{-1}, and then filtered through a 0.2 μm PTFE syringe filter.

2.6. ESI-MS^n analysis

The HPLC was coupled to a LCQ Fleet ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA), equipped with an electrospray ionization source and operating in negative mode. The nitrogen sheath and auxiliary gas were 50 and 10 (arbitrary units), respectively. The spray voltage was 5 kV and the capillary temperature was 360 °C. The capillary and tune lens voltages were set at −28 V and −115 V, respectively. CID-MS^n experiments were performed on mass-selected precursor ions in the range of *m/z* 100–1500. The isolation width of precursor ions was 1.0 mass units and the scan time was equal to 100 ms and the collision energy was optimized between 15 and 40 (arbitrary units), using helium as collision gas. The data acquisition was carried out by using Xcalibur® data system (ThermoFinnigan, San Jose, CA, USA).

2.7. Phenolic compounds quantification by UHPLC–UV

Catechin was used as reference compound for quantitative analysis, with the UV detector monitored at 280 nm. Calibration curve was obtained by UHPLC injection of standard solutions in MeOH:H_2O, with concentrations between 5 and 80 μg mL^{-1}. Besides the linearity, the limits of detection (LOD = 8.01 μg mL^{-1}) and quantification (LOQ = 26.72 μg mL^{-1}) were also estimated using the S/N approach (n = 5). The calibration curve obtained was $y = 45783x - 68{,}057$ (y = area, x = C (μg mL^{-1}); $R^2 = 0.9943$). The concentrations were calculated based on triplicate injections and the mean value determined in each case (less than 5% variation between injections).

3. Results and discussion

3.1. Physicochemical characterization of cherimoya fruit

Cherimoya fruits used in this study were comparable in weight, water content and firmness (Table 1). In general, fruit weight ranged from 522.1 g to 587.1 g in 'Funchal' and 'Perry Vidal', respectively. In what concerns water content, cultivars 'Mateus III' (66.7%) and 'Perry Vidal' (62.0%) were quite similar, showing a slightly higher content than 'Mateus I' (57.9%) and 'Funchal' (56.3%). The pulp firmness was evaluated in the mature stage, and 'Perry Vidal' displayed the highest pulp firmness with 1.09 N. Regarding the total soluble solids (TSS), which are predominantly made of sugars, the lowest value was observed for 'Funchal' (17.0 °Brix) and the highest one for 'Perry Vidal' (26.5 °Brix). All the values obtained are comparable to data reported elsewhere for other species and cultivars (Andrade, Zoghbi, Maia, Fabricius, & Marx, 2001; Arun Jyothi et al., 2011; Pareek et al., 2011), except the TSS of 'Perry Vidal' which indicates that this cultivar is sweeter than the others evaluated in this study, and in other previously studied varieties (Caldeira et al., 1995).

The ripe mesocarp of the four cherimoya cultivars studied here presented similar lipophilic extractives yields (Table 1) with values ranging from 0.57 to 0.94% of dry material for 'Mateus III' and 'Mateus I, respectively. These contents are of the same order of those found on the pulp of *Annona muricata* (Melot, Fall, Gleye, & Champy, 2009) and, also, on other tropical fruits, *e.g.* in the ripe pulp of mango fruits (Vilela et al., 2013) and in the unripe pulp of banana (Lúcia Oliveira, Freire, Silvestre, & Cordeiro, 2008).

The yields of the MeOH:H_2O ripe mesocarp extracts of the studied cherimoya cultivars ranged between 58.53% of dry weight for 'Perry Vidal' and 72.21% for 'Mateus III' (Table 1). The high yield of polar extractives obtained from fruit pulp was expectable, due to the presence of sugars or other water-soluble components, as suggested by the TSS values. In fact, an extraction yield of about 45% was already described for loquat fruit pulp (Delfanian, Esmaeilzadeh Kenari, & Sahari, 2015).

3.2. Composition of the lipophilic extracts

The chemical composition of the lipophilic extracts of the four cherimoya cultivars was studied by GC–MS analysis; the detailed identification and quantification of the main lipophilic compounds present in the ripe mesocarps are summarized in Table 2. The predominant compounds present in these extracts were two kaurene diterpenes, followed by a series of free fatty acids (C12 to C22), sterols and minor amounts of long-chain aliphatic alcohols (C16 to C30), among others. The relative abundance of the identified compounds and their families differ somewhat between cultivars, as depicted in Fig. 1.

As evoked above, two kaurene diterpenes (597–1560 mg kg^{-1} DW) are the most abundant lipophilic compounds present in the ripe cherimoya mesocarps, which account for 42.2% ('Mateus III') and 59.6% ('Funchal') of the total lipophilic extractives identified. This family of compounds have already been reported in the leaves, bark and fruits of *A. glabra* (Chang, Yang, Lin, Lee, & Wu, 1998; Hsieh, Wu, Chen, Huang, & Chen, 2004; Oliveira, Sant'Ana, & Bastos, 2002), in the fruit of *A. cherimola* (Miyashita, Nishida, Okawa, Nohara, & Yoshimitsu, 2010), in the leaves of *A. reticulata* (Thang et al., 2013) and in the stem bark of *A. vepretorum* (Dutra et al., 2014).

Kaurenoic acid (*ent*-kaur-16-en-19-oic acid, Fig. 2) is definitely the major component of this family in all mesocarp samples, representing between 84.7% ('Perry Vidal') and 92.8% ('Mateus III') of total kaurene diterpene contents and between 36.4% ('Mateus I') and 52.1% ('Mateus III') of the total lipophilic extractives (Table 2). In terms of edible portion, these cultivars can contribute to the intake of *ca.* 18.5–58.9 mg of kaurenoic acid per 100 g of fresh fruit. These values, although lower than the one reported for *A. squamosa* (90 mg per 100 g of fresh fruit) from the Amazon (Andrade et al., 2001), are significantly higher than those found for the specie *A. cherimola* (2.03 mg per 100 g of fresh fruit) (Miyashita et al.,

Table 1
Physicochemical characteristics and extraction yields (% of dry weight) of the four cherimoya cultivars.

Cultivar	Weight (g)	Moisture (%)	Firmness (N)	TSS (°Brix)	Extraction yields (%)	
					CH_2Cl_2	MeOH:H_2O
'Perry Vidal'	587.1 ± 136.2[a]	62.0 ± 3.2[ab]	1.09 ± 0.21[a]	26.5 ± 2.2[a]	0.67	58.53
'Mateus I'	566.1 ± 117.7[a]	57.9 ± 4.0[a]	0.76 ± 0.18[a]	22.2 ± 4.4[ab]	0.94	67.12
'Mateus III'	552.1 ± 112.8[a]	66.7 ± 5.6[b]	0.76 ± 0.23[a]	18.5 ± 3.2[b]	0.57	72.21
'Funchal'	522.1 ± 159.3[a]	56.3 ± 2.1[a]	0.49 ± 0.92[a]	17.0 ± 2.3[b]	0.73	71.86

[a,b] Distinct letters in the same column represent means significantly different ($p < 0.05$).

Table 2
Compounds identified in the lipophilic extracts of ripe mesocarp from cherimoya cultivars expressed in mg kg^{-1} of dry material.[a]

Compound	'Perry Vidal'	'Mateus I'	'Mateus III'	'Funchal'
Fatty acids	**854**	**773**	**191**	**737**
Saturated	*549*	*507*	*116*	*479*
Dodecanoic acid	39	4	1	14
Tetradecanoic acid	17	19	4	14
Pentadecanoic acid	6	17	2	15
Hexadecanoic acid	272	272	84	285
Heptadecanoic acid	12	20	4	19
Octadecanoic acid	109	99	15	79
Eicosanoic acid	86	68	4	49
Docosanoic acid	8	8	2	4
Unsaturated	*263*	*251*	*61*	*241*
Hexadec-9-enoic acid	2	2	1	3
Heptadec-9-enoic acid	12	18	8	22
Octadec-9-enoic acid (*cis* and *trans*)	42	66	11	50
Octadeca-9,12-dienoic acid (ω-3)	107	85	24	107
Octadeca-9,12,15-trienoic acid (ω-6)	100	80	17	59
Diacids	*2*	*5*	*2*	*7*
Nonadioic acid	2	5	2	7
ω-Hydroxyacids	*40*	*10*	*12*	*10*
22-Hydroxydocosanoic acid	40	10	12	10
Long chain aliphatic alcohols	**26**	**27**	**15**	**48**
Hexadecan-1-ol	6	8	4	19
Octadecan-1-ol	2	4	2	6
Octacosan-1-ol	6	9	4	12
Triacontan-1-ol	12	6	5	11
Sterols	**254**	**555**	**252**	**252**
24-Methylenecholesterol	2	1	0	3
Campesterol	42	70	38	28
Stigmasterol	64	183	54	55
β-Sitosterol	124	277	148	137
Fucosterol	16	11	4	18
24-Methylenecycloartenol	6	13	8	11
Diterpenes	**1238**	**1105**	**597**	**1560**
Kaurenoic acid	1048	953	554	1350
Kaurenol	190	152	43	210
Others	**25**	**159**	**8**	**19**
1-Monopalmitin	6	11	5	12
Squalene	13	136	1	4
α-Tocopherol	6	12	2	3

[a] Results are the average of concordant values obtained (less than 5% variation between injections) for the two aliquots of each sample injected in triplicate.

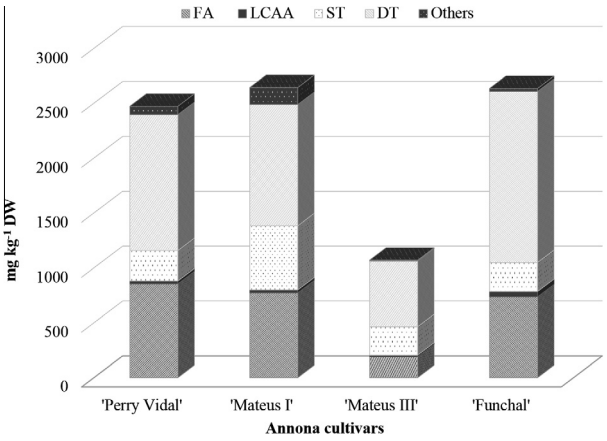

Fig. 1. Major families of lipophilic compounds identified in the cherimoya mesocarp extracts. FA, fatty acids; MG, monoglycerides; LCAA, long-chain aliphatic alcohols; ST, sterols; DT, diterpenes.

2010) and *A. glabra* (3.9 mg per 100 g of fresh fruit) (Hsieh et al., 2004). The role of kaurenoic acid is mostly associated with the inhibition of inflammatory pain (Mizokami et al., 2012), antibacterial activity (Okoye et al., 2012) and anticonvulsant effect (Okoye, Akah, Omeje, Okoye, & Nworu, 2013), among other properties (Ambrosio, Tirapelli, da Costa, & de Oliveira, 2006).

Another abundant identified kaurene diterpene included kaurenol (*ent*-kaur-16-en-19-ol, Fig. 2) with 43–210 mg kg^{-1} DW, which corresponds to 7.2% ('Mateus III') and 15.3% ('Perry Vidal') of total kaurene diterpenes contents, and 4.0% ('Mateus III') and 8.0% ('Funchal') of the total lipophilic extractives (Table 2). Although this compound was already identified in the fruits of *A. glabra* (Chang et al., 1998; Oliveira et al., 2002), no content values are available for comparison.

Free fatty acids represent about 18.0–35.6% of the total identified lipophilic components of the ripe cherimoya mesocarp, among which 60.7–65.6% correspond to saturated fatty acids and 30.8–32.7% unsaturated ones. The fatty acids ranged from dodecanoic (C12) to docosanoic (C22) acids, with hexadecanoic acid (palmitic acid, Fig. 2) as the most abundant saturated fatty acid (84–285 mg kg^{-1} DW) and octadeca-9,12-dienoic acid (linoleic acid, Fig. 2) as the most abundant unsaturated fatty acid (24–107 mg kg^{-1} DW). The presence of the identified saturated and unsaturated fatty acids in the cherimoya mesocarp has been previously reported in the fruits of *A. squamosa* (Andrade et al., 2001), and *A. Cherimola* 'Madeira' (Cordeiro et al., 2013) and 'Fino de Jete' (Gutiérrez, Mar Sola, & Vargas, 2005) cultivars. Minor amounts of 22-hydroxydocosanoic (10–40 mg kg^{-1} DW) and nonadioic acids (2–7 mg kg^{-1} DW) were also found in all extracts of ripe cherimoya mesocarps.

Among the fatty acids family, polyunsaturated fatty acids, like octadeca-9,12-dienoic (ω-6) and octadeca-9,12,15-trienoic (ω-3) acids, are essential nutrients that must be obtained from the diet since they are not produced in the human body (Sánchez-Moreno, De Pascual-Teresa, De Ancos, & Cano, 2012). The role of fatty acids, particularly ω-3 and ω-6 fatty acids, in the human health is essentially related with the prevention, delay, or treatment of chronic and acute diseases, including cancer, cardiovascular diseases, osteoporosis, and immune disorders (Chen, McClements, & Decker, 2013; Simopoulos, 1999; Simopoulos, 2008). The studied cherimoya fruits can contribute to the intake of *ca.* 3.8 mg ('Perry Vidal') and 4.7 mg ('Funchal') per 100 g of fresh

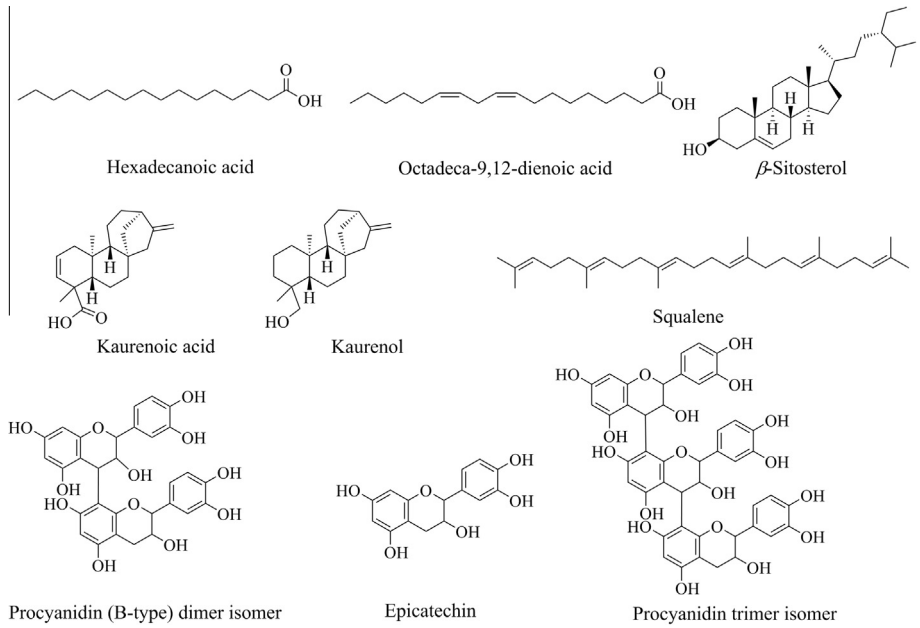

Fig. 2. Structures of the main lipophilic and phenolic compounds identified in the ripe mesocarp of the studied cherimoya cultivars.

fruit of ω-3 and ω-6 fatty acids, respectively. When compared with other fruits, the ripe cherimoya mesocarp has a higher content than *e.g.* mangoes (*ca.* 3.4 mg of ω-3 and 0.9 mg of ω-6 fatty acids per 100 g of fresh fruit) (Vilela et al., 2013), but a lower content than *e.g.* bananas (*ca.* 12.1 mg of ω-3 and 5.9 mg of ω-6 fatty acids per 100 g of fresh fruit) (Vilela et al., 2014). Notwithstanding, as expected, the content of ω-3 and ω-6 fatty acids in ripe cherimoya mesocarp is far from those found in well-known edible sources of these components, such as for example purslane and spinach (Simopoulos, 2004).

Free sterols, a very important group of lipophilic compounds given their broad range of biological functions and activities (Piironen, Lindsay, Miettinen, Toivo, & Lampi, 2000), account for 9.6–23.7% of the identified lipophilic components of ripe cherimoya mesocarps. β-Sitosterol is the major component of this family (124–277 mg kg^{-1} DW) present in all mesocarp samples, representing between 48.8% ('Perry Vidal') and 58.7% ('Mateus III') of total sterols contents and between 5.2% ('Perry Vidal' and 'Funchal') and 13.9% ('Mateus III') of the total lipophilic extractives (Table 2). Other sterols include stigmasterol (54–183 mg kg^{-1} DW) and campesterol (28–70 mg kg^{-1} DW). These three sterols have been detected in the fruits of the cultivar 'Madeira' (Cordeiro et al., 2013), and β-sitosterol along with stigmasterol were isolated from the fresh fruits of *A. glabra* (Hsieh et al., 2004). Smaller amounts of 24-methylenecholesterol (0–3 mg kg^{-1} DW), fucosterol (4–18 mg kg^{-1} DW) and 24-methylenecycloartenol (6–13 mg kg^{-1} DW) were also identified in all cultivars investigated. As far as our literature survey could ascertain, these three sterols were identified for the first time in *Annona* species. Still, they were already reported in mangoes (Vilela et al., 2013). Noteworthy is the fact that these cherimoya fruits can contribute to the daily intake of free phytosterols with *ca.* 8.7–24.6 mg per 100 g of fresh fruit, which seem to be a practical and safe option for reducing cholesterol levels in the population as discussed elsewhere (Piironen et al., 2000; Quílez, García-Lorda, & Salas-Salvadó, 2003).

Additionally, other compounds like long-chain aliphatic alcohols (LCAA), 1-monopalmitin, squalene and α-tocopherol were also detected in the extracts, although in minor amounts (Table 2). Only four LCAA were identified, namely hexadecan-1-ol, octadecan-1-ol, octacosan-1-ol and triacontan-1-ol, representing between 15 and 48 mg kg^{-1} of dry material. The presence of LCAA in the cherimoya mesocarp has been previously identified in the fruits of the cultivar 'Madeira' (Cordeiro et al., 2013). This class of compounds was reported to lower the plasma cholesterol in humans with their regular consumption (Hargrove, Greenspan, & Hartle, 2004). Squalene (Fig. 2), a natural polyunsaturated triterpene with the ability to inhibit the development of various tumours (Reddy & Couvreur, 2009), was only detected in significant amounts (136 mg kg^{-1} DW) in the cultivar 'Mateus I'. α-Tocopherol, the most bioactive form of vitamin E (Bramley et al., 2000), was the only tocopherol detected in the all the studied cherimoya mesocarps, accounting for 2–12 mg kg^{-1} DW, with the highest values observed for 'Mateus III' and 'Mateus I', respectively (Table 2).

Lastly, the lipophilic extracts of all cherimoya mesocarps were also analyzed by GC–MS with a short length (15 m) column, using chromatographic conditions that enable the elution and detection of esterified fatty acids (diglycerides, triglycerides and steryl esters) and steryl glucosides (Freire et al., 2002). As an illustrative example, Fig. S1 (Supplementary material) shows the typical GC–MS chromatogram of the derivatized lipophilic extract of the ripe mesocarp of 'Mateus I' cultivar, obtained by a short length column. Only small amounts of steryl glucosides, namely campesteryl 3β-D-glucopyranose, stigmasteryl 3β-D-glucopyranoside and sitosteryl 3β-D-glucopyranoside (41.97, 42.22 and 42.67 min, respectively), and steryl esters were detected in the four cultivars. Worth noting is the fact that these compounds are also present in small amounts in fruits like bananas (Vilela et al., 2014) and mangoes (Vilela et al., 2013). In addition, stigmasteryl 3β-D-glucopyranoside along with sitosteryl 3β-D-glucopyranoside were isolated from the fresh fruits of *A. glabra* (Hsieh et al., 2004). The presence of free and esterified sterols is important to improve lipid metabolism and immune function (Moreau, Whitaker, & Hicks, 2002).

3.3. Composition of the phenolic extracts

The identification of the main components of the MeOH:H_2O extracts of ripe mesocarp from the cherimoya cultivars 'Perry Vidal', 'Mateus', 'Mateus III' and 'Funchal' was carried out by UHPLC-DAD and UHPLC-MS^n analysis. Table 3 summarizes the phenolic compounds identified in each extract, their retention

Table 3
UV data and MS^n fragmentation profile of compounds identified in the phenolic extracts of ripe mesocarp from cherimoya cultivars.

Peak No.	Rt (min)	λ_{max} (nm)	Compound	$[M-H]^-$ (*m/z*)	MS^n product ions (*m/z*)	Id.
1	2.6	232, 278	Catechin	289	245	Co
2	2.9	232, 278	Procyanidin (B-type) dimer isomer	577	MS^2: 559, 451, 425, 407, **289**; MS3: 245	Rockenbach et al. (2012)
3	3.2	234, 278	(epi)Catechin-(epi)gallocatechin	593	MS^2: 575, 467, 441, 423, 289	Tala et al. (2013)
4	6.0	232, 279	Procyanidin (B-type) dimer isomer	577	MS^2: 559, 451, 425, 407, **289**; MS3: 245	Rockenbach et al. (2012)
5	6.8	234, 279	(epi)Gallocatechin	305	MS^2: 287, 261, 221, 219, 179, 169, 125	Tala et al. (2013)
6	7.3	235, 278	Epicatechin	289	245	Co
7	7.5	232, 278	Procyanidin (B-type) dimer isomer	577	MS^2: 559, 451, 425, 407, **289**; MS^3: 245	Rockenbach et al. (2012)
8	8.3	232, 279	Procyanidin trimer isomer	865	MS^2: 847, 829, 695, **577**, 289; MS^3: 451, 289	Rockenbach et al. (2012)
9	8.7	232, 279	Procyanidin trimer isomer	865	MS^2: 847, 829, 695, **577**, 289; MS^3: 451, 289	Rockenbach et al. (2012)
10	9.0	232, 279	(epi)Afzelechin-(epi)catechin	561	MS^2: 543, 435, 407, **289**	de Souza et al. (2008)
11	12.8	233, 279	Procyanidin trimer isomer	865	MS^2: 847, 829, 739, 713, 695, **577**, 289; MS^3: 451, 289	Rockenbach et al. (2012)
12	15.0	235, 279	Procyanidin tetramer	1153	MS^2: 1135, 863	Rockenbach et al. (2012)
13	19.4	234, 279	Procyanidin (B-type) dimer isomer	577	MS^2: 559, 451, 425, 407, **289**; MS3: 245	Rockenbach et al. (2012)
14	20.3	231, 279	Procyanidin trimer isomer	865	MS^2: 847, 829, 739, 713, 695, **577**, 289; MS^3: 289	Rockenbach et al. (2012)

time, the molecular ion $[M-H]^-$ and the main product ions obtained by MS^n. Compounds were identified by comparing their fragmentation profiles with reference compounds run under the same experimental conditions, or, when standard were not available, their identifications were corroborated with the literature, as indicated in Table 3 and discussed below.

Several flavan-3-ols derivatives were identified in all cultivars of cherimoya, with all UV spectra being coincident with that of catechin standard. Catechin **1** and epicatechin **6** were identified by co-injection of standards. Procyanidin (B-type) dimer isomers (compounds **2**, **4**, **7** and **13**) were identified, based on their characteristic fragmentation profile at *m/z* 559 ($[M-H-18]^-$, loss of water), 451 ($[M-H-126]^-$, retro-Diels-Alder fission), 425 ($[M-H-152]^-$, $-C_8H_8O_3-H_2O$), 407 ($[M-H-152-18]^-$) and also the two product ions at *m/z* 289 and 287, corresponding to the two flavonol monomeric units (Rockenbach et al., 2012). Furthermore, the MS^3 fragmentation profile of the ion at *m/z* 289 matched to the characteristic fragmentation of authentic standard catechin.

$[M-H]^-$ ion at *m/z* 593 was tentatively assigned to (epi)catechin-(epi)gallocatechin **3** based on its MS^2 fragmentation profile with product ions at *m/z* 575 ($[M-H-18]^-$, loss of water), 467 ($[M-H-126]^-$, resulted from a heterocyclic ring fission, 441 ($[M-H-152]^-$, loss of a galloyl moiety), 423, ($[M-H-152-18]^-$, a further loss of water) and at 289 ([(epi)catechin-H]$^-$) (Tala et al., 2013). Compound **5** was identified as (epi)gallocatechin based on its molecular ion at *m/z* 305 and corresponding MS^2 product ions at *m/z* 287 ($[M-H-18]^-$, loss of water), 269 $[M-H-2H_2O]^-$), 261 ($[M-H-CH{=}CHOH]^-$ and 169 ([gallic acid-H]$^-$) (Tala et al., 2013).

Compound **10** was assigned to (epi)afzelechin-(epi)catechin, based on its molecular ion at *m/z* 561 and corresponding MS^2 fragmentation profile, where the major product ions are observed at *m/z* 543 ($[M-H-18]^-$, loss of water), 435 ($[M-H-126]^-$, resulting from the loss of a phloroglucinol molecule (A-ring)), 407 ($[M-C_8H_8O_2-H_2O]^-$, heterocyclic ring fission, followed by a loss of water) and 272 ([catechin-H]$^-$, loss of the (epi)afzelechin unit) (De Souza, Cipriani, Iacomini, Gorin, & Sassaki, 2008).

Procyanidins trimers and tetramers, presenting single charged negative ions at *m/z* 865 (compounds **8**, **9**, **11** and **14**) and 1153 (**12**), respectively, were also identified, with basis on the MS^n consecutive losses of catechin units (−288 Da) (Rockenbach et al., 2012).

Fourteen phenolic compounds were identified in the cherimoya mesocarp fruits of 'Perry Vidal', 'Mateus I', 'Mateus III' and 'Funchal' cultivars. Some of these compounds were previously reported as constituents of cherimoya pulp (Barreca et al., 2011), although the variety involved is unknown. Nonetheless, five phenolic compounds are reported here for the first time as constituents of cherimoya fruits, namely, catechin **1**, (epi)catechin-(epi)gallocatechin **3**, (epi)gallocatechin **5**, (epi)afzelechin-(epi)catechin **10** and procyanidin tetramer **12**. Flavan-3-ols are well known constituents of several fruit pulps, such as apples (Alonso-Salces et al., 2001) and grapes (Perestrelo et al., 2012).

The quantification of the phenolic compounds detected in the ripe mesocarp of the four cherimoya cultivars is presented in Table 4. 'Mateus I' and 'Mateus III' cultivars show the higher phenolic compounds content, accounting 9603 and 6299 mg kg^{-1} of dry weight, respectively. There is no information regarding the content of flavan-3-ols on cherimoya fruits, for comparative purposes. However, when compared with those described for the pulp of other tropical fruits, such as avocado (which also presented catechin derivatives and procyanidins as the major constituents), these contents are considerably higher (Rodríguez-Carpena, Morcuende, Andrade, Kylli, & Estévez, 2011). The phenolic fractions of the mesocarp of 'Mateus I' and 'Mateus III' cultivars are very similar, with procyanidin dimer isomer **7** as the major component, followed by a procyanidin trimer isomer **11** and epicatechin **6** (Fig. 2). Besides the procyanidin isomers, it was also identified (epi)catechin-(epi)gallocatechin **3** and (epi)gallocatechin **5** in 'Mateus I' cultivar and (epi)afzelechin-(epi)catechin **10** in 'Mateus III' cultivar. Procyanidin dimer isomer **7** was also identified as the major component of the phenolic fraction of 'Funchal' cultivar mesocarp (706 mg kg^{-1} DW), followed by a procyanidin trimer isomer **11** (404 mg kg^{-1} DW) and procyanidin tetramer **12** and epicatechin **6** (both with 147 mg kg^{-1} DW). 'Perry Vidal' is the cultivar presenting the lowest

Table 4
Compounds identified in the phenolic extracts of ripe mesocarp from cherimoya cultivars expressed in mg kg^{-1} of dry material.[a]

Compound	'Perry Vidal'	'Mateus I'	'Mateus III'	'Funchal'
Catechin	–	230	425	–
Procyanidin (B-type) dimer isomer	87	936	210	76
(epi)Catechin-(epi)gallocatechin	–	294	–	–
Procyanidin (B-type) dimer isomer	117	524	296	124
(epi)Gallocatechin	–	150	–	–
Epicatechin	189	1211	630	147
Procyanidin (B-type) dimer isomer	–	2915	1854	706
Procyanidin trimer isomer	–	266	239	–
Procyanidin trimer isomer	–	377	302	82
(epi)Afzelechin-(epi)catechin	–	–	300	–
Procyanidin trimer isomer	92	1202	1061	404
Procyanidin tetramer	–	625	440	147
Procyanidin (B-type) dimer isomer	–	444	274	66
Procyanidin trimer isomer	–	429	270	53
Total (mg kg^{-1} DW)	485	9603	6299	1804

[a] Results are the average of the concordant values obtained (less than 5% variation between injections).

phenolic compounds content, accounting for 485 mg kg^{-1} DW. Epicatechin **6** (189 mg kg^{-1} DW), two procyanidin dimer isomers **2** and **4** (87 and 117 mg kg^{-1} DW, respectively) and a procyanidin trimer isomer **11** (92 mg kg^{-1} DW) were the only detected compounds in the phenolic fraction of this mesocarp.

Reports on the role of these bioactive phenolic compounds on human health suggest a relevant contribution to prevent cardiovascular health problems (De Pascual-Teresa, Moreno, & García-Viguera, 2010). Higher intakes of flavan-3-ols and their polymers were also associated with a significant reduction in the concentration of oxidative stress biomarkers (Cassidy et al., 2015).

4. Conclusions

The lipophilic fractions of the four cherimoya cultivars used in this study are mainly composed of kaurane diterpenes, namely kaurenoic acid and kaurenol, followed by fatty acids and sterols. Considerable amounts of unsaturated fatty acids (ω-3 and ω-6), with well-established health-promoting benefits, were also detected. Kaur-16-en-19-oic acid is the major lipophilic component of all cherimoya cultivars accounting between 554 ('Mateus III') and 1350 ('Funchal') mg $kg^{-1}$of dry material.

In addition, the mesocarp of cherimoya fruits from the investigated cultivars also contains a high variety of flavan-3-ols, including both galloylated and non-galloylated components. Five phenolic compounds are identified for the first time as constituents of cherimoya fruits, *viz.* catechin, (epi)catechin-(epi)gallocatechin, (epi)gallocatechin, (epi)afzelechin-(epi)catechin and procyanidin tetramer. In general, the mesocarp of 'Mateus I' contains a higher quantity of lipophilic and phenolic compounds. Our results support the use of this exotic fruit as a rich source of health-promoting components, with the capacity to prevent or slow down the progress of various oxidative-stress related disorders.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.05.123.

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