Research Article

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Exploring the potential of phenolic and antioxidant compounds in new Rosaceae fruits

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Abstract

BACKGROUND: Rosaceae fruits have been used in traditional medicine for the prevention and treatment of diseases. However, these fruits have not extensively been studied regarding their phenolic composition. Thus, this research focuses on the determination of phenolic compounds by high-performance liquid chromatography electrospray ionization time-of-flight mass spectrometry, flavan-3-ols by high-performance liquid chromatography with fluorescence detection, and the antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), and ferric reducing antioxidant power of the fruits of five species of genera *Crataegus* and *Sorbus* (Rosaceae).

RESULTS: We found a total of 71 phenolic compounds from which 30 were identified in these berries for the first time. *Crataegus monogyna* and *Crataegus laciniata* revealed higher total phenolic and flavan-3-ol contents than the other species and the highest antioxidant activities.

CONCLUSIONS: Therefore, the fruits evaluated have demonstrated to be important sources of bioactive compounds with huge potential for being used in nutraceutical or food scopes. Additional studies could be needed to evaluate the influence of the different production areas on the phenolic content.

Supporting information may be found in the online version of this article.

Keywords: *Crataegus*; HPLC–ESI-TOF-MS; HPLC–FLD; phenolic compounds; procyanidins; *Sorbus*; ultrasound-assisted extraction

INTRODUCTION

Rosaceae, one of the largest families of flowering plants, includes from 95 to more than 100 genera and about 2830–3100 accepted species.[1,2] It is a cosmopolitan family, which ranges from semidesert to lowland rainforest and open, alpine vegetation in a large number of different habitats.[3] Despite having a fairly well researched phytochemistry, particularly rich in polyphenol, polyalcohol, and cyanogenic compounds,[4] its ethnobotanical value and usefulness to non-Western societies is relatively low.[3] A high amount of species with edible fruits belong to this family, some of great economic importance; for example, apples, pears, cherries, and apricots.[5] However, this family encompasses a multitude of genera, and not all of them are studied in depth regarding secondary metabolites such as phenolic compounds, known for their antioxidant activity and numerous health benefits. Among these genera are *Crataegus and Sorbus*; the species belonging to these genera are widely distributed in the Northern Hemisphere; thus, the strengthening and popularization of these fruits could be very interesting.[6-10]

Genus *Crataegus* L., with more than 1000 species, belongs to the Amygdaloideae subfamily and the Maleae tribe. It is distributed mainly in Asia, Europe, and North America.[11] In the Iberian Peninsula, it is represented by five wild species, growing along almost all its territory.[12,13] Members of genus *Crataegus* are commonly called hawthorn,[8] and *Crataegus* species are included in the European Pharmacopoeia.[14] *Sorbus* L. is classified within the subtribe Pyrinae and it is composed by over 250 trees and shrubs distributed in the Northern Hemisphere, with nine species native to the Iberian Peninsula.[15] The fruits of both genera are pones, and they can be consumed fresh or processed added to other products, such as jams, jellies, or soft drinks. Their nutritional value is

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very high owing to their protein, vitamins, and minerals contents.[16]

Crataegus fruits have been found to possess several biological effects, like antiobesity[17] antiproliferative, antitumor, anti-inflammatory, and apoptosis-inducing activities, among others, which could be associated with their polyphenols content.[8,13,18,19] [8,13,18,19] Phenolic compounds are the principal bioactive compounds in *Crataegus* and *Sorbus* species.[16,20] For example, one of the best distributed species, *Crataegus monogyna* Jacq., is considered as a source of bioactive molecules, mainly polyphenols like procyanidins, epicatechin, hyperoside, isoquercitrin, and chlorogenic acid, but also various triterpenic acids, such as ursolic acid and oleanolic acid, and other important organic molecules. Moreover, they have vitamin C as well as parietal polysaccharides. Therefore, they have demonstrated a very high antioxidant activity.[8]

Some research has previously been done on the bioactive composition of Rosaceae fruits from *Crataegus pinnatifida* fruit, focusing on its terpenoids,[21] pectins,[22] or metabolome, including polyphenols.[23] However, many *Crataegus* and *Sorbus* fruits are still undervalued and not extensively studied regarding their phenolic composition.

For these reasons, the goals of this article were (i) to do a complete characterization of single phenolic compounds by high-performance liquid chromatography (HPLC) coupled with electrospray ionization (ESI) time-of-flight (TOF) mass spectrometry (MS) and flavan-3-ols by HPLC coupled with fluorescence detection (FLD), of the fruits of five different species (generally regarded as edible) of *Crataegus* and *Sorbus* genera, and to compare the polyphenol profiles among them; and (ii) to study their antioxidant activity using three methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP).

MATERIALS AND METHODS

Chemicals

Double-deionized water used in the analysis was obtained with a Milli-Q system (Millipore, Bedford, MA, USA). DPPH, ABTS, potassium persulfate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and 2,4,6-tri(2-pyridyl)-s-triazine were supplied by Sigma-Aldrich (St Louis, MO, USA). Ethanol, methanol, and hydrochloric acid were provided by Panreac (Barcelona, Spain). Analytical standards were also purchased from Sigma-Aldrich. Other reagents were purchased from Merck KGaA (Darmstadt, Germany).

Samples

All fruits of the studied species were collected from Andalusia (Spain). The following species were selected: *Crataegus azarolus* L. came from a cultivated specimen at Pulianas municipality in the province of Granada (37° 13′ 25″ N, 3° 36′ 0″ W; 750 m above sea level (a.s.l.)). *Crataegus monogyna* Jacq. fruit was collected from the wild at Sierra de Huétor Santillán (Granada province; 37° 13′ 07″ N, 3° 31′ 02″ W; 1200 m a.s.l.). *Crataegus granatensis* Boiss fruit was collected from the wild at Sierra Arana (Granada province; 37° 19′ 31″ N, 3° 28′ 31″ W; 1580 m a.s.l.). *Crataegus laciniata* Ucria fruits were collected from a cultivated specimen at Torre del Vinagre Botanical Garden in Sierra de Cazorla (Jaen province; 38° 00′ 51″ N, 2° 52′ 22″ W; 700 m a.s.l.). *Sorbus domestica* L. was collected from a cultivated specimen in Monachil municipality (Granada province; 37° 7′ 37″ N, 3° 31′ 32″ W, 850 m a.s.l.).

For plant material gathered from the wild, vouchers of the same specimens studied were collected, processed, and deposited in the University of Granada Herbarium (GDA), with voucher codes: *C. monogyna* (GDA70854); *C. granatensis* (GDA70798-1). All plant names in this text have been reviewed with the POWO database.[12,24] A total amount of 30–50 fruits of each variety were considered for the analysis. The edible part of the fruits was separated manually from the non-edible parts (petioles and seeds). The fresh fruit edible parts with moistures from 45 to 80 g kg^{-1} were freeze-dried using a Zirbus lyophilizer (Bad Grund, Germany) for 120 h at −50 °C with a pressure of 0.4 mbar. To avoid the oxidation of light-sensitive compounds, the freeze-dryer camera was covered to create a dark environment. After the lyophilization the samples were ground with a knife mill provided by IKA Werke GmbH & Co. KG (Staufen, Germany) until reaching an average particle size of 0.2 mm.

Ultrasound extraction

An ultrasonic bath (Sonorex RK52; Bandelin, Berlin, Germany) that operates with a frequency of 35 kHz was used for the extractions of phenolic compounds. Briefly, 0.5 g of sample was added to 10 mL ethanol:water, 80:20 (v/v) in Falcon tubes and sonicated 15 min in the ultrasound bath. After that, the samples were centrifuged at 9960 × *g* for 10 min, the supernatant was collected, and then the extraction was repeated twice more. Finally, the collected supernatants were evaporated and reconstituted in 2 mL of methanol:water (1:1, v/v).[25] The final extracts were filtered through 0.2 μm nylon syringe filters and stored at −18 °C until the analyses.

Determination of phenolic compounds by HPLC–MS-ESI-TOF

The edible part fruit extracts from the Rosaceae family obtained by ultrasonic-assisted extraction were analyzed using an ACQUITY Ultra Performance LC system (Waters Corporation, Milford, MA, USA) coupled to an ESI source operating in the negative mode and a TOF mass detector (Waters Corporation). Phenolic compounds were separated on an ACQUITY UPLC BEH Shield RP18 column (1.7 μm, 2.1 mm × 100 mm; Waters Corporation) at 40 °C using a gradient previously stated[26] using water containing 1% acetic acid as mobile phase A and acetonitrile as mobile phase B. Phenolic compounds were identified according to their mass-to-charge ratio (*m/z*), molecular formula and *m/z* in source fragments and searching the literature. Calibration curves of chlorogenic acid, ferulic acid, quercetin, rutin, and phlorizin in the range 0.5–60 μg g^{-1} were used for quantifying the identified phenolic compounds.

Determination of flavan-3-ols by HPLC–FLD

The methodology used for the determination of flavan-3-ols was previously reported.[27] An Agilent 1200 Series (Agilent Technologies, Palo Alto, CA, USA), equipped with a quaternary pump delivery system, a degasser, an autosampler, and a fluorometric detector, was used for the analyses. A Develosil Diol 100 Å column 5 mm, 250 mm × 4.6 mm ID (Phenomenex, Torrance, CA, USA) was used. Mobile phase A consisted of acidic acetonitrile (CH_3CN:CH_3COOH, 98:2; v/v) and mobile phase B consisted of acidic aqueous methanol (CH_3OH:H_2O:CH_3COOH, 95:3:2; v/v/v). The elution gradient was slightly modified as follows: 3% B for 50 min, 38% B for 3 min, 100% B for 13 min, and 100% B

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for 10 min. FLD was performed with an excitation wavelength of 230 nm and an emission wavelength of 321 nm. The injection volume was 10 μL. All the analyses were carried out at 35 °C. The identification of flavan-3-ols was done according to the previous HPLC–MS-ESI-TOF analyses and according to the elution of the compounds, because they elute according to their degree of polymerization, firstly eluting the monomers and then the different oligomers.[28] A standard curve of catechin at six concentration levels from 10 to 650 μg g^{-1} was carried out for the quantification of flavan-3-ols. In addition, correction factors suggested by Robbins *et al*.[28] were used for quantification. The results are expressed as micrograms catechin equivalents (CE)/gram dry weight (dw).

Antioxidant assays

The *in vitro* antioxidant activity of the fruits was performed using three different methods: DPPH, ABTS, and FRAP. A calibration curve of Trolox in methanol:water (4:1, v/v) was carried out at a concentration between 1 and 200 μg g^{-1} to determine the analyte concentration. The results of the three assays were expressed as milligrams Trolox equivalents (TE)/g dw. The DPPH assay was done according to Brand-Williams *et al*.:[29] 100 μL of the extract was added of 2.9 mL of DPPH and then vortexed and kept in a dark place for 30 min. Subsequently, it was measured in a spectrophotometer (Spectrophotometer 300 Array, ultraviolet–visible, single beam; Shimadzu, Duisburg, Germany) at 517 nm. To carry out the ABTS technique,[30] the ABTS reagent was prepared in ethanol 12 h earlier and adjusted to an absorbance of 0.7 ± 0.2 at 734 nm. To undertake the antioxidant capacity determinations, 2 mL of the ABTS solution and 0.2 mL of sample were mixed and the absorbance was recorded after 30 min at 734 nm. The FRAP technique was carried out as described by Benzie and Strain.[31] Briefly, 30 μL of sample, 90 μL of distilled water, and 900 μL of the FRAP solution were mixed and kept at 37 °C for 30 min. The absorbance at 595 nm was measured in a spectrophotometer.

Data processing

HPLC–ESI-TOF-MS data for the identification of polar compounds and the identification of phenolic compounds were elaborated using MassLynx 4.1 software (Waters Corporation). A Pearson correlation analysis between the fruit samples and the analyzed feature, hierarchical clustering analysis, and least-square discriminant analysis were performed using MetaboAnalyst 5.0. One-way ANOVA analysis (Tukey's honestly significant difference test) was performed using Statistica 8.0 software (StatSoft, Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Identification of phenolic compounds by HPLC–MS

All the extracts were analyzed in triplicate using HPLC–ESI-TOF-MS. The identified compounds are collected in Table 1 with their retention time (minutes), experimental and calculated *m/z*, molecular formula, in source fragments (*m/z*). In all cases the error was kept lower than 5 μg g^{-1} and the score higher than 90%. In addition, the chromatograms obtained for each fruit studied are presented in Supporting Information Fig. S1. In total, 71 polar compounds were identified in the Rosaceae fruits studied. To our knowledge, 30 of them have been elucidated in these fruits for the first time.

Phenolic acids

Żurek *et al*. characterized berries, leaf, and flower extracts of some *Crataegus* species from Poland, including *C. monogyna*.[32] According to them, some derivatives of quinic acid were identified, corresponding with peaks 7, 23, and 14 and called coumaroylquinic acids isomers a and b and dicaffeoylquinic acid respectively.[32] The compound caffeoylquinic acid has also been previously extracted and isolated from *S. domestica* fruits, and reported for reducing both the glucose and cholesterol uptakes by the cell line HepG2.[33] In addition, another four compounds have been found for the first time in these fruits. Two isomers, corresponding with peaks 10 and 25, that were tentatively named as 3-*O*-feruloylquinic acid according to their *m/z* 367, and fragment 191, reported previously by Parejo *et al*. in fennel.[34] They had also been found in sweet cherry, apricot, redcurrant, and peach.[35,36] The other two compounds at peaks 13 and 15 were named as unidentified quinic acid derivative isomers, as fragment 191 was also found.

Chlorogenic acid isomers have been found at 2.90 min, 4.86 min, and 5.10 min (peaks 4, 11, and 12 respectively) in concordance with several researchers.[32,37-39] They have been named as chlorogenic acid isomer a, b, and c, but they could be also named as neochlorogenic, chlorogenic, and cryptochlorogenic acids, as Bujor *et al*. did for *Crataegus pentagyna* Waldst. & Kit. ex Willd. fruits.[38] Chlorogenic acid from *C. pinnatifida* Bunge fruits has been previously reported to prevent stress-hormone-induced depressive behavior in mice.[40] Moreover, the compound caffeic acid-3-glucoside was detected at 2.96 min (peak 5) in concordance with Bekbolatova *et al*., who found it previously in *Crataegus almaatensis* Pojark. flowers,[37] but not in fruits. At time 2.33 min (peak 3) with fragments at *m/z* 125, 135, 151, and 169, a compound called beta-glucogallin according to Allen *et al*.,[41] has been detected for the first time in these fruits, which has been reported to be in high amounts in tea (*Camellia sinensis* (L.) Kuntze). It has also been reported in gooseberries (*Phyllanthus emblica* L.; syn. *Emblica officinalis* Gaertn.) as a novel antidiabetic compound demonstrated to inhibit sorbitol accumulation by 73%.[42] The compound found in peak 6 at *m/z* 325 and molecular formula $C_{15}H_{19}O_8$ was named as 1-*O*-*p*-coumaroyl-beta-D-glucose according to Wang *et al*.[43] It has been found in a few different foods, such as strawberries, jostaberries, and blackcurrants,[35,36] but not previously in these fruits from the Rosaceae family.

Flavonoids

At times 4.74 min and 5.91 min (peaks 9 and 21 respectively) two isomers of dihydromyrcetin-3-*O*-rhamnoside were found. In addition, the compound dihydromyricetin has been detected at 6.78 min (peak 29) and *m/z* 319. They were tentatively identified according to their *m/z* in source fragments (Table 1).[35,36] This compound and its derivatives were first isolated from a traditional Chinese medicinal plant called *Ampelopsis grossedentata*, and they were also found in various plant-based foods, such as grapes and red bayberry. Moreover, they have been attributed with several health-benefiting activities, such as antioxidant, antimicrobial, anti-inflammatory, anticancer, antidiabetic, and neuroprotective activities.[44] Additionally, Żurek *et al*. reported another myricetin derivate called myrcetin-3-*O*-rhamnoside in *Crataegus* fruits.[32] It is the first time that these myricetin derivatives have been described in these fruits.

The compound detected at 5.43 min (peak 16) has been named eriodictyol-7-*O*-glucoside according to its fragments and the PubChem database (PubChem CID: 13254473). It has been previously

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Table 1. Compounds tentatively identified in the studied fruits by high-performance liquid chromatography electrospray ionization time-of-flight mass spectrometry and tandem mass spectrometry

	Time (min)	Experimental mass (*m/z*)	Calculated mass (*m/z*)	Error (ppm)	Score (%)	Molecular formula	Fragments (*m/z*)	Tentative compound
1	0.52	191.0543	191.0556	−1.2	99.99	$C_7H_{12}O_6$	127.0365; 93.0301; 85.0277	Quinic acid
2	1.64	255.0497	255.0505	−4.1	99.99	$C_{11}H_{12}O_7$	165.0531; 179.0325; 193.0513; 221.0102	Piscidic acid
3	2.33	331.0666	331.0665	0.3	98.18	$C_{13}H_{16}O_{10}$	169.0069; 125.0230; 135.0446; 151.0015	beta-Glucogallin
4	2.90	353.0855	353.0873	−4.1	99.55	$C_{16}H_{18}O_9$	191.0532; 179.0327; 135.0426	Chlorogenic acid isomer a
5	2.96	341.0858	341.0873	−4.4	97.30	$C_{15}H_{18}O_9$	161.0224; 133.0265; 179.0331	Caffeic acid-3-glucoside
6	3.68	325.091	325.0923	−4.0	99.83	$C_{15}H_{18}O_8$	119.0472; 163.0370	1-*O*-*p*-Coumaroyl-beta-D-glucose
7	4.08	337.0901	337.0923	−4.5	99.59	$C_{16}H_{18}O_8$	163.0375; 119.0474; 191.0533	Coumaroylquinic acid isomer a
8	4.28	289.0695	289.0712	−3.9	97.23	$C_{15}H_{14}O_6$	125.0233; 203.0714	Catechin
9	4.74	465.1021	465.1033	−2.6	98.01	$C_{21}H_{22}O_{12}$	447.0919; 329.0845	Dihydromyricetin 3-*O*-rhamnoside isomer a
10	4.81	367.1013	367.1029	−4.4	99.99	$C_{17}H_{20}O_9$	119.0780; 163.0372; 191.0530	3-*O*-Feruloylquinic acid isomer a
11	4.86	353.0866	353.0873	−2.0	91.68	$C_{16}H_{18}O_9$	173.0432; 285.0378; 135.0437	Chlorogenic acid isomer b
12	5.10	353.0868	353.0873	−1.4	100.00	$C_{16}H_{18}O_9$	173.0432; 285.0378; 135.0437	Chlorogenic acid isomer c
13	5.33	381.1761	381.1761	0.0	94.49	$C_{16}H_{30}O_{10}$	223.0586; 191.0533; 149.0213	Unidentified quinic acid derivate isomer a
14	5.36	515.1395	515.1401	−1.2	97.12	$C_{22}H_{28}O_{14}$	191.0533; 323.0752; 161.0230	Dicaffeoylquinic acid
15	5.41	381.175	381.1761	−2.9	98.60	$C_{16}H_{30}O_{10}$	223.0586; 191.0533; 149.0213	Unidentified quinic acid derivate isomer b
16	5.43	449.1076	449.1084	−1.8	99.98	$C_{21}H_{22}O_{11}$	259.0500; 269.0437; 287.0526; 150.9996; 178.9963; 125.0224	Eriodictyol-7-*O*-glucoside
17	5.46	289.0699	289.0712	−4.5	99.82	$C_{15}H_{14}O_6$	245.0795; 203.0699; 123.0427	Epicatechin
18	5.65	577.1346	577.1346	0.0	98.42	$C_{30}H_{26}O_{12}$	407.0750; 289.0694; 125.0221	Procyanidin dimer
19	5.71	481.0979	481.0982	−0.6	95.17	$C_{21}H_{22}O_{13}$	407.0756; 345.0800; 289.0696; 139.0377	Epigallocatechin-7-glucuronide
20	5.83	517.2288	517.2285	0.6	92.89	$C_{24}H_{38}O_{12}$	385.1910; 205.1235; 153.0919	Vomifoliol-xylosyl-glucoside
21	5.91	465.1006	465.1033	−3.8	99.07	$C_{21}H_{22}O_{12}$	447.0924; 329.0857	Dihydromyricetin 3-*O*-rhamnoside isomer b
22	6.00	577.1335	577.1346	−1.9	95.23	$C_{30}H_{26}O_{12}$	407.0742; 289.0691; 125.0204	Procyanidin dimer
23	6.22	337.0909	337.0923	−4.2	91.54	$C_{16}H_{18}O_8$	191.0535; 173.0501	Coumaroylquinic acid isomer b
24	6.39	865.1991	865.198	1.3	98.92	$C_{45}H_{38}O_{18}$	577.1331; 407.0760; 289.0696; 125.0220	Procyanidin trimer
25	6.40	367.1013	367.1029	−4.4	99.99	$C_{17}H_{20}O_9$	119.0780; 163.0372; 191.0530	3-*O*-Feruloylquinic acid isomer b
26	6.59	577.1336	577.1346	−1.7	94.69	$C_{30}H_{26}O_{12}$	407.0750; 287.0534; 125.0212	Procyanidin dimer
28	6.70	1153.2677	1153.2614	3.5	96.24	$C_{60}H_{50}O_{24}$	863.1866; 575.1213; 287.0548; 125.0215	Procyanidin tetramer
29	6.78	319.0438	319.0454	−5.0	96.33	$C_{15}H_{12}O_8$	313.0700	Dihydromyricetin
30	6.83	593.1508	593.1506	0.3	99.00	$C_{27}H_{30}O_{15}$	353.0650; 383.0756; 473.1089; 297.0740	Vitexin 4″-*O*-glucoside
31	7.00	433.1122	433.1135	−3.0	91.60	$C_{21}H_{22}O_{10}$	313.0697; 343.0797; 243.0271	Naringenin 7-*O*-glucoside
32	7.22	865.1978	865.198	−0.2	96.88	$C_{45}H_{38}O_{18}$	577.1345; 407.0751; 289.0699; 125.0212	Procyanidin trimer
33	7.52	577.1385	577.1346	−0.7	98.00	$C_{30}H_{26}O_{12}$	407.0734; 289.0687; 125.0226	Procyanidin dimer
34	7.66	263.127	263.1283	−3.9	100.00	$C_{15}H_{20}O_4$	153.0890; 203.1045; 219.1354; 201.1255	Abscisic acid
35	7.70	1153.2639	1153.2614	2.2	98.92	$C_{60}H_{50}O_{24}$	865.1976; 575.1196; 407.0757; 289.0537; 125.0224	Procyanidin tetramer
36	7.74	447.0918	447.0927	−2.0	91.76	$C_{21}H_{20}O_{11}$	285.0379; 297.0301; 327.0498; 357.0503	Luteolin-8-glucoside (orientin) isomer a
37	8.05	447.0919	447.0927	−1.8	99.31	$C_{21}H_{20}O_{11}$	285.0379; 297.0301; 327.0498; 357.0503	Luteolin-8-glucoside (orientin) isomer b
38	8.27	451.1014	451.1029	−3.3	99.64	$C_{24}H_{20}O_9$	341.0633; 217.0108; 177.0163	Epigallocatechin 3-*O*-*p*-coumarate isomer a
39	8.31	431.0968	431.0978	−2.3	99.36	$C_{21}H_{20}O_{10}$	283.0583; 311.0543; 341.0547	Apigenin 8-*C*-glucoside (vitexin) isomer a
40	8.38	577.1552	577.1557	−0.9	99.94	$C_{27}H_{30}O_{14}$	413.0846; 293.0426	Vitexin 2″-*O*-rhamnoside
41	8.49	563.1401	563.1401	0.0	98.31	$C_{26}H_{28}O_{14}$	293.0432; 311.0542; 413.0807	Isorhamnetin-*O*-malonylhexoside/apigenin 6-arabinoside 8-*C*-glucoside isomer a
42	8.75	1153.2573	1153.2614	−0.5	93.03	$C_{60}H_{50}O_{24}$	865.1976; 575.1196; 407.0757; 289.0537; 125.0224	Procyanidin tetramer

Table 1. Continued

	Time (min)	Experimental mass (*m/z*)	Calculated mass (*m/z*)	Error (ppm)	Score (%)	Molecular formula	Fragments (*m/z*)	Tentative compound
43	8.81	451.1017	451.1029	−2.7	99.85	$C_{24}H_{20}O_9$	407.0747; 341.0644; 289.0708	Epigallocatechin 3-*O*-*p*-coumarate isomer b
44	8.86	563.1409	563.1401	1.4	99.89	$C_{26}H_{28}O_{14}$	293.0432; 311.0542; 413.0807	Isorhamnetin-*O*-malonylhexoside/apigenin 6-arabinoside 8-*C*-glucoside isomer b
45	8.90	597.1823	597.1819	0.7	95.93	$C_{27}H_{34}O_{15}$	575.1180; 357.0967; 289.0697	Catechin 3-*O*-rutinoside
46	8.98	435.1269	435.1291	−4.1	93.58	$C_{21}H_{24}O_{10}$	125.0235; 167.0333; 255.0280; 273.0683	Phlorizin isomer a
47	9.01	577.134	577.1346	−1.0	99.01	$C_{30}H_{26}O_{12}$	425.0856; 407.0745; 289.0688; 125.0217	Procyanidin dimer
48	9.07	315.0854	315.0869	−4.8	94.32	$C_{17}H_{16}O_6$	125.0217; 177.0159; 165.0164; 151.0371	Dihydroxy-dimethoxyisoflavanone
49	9.19	431.0955	431.0978	−4.3	99.14	$C_{21}H_{20}O_{10}$	283.0582; 311.0531; 341.0637	Apigenin 8-*C*-glucoside (vitexin) isomer b
50	9.33	609.1445	609.1456	−1.8	98.22	$C_{27}H_{30}O_{16}$	300.0247; 271.0223	Quercetin 3-*O*-rutinoside isomer a
51	9.46	463.0865	463.0877	−2.6	96.90	$C_{21}H_{20}O_{12}$	300.0249; 271.0223; 255.0272	Quercetin 3-*O*-glucoside isomer a (hyperoside)
52	9.47	609.1454	609.1456	−0.3	93.16	$C_{27}H_{30}O_{16}$	300.0247; 271.0223	Quercetin 3-*O*-rutinoside isomer b
53	9.66	463.0873	463.0877	−0.9	99.25	$C_{21}H_{20}O_{12}$	300.0253; 271.0223; 255.0276	Quercetin 3-*O*-glucoside isomer b (isoquercitrin)
54	9.91	435.1072	435.108	−1.8	91.13	$C_{24}H_{20}O_8$	341.0648; 287.0545; 189.0175; 125.0227	Epigallocatechin 3-*O*-cinnamate isomer a
55	10.01	577.1346	577.1346	0.0	99.10	$C_{30}H_{26}O_{12}$	289.0709; 125.0215	Procyanidin dimer
56	10.05	435.1270	435.1291	3.7	98.92	$C_{21}H_{24}O_{10}$	273.0744; 167.0327	Phlorizin isomer b
57	10.21	433.0759	433.0771	−2.8	98.01	$C_{20}H_{18}O_{11}$	300.0261; 271.0226; 255.0276; 243.0266; 227.0323	Quercetin 3-alpha-L-arabinofuranoside (avicularin) isomer a
58	10.25	447.0918	447.0927	−2.0	99.39	$C_{21}H_{20}O_{11}$	255.0279; 284.0309; 300.0249; 227.0324	Kaempferol-3-*O*-glucoside (astragalin)
59	10.32	433.0752	433.0771	−4.4	96.03	$C_{20}H_{18}O_{11}$	300.0250; 271.0228; 255.0274	Quercetin 3-alpha-L-arabinofuranoside (avicularin) isomer b
60	10.36	505.0977	505.0982	−1.0	99.93	$C_{23}H_{22}O_{13}$	300.0254; 271.0218; 255.0266	Quercetin 3-*O*-(6″-acetyl-glucoside) isomer a
61	10.38	623.1589	623.1612	−3.7	97.77	$C_{28}H_{32}O_{16}$	315.0504; 300.0266; 285.0412; 271.0262	Isorhamnetin 3-rutinoside isomer a
62	10.57	505.0978	505.0982	−0.8	94.91	$C_{23}H_{22}O_{13}$	300.0253; 271.0230; 255.0278	Quercetin 3-*O*-(6″-acetyl-glucoside) isomer b
63	10.59	477.1033	477.1033	0.0	99.62	$C_{22}H_{22}O_{12}$	314.0436;271.0238; 243.0289; 285.0407; 341.0667; 257.0428	Isorhamnetin-3-*O*-glucoside isomer a
64	10.62	623.1617	623.1612	0.8	97.08	$C_{28}H_{32}O_{16}$	315.0504; 300.0266; 285.0412; 271.0262	Isorhamnetin 3-rutinoside isomer b
65	10.71	477.1029	477.1033	−0.8	95.24	$C_{22}H_{22}O_{12}$	314.0436;271.0238; 243.0289; 285.0407; 341.0667; 257.0428	Isorhamnetin-3-*O*-glucoside isomer b
66	10.78	451.1022	451.1029	−1.6	96.32	$C_{24}H_{20}O_9$	341.0646; 189.0170; 217.0116	Epigallocatechin 3-*O*-*p*-coumarate isomer c
67	10.99	505.0982	505.0982	0.0	98.01	$C_{23}H_{22}O_{13}$	300.0242; 271.0211; 243.0268; 163.0744	Quercetin 3-*O*-(6″-acetyl-glucoside) isomer c
68	11.21	505.0981	505.0982	−0.2	90.30	$C_{23}H_{22}O_{13}$	300.0258; 271.0226; 255.0289	Quercetin 3-*O*-(6″-acetyl-glucoside) isomer d
69	11.35	489.1024	489.1033	−1.8	96.14	$C_{23}H_{22}O_{12}$	407.0762; 285.0292; 255.0281; 243.0235; 125.0206	Kaempferol 3-*O*-acetyl-glucoside
70	11.64	435.1058	435.108	−4.1	91.93	$C_{24}H_{20}O_8$	341.0645; 285.0370; 255.0268; 189.0164	Epigallocatechin 3-*O*-cinnamate isomer b
71	11.73	575.1183	575.119	−1.2	99.64	$C_{30}H_{24}O_{12}$	407.0724; 289.0693; 243.0286; 271.0215	Procyanidin dimer (A type)

detected, but not quantified, in several different foods, such as pomegranates, herbs and spices, spearmints, orange mints, and winter savories.[35,36] So, it is the first time that this compound had been identified in Rosaceae fruits. The compound vormifoliol-xylosyl-glucoside (peak 20) with molecular formula $C_{24}H_{38}O_{12}$ and fragments at *m/z* 153, 205, and 385 was tentatively identified as previously described by Allen *et al*[41] This compound has been detected, but not quantified in *Malus* (crab apple) and pomes,[35,36] and first described in the studied fruits. Peak 31 was named as naringenin-7-*O*-glucoside, also known as prunin according to its molecular formula $C_{21}H_{22}O_{10}$. It is a compound with a bitter taste previously reported in almond, tomato, peach, pine, nut, and immature citrus fruits.[35,36] It has also been found previously in *Crataegus* species fruits.[32]

Peaks 39 and 49 with molecular formula $C_{21}H_{21}O_{10}$ have been detected as two isomers of apigenin 8-*C*-glucoside, also known as vitexin.[32,37,38] In concordance, two vitexin derivatives have been found at 6.83 min and 8.38 min (peaks 30 and

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40 respectively) named as vitexin 4″-*O*-glucoside and vitexin 2″-*O*-rhamnoside respectively, previously described in *C. almaatensis* flowers and leaves.[37] These compounds have been previously studied in *C. pinnatifida* leaves for having several bioactivities as cardioprotective, antioxidant, antiviral, anti-*Helicobacter pylori*, anti-tumor and anti-inflammatory activities.[45] However, those vitexin derivatives have never been described before in the studied fruits.

At times 7.64 min and 8.05 min (peaks 36 and 37 respectively), two isomers of luteolin-8-glucoside were found, also known as orientin thanks to its fragments at *m/z* 285, 297, 327, and 357.[41] This compound has been attributed several bioactivities, such as antioxidant, antiaging, antiviral, antibacterial, anti-inflammation, cardioprotective, and neuroprotective activity, among others.[46] Previously, it has been detected but not quantified in several different foods, such as flour, wild rice, corn, fenugreek, and quinoa, but isolated from various medicinal plants such as bamboo leaves and passion flowers.[35,46] In addition, a similar compound, luteolin 6,8-*C*-diglucoside, was reported by Żurek *et al*. in *Crataegus* berries.[32] These luteolin derivatives have been described in fruits of the Rosaceae family here for the first time.

Flavan-3-ols and their polymeric products, the procyanidins, are regarded as functional ingredients in various beverages, whole and processed foods, herbal remedies, and supplements.[47] So in these fruits, catechin (peak 8), epicatechin (peak 17), procyanidin dimers (peaks 18, 22, 26, 33, 47, 55, and 71), trimers (peaks 24 and 32), and tetramers (peaks 28, 35, and 42) have been found.[32,37,38] However, owing to the *m/z* working range of the mass spectrometer, the complete determination of procyanidins has been carried out using HPLC–FLD.

Peak 45, with molecular formula $C_{27}H_{34}O_{15}$, *m/z* 597 and according to its fragment at *m/z* 289 that corresponds to catechin, has been tentatively named as catechin-3-*O*-rutinoside (PubChem CID: 44257079). The compounds found at peaks 54 and 70 were named as epillocatechin-3-*O*-cinnamate isomers according to its molecular formula and *m/z* in source fragments [41] (PubChem CID: 21629801). This compound has been reported at high concentration in teas (*C. sinensis*) as red tea, herbal tea and, in a lower concentration, in green tea and black tea,[48] but not described previously in the fruits studied. Peak 19, with fragments at *m/z* 139, 289, 345, and 407 and molecular ion at *m/z* 481, was tentatively identified as epigallocatechin-7-glucuronide.[35,41,43] Similarly, at 8.27 min, 8.81 min, and 10.78 min (peaks 38, 43, and 66 respectively), three isomers of epigallocatechin 3-*O*-*p*-coumarate were detected. This compound was also named cinchonain by Żurek *et al*.[32]

Corresponding with peaks 46 and 56, two isomers of phlorizin were identified in concordance with their molecular ion at *m/z* 435 and their fragments at *m/z* 273.[41] This compound had been described at high concentration in a few different foods, such as oregano, plum, and apple, and at lower concentration in pomegranate and apricot. Additionally, it had also been detected in tamarind, millet, fig, black-eyed pea, and chamomile,[35,49] but not in Rosaceae fruits. The main bioactivity reported of phlorizin is antidiabetic activity.[49]

Dihydroxy-dimethoxyisoflavanone (peak 48) was detected at *m/z* 315 and molecular formula $C_{17}H_{16}O_6$ and was previously reported in some leaves of plants such as *Uraria lacei* Craib[50] and *Desmodium oojeinense* (Roxb.) H.Ohashi (syn. *Ougeinia oojeinensis* (Roxb.) Hochr.).[51] Regarding fruits, this compound has already been detected, but not quantified, in peaches (*Prunus persica* (L.) Batsch),[35,36] but it is the first time that this compound is described in Rosaceae fruits.

Peaks 50 and 52 were identified at 9.33 min and 9.47 min respectively as quercetin-3-*O*-rutinoside (also named rutin) with molecular formula $C_{27}H_{30}O_{16}$ and *m/z* 609. Peaks 51 and 53, with *m/z* 463, were reported as two isomers of quercetin-3-*O*-glucoside (also called hyperoside and isoquercetin) in concordance with other researchers.[32,37,38] Four isomers of quercetin 3-*O*-(6″-acetyl-glucoside) (peaks 60, 62, 67, and 68) with *m/z* 505 were also identified as previously described in *C. monogyna* flowers,[32] *C. almaatensis* leaves[37] and *C. pentagyna* fruits.[38] At 10.21 min and 10.32 min (peaks 57 and 59 respectively) two isomers of the same compound were found with molecular ion at *m/z* 433 and fragments at *m/z* 300, 271, 255, and 243, which indicate that they could be a quercetin derivative. So, these compounds were identified as quercetin-3-alpha-L-arabinofuranoside isomers, also known as avicularin. They had been previously found in *Taxillus kaempferi* (DC.) Danser, *Polygonum aviculare* L.,[52] and fruits such as apples,[53] but not previously in the fruits studied here.

Two kaempferol derivatives were found corresponding with peaks 58 and 69. They both had the *m/z* fragment of 285 related to kaempferol and were named kaempferol-3-*O*-glucoside and kaempferol-3-*O*-acetyl-glucoside respectively. The first of them is also known as astragalin, a well-known bioactive natural flavonoid with antioxidant, anti-inflammatory, anticancer, neuroprotective, and cardioprotective activity. Previously, it had been identified in different plants, such as *Cuscuta chinensis* Lam., and species within Ebenaceae, Rosaceae, and Eucommiaceae families.[54] Moreover, both compounds have also been found in lingonberry (*Vaccinium vitis-idaea* L. 'Amberland'), cranberry (*Vaccinium macrocarpon* Aiton 'Ben Lear'),[55] red raspberry (*Rubus idaeus* L.),[56] and strawberry (*Fragaria* × *ananassa* (Duchesne ex Weston) Duchesne ex Rozier),[57] but it is the first time they have been described in fruits of the Rosaceae family.

Isorhamnetin is a well-known flavonoid present in fruits. At 8.49 min and 8.86 min (peaks 41 and 44 respectively), two isomers of an isorhamnetin derivative were identified as isorhamnetin-*O*-malonylhexoside according to Bujor *et al*.,[38] who found it in *C. pentagyna* flowers. These compounds could also be named as apigenin 6-*C*-arabinoside-8-*C*-glucoside described in *Crataegus* species flowers by Żurek *et al*.[32] Moreover, another four isorhamnetin derivates were detected (peaks 61, 63–65). According to its fragments (*m/z* 285 and 271), those with molecular formula $C_{28}H_{32}O_{16}$ were tentatively identified as isorhamnetin-3-*O*-glucoside isomers, and those with molecular formula $C_{22}H_{22}O_{12}$ were named isorhamnetin-3-*O*-rutinoside isomers. They had been previously reported in grapes[58] and pears[59] and have been described here for the first time in Rosaceae fruits.

Other polar compounds

First, peak 1 corresponded to an organic acid, quinic acid. Peak 2 with *m/z* 255 and fragments at *m/z* 165, 179, and 193 was tentatively named piscidic acid according to Yannai.[60] This compound had been previously described in *Opuntia ficus-indica* (L.) Mill. as a potential anticholesterolemic.[61] Abscisic acid (peak 34) was detected at *m/z* 263 and according to its fragments 153, 201, and 219 as related by Sawada *et al*.[62] It is an important sesquiterpenoid plant hormone that acts as a regulator of plant responses to environmental stresses such as drought and cold and additionally plays an important role in managing glucose homeostasis in humans. It has been previously reported in several fruit and vegetables, such as avocado, citrus, soybean, fig, maize, and apricot, among others.[63] To our knowledge, these

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compounds have been detected in fruits of the Rosaceae family here for the first time.

Quantification of phenolic compound

Phenolic compounds were quantified in all samples and the results are presented in Table 2.

C. monogyna was the fruit that had the highest content of total phenolic compounds, followed by *S. domestica* and then by *C. azarolus*, *C. granatensis*, and *C. laciniata* that did not present significant differences among them. Regarding the phenolic acids, *S. domestica* revealed the highest content, whereas *C. granatensis* and *C. laciniata* had the lowest concentration of phenolic acids. *C. azarolus* showed some phenolic acids that could not be quantified in the other Rosaceae fruits, such as two caffeic acid derivatives (caffeic acid-3-glucoside and dicaffeoylquinic acid), 1-*O*-*p*-coumaroyl-beta-D-glucose and 3-*O*-feruloylquinic acid isomer b. In addition, beta-glucogallin was only quantified in *C. laciniata*. In all the fruits analyzed the major phenolic acid was chlorogenic acid and the second one was coumaroylquinic acid. Concerning the sum of flavonoids, *C. monogyna* was the fruit with the highest amount of these compounds, followed in this case by *C. laciniata* and *C. granatensis*. In contrast to what happened for phenolic acids, the lowest concentration of flavonoids was found in *S. domestica*, but also in *C. azarolus*. The compounds that presented higher concentrations in all Rosaceae fruits were hyperoside, isoquercitrin, rutin, and other quercetin derivatives. *C. laciniata* was the only fruit studied where isorhamnetin 3-rutinoside (isomers a and b), kaempferol 3-*O*-acetyl-glucoside, and phloridzin isomer a were quantified. The compound apigenin 8-*C*-glucoside (vitexin) isomer a, apigenin 6,8-di-*C*-glucoside (vicenin 2), vomifoliol-xylosyl-glucoside, luteolin-8-glucoside (orientin) isomer a, and phloridzin isomer b could only be quantified in *C. azarolus*, whereas quercetin 3-*O*-(6″-acetyl-glucoside) isomer c was found in *C. monogyna*. Luteolin-8-glucoside (orientin) and isorhamnetin-*O*-malonylhexoside were found in *C. azarolus* and *C. laciniata*, isorhamnetin 3-rutinoside in *C. laciniata*, and isorhamnetin-3-*O*-glucoside in *C. granatensis* and *C. laciniata*. So, *C. laciniata* was revealed to be the best source of quercetin and isorhamnetin derivatives and *C. azarolus* for apigenin derivatives, among the fruits analyzed.

Other researchers have previously studied the total phenolic content of some of these fruits using spectrophotometric methods such as Folin–Ciocalteu, not focusing on specific compounds.[64-67] Moreover, other researchers have studied the polyphenol content of some *Crataegus* and *Sorbus* species similar and different from those studied here by HPLC. In *C. monogyna* fruit ethanolic extracts, Bernatoniene *et al*. reported the following major compounds (ordered from higher to lower concentration): chlorogenic acid, hyperoside, rutin, quercetin, vitexin-2-*O*-rhamnoside, and procyanidins.[68] Belkhir *et al*. analyzed fruit extracts of *C. azarolus* and *C. monogyna*, finding that *C. azarolus* had a higher total phenolic content than the results found in our extracts.[69] Abuashwashi *et al*. studied the phenolic compounds by HPLC of nine *C. monogyna* fruits spontaneously grown in Spain (Cuenca and Guadalajara provinces) that were harvested in May 2011. They reported a total phenolic content ranging from 23.3 to 143.26 μg g^{-1} dw.[13] Alirezalu *et al*. reported a content of 0.50 mg g^{-1} dw of chlorogenic acid, 0.18 mg g^{-1} dw of vitexin, 1.15 mg g^{-1} dw of hyperoside (quercetin-3-*O* glucoside isomer (i)), and 0.68 mg g^{-1} dw of isoquercetin (quercetin-3-*O* glucoside isomer (ii)) in *C. monogyna* fruit. Moreover, they reported that they did not find vitexin 2-*O*-rhamnoside and rutin.[70] Mikulic-Petkovsek *et al*. characterized nine *Sorbus* genotypes, all different to those studied here, and they obtained that quercetin derivatives represented more than 95% of total flavonols, according to our results. In addition, they reported that chlorogenic acid was the major hydroxycinnamic acid, accounting from 33% to 73% of total hydroxycinnamic acid derivatives analyzed.[71] In our study, chlorogenic acid was also the major hydroxycinnamic acid, but represented 95% of the hydroxycinnamic acid derivatives. Becerra-Herrera *et al*. determined chlorogenic acid and quercetin-3-*O*-glucoside as major compounds in *Sorbus americana* Marshall fruits.[72] They also found quercetin-3-*O*-rutinoside (rutin) as a major compound in *S. domestica* fruit. Other researchers[73] also determined phenolic compounds by HPLC in different *Sorbus* species fruits, finding as the major compounds chlorogenic acid ranging 0.55–7.50 mg g^{-1}, quercetin-3-*O*-rutinoside 0.02–0.39 mg g^{-1}, quercetin-3-*O* glucoside isomer a 0.02–1.19 mg g^{-1}, and quercetin-3-*O* glucoside isomer b 0.02–0.65 mg g^{-1}. Šavikin *et al*. also corroborates these major compounds, and their results obtained from ten fruit samples of *Sorbus aucuparia* L. and *S. aria* (L.) Crantz were in the same range of magnitude found in our samples.[74] Similar results were reported by Liu *et al*. in *C. pinnatifida* var. *major* fruits[75] and by Liu *et al*. in *Crataegus grayana* Eggl. (syn. *Crataegus flabellata* (Bosc ex Spach) K.Koch) fruits.[76]

Quantification of flavan-3-ols by HPLC–FLD

Flavan-3-ols and the derived procyanidins, are bioactive ingredients with huge relevance in human health, as they have been reported to exhibit several health beneficial effects, such as antioxidant, antimicrobial, anti-viral, cardioprotective, and neuroprotective activities. Moreover, their presence in food affects food quality characteristics such as taste, astringency, aroma, and appearance. In addition, flavan-3-ols also have been attributed to help food preventing microbial, oxidative, and thermal degradations.[47] According to Yang *et al*., the content and profile of procyanidin may be used as a chemotaxonomic marker to distinguish different *Crataegus* species. They established significant correlations between contents of epicatechin/catechin and oligomeric procyanidins of different degree of polymerization values and different fruits than those studied here.[20]

Therefore, according to the results of the HPLC–MS analysis, the presence of flavan-3-ols was confirmed and the samples of *Crataegus* and *Sorbus* fruits were analyzed by HPLC–FLD. Figure 1 shows a chromatogram of the procyanidin profile of these fruits. In addition, Table 3 summarizes the results relative to the quantification of the flavan-3-ols present in the samples, detailing the amounts of each oligomer and the total content.

Comparing the total content of flavan-3-ols of the samples, it was observed that *C. monogyna* fruit had the highest concentration of these compounds, followed by *C. laciniata*, *S. domestica*, and *C. granatensis*, with *C. azarolus* being the one with the lowest content. Rodrigues *et al*. reported that *C. monogyna* fruit flavan-3-ols exhibited growth inhibitory activity on human tumor cell lines.[77] Comparing with other fruits studied by Hellström *et al*., it was observed that red fruits such as strawberries, raspberries, blackberries, and cherries have concentrations from 270 to 760 μg procyanidins/gram of fresh fruit.[78] In other fruits like ours, such as apple, the results varied between 430 and 1620 μg g^{-1} of fresh fruit depending on the cultivar. In addition, avocado, banana, kiwifruit, peach, nectarine, and grapes also revealed values lower than 650 μg g^{-1} fresh fruit.[78] Similar results were

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Table 2. Content of the phenolic compounds in the fruits studied (Rosaceae family) expressed as μg g^{-1} dry weight with the average plus/minus standard deviation

Compound	*Crataegus azarolus*	*Crataegus granatensis*	*Crataegus laciniata*	*Crataegus monogyna*	*Sorbus domestica*
Chlorogenic acid isomer a	187.72 ± 20.11^{d}	179.34 ± 5.95^{d}	310.71 ± 2.02^{c}	757.71 ± 52.67^{b}	1592.67 ± 18.09^{a}
Caffeic acid-3-glucoside	111.97 ± 3.70	n.d.	n.d.	n.d.	n.d.
1-*O*-*p*-Coumaroyl-beta-D-glucose	71.75 ± 1.66	n.d.	n.d.	n.d.	n.d.
Coumaroylquinic acid isomer a	n.d.	173.49 ± 13.44^{b}	n.d.	353.96 ± 39.62^{a}	20.59 ± 2.39^{c}
3-*O*-Feruloylquinic acid isomer a	46.80 ± 2.78^{a}	n.d.	n.d.	n.d.	37.84 ± 1.84^{b}
Chlorogenic acid isomer b	n.d.	35.26 ± 11.70^{c}	63.64 ± 4.26^{b}	136.19 ± 12.28^{a}	69.68 ± 7.29^{b}
Chlorogenic acid isomer c	334.73 ± 4.01^{a}	133.72 ± 25.09^{c}	210.51 ± 16.18^{b}	246.68 ± 18.50^{b}	n.d.
Unidentified quinic acid derivate isomer a	32.60 ± 2.61^{a}	10.67 ± 4.29^{c}	n.d.	22.58 ± 1.80^{b}	22.69 ± 1.45^{b}
Dicaffeoylquinic acid	32.66 ± 2.14	n.d.	n.d.	n.d.	n.d.
Unidentified quinic acid derivate isomer b	25.65 ± 2.48^{a}	n.d.	n.d.	n.d.	11.13 ± 0.13^{b}
Coumaroylquinic acid isomer b	20.45 ± 7.7^{a}	15.28 ± 3.90^{a}	18.78 ± 1.14^{a}	25.71 ± 5.77^{a}	n.d.
3-*O*-Feruloylquinic acid isomer b	46.80 ± 2.78	n.d.	n.d.	n.d.	n.d.
beta-Glucogallin	n.d.	n.d.	27.40 ± 2.23	n.d.	< LOQ
Dihydromyricetin 3-*O*-rhamnoside isomer a	n.d.	55.40 ± 2.68^{a}	19.90 ± 2.17^{c}	48.68 ± 3.14^{b}	7.89 ± 0.37^{d}
Eriodictyol-7-*O*-glucoside	n.d.	n.d.	n.d.	14.92 ± 0.42^{b}	27.80 ± 1.78^{a}
Vomifoliol-xylosyl-glucoside	9.96 ± 3.5	n.d.	n.d.	n.d.	n.d.
Dihydromyricetin 3-*O*-rhamnoside isomer b	< LOQ	20.11 ± 1.11^{a}	< LOQ	16.81 ± 1.43^{b}	n.d.
Dihydromyricetin	n.d.	< LOQ	n.d.	n.d.	n.d.
Apigenin 6,8-di-*C*-glucoside (vicenin 2)	33.89 ± 1.39	n.d.	n.d.	n.d.	n.d.
Naringenin 7-*O*-glucoside	n.d.	6.82 ± 0.45^{b}	n.d.	8.77 ± 1.01^{a}	n.d.
Luteolin-8-glucoside (orientin) isomer a	8.85 ± 1.20	n.d.	< LOQ	n.d.	n.d.
Luteolin-8-glucoside (orientin) isomer b	18.98 ± 1.79^{a}	n.d.	11.55 ± 1.12^{b}	n.d.	n.d.
Apigenin 8-*C*-glucoside (vitexin) isomer a	34.29 ± 1.71	n.d.	< LOQ	n.d.	n.d.
Vitexin 2″-*O*-rhamnoside	5.59 ± 0.05^{c}	17.90 ± 1.05^{b}	n.d.	35.17 ± 6.45^{a}	< LOQ
Isorhamnetin-*O*-malonylhexoside isomer a	4.59 ± 0.12^{b}	n.d.	7.31 ± 0.98^{a}	n.d.	n.d.
Isorhamnetin-*O*-malonylhexoside isomer b	< LOQ	n.d.	n.d.	n.d.	n.d.
Phlorizin isomer a	n.d.	n.d.	9.71 ± 0.09	n.d.	n.d.
Dihydroxy-dimethoxyisoflavanone	5.83 ± 0.12^{a}	n.d.	4.06 ± 0.51^{c}	4.78 ± 0.14^{b}	n.d.
Apigenin 8-*C*-glucoside (vitexin) isomer b	42.44 ± 3.85^{a}	n.d.	9.81 ± 1.62^{b}	0.72 ± 0.25^{c}	n.d.
Quercetin 3-*O*-rutinoside isomer a	16.53 ± 1.56^{c}	31.11 ± 1.45^{b}	59.20 ± 5.35^{a}	67.33 ± 7.21^{a}	30.23 ± 3.66^{b}
Quercetin 3-*O*-glucoside isomer a (hyperoside)	110.42 ± 9.61^{b}	53.24 ± 1.37^{c}	116.07 ± 10.27^{b}	287.85 ± 39.47^{a}	55.45 ± 8.03^{c}
Quercetin 3-*O*-rutinoside isomer b	n.d.	n.d.	25.68 ± 2.48^{b}	n.d.	118.63 ± 4.65^{a}
Quercetin 3-*O*-glucoside isomer b (isoquercitrin)	42.92 ± 3.76^{c}	214.50 ± 3.21^{a}	113.16 ± 9.79^{b}	188.61 ± 26.96^{a}	3.30 ± 2.19^{d}
Phlorizin isomer b	3.33 ± 0.04	< LOQ	n.d.	n.d.	n.d.
Quercetin 3-alpha-L-arabinofuranoside (avicularin) isomer a	4.69 ± 0.06^{b}	3.87 ± 0.02^{c}	3.41 ± 0.05^{c}	8.26 ± 0.42^{a}	n.d.
Kaempferol-3-*O*-glucoside (astragalin)	<LOQ	12.04 ± 0.05^{a}	8.06 ± 0.10^{b}	11.07 ± 0.91^{a}	n.d.
Quercetin 3-alpha-L-arabinofuranoside (avicularin) isomer b	4.69 ± 0.07^{c}	9.09 ± 0.79^{b}	8.52 ± 1.05^{b}	25.77 ± 1.88^{a}	n.d.
Quercetin 3-*O*-(6″-acetyl-glucoside) isomer a	n.d.	6.64 ± 0.08^{b}	n.d.	12.85 ± 0.39^{a}	n.d.
Isorhamnetin 3-rutinoside isomer a	n.d.	n.d.	8.23 ± 0.12	n.d.	n.d.
Quercetin 3-*O*-(6″-acetyl-glucoside) isomer b	n.d.	50.73 ± 2.27^{c}	67.91 ± 0.54^{b}	121.98 ± 8.00^{a}	n.d.
Isorhamnetin-3-*O*-glucoside isomer a	<LOQ	8.42 ± 0.12^{a}	3.88 ± 0.07^{b}	n.d.	n.d.
Isorhamnetin 3-rutinoside isomer b	n.d.	n.d.	3.91 ± 0.14	n.d.	n.d.
Isorhamnetin-3-*O*-glucoside isomer b	n.d.	10.23 ± 0.67^{a}	7.04 ± 0.91^{b}	n.d.	n.d.
Quercetin 3-*O*-(6″-acetyl-glucoside) isomer c	n.d.	n.d.	< LOQ	1.45 ± 0.23	n.d.
Quercetin 3-*O*-(6″-acetyl-glucoside) isomer d	n.d.	1.80 ± 0.01^{b}	< LOQ	5.58 ± 0.86^{a}	n.d.
Kaempferol 3-*O*-acetyl-glucoside	n.d.	< LOQ	4.04 ± 0.85	< LOQ	n.d.
Sum of phenolic acids	911.13 ± 50.08^{c}	547.77 ± 64.32^{d}	603.63 ± 23.61^{d}	1542.83 ± 130.64^{b}	1754.60 ± 31.19^{a}
Sum of flavonoids†	347.99 ± 28.83^{c}	501.90 ± 34.17^{b}	518.86 ± 39.06^{b}	860.60 ± 100.18^{a}	243.30 ± 20.68^{c}
Sum of phenolic compounds	1259.12 ± 78.91^{c}	1049.67 ± 98.49^{c}	1122.49 ± 62.66^{c}	2403.44 ± 230.82^{a}	1997.90 ± 51.87^{b}

Different letters (a–d) in the same line indicate significant differences ($P < 0.05$).
† Flavan-3-ols and derivatives were not taken into account for the quantification. n.d.: not detected; LOQ: limit of quantification.

obtained in the study carried out by Gu and *et al.*,[79] in which a total procyanidin content of 270 μg g^{-1} fresh weight was found in blackberries, 302 μg g^{-1} in raspberries, 81 μg g^{-1} in cherries, 1450 μg g^{-1} in strawberries, and around 920 μg g^{-1} in apples. The disparity between the different fruits may also be due, in addition to natural variability, to analytical differences or to the

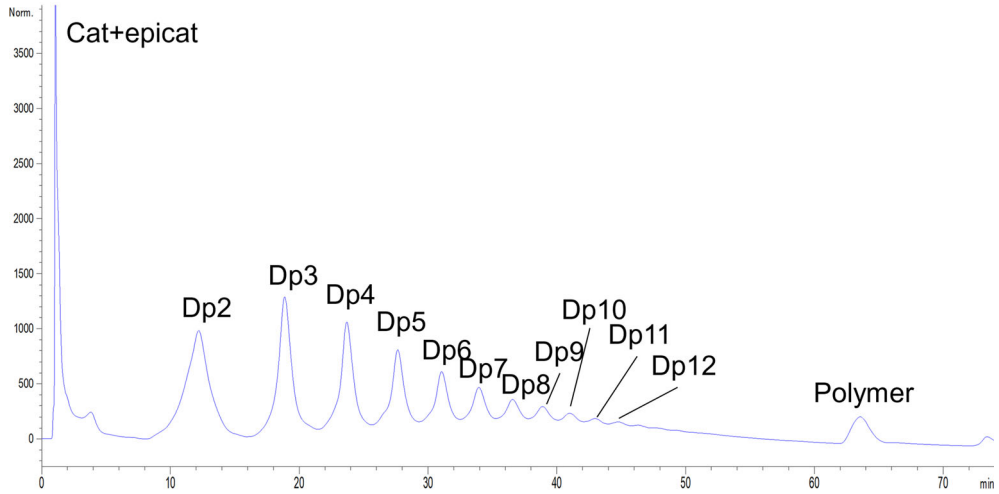

Figure 1. Chromatogram of the procyanidin profile of the wild fruits (Rosaceae family)). Cat+epicat: Catechin + epicatechin; Dp: degree of polymerization.

growing and harvesting conditions of each case. In our case, the values obtained for the total procyanidin content are in the same range of magnitude, ranging from 61 to 9970 μg g^{-1} of fresh fruit.

The degree of polymerization affects the bioavailability and bioactivity of procyanidins; therefore, not only the total content but also the polymers distribution should be taken into account to assess the potential health effects of foods rich in these compounds.[80] Low molecular weight oligomers can be absorbed intact in the gastrointestinal tract; however, the permeability of higher molecular weight polymers is lower, although recent studies have shown that the polymers can be metabolized by the intestinal microbiota.[79] There is a wide variation in the size distribution of the procyanidins among the fruit analyzed. In general, the smallest oligomers, those ranging from monomers to tetramers, are the predominant in all *Crataegus* fruits samples, constituting in some cases more than 80% of the total procyanidin content. Cui *et al.* reported yields of procyanidin monomer, dimer, trimer, tetramer, and pentamer of around 50.5%, 30.3%, 23.0%, 14.6%, and 12.5% respectively in *C. pinnatifida* fruit.[81] In our case, the *Crataegus* spp. fruits analyzed were in the range 24.8–47.8%, 10.7–28.4%, 4.9–19.2%, 2.9–10.8%, 1.5–6.5%, and 0.4–3.5% for the monomer, dimer, trimer, tetramer, and pentamer respectively (Supporting Information Fig. S2). In addition, other polymers were quantified in minor amounts, and the polymers that accounted from 2.5 to 31.3% of the total procyanidins was identified. The *S. domestica* fruit flavan-3-ols profile was the most different among the samples analyzed. It had the highest amount of polymers and the lowest of the rest of the oligomers detected. This confirms the astringency of this fruit due to the very high content of these polymers (condensed tannins).

Evaluation of the antioxidant activity

Table 4 shows the results (expressed as mean plus/minus standard deviation) of the determination of antioxidant capacity using

Table 3. Content of flavan-3-ols in fruits studied (Rosaceae family) expressed as micrograms catechin equivalents/gram dry weight with the average plus/minus standard deviation

	Crataegus azarolus	*Crataegus granatensis*	*Crataegus laciniata*	*Crataegus monogyna*	*Sorbus domestica*
Monomers	1204.32 ± 1.84^{d}	4122.91 ± 6.17^{c}	5 272.29 ± 7.90^{a}	4457.50 ± 6.74^{b}	135.01 ± 0.13^{e}
Dimers	269.49 ± 1.03^{d}	2583.39 ± 9.84^{c}	3 886.97 ± 14.80^{b}	5112.72 ± 19.47^{a}	<LOQ
Dp3	122.95 ± 0.39^{d}	1556.59 ± 4.95^{c}	3 017.55 ± 9.59^{b}	3450.84 ± 10.96^{a}	<LOQ
Dp4	72.21 ± 0.33^{d}	805.81 ± 3.64^{c}	1 839.28 ± 8.32^{b}	1951.44 ± 8.82^{a}	<LOQ
Dp5	37.62 ± 0.19^{d}	453.28 ± 2.32^{c}	1 135.80 ± 5.82^{a}	1051.91 ± 5.39^{b}	n.d.
Dp6	9.03 ± 0.08^{d}	176.90 ± 1.53^{c}	616.08 ± 5.32^{a}	541.85 ± 4.68^{b}	n.d.
Dp7	16.35 ± 0.07^{d}	225.20 ± 0.98^{c}	559.51 ± 2.42^{a}	474.51 ± 2.06^{b}	n.d.
Dp8	<LOQ	135.48 ± 0.88^{c}	277.37 ± 1.81^{a}	271.49 ± 1.77^{b}	n.d.
Dp9	<LOQ	71.96 ± 0.90^{c}	126.14 ± 1.59^{a}	96.28 ± 1.21^{b}	n.d.
Dp10	<LOQ	38.65 ± 0.21^{c}	118.03 ± 0.66^{a}	89.26 ± 0.50^{b}	n.d.
Dp11	n.d.	n.d.	62.21 ± 0.54^{a}	50.21 ± 0.43^{b}	n.d.
Dp12	n.d.	n.d.	69.39 ± 0.39^{a}	n.d.	n.d.
Polymers	787.31 ± 6.80^{b}	341.01 ± 2.95^{e}	491.25 ± 4.25^{c}	441.76 ± 3.82^{d}	11 228.14 ± 9.70^{a}
Sum of procyanidins	2519.30 ± 10.73^{e}	10 511.17 ± 31.42^{d}	17 471.85 ± 63.41^{b}	17 989.77 ± 65.85^{a}	11 363.15 ± 9.83^{c}

Different letters (a–e) in the same line indicate significant differences ($P < 0.05$). Dp: degree of polymerization; n.d.: not detected; LOQ: limit of quantification.

Table 4. Antioxidant activity of Rosaceae fruits evaluated by different antioxidant assays with the average plus/minus standard deviation

Sample	DPPH (mg TE/g dw)	ABTS (mg TE/g dw)	FRAP (mg TE/g dw)
Crataegus azarolus	5.29 ± 0.08^{e}	14.00 ± 0.02^{d}	18.73 ± 0.48^{c}
Crataegus granatensis	25.19 ± 0.44^{d}	41.02 ± 0.08^{c}	59.89 ± 0.64^{b}
Crataegus laciniata	31.20 ± 0.38^{a}	57.36 ± 0.38^{a}	71.8 ± 0.39^{a}
Crataegus monogyna	29.64 ± 0.76^{b}	56.49 ± 2.17^{a}	70.78 ± 1.78^{a}
Sorbus domestica	27.59 ± 0.72^{c}	50.03 ± 1.74^{b}	63.11 ± 1.81^{b}

Different letters (a-d) for the same method indicate significant differences ($P < 0.05$). DPPH, 2,2-diphenyl-1-picrylhydrazy; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); FRAP, ferric reducing antioxidant power; TE, Trolox equivalent; dw, dry weight.

the DPPH, ABTS, and FRAP techniques in the five fruits analyzed. The results ranged 5.29–31.20 mg TE/g dw, 14.00–57.36 mg TE/g dw, and 18.73–71.8 mg TE/g dw for the DPPH, ABTS, and FRAP assays respectively. The three methods had a high significant correlation at $P < 0.05$ ($r = 0.9853$–0.9984; Supporting Information Fig. S3). As can be seen, between all the fruits evaluated, *C. laciniata* had the highest antioxidant activity, followed by *C. monogyna*. After these were *S. domestica* and *C. granatensis*, with *C. azarolus* fruit exhibiting the lowest antioxidant activity.

Among the fruits studied, *C. monogyna* and *C. azarolus* were previously studied by others, whereas, to our knowledge, no previous research has been done in *C. granatensis* and *C. laciniata*. These species occupy large areas in forested upland areas, and where they occur they are locally abundant, forming their own plant community (hawthorn formations). They also bear fruit abundantly, and the fruits are easily harvested, so that harvesting can be economically profitable. Given its proven high antioxidant capacity, we believe they could be used as a nutraceutical in the fortification of other food products. Belkhir *et al.*[82] evaluated the antioxidant activity by the FRAP method and revealed a higher antioxidant activity in *C. azarolus* than in *C. monogyna*. In addition, in posterior *in vitro* biomolecular and cellular models, Belkhir *et al.*[69] found that *C. monogyna* had a higher protective effect against oxidative damage than *C. monogyna* did. In contrast, Mraihi *et al.*[83] found a lower IC50 by DPPH in *C. azarolus* than in *C. monogyna*, but the opposite by the FRAP method. Alirezalu *et al.*[70] studied the antioxidant activity of eight different *Crataegus* fruits, finding that *C. monogyna* had stronger antioxidant activity than *C. azarolus*. The differences in tendency between studies could be attributed to the differences in growth location and harvesting data. Thus, in this study, it was *C. monogyna* that stood out the most between those two fruits regarding their antioxidant activity by the three measurment methods.

Other researchers also studied the antioxidant activity in other *Crataegus* species, showing their potential and differences in their antioxidant activity, comparing different extracting methods,[84] drying techniques,[85] different harvesting dates,[86] and different species.[87] Those studies corroborate the huge diversity and differences in antioxidant activities between different species of *Crataegus* fruits, as showed in this study.

Moreover, other workers compared different *Crataegus* and *Sorbus* fruits. Egea *et al.*[65] compared the antioxidant activity of *C. monogyna*, *C. azarolus*, and *S. domestica* fruits, among others, and reported that *S. domestica* had the lowest radical scavenging activity among them. Our results are in agreement, as among those three mentioned fruits it was *C. monogyna* that had the highest antioxidant activity, but *S. domestica* antioxidant activity was higher than in *C. azarolus*. Sagdic *et al.*[88] also compared *Crataegus* spp. and *S. domestica* fruits' antioxidant activity, finding that the former had between 8 and 94% higher antioxidant activity than the latter. In our case, *S. domestica* had significantly higher antioxidant activity than *C. azarolus* and *C. granatensis* by the DPPH and ABTS assays.

Focusing only in *Sorbus* fruits, there also are some researchers who reported some results comparing different maturity stages,[89] different parts of the fruit,[90] different genotypes,[67,71] different harvesting dates,[91] and different locations,[92,93] all reporting values of the same range of magnitude as the results reported here.

Comparing with other fruits, the fruits of the five Rosaceae species studied here have an antioxidant activity that is in the same range of magnitude as apple, apricot, blackberry, red grape, nectarine, peach, red plum, pomegranate, or strawberry.[94]

The correlation between the phenolic composition and the antioxidant activity was evaluated by Pearson correlation (Supporting Information Fig. S3). In general, the antioxidant activity presented a strong positive correlation to the total phenolic content (0.5180–0.6003) and, specially, to the total flavonoid content (0.5179–0.5650). However, a weak positive correlation was found to the total phenolic acids (0.1737–0.2577). Among them, total chlorogenic acid (0.3398–0.4210) and total 3-*p*-coumaroylquinic acid (0.3447–0.3920) had a moderate positive correlation. Among flavonoids, the total procyanidin content (flavan-3-ols) measured using HPLC–FLD presented a strong positive correlation with antioxidant activity (0.6867–0.7298). Apart from them, the sum of quercetin derivatives exhibited the strongest positive correlation with the antioxidant activity (0.8691–0.9077). In addition, total rutin (0.5703–0.6345) and total kaempferol derivatives (0.5897–0.6579) had a strong positive correlation, and total isorhamnetin 3-rutinoside (0.3812–0.4244), total dihydromyricetin 3-*O*-rhamnoside (0.3827–0.4925), and isoquercitrin (0.3209–0.4212) had a moderate positive influence. In contrast, some compounds had a strong negative correlation with the antioxidant activity, such as total vitexin (0.8968–0.9494), total apigenin derivatives (0.8952–0.9511), total luteolin-8-glucoside (orientin; 0.7683–0.8397), and vomifoliol 9-[xylosyl-(1→6)-glucoside] (0.9308–0.9770). Moreover, total isorhamnetin-*O*-malonylhexoside (0.2610–0.3299) and persicogenin (0.2929–0.4263) presented a moderate negative correlation.

Clustering analysis

To provide an intuitive visualization of all the data obtained for the fruits studied, a hierarchical heatmap was performed (Fig. 2). Briefly, data were normalized, the distance was the Pearson statistical measure, and the selected clustering method was the average. Each colored cell on the map corresponds to a concentration value normalized from +1 (intense red) to −1

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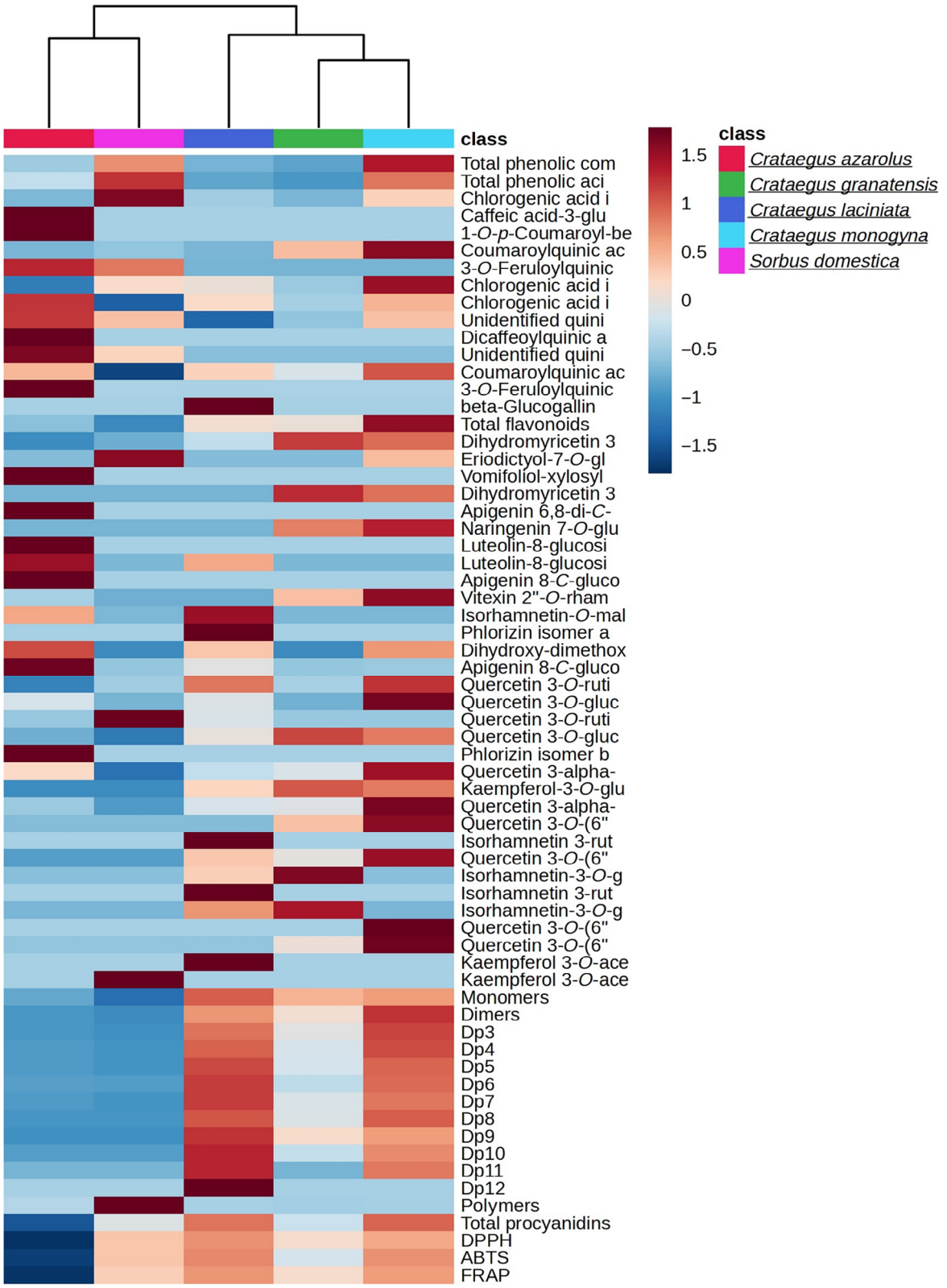

Figure 2. Clustering heatmap of the fruits studied for the features measured.

(intense blue), with the features (phenolic compounds, flavan-3-ols, and antioxidant activities) in rows and samples in columns. Each sample has an associated color. As can be seen from the figure, there is a clustered group composed of *C. monogyna* and *C. granatensis*, linked in a further way with *C. laciniata*. Moreover, another group can be appreciated formed by *C. azarolus* and *S. domestica*. Thus, some compounds could be mentioned as possible markers for each group. The compounds that liked *C. azarolus*

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and *S. domestica* together seemed to be 3-*O*-feruloylquinic acid isomer a and unidentified quinic acid derivative isomer b. In contrast, *C. granatensis* and *C. monogyna* were clustered by the compounds coumaroyl, quinic acid isomer a, dihydromyricetin 3-*O*-rhamnoside isomers a and b, naringenin-7-*O*-glucoside, and vitexin 2″-*O*-rhamnoside. These two fruits were then clustered to *C. laciniata* according to the sum of flavonoids, and the procyanidins from monomer to degree of polymerization 9. Moreover, this was confirmed by a least-square discriminant analysis, presented in Supporting Information Fig. S4.

CONCLUSIONS

The fruits of the Rosaceae family analyzed in this work have been demonstrated to be a high source of phenolic compounds, especially flavan-3-ols, which directly correlates with their antioxidant activity. A total of 71 phenolic compounds were identified using HPLC–ESI-TOF-MS; and as far as we are concerned, 30 of them have been identified in *Crataegus* or *Sorbus* berries for the first time. *C. monogyna* revealed the highest total phenolic content and *C. azarolus* the lowest one. The highest content of phenolic acids was found in *S. domestica*, and the highest concentration of flavonoids was seen in *C. monogyna*. The major phenolic compounds found were chlorogenic acid, rutin, hyperoside, and isoquercitrin, among others, which demonstrated to have a moderate or strong correlation with the antioxidant activity. The content of flavan-3-ols of the samples measured using HPLC–FLD was highest in *C. monogyna* fruit and the lowest in *C. azarolus*. Similarly, regarding the antioxidant activity measured using DPPH, ABTS, and FRAP assays, *C. laciniata* exhibited highest activity, followed by *C. monogyna*, and the lowest was *C. azarolus*. To sum up, *C. monogyna* and *C. laciniata* have demonstrated to be the most promising fruits according to the obtained results. In general, the phenolic and procyanidin contents, as well as the antioxidant activity, in all the fruits analyzed are as high as many fruits marketed nowadays in Spain, so this aspect can be an important starting point for their valorization as snacks or to produce fruit-based products. However, additional studies are necessary to evaluate the influence of the different production areas on the content of bioactive compounds.

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DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Judd WS, Campbell CS, Kellogg EA, Stevens PF and Donoghue MJ, *Plant Systematics: A Phylogenetic Approach*. Sinauer Associates, Sunderland, MA, USA (2002).
2 Hummer KE and Janick J, Rosaceae: taxonomy, economic importance, genomics, in *Genetetics and Genomics of Rosaceae*, ed. by Folta KM and Gardiner SE. Springer, New York, NY, USA, pp. 1–17 (2009).
3 Kallman C, Roseaceae, in *Flowering Plants. Dicotyledons: Celastrales, Oxalidales, Rosales, Cornales, Ericales*, ed. by Kubitzki K. Springer, Berlin, Germany, pp. 343–386 (2004).
4 Hegnauer R, Rosaceae, in *Chemotaxonomie der Pflanzen: Eine Übersicht über die Verbreitung und die systematische Bedeutung der Pflanzenstoffe*. Birkhäuser, Basel, Switzerland, pp. 84–130 (1973).
5 Hamzeh'ee B, Attar F, Assareh MH, Maassoumi AA, Kazempour Osaloo S and Christensen KI, Taxonomic notes on *Crataegus*, ser. *Crataegus*, subser. *Erianthae* (Rosaceae), new species and record, using morphology and micromorphological evidence. *Nord J Bot* **32**:26–37 (2014).
6 Benítez G, Molero-Mesa J and González-Tejero MR, Gathering an edible wild plant: food or medicine? A case study on wild edibles and functional foods in Granada, Spain. *Acta Soc Bot Pol* **86**:3550 (2017).
7 Redzic SJ, Wild edible plants and their traditional use in the human nutrition in Bosnia-Herzegovina. *Ecol Food Nutr* **45**:189–232 (2007).
8 Martinelli F, Perrone A, Yousefi S, Papini A, Castiglione S, Guarino F *et al.*, Botanical, phytochemical, anti-microbial and pharmaceutical characteristics of hawthorn (*Crataegus monogyna* Jacq.), Rosaceae. *Molecules* **26**:7266 (2021).
9 Łuczaj Ł and Łuczaj Ł, Ethnobotanical review of wild edible plants of Slovakia. *Acta Soc Bot Pol* **81**:245–255 (2012).
10 Sołtys A, Galanty A and Podolak I, Ethnopharmacologically important but underestimated genus *Sorbus*: a comprehensive review. *Phyochem Rev* **19**:491–526 (2020).
11 Christensen KI, *Revision of Crataegus sect. Crataegus and Nothosect. Crataeguineae (Rosaceae-Maloideae) in the Old World*. Systematic Botany Monographs, Vol. **35**. American Society of Plant Taxonomists, Laramie, WY, USA (1992).
12 Muñoz-Garmendia F, Navarro C and Aedo C, *Crataegus* L., in *Flora Iberica: Plantas Vasculares de la Península Ibérica e Islas Baleares Rosaceae*, ed. by Catroviejo S, Muñoz-Garmendia F and Navarro C. Real Jardín Botánico, Madrid, Spain, pp. 404–414 (1998).
13 Abuashwashi MA, Palomino OM and Gómez-Serranillos MP, Geographic origin influences the phenolic composition and antioxidant potential of wild *Crataegus monogyna* from Spain. *Pharm Biol* **54**:2708–2713 (2016).
14 Council of Europe, *European Pharmacopoeia*. Council of Europe, Strasbourg, France (2001).
15 Aedo C and Aldasoro J, *Sorbus* L., in *Flora Iberica: Plantas Vasculares de la Península Ibérica e Islas Baleares Rosaceae*, ed. by Catroviejo S, Muñoz-Garmendia F and Navarro C. Real Jardín Botánico, Madrid, Spain, pp. 414–429 (1998).
16 Küpeli Akkol E, Gürağaç Dereli FT, Taştan H, Sobarzo-Sánchez E and Khan H, Effect of *Sorbus domestica* and its active constituents in an experimental model of colitis rats induced by acetic acid. *J Ethnopharmacol* **251**:112521 (2020).
17 Wahabi S, Rtibi K, Atouani A and Sebai H, Anti-obesity actions of two separated aqueous extracts from arbutus (*Arbutus unedo*) and hawthorn (*Crataegus monogyna*) fruits against high-fat diet in rats via potent antioxidant target. *Dose-Response* **21**:1–14 (2023). https://doi.org/10.1177/15593258231179904.
18 Lu M, Zhang L, Pan J, Shi H, Zhang M and Li C, Advances in the study of the vascular protective effects and molecular mechanisms of hawthorn (*Crataegus anamesa* Sarg.) extracts in cardiovascular diseases. *Food Funct* **14**:5870–5890 (2023).
19 Basavarajappa GM, Rehman A, Shiroorkar PN, Sreeharsha N, Anwer MK and Aloufi B, Therapeutic effects of *Crataegus monogyna* inhibitors against breast cancer. *Front Pharmacol* **14**:1187079 (2023).
20 Yang B and Liu P, Composition and health effects of phenolic compounds in hawthorn (*Crataegus* spp.) of different origins. *J Sci Food Agric* **92**:1578–1590 (2012).
21 Lyu Q, Zheng W, Shan Q, Huang L, Wang Y, Wang L *et al.*, Expanding annotation of chemical compounds in hawthorn fruits and their variations in thermal processing using integrated mass spectral similarity networking. *Food Res Int* **172**:113114 (2023).
22 Tian H, Xiong J, Yu H, Chen C, Xu H and Lou X, Characterize the physicochemical properties and microstructure of pectin from

high-pressure and thermal processed cloudy hawthorn (*Crataegus pinnatifida*) juice based on acid heating extraction. *Food Chem* **407**:135199 (2023).
23 Wang Y, Hao R, Guo R, Nong H, Qin Y and Dong N, Integrative analysis of metabolome and transcriptome reveals molecular insight into metabolomic variations during hawthorn fruit development. *Metabolites* **13**:423 (2023).
24 Angelica L and Hoffm AL, Plants of the World Online | Kew Science. 2017 Available: https://powo.science.kew.org/ [15 July 2022].
25 Razola-Díaz MC, Guerra-Hernández EJ, Rodríguez-Pérez C, Gómez-Caravaca AM, García-Villanova B and Verardo V, Optimization of ultrasound-assisted extraction via sonotrode of phenolic compounds from orange by-products. *Foods* **10**:1120 (2021).
26 Verni M, Pontonio E, Krona A, Jacob S, Pinto D, Rinaldi F *et al*., Bioprocessing of brewers' spent grain enhances its antioxidant activity: characterization of phenolic compounds and bioactive peptides. *Front Microbiol* **11**:1831 (2020).
27 López-Cobo A, Gómez-Caravaca AM, Pasini F, Caboni MF, Segura-Carretero A and Fernández-Gutiérrez A, HPLC–DAD-ESI-QTOF-MS and HPLC–FLD–MS as valuable tools for the determination of phenolic and other polar compounds in the edible part and by-products of avocado. *LWT* **73**:505–513 (2016).
28 Robbins RJ, Leonczak J, Johnson JC, Li J, Kwik-Uribe C, Prior RL *et al*., Method performance and multi-laboratory assessment of a normal phase high pressure liquid chromatography–fluorescence detection method for the quantitation of flavanols and procyanidins in cocoa and chocolate containing samples. *J Chromatogr A* **1216**:4831–4840 (2009).
29 Brand-Williams W, Cuvelier ME and Berset C, Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci Technol* **28**: 25–30 (1995).
30 Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C, Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* **26**:1231–1237 (1999).
31 Benzie IF and Strain JJ, The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. *Anal Biochem* **239**: 70–76 (1996).
32 Żurek N, Karatsai O, Rędowicz MJ and Kapusta IT, Polyphenolic compounds of *Crataegus* berry, leaf, and flower extracts affect viability and invasive potential of human glioblastoma cells. *Molecules* **26**: 2656 (2021).
33 Forino M, Tenore GC, Tartaglione L, Carmela D, Novellino E and Ciminiello P, (1*S*,3*R*,4*S*,5*R*)5-*O*-Caffeoylquinic acid: isolation, stereostructure characterization and biological activity. *Food Chem* **178**: 306–310 (2015).
34 Parejo I, Jauregui O, Sánchez-Rabaneda F, Viladomat F, Bastida J and Codina C, Separation and characterization of phenolic compounds in fennel (*Foeniculum vulgare*) using liquid chromatography–negative electrospray ionization tandem mass spectrometry. *J Agric Food Chem* **52**:3679–3687 (2004).
35 Neveu V, Perez-Jiménez J, Vos F, Crespy V, du Chaffaut L, Mennen L *et al*., Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. *Database (Oxford)* **2010**:bap024 (2010).
36 Rothwell JA, Perez-Jimenez J, Neveu V, Medina-Remón A, M'hiri N, García-Lobato P *et al*., Phenol-Explorer 3.0: a major update of the Phenol-Explorer database to incorporate data on the effects of food processing on polyphenol content. *Database (Oxford)* **2013**:bat070 (2013).
37 Bekbolatova E, Kukula-Koch W, Baj T, Stasiak N, Ibadullayeva G, Koch W *et al*., Phenolic composition and antioxidant potential of different organs of Kazakh *Crataegus almaatensis* Pojark: a comparison with the European *Crataegus oxyacantha* L. flowers. *Open Chem* **16**: 415–426 (2018).
38 Bujor A, Miron A, Luca SV, Skalicka-Wozniak K, Silion M, Trifan A *et al*., Vasorelaxant effects of *Crataegus pentagyna*: links with arginase inhibition and phenolic profile. *J Ethnopharmacol* **252**:112559 (2020).
39 Barros L, Dueñas M, Carvalho AM, Ferreira ICFR and Santos-Buelga C, Characterization of phenolic compounds in flowers of wild medicinal plants from northeastern Portugal. *Food Chem Toxicol* **50**:1576–1582 (2012).
40 Lim DW, Han T, Jung J, Song Y, Um MY, Yoon M *et al*., Chlorogenic acid from hawthorn berry (*Crataegus pinnatifida* fruit) prevents stress hormone-induced depressive behavior, through monoamine oxidase B-reactive oxygen species signaling in hippocampal astrocytes of mice. *Mol Nutr Food Res* **62**:1800029 (2018).
41 Allen F, Greiner R and Wishart D, Competitive fragmentation modeling of ESI-MS/MS spectra for putative metabolite identification. *Metabolomics* **11**:98–110 (2015).
42 Puppala M, Ponder J, Suryanarayana P, Reddy GB, Petrash M and LaBarbera DV, The isolation and characterization of *β*-glucogallin as a novel aldose reductase inhibitor from *Emblica officinalis*. *PloS One* **7**:e31399 (2012).
43 Wang F, Liigand J, Tian S, Arndt D, Greiner R and Wishart DS, CFM-ID 4.0: more accurate ESI-MS/MS spectral prediction and compound identification. *Anal Chem* **93**:11692–11700 (2021).
44 Liu D, Mao Y, Ding L and Zeng XA, Dihydromyricetin: a review on identification and quantification methods, biological activities, chemical stability, metabolism and approaches to enhance its bioavailability. *Trends Food Sci Technol* **91**:586–597 (2019).
45 Han F, Guo Y, Gu H, Li F, Hu B and Yang L, Application of alkyl polyglycoside surfactant in ultrasonic-assisted extraction followed by macroporous resin enrichment for the separation of vitexin-2″-*O*-rhamnoside and vitexin from *Crataegus pinnatifida* leaves. *J Chromatogr B Anal Technol Biomed Life Sci* **1012–1013**:69–78 (2016).
46 Lam KY, Ling APK, Koh RY, Wong YP and Say YH, A review on medicinal properties of orientin. *Adv Pharmacol Sci* **2016**:4104595 (2016).
47 Aron PM and Kennedy JA, Flavan-3-ols: nature, occurrence and biological activity. *Mol Nutr Food Res* **52**:79–104 (2008).
48 Engelhardt UH, 3.23–Chemistry of tea, in *Comprehensive Natural Products II*, ed. by Liu H-W(B) and Mander L. Elsevier, Oxford, UK, pp. 999–1032 (2010).
49 Ehrenkranz JRL, Lewis NG, Kahn CR and Roth J, Phlorizin: A review. *Diabetes Metab Res Rev* **21**:31–38 (2005).
50 Wang C, Liang H, Guo J, Huang X, Liu X and Wang J, Studies on chemical constituents from leaves of *Uraria lacei*. *China J Chin Mater Med* **36**:2676–2679 (2011).
51 Zhao M, Duan JA and Che CT, Isoflavanones and their *O*-glycosides from *Desmodium styracifolium*. *Phytochemistry* **68**:1471–1479 (2007).
52 Fujimori K and Shibano M, Avicularin, a plant flavonoid, suppresses lipid accumulation through repression of C/EBP*α*-activated GLUT4-mediated glucose uptake in 3T3-L1 cells. *J Agric Food Chem* **61**:5139–5147 (2013).
53 Tsao R, Yang R, Young JC and Zhu H, Polyphenolic profiles in eight apple cultivars using high-performance liquid chromatography (HPLC). *J Agric Food Chem* **51**:6347–6353 (2003).
54 Riaz A, Rasul A, Hussain G, Zahoor MK, Jabeen F, Subhani Z *et al*., Astragalin: a bioactive phytochemical with potential therapeutic activities. *Adv Pharmacol Sci* **2018**:9794625 (2018).
55 Zheng W and Wang SY, Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. *J Agric Food Chem* **51**:502–509 (2003).
56 Zafrilla P, Ferreres F and Tomás-Barberán FA, Effect of processing and storage on the antioxidant ellagic acid derivatives and flavonoids of red raspberry (*Rubus idaeus*) jams. *J Agric Food Chem* **49**:3651–3655 (2001).
57 Wang SY, Zheng W and Galletta GJ, Cultural system affects fruit quality and antioxidant capacity in strawberries. *J Agric Food Chem* **50**: 6534–6542 (2002).
58 Cantos E, Espín JC and Tomás-Barberán FA, Varietal differences among the polyphenol profiles of seven table grape cultivars studied by LC–DAD-MS–MS. *J Agric Food Chem* **50**:5691–5696 (2002).
59 Proteggente AR, Pannala AS, Paganga G, Van Buren L, Wagner E, Wiseman S *et al*., The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radic Res* **36**:217–233 (2002).
60 Yannai S, *Dictionary of Food Compounds with CD-ROM: Additives, Flavors, and Ingredients*. Chapman & Hall/CRC, Boca Raton, FL, USA (2004).
61 Ressaissi A, Attia N, Falé PL, Pacheco R, Victor BL, Machuqueiro M *et al*., Isorhamnetin derivatives and piscidic acid for hypercholesterolemia: cholesterol permeability, HMG-CoA reductase inhibition, and docking studies. *Arch Pharm Res* **40**:1278–1286 (2017).
62 Sawada Y, Nakabayashi R, Yamada Y, Suzuki M, Sato M, Sakata A *et al*., RIKEN tandem mass spectral database (ReSpect) for phytochemicals: a plant-specific MS/MS-based data resource and database. *Phytochemistry* **82**:38–45 (2012).

63 Zocchi E, Hontecillas R, Leber A, Einerhand A, Carbo A, Bruzzone S *et al.*, Abscisic acid: a novel nutraceutical for glycemic control. *Front Nutr* **4**: 24 (2017).
64 Barbieri C, Bignami C, Cristofori V, Paolocci M and Bertazza G, Characterization and exploitation of minor pome fruits in Italy. *Acta Hortic* **918**:953–959 (2011).
65 Egea I, Sánchez-Bel P, Romojaro F and Pretel MT, Six edible wild fruits as potential antioxidant additives or nutritional supplements. *Plant Foods Hum Nutr* **65**:121–129 (2010).
66 Matczak M, Marchelak A, Michel P, Owczarek A, Piszczan A, Kolodziejczyk-Czepas J *et al.*, *Sorbus domestica* L. leaf extracts as functional products: phytochemical profiling, cellular safety, pro-inflammatory enzymes inhibition and protective effects against oxidative stress *in vitro*. *J Funct Foods* **40**:207–218 (2018).
67 Olszewska MA and Michel P, Antioxidant activity of inflorescences, leaves and fruits of three sorbus species in relation to their polyphenolic composition. *Nat Prod Res* **23**:1507–1521 (2009).
68 Bernatoniene J, Masteikova R, Majiene D, Savickas A, Kevelaitis E, Bernatoniene R *et al.*, Free radical-scavenging activities of *Crataegus monogyna* extracts. *Medicina (Kaunas)* **44**:706–712 (2008).
69 Belkhir M, Dhaouadi K, Rosa A, Atzeri A, Nieddu M, Tuberoso CIG *et al.*, Protective effects of azarole polyphenolic extracts against oxidative damage using *in vitro* biomolecular and cellular models. *Ind Crops Prod* **86**:239–250 (2016).
70 Alirezalu A, Ahmadi N, Salehi P, Sonboli A, Alirezalu K, Khaneghah AM *et al.*, Physicochemical characterization, antioxidant activity, and phenolic compounds of hawthorn (*Crataegus* spp.) fruits species for potential use in food applications. *Foods* **9**:436 (2020).
71 Mikulic-Petkovsek M, Krska B, Kiprovski B and Veberic R, Bioactive components and antioxidant capacity of fruits from nine *Sorbus* genotypes. *J Food Sci* **82**:647–658 (2017).
72 Becerra-Herrera M, Lazzoi MR, Sayago A, Beltrán R, Del Sole R and Vasapollo G, Extraction and determination of phenolic compounds in the berries of *Sorbus americana* Marsh and *Lonicera oblongifolia* (Goldie) hook. *Food Anal Methods* **8**:2554–2559 (2015).
73 Gaivelyte K, Jakstas V, Razukas A and Janulis V, Variation in the contents of neochlorogenic acid, chlorogenic acid and three quercetin glycosides in leaves and fruits of rowan (*Sorbus*) species and varieties from collections in Lithuania. *Nat Prod Commun* **8**:1105–1110 (2013).
74 Šavikin KP, Zdunić GM, Krstić-Milošević DB, Šircelj HJ, Stešević DD and Pljevljakušić DS, *Sorbus aucuparia* and *Sorbus aria* as a source of antioxidant phenolics, tocopherols, and pigments. *Chem Biodivers* **14**: e1700329 (2017).
75 Liu P, Kallio H, Lü D, Zhou C and Yang B, Quantitative analysis of phenolic compounds in Chinese hawthorn (*Crataegus* spp.) fruits by high performance liquid chromatography–electrospray ionisation mass spectrometry. *Food Chem* **127**:1370–1377 (2011).
76 Liu P, Kallio H and Yang B, Phenolic compounds in hawthorn (*Crataegus grayana*) fruits and leaves and changes during fruit ripening. *J Agric Food Chem* **59**:11141–11149 (2011).
77 Rodrigues S, Calhelha RC, Barreira JCM, Dueñas M, Carvalho AM, Abreu RMV *et al.*, *Crataegus monogyna* buds and fruits phenolic extracts: growth inhibitory activity on human tumor cell lines and chemical characterization by HPLC–DAD-ESI/MS. *Food Res Int* **49**: 516–523 (2012).
78 Hellström JK, Törrönen AR and Mattila PH, Proanthocyanidins in common food products of plant origin. *J Agric Food Chem* **57**:7899–7906 (2009).
79 Gu L, Kelm MA, Hammerstone JF, Beecher G, Holden J, Haytowitz D *et al.*, Concentrations of proanthocyanidins in common foods and estimations of normal consumption. *J Nutr* **134**:613–617 (2004).
80 Schmidt BM, Howell AB, McEniry B, Knight CT, Seigler D, Erdman JW *et al.*, Effective separation of potent antiproliferation and antiadhesion components from wild blueberry (*Vaccinium angustifolium* Ait.) fruits. *J Agric Food Chem* **52**:6433–6442 (2004).
81 Cui T, Nakamura K, Tian S, Kayahara H and Tian YL, Polyphenolic content and physiological activities of Chinese hawthorn extracts. *Biosci Biotechnol Biochem* **70**:2948–2956 (2006).
82 Belkhir M, Rebai O, Dhaouadi K, Congiu F, Tuberoso CIG, Amri M *et al.*, Comparative analysis of Tunisian wild *Crataegus azarolus* (yellow azarole) and *Crataegus monogyna* (red azarole) leaf, fruit, and traditionally derived syrup: phenolic profiles and antioxidant and antimicrobial activities of the aqueous–acetone extracts. *J Agric Food Chem* **61**:9594–9601 (2013).
83 Mraihi F, Journi M, Chérif JK, Sokmen M, Sokmen A and Trabelsi-Ayadi M, Phenolic contents and antioxidant potential of *Crataegus* fruits grown in Tunisia as determined by DPPH, FRAP, and β-carotene/linoleic acid assay. *J Chem* **2013**:378264 (2013).
84 Liu JL, Yuan JF and Zhang ZQ, Microwave-assisted extraction optimised with response surface methodology and antioxidant activity of polyphenols from hawthorn (*Crataegus pinnatifida* Bge.) fruit. *Int J Food Sci Technol* **45**:2400–2406 (2010).
85 Coklar H, Akbulut M, Kilinc S, Yildirim A and Alhassan I, Effect of freeze, oven and microwave pretreated oven drying on color, browning index, phenolic compounds and antioxidant activity of hawthorn (*Crataegus orientalis*) fruit. *Not Bot Horti Agrobot Cluj-Napoca* **46**: 449–456 (2018).
86 Pavlovic J, Mitic S, Mitic M, Kocic G, Pavlovic A and Tosic S, Variation in the phenolic compounds profile and antioxidant activity in different parts of hawthorn (*Crataegus pentagyna* Willd.) during harvest periods. *Polish J Food Nutr Sci* **69**:367–378 (2019).
87 Bardakci H, Celep E, Gözet T, Kan Y and Kırmızıbekmez H, Phytochemical characterization and antioxidant activities of the fruit extracts of several *Crataegus* taxa. *South African J Bot* **124**:5–13 (2019).
88 Sagdic O, Polat B and Yetim H, Bioactivities of some wild fruits grown in Turkey. *Erwerbs-Obstbau* **64**:299–305 (2022).
89 Termentzi A, Kefalas P and Kokkalou E, Antioxidant activities of various extracts and fractions of *Sorbus domestica* fruits at different maturity stages. *Food Chem* **98**:599–608 (2006).
90 Majić B, Šola I, Likić S, Cindrić IJ and Rusak G, Characterisation of *Sorbus domestica* L. bark, fruits and seeds: nutrient composition and antioxidant activity. *Food Technol Biotechnol* **53**:463–471 (2015).
91 Wolf J, Göttingerová M, Kaplan J, Kiss T, Venuta R and Nečas T, Determination of the pomological and nutritional properties of selected plum cultivars and minor fruit species. *Hortic Sci* **47**:181–193 (2020).
92 Poljak I, Vahčić N, Liber Z, Tumpa K, Pintar V, Zegnal I *et al.*, Morphological and chemical diversity and antioxidant capacity of the service tree (*Sorbus domestica* L.) fruits from two eco-geographical regions. *Plants* **10**:1691 (2021).
93 Tamayo-Vives C, García-Herrera P, Sánchez-Mata MC, Cámara-Hurtado RM, Pérez-Rodríguez ML, Aceituno L *et al.*, Wild fruits of *Crataegus monogyna* Jacq. and *Sorbus aria* (L.) Crantz: from traditional foods to innovative sources of pigments and antioxidant ingredients for food products. *Foods* **12**:2427 (2023).
94 Ruiz-Torralba A, Guerra-Hernández EJ and García-Villanova B, Antioxidant capacity, polyphenol content and contribution to dietary intake of 52 fruits sold in Spain. *CYTA-J Food* **16**:1131–1138 (2018).

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