Journal of Food Composition and Analysis 98 (2021) 103844

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

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

Original Research Article

A high-throughput analytical strategy based on QuEChERS-dSPE/HPLC–DAD–ESI-MS^n to establish the phenolic profile of tropical fruits

Catarina Silva [a], José S. Câmara [b], Rosa Perestrelo [a,*]

[a] CQM - Centro de Química da Madeira, Universidade da Madeira, Campus da Penteada, 9020-105, Funchal, Portugal
[b] Departamento de Química, Faculdade de Ciências Exatas e Engenharia, Universidade da Madeira, Campus da Penteada, 9020-105, Funchal, Portugal

ARTICLE INFO

Keywords:
Phenolic profile
Tropical fruits
QuEChERS-dSPE
HPLC–DAD–ESI-MS^n
Method validation

ABSTRACT

Tropical fruits are a rich source of phenolic compounds which are favorable in defending the human body against damage induced by free radicals (*e.g.*, ROS, RNS). In the current work, a high throughput analytical approach based on a simple extraction procedure (QuEChERS-dSPE) combined with high-performance liquid chromatography-diode array detector-electrospray ionization-mass spectrometry (HPLC-DAD-ESI-MS^n) was used to establish the phenolic profile of tropical fruits. The proposed method showed good linearity ($r^2 \geq 0.991$), precision (RSD < 8 %), as well as low limits of detection (LOD ≤ 19.7 μg/L) and quantification (LOQ ≤ 65.8 μg/L). Thirty-four phenolic compounds were identified as belonging to different chemical groups, from which only 6 were common to all tropical fruits. Pitanga showed the highest relative phenolic concentration (99.5 mg/100 g of fruit), with the passion fruit (17.5 mg/100 g of fruit) the lowest. Flavonols were the most predominant chemical group in tropical fruits, representing 77.9, 60.1, and 55.8% of the phenolic composition of pitanga, passion fruit and mango, respectively. The data obtained allow deep and comprehensive insights into the phenolic composition of tropical fruits in order to explore its potential bioactive activity. Nevertheless, *in vivo* assays using fruit extracts will be essential to recognize their potential health-promoting properties.

1. Introduction

Numerous health-promoting properties (*e.g.*, anticarcinogenic, antiallergic, antiaging, antiatherosclerotic, antioxidative) have been attributed to phenolic compounds present in fruits since they reduce the oxidative stress produced by free radicals and other reactive oxygen (ROS) and nitrogen (RNS) species (Barnes et al., 2020; Spínola et al., 2015). Tropical fruits, including passion fruit (*Passiflora edulis* L.), pitanga (*Eugenia uniflora* L.) and mango (*Mangifera indica* L.) constitute a rich source of phenolic compounds, as flavonoids (*e.g.*, rutin, quercetin, kaempferol), anthocyanins (*e.g.*, cyanidin-3-glucoside, delphinidin-3--glucoside), and carotenoids (*e.g.*, β-carotene, β-cryptoxanthin) (Celli et al., 2011; Russo et al., 2018; Spínola et al., 2015; Vu et al., 2019). Moreover, these tropical fruits are cultivated throughout the Madeira Island since the edaphoclimatic conditions are propitious for their production.

Specific attention has been paid to the extraction of phenolic compounds from fruits, which represents a critical step in the establishment of the phenolic profile of fruits. Among the extraction procedure, organic solvents (*e.g.*, acetonitrile, ethyl acetate, methanol) and/or solid-phase extraction (SPE) with reversed-phase C_{18} are the most commonly used (Barnes et al., 2020; Celli et al., 2011; Russo et al., 2018). Nevertheless, these extraction procedures are solvent- and time-consuming and involve extra steps (*e.g.*, clean-up, solvent evaporation) due to chromatographic incompatibility and/or sample concentration (Rotta et al., 2019). More recently, a quick, easy, cheap, effective, rugged and safe (QuEChERS) method with C_{18} as dispersive cleaning sorbent followed by ultra-pressure liquid chromatographic tandem mass spectrometry (UHPLC-MS/MS) has been proposed to identify and quantify nine phenolic compounds in passion fruit pulp (*Passiflora* spp.). This extraction procedure comprises two steps: (*i*) an extraction step based on partitioning *via* salting-out extraction where an equilibrium between an aqueous and an organic layer is promoted, and (*ii*) a dispersive solid-phase extraction (d-SPE) step that includes a clean-up process applying numerous mixtures of porous sorbents and salts to eliminate matrix interfering constituents (Perestrelo et al., 2019). On the other hand, regarding phenolic compound identification and quantification, several analytical platforms have been purposed such as spectrophotometry (Vasco et al., 2008) and/or high-performance liquid chromatography (HPLC) coupled to ultraviolet

* Corresponding author.
E-mail address: rmp@staff.uma.pt (R. Perestrelo).

https://doi.org/10.1016/j.jfca.2021.103844
Received 27 November 2020; Received in revised form 2 February 2021; Accepted 7 February 2021
Available online 9 February 2021

(UV) or diode array (DAD) detectors (Dorta et al., 2014; Garmus et al., 2014). Nevertheless, these analytical platforms showed limitations such as coelutions, similar UV-absorption spectra and require standards reference to provide target identification. High-performance liquid chromatography-diode array detector-electrospray ionization-mass spectrometry (HPLC-DAD-ESI-MS^n) appears to be the most suitable analytical platform to establish the phenolic profile of fruits, since it provides useful structural information and allows a tentative target identification when the reference standards are commercially unavailable (Celli et al., 2011; Russo et al., 2018; Spínola et al., 2015; Vu et al., 2019).

Most of the published investigations related to tropical fruits only provide a screening of the phenolic profile and/or reported the total phenolic compounds (TPC), tannins and flavonoids through colorimetric assays (*e.g.*, Folin-Ciocalteu), as well as antioxidant activity through *in vitro* chemical tests (Hu et al., 2018; Sobeh et al., 2020; Spínola et al., 2015). As far as we know, QuEChERS-dSPE/HPLC-DAD-ESI-MS^n is scarcely used to establish the phenolic profile of tropical fruits (Rotta et al., 2019). Therefore, the main goal of this research is to validate a high-throughput analytical approach based on HPLC-DAD-ESI-MS^n combined with QuEChERS-dSPE to separate, identify and semi-quantify the phenolic compounds of passion fruit (*Passiflora edulis* L.), pitanga (*Eugenia uniflora* L.) and mango (*Mangifera indica* L.) habitually part of Madeiran diet. Considering the scarce application of QuEChERS-dSPE for the extraction of phenolic compounds from fruits, the current research represents an added value and improved alternative to the most conventional extraction procedures.

2. Materials and methods

2.1. Chemical and materials

All reagents and standards were of analytical grade (purity > 98%). The solvents used for the extraction procedure and HPLC-MS analysis, acetonitrile (MeCN) was purchased from Sigma–Aldrich (Madrid, Spain). Sodium chloride (NaCl), trisodium citrate dehydrate ($C_6H_5Na_3O_7.2H_2O$), disodium hydrogen citrate sesquihydrate ($C_6H_8Na_2O_8$), magnesium sulfate ($MgSO_4$) and formic acid (FA) were supplied from Panreac (Barcelona, Spain). The sorbents, Bondesil primary secondary amine (PSA, 40 μm) was obtained from Agilent Technologies (USA), whereas octadecylsilane (C18) were from Supelco (Bellefonte, PA, USA). QuEChERS extraction/partitioning tubes and the clean-up tubes were obtained from Waters (Milford, MA, USA).

Six of the phenolic standards that were used for identification and semi-quantification purposes, namely protocatechuic acid, *p*-coumaric acid, kaempferol and *trans*-resveratrol were supplied by Sigma–Aldrich (St. Louis, MO, USA), whereas (+)-catechin and rutin were supplied by Fluka (Buchs, Switzerland). Ultrapure water (18 MΩ cm at 23 °C) was obtained by means of a Milli-Q water purification system (Millipore, Milford, MA, USA). All the eluates were filtered through 0.22 μm PTFE membrane filters (Millipore) supplied by *via* Athena - Gestão de Laboratórios, Lda. (Sacavém, Lisbon, Portugal).

2.2. Fruit samples

Fresh samples of passion fruit (*Passiflora edulis* L.), pitanga (*Eugenia uniflora* L.) and mango (*Mangifera indica* L.) were purchased from a local market in Funchal, Portugal (32°38′55″ N, 16°54′14″ W). For each fruit sample, approximately 1 kg was randomly sampled from the market shelves, simulating consumer shopping behavior. Fruits were washed in water and all inedible parts were removed manually or using a steel knife. Bruised and/or wounded fruits were discarded. Passion fruit, pitanga and mango were peeled and only the pulp was analyzed. For each independent analysis, at least 250 g of fruit sample, with 50 mL of Milli-Q water added, were put in a commercial juice extractor (Instant pulp, 200 W, Worten, Portugal), obtaining a fluid fruit extract which was stored at −20 °C until QuEChERS-dSPE extraction procedure.

2.3. QuEChERS procedure for extraction of phenolic compounds

A homogenized fluid fruit sample (5 ± 0.1 *g*) was weighed into a 50 mL PTFE centrifuge tube, followed by addition of 5 mL of MeCN. Then, the tube was shaken vigorously for 2 min with vortex mixer ensuring that the solvent interacted well with the entire sample. Buffered salts, $C_6H_5Na_3O_7.2H_2O$ (0.5 *g*), $C_6H_8Na_2O_8$ (0.25 *g*), NaCl (0.5 *g*) and $MgSO_4$ (2 *g*) were added into the homogenized mixture and the shaking step was repeated for 1 min followed by centrifugation at 5000 rpm for 3 min, Fig. 1.

For the dSPE step, an aliquot of the MeCN phase was transferred into a 2 mL PTFE single-use centrifugation tube already containing 25 mg of PSA (removes various polar organic acids, polar pigments, some sugars and fatty acids), 25 mg of C_{18} sorbent (removes non-polar interfering substances like lipids) and 150 mg $MgSO_4$. The mixture was shaken in a vortex and centrifuged for 2 min at 3000 rpm. Then, a 700 μL aliquot of the extract was evaporated under nitrogen (N_2) flow to dryness and the residue was dissolved in 100 μL of mobile phase A. All the samples were filtered through a 0.22 μm Millipore PTFE filter membrane prior to HPLC–DAD–ESI-MS^n analysis.

2.4. Method validation

The proposed method was validated based on the linearity, sensitivity, precision and selectivity. As not all the phenolic compounds identified in fruits are commercially available and using a regularly adopted approach (Perestrelo et al., 2012; Spínola et al., 2015), six representative standards of the phenolic compounds under study were chosen. These phenolic standards were used to construct the calibration curves and the results for each target phenolic compound were expressed in equivalents of the respective standard. For each of the six standards, an ethanolic stock solution was prepared (200 mg/L). All solutions were stored at −20 °C in the dark. Then, seven different concentrations, covering the concentration range predictable for each phenolic compound (Table 1), were prepared by diluting suitable amounts of each stock solution in mobile phase A. Each one of these solutions was analyzed in triplicate, using the QuEChERS-d-SPE/HPLC–DAD–ESI-MS^n method.

The method sensitivity was measured based on limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ were determined through the multiplication by 3 and 10 of the ratios of standard deviation(s) of calibration curve interception and the slope of the regression curve, respectively.

The accuracy of the method was assessed by spiking pitanga sample in triplicate at three concentration levels (low (LL), middle (ML) and high (HL)) and subjecting them to QuEChERS-dSPE procedure. The repeatability of the method was evaluated by analyzing three replicates of standard solutions (5 mg/L) under the same conditions on the same

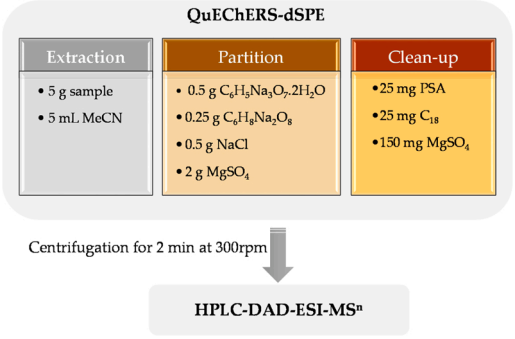

Fig. 1. Schematic representation of the QuEChERS-dSPE extraction procedure.

Table 1
Validation data used for the HPLC-DAD semi-quantification of phenolic compounds in tropical fruits.

RT (min)	Phenolic compound	λ_{max} (nm)	$[M-H]^-$	MS^2 (m/z)	Concentration range (mg/L)	Calibration curve	R^2	LOD (μg/L)	LOQ (μg/L)	Recovery (% RSD)			Precision (% RSD)	
										LL	ML	HL	Intra-day	Inter-day
13.7	Protocatechuic acid	259	153	**109**[a]	2.0 – 25	y = 33550.1x – 76177.2	0.998	15.3	51.0	76 (12)	90 (3)	95 (7)	4.25	5.04
18.8	Catechin	278	289	**245**, 205, 179	2.0 – 30	y = 1515.4x + 259.6	0.991	11.3	37.7	84 (9)	96 (2)	99 (3)	6.32	7.18
19.4	*p*-Coumaric acid	309	163	153, **119**	0.05 – 0.8	y = 151259.3x – 420.9	0.996	0.99	3.30	90 (4)	89 (6)	102 (3)	3.19	4.03
25.3	Rutin	354	609	**301**, 179, 151	0.5 – 20	y = 23268.4x – 10099.2	0.997	10.1	33.8	97 (2)	105 (4)	93 (2)	2.98	3.14
39.4	*trans*-resveratrol	306	227	**185**, 159	0.1 – 7.0	y = 129510.2x – 2680.3	0.996	6.13	20.4	80 (3)	83 (5)	85 (2)	1.76	2.85
46.7	Kaempferol	366	285	**257**, 169, 151	1.0 – 12	y = 7308.9x – 836.9	0.996	19.7	65.8	119 (4)	99 (7)	93 (9)	4.09	5.21

λ_{max} – Maximum wavelength; R^2 – Correlation coefficient; LOD – Limit of detection; LOQ – Limit of quantification.
[a] Ions in boldface indicate the more abundant *m/z* ratio.

day (intra-day) and over a period of one week (inter-day). The results obtained were expressed as the relative standard deviation (% RSD).

The selectivity was assessed by the absence of interfering peaks at the analyte retention time (RT). To demonstrate the nonexistence of any carryover during injection, a pure solvent was injected directly using the highest calibration point of each phenolic standard.

2.5. Phenolic profile by HPLC–DAD–ESI-MS^n

The qualitative and semi-quantitative analysis of the phenolic compounds was carried out on a HPLC system of Dionex ultimate 3000 series (Sunnyvale, CA) instrument equipped with a binary pump, diode array detector (DAD), autosampler and column compartment according to the method previously described (Perestrelo et al., 2012). The equipment was equipped with an Atlantis dC18 column (250 mm × 4.6 mm i.d. × 5 μm) supplied from Waters (Milford, Ma, USA) at controlled temperature (25 °C). The elution was performed using mobile phase A (0.1% FA in aqueous solution) and mobile phase B (0.1% FA in acetonitrile). The flow rate was 300 μL/min. The gradient program was used as follows: 0−3 min, 100 % A; 3−10 min, 100−90% A; 10−30 min, 90−80% A; 30−35 min, 80−75% A; 35−50 min, 75–100% A. The fruit extract obtained was dissolved in the initial HPLC mobile phase A and were filtered through 0.22 μm micropore membranes prior to injection into the HPLC system (injection volume of 10 μL). The detection by DAD was carried out by scanning 210–520 nm, with a resolution of 1.2 nm, and the semi-quantification was performed at 280, 320 and 360 nm for phenolic acids, stilbenes and flavonols, respectively.

For identification purposes, mass spectrometry analysis was performed using a Bruker Esquire model 6000 ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with an electrospray ionization (ESI) source. Data acquisition and processing were performed using Esquire control software. The mass spectrometer was operated in the negative mode, and the mass range from 50 to 3000 *m/z*, under the following conditions: nebulizer gas pressure of 50 psi, drying gas flow of 10 mL/min, desolvation temperature of 350 °C, cone voltage between 30 and 50 V, collision energy set between 10 and 45 V, and the capillary voltage ranged from 2.6 to 2.9 kV.

3. Results and discussion

3.1. Phenolic identification by HPLC–DAD–ESI-MS^n

A total of 34 phenolic compounds were tentatively identified in tropical fruits that included 5 hydroxybenzoic acids, 6 hydroxycinnamic acids, 15 flavonols, 3 stilbenes and 5 others. The peak assignment of phenolic compounds extracted from tropical fruits was carried out by comparison of their retention time (RT) and MS^n fragmentation profiles with reference standards, analyzed under the same experimental conditions and/or with published data. In general, in the MS^1spectrum the most intense peak corresponded to the deprotonated molecular ion $[M-H]^-$. The identification of the phenolic compounds detected in tropical fruit extracts is presented in Table 2. The resulting chromatograms of investigated tropical fruits obtained by HPLC-DAD analysis are shown in Fig. 2.

3.1.1. Hydroxybenzoic acid

Compound 1 was identified as hydroxybenzoyl glucose based on fragmentation pattern, with $[M-H]^-$ at *m/z* 299 showing product ions at *m/z* 137 (loss of glucose moiety, 162 Da) and *m/z* 93 (loss of CO_2 ([4-hydroxybenzoic acid − CO_2−H^+]$^-$). Compound 2 was assigned as galloyl glucose presenting a pseudomolecular ion $[M-H]^-$ at *m/z* 331 that released an MS^2 fragment at *m/z* 169 ([M − 162]$^-$, loss of a glucose moiety) corresponding to gallic acid (Fig. 3). Compound 3 showed the characteristic fragmentation pathway of an galloyl−HHDP-glucoside with a $[M-H]^-$ at 633 and the MS^2 spectrum showed ions at *m/z* 463 by loss of galloyl + CO_2 unit (170 Da) and *m/z*301 as main fragment (loss of galloylglucose, 332 Da). Orsellinic acid glucoside (compound 4) exhibited a pseudomolecular $[M-H]^-$ ion at *m/z* 329, yielding MS^2 fragments at *m/z* 167 corresponding to [orsellinic acid−H]$^-$ due to a loss of glucose (−162 Da). Compound 8 with a pseudomolecular ion $[M-H]^-$ at *m/z* 635 and MS^2 fragment ions at *m/z* 465 (loss of gallic acid moiety), *m/z* 313 (loss of a galloyl moiety) and *m/z* 169 ([gallic acid−H]$^-$) was identified as trigalloyl glucose

3.1.2. Hydroxycinnamic acid

Mass spectra of compound 5 displayed a parent ion at *m/z* 341 and three fragment ions with one at *m/z* 179 and 135 for caffeic acid through the loss of a glucose and CO_2moiety. Compound 9 showed a pseudomolecular ion $[M-H]^-$ at *m/z* 325 and MS^2 fragment ions at *m/z* 163 ([coumaric acid−H]$^-$) corresponding to a loss of glucose moiety, which allow identification of this compounds as *p*-coumaric acid glucoside. Compound 10 presented a pseudomolecular ion $[M-H]^-$ at *m/z* 385, yielding MS^2 fragments at *m/z* 223 (loss of a glucose moiety; [sinapic acid−H]$^-$), suggesting that it could be a sinapic acid glucoside. Compound 17 was tentatively identified as coumaric acid derivate due to its typical MS^2 fragments (*m/z* 163 and 119), whereas compounds 6 and 19

Table 2
Relative concentration of phenolic compounds identified in tropical fruits using QuEChERS-dSPE/HPLC-DAD-ESI-MS^n.

Peak nº	RT (min)	$[M-H]^-$	MS^2 (m/z)	Phenolic compounds	Concentration (mg/100 g fruit) ± standard deviation		
					Passion fruit	Pitanga	Mango
1	2.52	299	239, 179, 137, 93	Hydroxybenzoyl glucose[a]	< LOQ	< LOQ	2.88 ± 7.64E-4
2	6.36	331	271, 169, 129	Galloyl glucose[a]	2.60 ± 3.70E-3	3.01 ± 4.86E-2	1.96 ± 5.14E-3
3	6.64	633	463, 301	Galloyl-HHDP-glucoside[a]	–	4.56 ± 6.15E-5	2.86 ± 4.76E-3
4	6.92	329	167, 123, 108	Orsellinic acid glucoside[a]	< LOQ	–	–
5	7.11	341	179, 163	Caffeoyl glucose[c]	< LOQ	–	–
6	7.75	395	349, 179,135	Caffeic acid derivate[c]	–	0.11 ± 8.54E-6	–
7	8.08	401	269, 179, 161	Apigenin pentoside[f]	< LOQ		
8	8.15	635	465, 313, 169	Trigalloyl-glucose[a]	–	5.12 ± 3.12E-4	–
9	8.24	325	187, 163	Coumaric acid glucoside[c]	–	0.14 ± 2.65E-3	–
10	8.76	385	223, 208, 179, 164	Sinapic acid glucoside[c]	0.15 ± 5.51E-4	0.13 ± 3.31E-3	0.20 ± 3.93E-3
11	9.44	457	331, 305, 169	Epigallocatechin gallate[b]	–	6.21 ± 0.18	–
12	10.5	479	317, 316, 179	Myricetin glucoside[d]	–	1.53 ± 1.78E-2	0.96 ± 2.04E-3
13	11.2	449	317, 316, 179	Myricetin arabinopyranoside[d]	0.52 ± 1.14E-3	1.08 ± 5.58E-3	0.83 ± 2.18E-3
14	11.5	405	243	Piceatannol glucoside[e]	< LOQ		
15	11.7	521	329	Vanilloyl glucose derivate[c]	–	–	< LOQ
16	12.1	449	317, 316, 179	Myricetin arabinopyranoside[d]	5.41 ± 8.76E-5	18.7 ± 0.01	8.98 ± 1.56E-3
17	13.1	501	454, 307, 163	Coumaric acid derivate[c]	2.01 ± 5.98E-3	–	–
18	13.3	463	301, 300	Quercetin glucoside[d]	4.09 ± 7.93E-3	15.1 ± 0.04	1.12 ± 3.45E-5
19	13.6	415	409, 179, 135	Caffeic acid derivate[c]	1.46 ± 3.76E-2	0.14 ± 3.91E-3	0.76 ± 2.08E-3
20	14.1	579	417, 166	Syringaresinol glucoside[c]	–	–	0.15 ± 6.09E-4
21	14.3	433	301, 300	Quercetin pentoside[d]	0.98 ± 4.76E-3	< LOQ	–
22	14.8	447	285, 284, 255, 256	Kaempferol glucoside[f]	–	2.15 ± 1.63E-3	–
23	15.3	433	301	Quercetin pentoside[d]	–	15.6 ± 9.56E-4	3.27 ± 5.48E-5
24	15.9	447	301, 300	Quercetin rhamnose[d]	–	19.2 ± 7.43E-4	–
25	16.6	243	225, 201, 175, 159	Piceatannol[e]	0.17 ± 1.56E-5	–	–
26	17.1	447	301, 179, 151	Quercetin deoxyglucoside[d]	–	2.76 ± 4.06E-4	–
27	17.3	449	287, 269, 259	Dihydrokaempferol glucoside[f]	–	< LOQ	–
28	18.2	615	463, 317	Myricetin-galloyl-deoxyhexose[d]	–	0.56 ± 3.97E-4	–
29	19.5	317	179, 151	Myricetin[d]	–	0.78 ± 5.78E-4	–
30	20.3	431	285, 255, 151	Kaempferol rhamnose[f]	–	< LOQ	–
31	24.2	599	301, 179, 151	Quercetin-glucoside-protocatechuic acid[d]	–	< LOQ	–
32	24.7	227	185, 157, 143	*Trans*-resveratrol[e]	0.06 ± 1.56E-3	–	–
33	25.6	609	301, 179, 151	Quercetin rutinoside[d]	–	< LOQ	–
34	30.9	301	179, 151	Quercetin[d]	–	2.67 ± 6.09E-4	–

Values were expressed as mean ± standard deviation of three replicates (n = 3).
< LOQ : lower than limit of quantification; - : not detected.
[a] Expressed in equivalents of protocatechuic acid.
[b] Expressed in equivalents of catechin.
[c] Expressed in equivalents of *p*-coumaric acid.
[d] Expressed in equivalents of rutin.
[e] Expressed in equivalents of *trans*-resveratrol.
[f] Expressed in equivalents of kaempferol.

were assigned as caffeic acid derivates due to their typical MS^2 fragments (*m/z* 179 and 135).

3.1.3. Flavonols

Four conjugates of myricetin (compounds 12, 13, 16 and 28), seven of quercetin (compounds 18, 21, 23, 24, 26, 31 and 33) and three of kaempferol (compounds 22 and 30) were characterized according to the sugar moieties linked to their aglycones at *m/z* 317, 301 and 285, respectively.

Compound 12 with a pseudomolecular ion $[M-H]^-$ at *m/z* 479 produced product ions at *m/z* 317 [myricetin acid−H]$^-$ and minor ions at *m/z*271, 171 and 159, suggesting a loss of glucose. On the basis of mass fragments, this compound was identified as myricetin glucoside. Compounds 13 and 16 identified as myricetin arabinopyranoside, yielded an MS spectrum containing $[M-H]^-$ at *m/z* 449, which fragmented on MS^2 to produce a myricetin ion at *m/z*317 due to a loss of an arabinopyranoside moiety, 132 Da. The myricetin-galloyl-deoxyhexose (compound 28) was identified by its $[M-H]^-$ ion at *m/z* 615 and MS^2 fragment ions at *m/z* 463 and 317 by neutral loss of galloyl (−152 Da) and deoxyhexose (−146 Da) moiety.

Quercetin glucoside (compound 18), quercetin pentoside (compound 21 and 23), quercetin rhamnose (compound 24), quercetin deoxyglucoside (compound 26), quercetin-glucoside-protocatechuic acid (compound 31) and quercetin-rutinoside (compound 33) yielded MS spectra containing $[M-H]^-$ at *m/z* 463, 433, 447, 599 and 609, respectively. The MS^2 spectrum showed fragment ion at *m/z* 301 by neutral loss of glucose (-162 Da), pentose (-132 Da), rhamnose (−146 Da), deoxyglucose (−324 Da), glucose-protocatechuic acid (−298 Da) and rutinose (−308 Da) moiety, respectively. To clarify the aglycone, a product ion scan of *m/z* 301 was performed, and fragment ions were observed at *m/z* 271, 151 and 121 evidenced that the aglycone was quercetin, Fig. 4.

Compounds 22 and 30 were assigned as kaempferol glucoside and kaempferol rhamnose presenting a $[M-H]^-$ at *m/z* 447 and 431, respectively, which released an MS^2 fragment at *m/z* 285 originating from kaempferol, resulting from a neutral loss of glucose (-162 Da) and rhamnose (−146 Da). Dihydrokaempferol glucoside (compound 27) exhibited a pseudomolecular $[M-H]^-$ ion at *m/z* 449, yielding MS^2 fragments at *m/z* 287 corresponding to [dihydrokaempferol−H]$^-$ due to a loss of glucose (−162 Da).

3.1.4. Flavan-3-ol

Compound 11 was assigned as epigallocatechin gallate presenting a $[M-H]^-$ at *m/z* 457 that released an MS^2 fragment at *m/z* 331 and 305 originating from epigallocatechin, while the signal at *m/z* 169 originated from [gallic acid−H]$^-$.

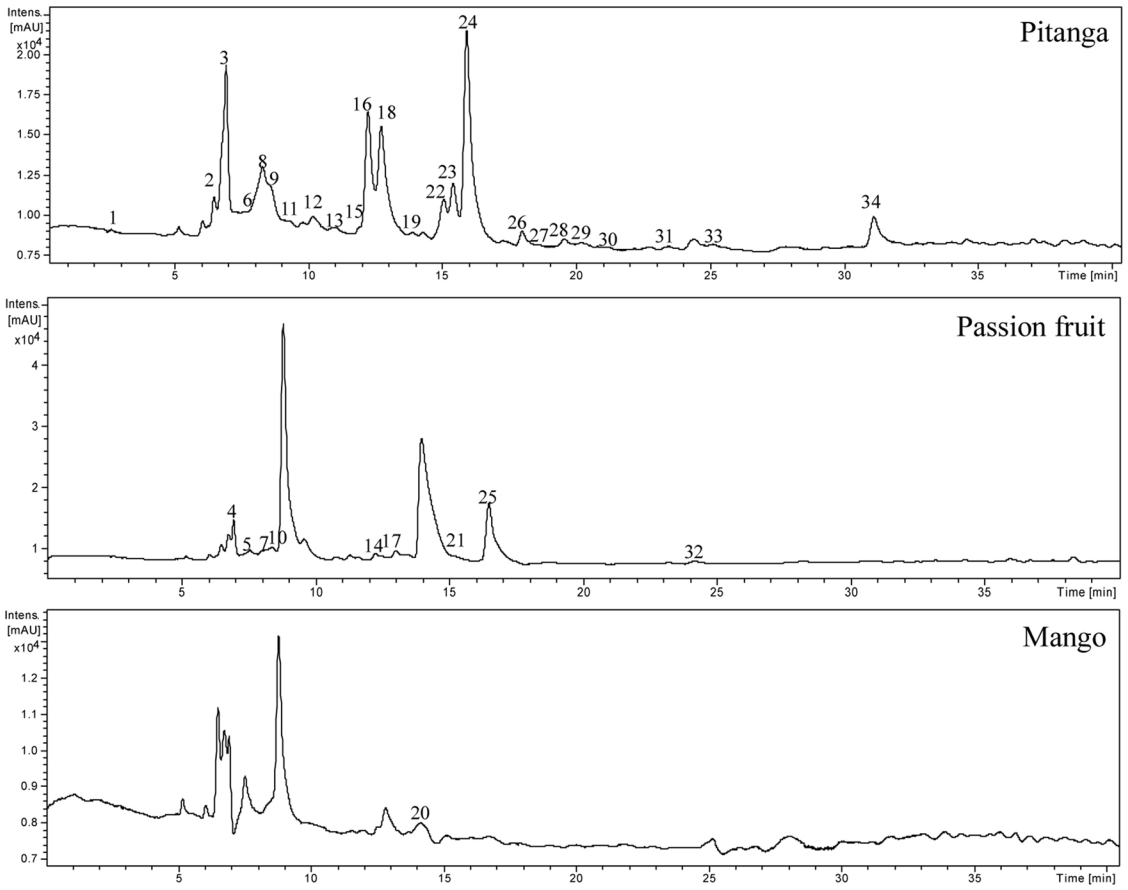

Fig. 2. Representative HPLC-DAD chromatograms from the investigated tropical fruits (peak numbers refer to Table 2).

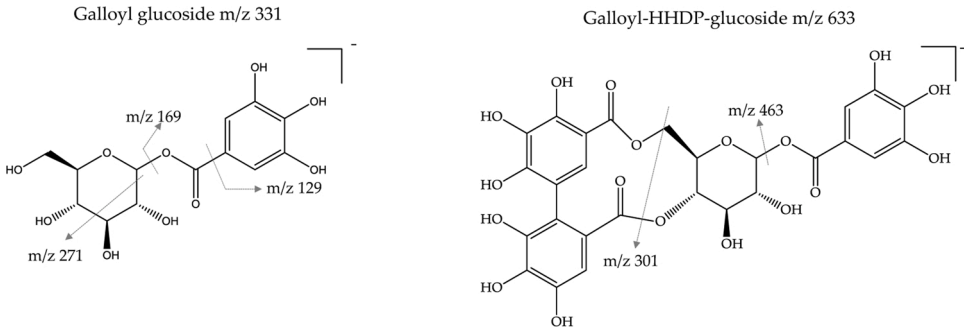

Fig. 3. Fragmentation of galloyl glucoside (compound 2) and galloy-HHDP-glucoside (compound 6) by negative ion mode ESI-MS^n.

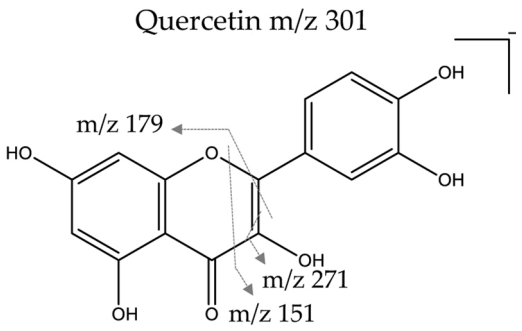

Fig. 4. Fragmentation of quercetin by negative ion mode ESI-MS^n.

3.1.5. *Flavone*

Compound 7 with a pseudomolecular ion $[M-H]^-$ at *m/z* 401 produced product ions at *m/z* 269, suggesting a loss of glucose (−162 Da). Based on the mass pattern, this compound was identified as apigenin pentoside.

3.1.6. *Stilbenes*

Piceatannol glucoside (compound 14) was identified based on the precursor $[M-H]^-$ ion at *m/z* 405, and released an MS^2 fragment at *m/z* 243 corresponds to the deprotonated [piceatannol−H]$^-$by a neutral loss of a glucose moiety. Compound 25 predominated in the phenolic profile and exhibited a pseudomolecular ion $[M-H]^-$ at *m/z* 243 and fragment ions at *m/z* 225, 201, 175 and 159. This fragmentation pattern is typical for the stilbene piceatannol. *Trans*-resveratrol (compound 32) identified as resveratrol showed a pseudomolecular ion $[M-H]^-$ at *m/z* 227 and MS^2 fragment ions at *m/z* 185 (loss of C_2H_2O moiety, −42 Da). This

product ion was further fragmented to form *m/z* 157 and 143 corresponding to the loss of CO (−28 Da) and C_2H_2O (−42 Da), respectively.

3.1.7. Lignans

Compound 20 has been identified as a possible syringaresinol glucoside, which exhibited a deprotonated molecular ion $[M-H]^-$ at *m/z* 579, and released MS^2 fragments at *m/z* 417 and 166. The same fragmentation pattern was observed for syringaresinol.

3.2. Method validation and semi-quantification of phenolic compounds in tropical fruits

According to the HPLC–DAD–ESI-MS^n analysis, as well as the distribution and structure of phenolic compounds in the chromatogram, the phenolic compounds tentatively identified in tropical fruits are organized into 4 main chemical families, namely hydroxybenzoic acids, hydroxycinnamic acids, flavonols and stilbenes. The tropical fruits revealed dissimilar phenolic profiles based on the phenolic compounds identified and their relative concentration. A total of 25 phenolic compounds were identified in pitanga fruit, whereas in passion fruit and mango 15 and 12 compounds, respectively, were identified. From these, only 6 were identified in all tropical fruits, namely galloyl glucose, sinapic acid glucoside, myricetin arabinopyranoside, quercetin glucoside and caffeic acid derivate. Most of the phenolic compounds identified in tropical fruits were already found in pitanga, passion fruit and mango fruits (Hu et al., 2018; Shanmugam et al., 2018; Sobeh et al., 2020). On the other hand, some phenolic compounds expected to be found in pitanga (*e.g.*, gallic acid, quinic acid, *p*-coumaryl quinic acid) (Sobeh et al., 2020), passion fruit (*e.g.*, gallic acid, artepellin C, daidzein, narigenin) (Shanmugam et al., 2018) and mango (*e.g.*, gallic acid, ellagic acid, mangiferin and mangiferin gallate) (Hu et al., 2018) were not identified may be the methodology applied in this research was not the most appropriate to identify and quantify trace amounts of phenolic compounds.

Each phenolic compound was relatively quantified using the calibration curves, using a set of 6 reference phenolic compounds chosen according to the principle of structure-related target analyte/standard (functional group and/or chemical structure). Each concentration level was processed following the proposed QuEChERS-dSPE procedure followed by HPLC–DAD–ESI-MS^n in triplicate. The important data regarding the method validation is displayed in Table 1. The method exhibited good linearity with a regression coefficient (r^2) higher than 0.991. The LOD values ranged from 0.99 to 19.7 μg/L, whereas the LOQ values ranged from 3.30–65.8 μg/L. Regarding the accuracy of the QuEChERS-dSPE/HPLC–DAD–ESI-MS^n method, satisfactory recovery values, for the three concentration levels (LL – low level, ML – middle level, HL – high level), were obtained, ranging from 76 to 119%. The repeatability was assessed in terms of by intra- and inter-days, and the values for both were lower than 8%. The literature has described that a quantitative method should be proved as being capable of showing mean recoveries with the range of 70–120%, and precision with %RSD values lower or equal to 20% (Nantia et al., 2017; Rotta et al., 2019). Thus, the data obtained for repeatability and accuracy indicating the stability and robustness of the proposed method.

The analytical method developed was compared with other liquid chromatography (LC) methods reported in the literature for phenolic compounds quantification in tropical fruits (Barnes et al., 2020; Celli et al., 2011; Hu et al., 2018; Nguyen et al., 2019; Rotta et al., 2019; Shanmugam et al., 2018). It should be pointed out that the proposed method required lower sample amount (5 g) and solvent volumes (5 mL), instead of the higher amount of sample (up to 10 g) and solvent volume (10 mL) applied in QuEChERS-dSPE (Rotta et al., 2019) and other extraction procedures for the determination of phenolic compound in fruits (Celli et al., 2011; Nguyen et al., 2019). Moreover, QuEChERS-dSPE contrarily to other extraction procedures does not require any previous sample treatment (*e.g.*, lyophilization) before the extraction procedure (Celli et al., 2011; Shanmugam et al., 2018). Regarding analytical performance, the LOD, LOQ, precision and recovery obtained with the proposed method were similar to the reference methods (Barnes et al., 2020; Rotta et al., 2019).

Table 2 reports the qualitative and semi-quantitative data related to the tentatively identified phenolic compounds, and it was performed at 280, 320 and 360 nm for the phenolic acids, stilbenes and flavonols, respectively. The relative quantified phenolic compounds accounted for 99.5 mg/100 g of fruit for pitanga, whereas for passion fruit and mango it was 17.5 mg/100 g and 24.0 mg/100 g of fruit, respectively.

Myricetin arabinopyranoside (18.7 mg /100 g of fruit), quercetin glucoside (15.1 mg/100 g of fruit), quercetin pentoside (15.6 mg/100 g of fruit) and quercetin rhamnose (19.2 mg/100 g of fruit) were the most abundant phenolic compounds identified in pitanga, which represent 68.9% of the total phenolic composition. This result is in agreement with a previous study that reported the quantification of phenolic compounds in two varieties of Brazilian cherry (*Eugenia uniflora* L.) using organic solvent followed by HPLC-MS/MS analysis (Celli et al., 2011). In addition, the phenolic content and antioxidant activity of tropical fruits (mango, passion fruit, range, acerola, longan, rambutan) were previously assessed, and the results showed that among of the investigated tropical fruits, mango presented the highest phenolic content and antioxidant activity (de C. Albuquerque et al., 2019; Nguyen et al., 2019). This is in agreement with the results obtained in this research, where mango showed a higher phenolic content compared to passion fruit.

Rotta et al. (2019) determine the phenolic composition in the pulp of three different *Passiflora species* using a QuEChERS-dSPE/UHPLC-MS/MS method. Differences phenolic profile were observed among the three species, with quercetin, vanillic aid and rutin the most abundant. The total concentration of phenolic compounds in these three species was lower than that determined in the current study (17.5 mg/100 g of fruit). On the other hand, the passion fruit analyzed in this study showed lower content of phenolic compounds compared to that reported by Reis et al. (2018) using an exhaustive extraction with 20 mL of ethanol.

From human health benefits point of view, quercetin glucoside showed remarkable growth-inhibitory effects in colon, breast, hepatocellular and lung cancer cells (Ju You et al., 2010). Moreover, quercetin (2.67 mg/100 g of fruit) only detected in pitanga fruits showed various biological activities, namely anti-inflammatory, anti-hypertensive, antioxidant, antiviral, anticancer, anti-microbial, anti-diabetic, gastroprotective effects, among others (Anand David et al., 2016; Carvalho et al., 2017).

For passion fruit, the most abundant phenolic compounds were myricetin arabinopyranoside and quercetin glucoside (representing 54.4% of the total phenolic composition), whereas for mango it was myricetin arabinopyranoside and quercetin pentoside representing 51.1% of the total phenolic composition. Nevertheless, it should be highlighted that stilbenes such as piceatannol glucoside (< LOQ), piceatannol (0.17 mg/100 g of fruit) and *trans*-resveratrol (0.06 mg/100 g of fruit) were only detected in passion fruits. Piceatannol similar to its precursor resveratrol showed health-promoting properties such as antiaging, anticarcinogenic, anti-diabetic, anti-inflammatory, anti-obesity properties, as well as cardio-, hepato- and neuro-protection, in several pre-clinical studies (Dai et al., 2020; Kershaw and Kim, 2017; Wen et al., 2018; Zhang et al., 2018).

These outcomes endorse that tropical fruits are a dietary source of phenolic compounds, mainly quercetin glucoside and piceatannol, consequently its ingesting can result in health-promoting benefits.

4. Conclusions

The QuEChERS-dSPE/HPLC–DAD–ESI-MS^n method was successfully validated and applied to establish the phenolic profile of tropical fruits from Madeira Island. A total of 34 phenolic compounds were identified in the investigated tropical fruits, including 5 hydroxybenzoic acids, 7

hydroxycinnamic acids, 16 flavonols, 3 stilbenes, 1 flavan-3-ol, 1 flavone, and 1 lignans, of which 6 were common to all fruits. Stilbenes were only found in passion fruit. The semi-quantification was performed using 6 standards representative of each chemical group, and good linearity ($r^2 \geq 0.991$), precision (RSD < 8%), lower LOD ($\leq$ 19.7 μg/L) and LOQs ($\leq$ 65.8 μg/L) were achieved.

Remarkable differences in the semi-quantitative profile of tropical fruits were observed, with the pitanga being richest in phenolic compounds (99.5 mg/100 g of fruit), followed by mango (24.0 mg/100 g of fruit) and passion fruit (17.5 mg/100 g of fruit). It is important to point out that flavonols were the most predominant chemical group found in investigated tropical fruits, representing 60.1, 77.9 and 55.8% of the phenolic composition of passion fruit, pitanga and mango, respectively. The data obtained could contribute to valorize the tropical fruits through the exploitation of the presence of potential bioactive compounds (*e.g.*, phenolic compounds) with several health benefits. Nevertheless, *in vivo* assays using fruit extracts will be performed to recognize their potential health-promoting properties.

CRediT authorship contribution statement

Catarina Silva: Investigation, Methodology, Validation, Writing - original draft. **José S. Câmara:** Methodology, Writing - review & editing, Funding acquisition. **Rosa Perestrelo:** Data curation, Conceptualization, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

This work was supported by FCT - Fundação para a Ciência e a Tecnologia through the CQM Base Fund - UIDB/00674/2020, and Programmatic Fund - UIDP/00674/2020, and by Madeira 14–20 Program, project PROEQUIPRAM - Reforço do Investimento em Equipamentos e Infraestruturas Científicas na RAM (M1420-01-0145-FEDER-000008) and by ARDITI - Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação, through the project M1420-01-0145-FEDER-000005 - Centro de Química da Madeira - CQM$^+$ (Madeira 14–20 Program).

References

Anand David, A.V., Arulmoli, R., Parasuraman, S., 2016. Overviews of biological importance of quercetin: a bioactive flavonoid. Pharmacogn. Rev. 10, 84–89.

Barnes, R.C., Kim, H., Mertens-Talcott, S.U., Talcott, S.T., 2020. Improved recovery of galloyl metabolites from mango (*Mangifera indica* L.) in human plasma using protein precipitation with sodium dodecyl sulfate and methanol. Food Res. Int. 129, 108812.

Carvalho, D., Paulino, M., Polticelli, F., Arredondo, F., Williams, R.J., Abin-Carriquiry, J. A., 2017. Structural evidence of quercetin multi-target bioactivity: a reverse virtual screening strategy. Eur. J. Pharm. Sci. 106, 393–403.

Celli, G.B., Pereira-Netto, A.B., Beta, T., 2011. Comparative analysis of total phenolic content, antioxidant activity, and flavonoids profile of fruits from two varieties of Brazilian cherry (*Eugenia uniflora* L.) throughout the fruit developmental stages. Food Res. Int. 44, 2442–2451.

Dai, Y., Lim, J.X., Yeo, S.C.M., Xiang, X., Tan, K.S., Fu, J.H., Huang, L., Lin, H., 2020. Biotransformation of piceatannol, a dietary resveratrol derivative: promises to human health. Mol. Nutr. Food Res. 64, 1900905.

de C. Albuquerque, M.A., Levit, R., Beres, C., Bedani, R., de Moreno de LeBlanc, A., Saad, S.M.I., LeBlanc, J.G., 2019. Tropical fruit by-products water extracts as sources of soluble fibres and phenolic compounds with potential antioxidant, anti-inflammatory, and functional properties. J. Funct. Foods 52, 724–733.

Dorta, E., González, M., Lobo, M.G., Sánchez-Moreno, C., de Ancos, B., 2014. Screening of phenolic compounds in by-product extracts from mangoes (*Mangifera indica* L.) by HPLC-ESI-QTOF-MS and multivariate analysis for use as a food ingredient. Food Res. Int. 57, 51–60.

Garmus, T.T., Paviani, L.C., Queiroga, C.L., Magalhães, P.M., Cabral, F.A., 2014. Extraction of phenolic compounds from pitanga (*Eugenia uniflora* L.) leaves by sequential extraction in fixed bed extractor using supercritical CO_2, ethanol and water as solvents. J. Supercrit. Fluids 86, 4–14.

Hu, K., Dars, A.G., Liu, Q., Xie, B., Sun, Z., 2018. Phytochemical profiling of the ripening of Chinese mango (*Mangifera indica* L.) cultivars by real-time monitoring using UPLC-ESI-QTOF-MS and its potential benefits as prebiotic ingredients. Food Chem. 256, 171–180.

Ju You, H., Jin Ahn, H., Ji, G.E., 2010. Transformation of rutin to antiproliferative quercetin-3-glucoside by aspergillus niger. J. Agric. Food Chem. 58, 10886–10892.

Kershaw, J., Kim, K.H., 2017. The therapeutic potential of piceatannol, a natural stilbene, in metabolic diseases: a review. J. Med. Food 20, 427–438.

Nantia, E.A., Moreno-González, D., Manfo, F.P.T., Gámiz-Gracia, L., García-Campaña, A. M., 2017. QuEChERS-based method for the determination of carbamate residues in aromatic herbs by UHPLC-MS/MS. Food Chem. 216, 334–341. https://doi.org/10.1016/j.foodchem.2016.08.038.

Nguyen, N.M.P., Le, T.T., Vissenaekens, H., Gonzales, G.B., Van Camp, J., Smagghe, G., Raes, K., 2019. In vitro antioxidant activity and phenolic profiles of tropical fruit by-products. Int. J. Food Sci. Technol. 54, 1169–1178.

Perestrelo, R., Lu, Y., Santos, S.A.O., Silvestre, A.J.D., Neto, C.P., Câmara, J.S., Rocha, S. M., 2012. Phenolic profile of Sercial and Tinta Negra *Vitis vinifera* L. Grape skins by HPLC–DAD–ESI-MSn: novel phenolic compounds in *Vitis vinifera* L. Grape. Food Chem. 135, 94–104.

Perestrelo, R., Silva, P., Porto-Figueira, P., J.A.M.J.A.M. Pereira, Silva, C., Medina, S., Câmara, J.S., 2019. QuEChERS - Fundamentals, relevant improvements, applications and future trends. Anal. Chim. Acta 1070, 1–28.

Reis, L.C.R.dos, Facco, E.M.P., Salvador, M., Flôres, S.H., de Oliveira Rios, A., 2018. Antioxidant potential and physicochemical characterization of yellow, purple and orange passion fruit. J. Food Sci. Technol. 55, 2679–2691.

Rotta, E.M., Rodrigues, C.A., Jardim, I.C.S.F., Maldaner, L., Visentainer, J.V., 2019. Determination of phenolic compounds and antioxidant activity in passion fruit pulp (*Passiflora* spp.) using a modified QuEChERS method and UHPLC-MS/MS. LWT 100, 397–403.

Russo, M., Fanali, C., Tripodo, G., Dugo, P., Muleo, R., Dugo, L., De Gara, L., Mondello, L., 2018. Analysis of phenolic compounds in different parts of pomegranate (*Punica granatum*) fruit by HPLC-PDA-ESI/MS and evaluation of their antioxidant activity: application to different Italian varieties. Anal. Bioanal. Chem. 410, 3507–3520.

Shanmugam, S., Gomes, I.A., Denadai, M., dos Santos Lima, B., de Souza Araújo, A.A., Narain, N., Neta, M.T.S.L., Serafini, M.R., Quintans-Júnior, L.J., Thangaraj, P., 2018. UHPLC-QqQ-MS/MS identification, quantification of polyphenols from *Passiflora subpeltata* fruit pulp and determination of nutritional, antioxidant, α-amylase and α-glucosidase key enzymes inhibition properties. Food Res. Int. 108, 611–620.

Sobeh, M., Hamza, M.S., Ashour, M.L., Elkhatieb, M., El Raey, M.A., Abdel-Naim, A.B., Wink, M., 2020. A polyphenol-rich fraction from *Eugenia uniflora* exhibits antioxidant and hepatoprotective activities in vivo. Pharmaceuticals 13, 84–96.

Spínola, V., Pinto, J., Castilho, P.C., 2015. Identification and quantification of phenolic compounds of selected fruits from Madeira Island by HPLC-DAD-ESI-MSn and screening for their antioxidant activity. Food Chem. 173, 14–30.

Vasco, C., Ruales, J., Kamal-Eldin, A., 2008. Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. Food Chem. 111, 816–823.

Vu, T.P.D., Cho, I.S., Kim, K.T., Yang, S.Y., Kim, Y.H., Kang, J.S., 2019. Evaluation of phenolic compounds from viroid-free and viroid-infected apples using HPLC-PDA-ESI-MS/MS. Phytochem. Anal. 30, 395–404.

Wen, J., Lin, H., Zhao, M., Tao, L., Yang, Y., Xu, X., Jia, A., Zhang, J., Weng, D., 2018. Piceatannol attenuates D-GalN/LPS-induced hepatoxicity in mice: involvement of ER stress, inflammation and oxidative stress. Int. Immunopharmacol. 64, 131–139.

Zhang, Y., Zhang, L.H., Chen, X., Zhang, N., Li, G., 2018. Piceatannol attenuates behavioral disorder and neurological deficits in aging mice: via activating the Nrf2 pathway. Food Funct. 9, 371–378.