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ORIGINAL ARTICLE

Wild grown red and yellow hawthorn fruits from Tunisia as source of antioxidants

Farouk Mraihi [a,*], Maria Hidalgo [b], Sonia de Pascual-Teresa [b], Malika Trabelsi-Ayadi [a], Jamila-Kaltoum Chérif [a,c]

[a] *Laboratoire d'Application de la Chimie aux Ressources et Substances Naturelles et à l'Environnement (LACReSNE), Université de Carthage, Faculté des Sciences de Bizerte, 7021 Zarzouna, Tunisia*
[b] *Department of Nutrition and Metabolism, Institute of Food Science and Technology and Nutrition (ICTAN-CSIC), C/José Antonio Novais, 10, 28040 Madrid, Spain*
[c] *Institut Préparatoire aux Etudes d'Ingénieurs de Tunis (IPEIT), Rue Taha Huseein, 5, 1008 Tunis, Tunisia*

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KEYWORDS

Phenolic acids;
Flavonoids;
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HPLC–DAD–MS

Abstract Hawthorn fruits (*Crataegus* spp.), may be a good source of antioxidants if is consumed as fresh fruit since we know that it produce a numerous beneficial effects for human health. In this study, two species of hawthorn fruit, *Crataegus monogyna* and *Crataegus azarolus* were analyzed by HPLC–DAD–MS and compared with respect to their phytochemical composition. Phenolic profiles of studied fruits showed some similarities and differences in terms of polyphenols between the two species. Twenty phenolics compounds distributed into four subclasses were identified: four phenolic acids including three hydroxycinnamic acids and one hydroxybenzoic acid, eight flavonoids representing the most abundant subclass including six glucosylated flavonols and two flavones, two anthocyanins are present as glycosides of cyanidin, with cyanidin-3-*O*-glucoside is the most abundant, only in *monogyna* peel fraction and four flavanols divided into a monomer (−)-epicatechin identified in all fruit parts of both species, a dimer B2 and two trimers (C1 and C2). These phenolic compounds are concentrated especially in peel fraction. These results indicate that hawthorn fruits should be recommended in dietary habits as a potential source of antioxidant and anticarcinogenic phenolic compounds.

1. Introduction

The genus *Crataegus* (Hawthorn), belonging to the Rosaceae family, is a genus of spiny trees or shrubs present in the northern hemisphere (Verma et al., 2007). They are usually multibranched shrubby trees that can reach a height of up to 10 m. The color of the ripe fruit ranges from yellow, through

* Corresponding author. Tel.: +216 97 75 42 65, +216 22 91 00 70; fax: +216 71 60 72 70.
E-mail address: mraihi_farouk@yahoo.fr (F. Mraihi).
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green to red and on to dark purple. Most of the species ripen their fruit in early to mid-autumn (Brown, 1995).

Beneficial effects of hawthorn fruit extracts have been confirmed by various studies, pharmacological data show that hawthorn fruits and its preparations enhance myocardial contraction and conductivity, protect against ischemia (Veveris et al., 2004). They have a sedative action, a protective effect against arrhythmia and increase of coronary vessel flow (Zhang et al., 2001). They have also positive effects on the cardiovascular system (Caliskan et al., 2012). Recent studies have focused on the health benefits of hawthorn fruits such as antioxidant, antimicrobial, antiproliferative and mutagen properties (Froehlicher et al., 2009; Caliskan et al., 2012; Rodrigues et al., 2012 and Mraihi et al., 2013). These pharmacological properties are the consequence of the benefic effect of active phenolic compounds of hawthorn fruits that modulate a variety of biological events.

Polyphenols are secondary compounds widely distributed in the plant kingdom. They are divided into several classes, phenolic acids (hydroxybenzoic and hydroxycinnamic acids) (Fig. 1), which is distributed in plants and foods of plant origin (Manach et al., 2005). Additionally, phenolics act as metal chelators, antimutagens or anticarcinogens antimicrobial and clarifying agents (Proestos et al., 2005).

The flavonoid family is divided into a number of subgroups. The six main classes are flavonols, flavones, flavan-3-ols, isoflavones, flavanones and anthocyanidins with similar structure having a C6–C3–C6 flavone skeleton (Fig. 1). Flavonoids are one of the most important bioactive polyphenols, showing a diverse structure and a broad range of biological activities (Naczk and Shahidi, 2004; De Rijke et al., 2006).

Flavonols and flavones are synthesized in plant tissues from a branch of the phenylpropanoid pathway. The major flavonol aglycones found in plant foods are quercetin, myricetin and kaempferol, while a more limited number of fruits and vegetables contain the structurally-related flavones, apigenin and luteolin (Fig. 1). In plant tissues, flavonols and flavones are found conjugated to sugars such as glucose, galactose, rhamnose, and rutinose (Herrmann, 1988). Most conjugations occur at the 3 position of the B ring, although it can also occur frequently at the 7 and 4′ positions.

Flavan-3-ols are a complex subclass of flavonoids encompassing the simple monomers (+)-catechin, its isomer (−)-epicatechin, oligomeric and polymeric procyanidins, commonly known as condensed tannins (Catherine et al., 2005) (Fig. 1). In particular, condensed tannins are usually associated with astringent perception (Porter, 1988).

Anthocyanins are the strong antioxidants, which may be related to the health benefits. Anthocyanidins are flavylium (2-phenylbenzopyrylium) structures with varying hydroxyl or methoxyl substitutions. The anthocyanin forms found in foods are glycosides and acylglycosides of six common aglycon anthocyanidins: pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin (Nicoue et al., 2007). Anthocyanin pigments are responsible for the reddish blue and purple color of many fruit (Martinelli et al., 1992).

The aim of the present study was to identify the main phenolic compounds and to provide an overview of the phytochemical composition of hawthorn fruit extracts using HPLC–DAD–MS. Additionally, it was established the compositional differences between the two *Crataegus* species very distributed in Tunisian flora.

2. Materials and methods

2.1. Samples

2 kg of *Crataegus azarolus* and *Crataegus monogyna* fruit samples was recollected in September 2009 from Kef en Nsour (Jendouba) northwestern Tunisia, located at 36° 33′ 2″ N latitude, 8° 25′ 29″ E longitude and 606 m altitude. Fruits were immediately transported after recollection to our laboratory. Fruits were peeled with a sharp knife, and the three parts: peel, pulp and seeds were lyophilized and stored at −20 °C until analyzed.

2.2. Standards and reagents

Analytical grade phenolic standards: phloroglucinol, gallic, protocatechuic, OH-benzoic, vanillic, caffeic, syringic, *p*-coumaric, sinapic, 3-(2′,5′-dimethoxybenzoyl) propionic (DMB propionic), homovanillic, homogentisic, ferulic and chlorogenic acids; phloridzin, catechin, epicatechin, quercetin, quercetin-3-*O*-glucoside, kaempferol, were purchased from Sigma–Aldrich Química S. A. (Madrid, Spain). Solvents used were HPLC-grade and were obtained from Merck (Darmstadt, Germany). Methanol, ethanol, chloroform and chlorhydric acid were supplied by Prolabo (Madrid, Spain).

2.3. Preparation of the phenolic extracts

The lyophilized peel, pulp and seed were processed separately; Approximately 2 g of dried parts from each *Crataegus* species was extracted three times by 15 ml of methanol/acidified water HCl 1.5 N, during 30 min in an ultrasonic bath (FALC Instruments, Italy) (Khanizadeh et al., 2008). The extracts were centrifuged and the three methanolic supernatants were combined and the methanol was removed *in vacuo*. The aqua resultant extracts were lyophilized until dried. Finally, 5 ml of ultrapure water (Millipore Milli-Q water purification system) was added into the dried extracts and filtered through a 0.45 μm membrane-filter.

2.4. HPLC–UV Analysis of Phenolics

A sample of 20 μL of the different supernatants above-obtained was analyzed using an Agilent 1200 Series liquid chromatography with a quaternary pump and a photodiode array detector (DAD) and an Ultrabase C18 column (5 μm; 4.6 mm × 150 mm) which was set thermostatically at 25 °C. Solvents used to analysis were acetic acid 2.5% (A), HPLC-grade acetonitrile (B), ultra-pure water (C) and acetic acid 2.5% HPLC-grade acetonitrile (90:10) (D) at a flow rate of 0.5 mL min^{-1}. Elution was performed as previously described by Pallaufa et al. (2008).

2.5. HPLC–MS analysis

In order to confirm the identity of the phenolic compounds that could not be done by HPLC–UV, additional analysis was carried out using HPLC with mass spectrometry detection Agilent 1100 Series liquid chromatography equipped with an API source and employing an ESI (electrospray ionization)

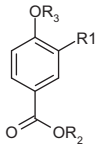
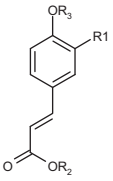
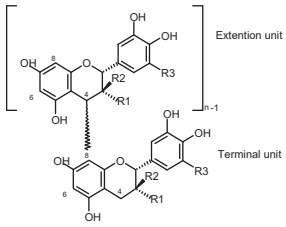
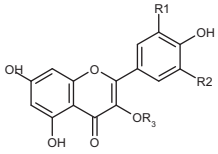
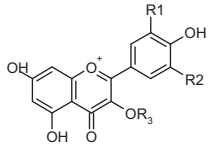
Hydroxybenzoic acids and derivatives		R1	R2	R3
	Procatechuic acid	OH	H	H
	Vanillic acid	OC H_3	H	H
Hydroxycinnamic acids				
	*p*-coumaric acid	H	H	H
	Chlorogenic acid	OH	$C_7H_{11}O_5$	H
Flavan-3-ols and proanthocyanidins				
	Epicatechin	OH	H	H
	Catechin	H	OH	H
	Dimer B2			
	Trimer C2			
	Trimer C3			
Flavonol aglycones and glycosides				
	Quercetin-3-O-glucoside	OH	H	glucose
	Quercetin-3-O-galactoside	OH	H	galactose
	Quercetin-3-5-diglucoside	OH	H	diglucose
	Kaempherol-3-O-galactoside	H	H	galactose
	Kaempherol-3-O-glucoside	H	H	glucose
Anthocyanins glycosides				
	Cyanidin-3-O- glucoside	OH	H	glucose
	Cyanidin-3-O-arabinoside	OH	H	arabinose

Figure 1 Chemical structure of antioxidants present in *Crataegus* extracts fruits.

interface. The HPLC system was connected to a DAD and a simple quadruple G1946D Q-LC/MS. Sheath as well as auxiliary gas was a mixture of helium and nitrogen. The capillary voltage was 3 V and the capillary temperature 180 °C. Solvents used were ultrapure water (A), HPLC-grade acetonitrile (B), formic acid 1% (C) and formic acid 1% HPLC-grade acetonitrile (90:10) (D) at a flow rate of 1 mL min^{-1}. Elution program start with 100% C, the gradient was the following: from 100% C to 100% D in 3 min, from 100% D to 1% B in 4 min, isocratically 1% B in 3 min, from 1% B to 12% B in 20 min, from 12% B to 50% B in 5 min, isocratically 50% B in 5 min, from 50% B to 100% C in 2 min (Pallaufa et al., 2008). Spectra were recorded in the positive ion mode and the MS detector was programmed to perform a series of consecutive scan: full scan from *m*/*z* 150 to 1500.

2.6. Phenolic identification and quantification

Chromatograms were recorded at 280, 330, 370 and 520 nm. Phenolic compounds were identified by their UV spectra recorded with a diode array detector and by LC–MS. Some of these phenolics have been previously identified with authentic

markers, and others were identified by their MS spectra and their corresponding daughter MS^2 fragments. Anthocyanins were quantified at 520 nm as cyanidin 3-glucoside, flavonols at 360 nm as quercetin-3-*O*-glucoside, hydroxycinnamic acid derivatives around 320 nm as chlorogenic, acid and flavan-3-ols at 280 nm as catechin.

3. Results and discussion

3.1. Antioxidant peaks identification

The method coupling high-performance liquid chromatography (HPLC) with diode-array detector (DAD) and electrospray ionization mass spectrometry with an ion trap analyser was optimized for the separation, identification and characterization of phenolic acids, flavonoid glycosides flavonoid aglycones and anthocyanins by data of the retention time, λ_{max}, pseudomolecular ion, main fragment ions in MS^2.

The Phenolic chromatographic profile at 280, 320, 370 and 520 nm of the two species of hawthorn is shown in Figs. 2 and 3. Four classes of phenolic compounds were identified, mass spectrometry analysis revealed that peaks PA1 to PA4 corresponded to different phenolic acids, A1 and A2 represented the anthocyanins glucoside, while peaks P1 to P4 were assigned to proanthocyanidins. The symbolized picks F1 to F8 belong to the flavonols and flavones subclasses.

3.2. Identification and structure characterization of phenolic acids and derivatives

The major phenolics compounds of *Crataegus* species studied by LC–MS in positive ionization mode are presented in Tables 1 and 2. Four phenolic acids are present in *Crataegus* corresponded to hydroxycinnamic acids derivatives such as 5-*O*-caffeoylquinic acid (PA1), chlorogenic acid (PA3) and *p*-coumaric acid (PA4). According to their UV spectra (λ_{max} between 314 and 328 nm) and pseudo molecular ions $[M-H]^+$ (m/z at 355, 355 and 165, all of them yielding a product ion at m/z 193, due to the deprotonated quinic acid) (Rodrigues et al., 2012). Hydroxybenzoic acid represented by protocatechuic acid peak PA2 at 8.6 min (pseudomolecular ion $[M-H]^+$ at m/z 155).

3.3. Identification of proanthocyanidins

The flavan-3-ols with a different degree of polymerization (i.e., catechins and proanthocyanidins) were other relevant flavonoids found in *Crataegus* species, especially, fruit extracts of the *C. monogyna* samples (Tables 1 and 2). The flavan-3-ols profiles, showed the presence of four peaks respectively symbolized in chromatograms by P1, P2, P3 and P4. The major positive ions containing structural information for peaks P1 to P4 m/z were respectively; 291, 579, and 867. Peak P4 was identified as (−)-epicatechin by comparison of its UV spectra and retention time with a commercial standard. Signal at m/z 579 (peak P2) is associated with B-type procyanidin dimer. Peaks P1 and P3 (same pseudomolecular ion $[M-H]^+$ at m/z 867) were assigned to two procyanidin trimer C1 and trimer C2 containing two B-type interflavonoid linkages.

The obtained fragmentation patterns of the condensed tannins (Peaks P1–P3) are 697, 579, 427, 409, 289 and 291. They may be the assumption of two fragmentation mechanisms for these types of compounds. Either by retro Diels–Alder

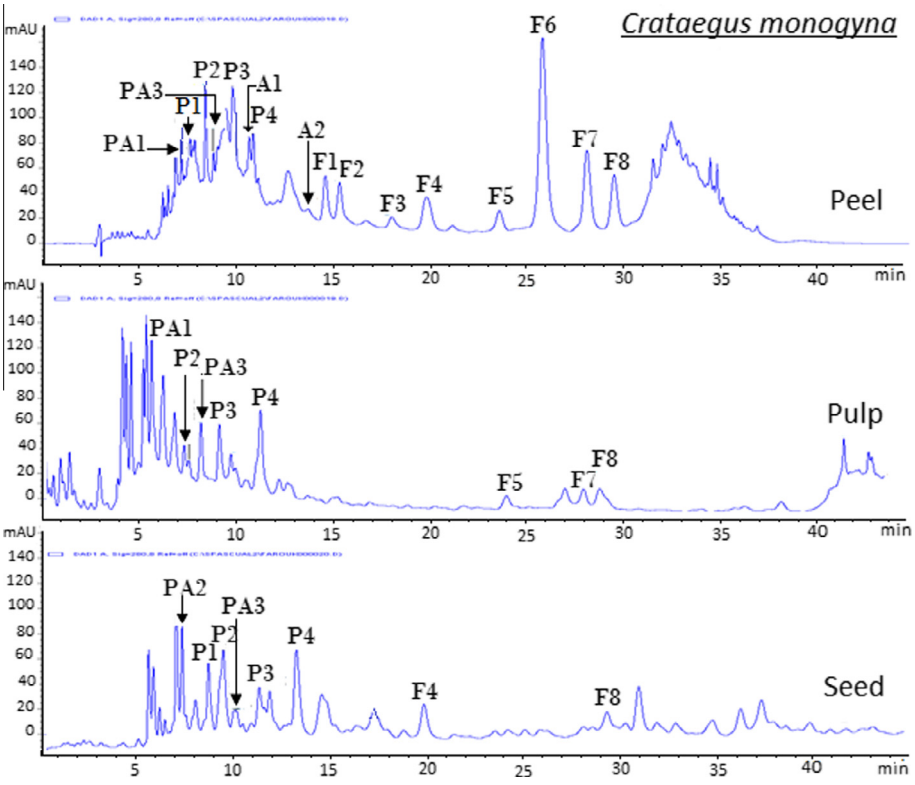


Figure 2 HPLC/MS chromatograms of different parts of *C. monogyna*.

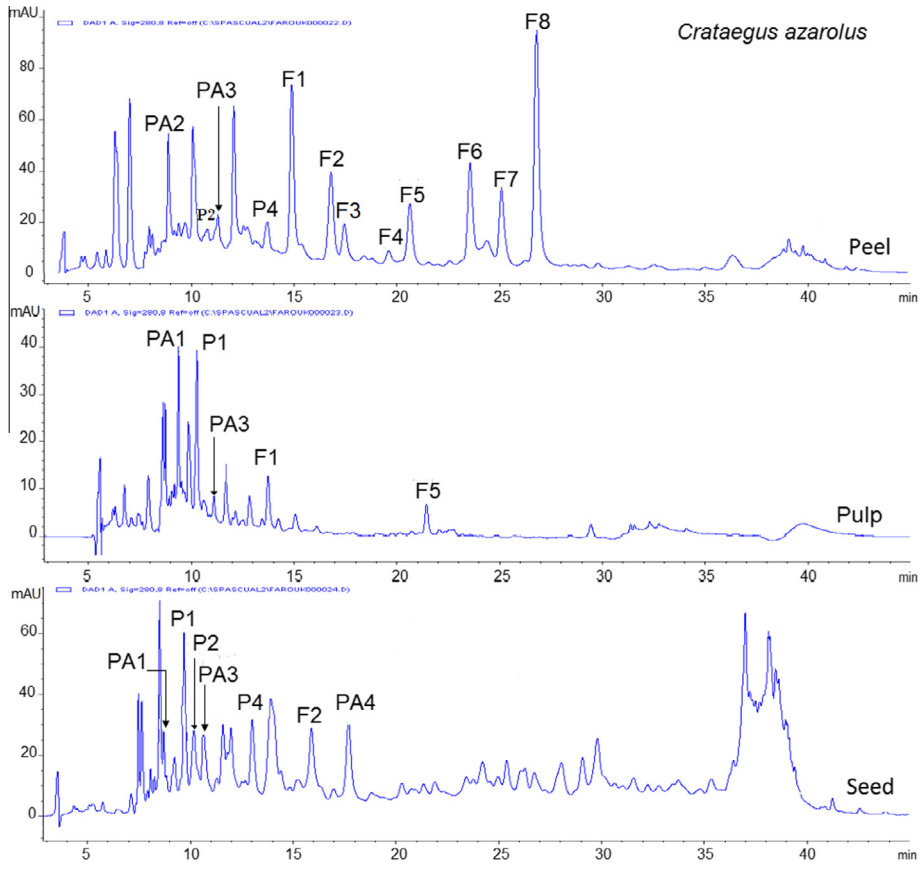


Figure 3 HPLC/MS chromatograms of different parts of *C. azarolus*.

Table 1 Characteristics; (Rt), (λ_{max}), mass spectral data, relative abundances of fragment ions and tentative identification of phenolic compounds in *Crataegus monogyna* fruit extracts.

Peak	Rt (min)	λ_{max} (nm)	Pseudomolecular ion $[M + H]^+$ (m/z)	MS/MS (m/z)	Tentative identification
PA1	7.6	326	355	193, 181, 175, 163, 137	5-*O*-caffeoylquinic acid
PA2	8.6	318	155	155, 119	Protocatechuic acid
P1	9.3	278	867	697, 579, 427, 409, 289, 291	Procyanidin trimer C1
P2	10.3	280	579	427, 409, 291, 289	Procyanidin dimer B2
PA3	10.8	328	355	193, 181, 175, 163, 137	Chorogenic acid
P3	11.9	278	867	697, 579, 427, 409, 289, 291	Procyanidin trimer C2
A1	13.1	516	449	287	Cyanidin-3-*O*-glucoside
P4	13.2	280	291	291	(−)-Epicatechin
F1	14.3	340	595	595, 287	Luteolin-7-*O*-rutinoside
A2	16.8	518	419	287	Cyanidin-3-*O*-arabinoside
F2	17.9	358	627	627, 465, 303	Quercitin-3,5-*O*-digaluctoside
F3	18.7	358	627	627, 465, 303	Quercitin-3,5-*O*-diglucoside
F4	22.2	348	449	449, 287	Kaempherol-3-*O*-galactoside
F5	24.3	348	449	449, 287	Kaempherol-3-*O*-glucoside
F6	28.9	338	433	433, 271	Apigenin-7-*O*-glucoside
F7	31.6	360	465	465, 303	Quercitin-3-*O*-galactoside
F8	34.4	360	465	465, 303	Quercitin-3-*O*-glucoside

fragmentation; loss of 152 amu followed by dehydration; loss 18 amu and loss of 122 amu not characterized correspond to $C_6H_2O_3$. Also by cleavage of the C–C bonds of the interflavonoid linkages by loss of a monomeric unit $[M - H\text{-}291]^+$ (Friedrich et al., 2000).

Results show considerable variations in the contents of these compounds between red and yellow fruits extracts. (−)-epicatechin is the monomer presents in all fruits parts of both *Crataegus* species extract, while the condensed tannins are present only in red fruit extracts.

Table 2 Characteristics; (Rt), (λ_{max}), mass spectral data, relative abundances of fragment ions and tentative identification of phenolic compounds in *Crataegus azarolus* fruit extracts.

Peak	Rt (min)	λ_{max} (nm)	Pseudomolecular ion $[M + H]^+$ (m/z)	MS/MS (m/z)	Tentative identification
PA1	7.6	326	355	193, 181, 175, 163, 137	5-*O*-caffeoylquinic acid
PA2	8.6	318	155	155, 119	Protocatechuic acid
PA3	10.8	328	355	193, 181, 175, 163, 137	Chorogenic acid
P4	13.2	280	291	291	(−)-Epicatechin
F1	14.3	340	595	595, 287	Luteolin-7-*O*-rutinoside
PA4	17.7	314	165	114, 102	*p*-coumaric acid
F2	17.9	358	627	627, 465, 303	Quercitin-3,5-*O*-digaluctoside
F3	18.7	358	627	627, 465, 303	Quercitin-3,5-*O*-diglucoside
F4	22.2	348	449	449, 287	Kaempherol-3-*O*-galactoside
F5	24.3	348	449	449, 287	Kaempherol-3-*O*-glucoside
F8	34.4	360	465	465, 303	Quercitin-3-*O*-glucoside

3.4. Identification of anthocyanins

The anthocyanin profiles obtained for *Crataegus* species were very different. Only two broad peaks (A1 and A2) detected around 517 nm were assigned to anthocyanin especially in red *Crataegus* fruits (peel extract), respectively at Rt = 13.1 and 16.8 min. Identification of individual anthocyanin was performed by comparison with standards, retention times, UV/vis, mass spectral data and the mass of the sugars bound to the aglycons and the specific fragmentation patterns of the compounds. The bound sugar moieties consist of hexoses with a mass unit of 162 (glucose) and pentose with a mass unit of 132 (arabinose).

Cyanidin aglycone mass is 287, the order of elution of the glycosides on the C18 column is glucoside before the arabinoside. The MS data of the molecular and product ions of compounds A_1 and A_2 (Fig. 4) were consistent with tow constituents of *Crataegus* anthocyanins previously reported: cyanidin-3-*O*-glucoside (449/287) and cyanidin-3-*O*-arabinoside (419/287).

The red color of *Crataegus* fruit was coherent with the presence of the two major anthocyanin pigments, however, in the other parts of fruits and the yellow specie, the anthocyanin was absent.

3.5. Identification of flavonols

Mass spectrometric methods can be used to obtain information on the carbohydrate sequence and the aglycone flavonols. Their identities were assigned based on their retention times (Rt), maximal UV wavelength (λ_{max}), pseudomolecular ions and MS^2 spectra, releasing fragments corresponding to the losses of sugar. Based on bibliographical sadies, fragmentation of O-glycosylated flavonoids produced the cleavage of the glycosidic bond with protons rearrangement resulting in the formation of the genin. The removing of monosaccharide residue is demonstrated by the loss of sugar. (Wolfender et al., 2000; Cuyckens and Claeys, 2004). In none of them the identity of the sugar and positions of location of the substituents could be established.

Furthermore, the pseudomolecular ions $[M - H]^+$ of the identified flavonols compounds have respectively at m/z 627, 449 and 465. Major diagnostic fragments of flavonols aglycone identification are those involving the cleavage of two C–C bonds of the C-ring giving two fragment ions which provide information about the number and type of substituents. Therefore the product ions, $[aglycon–H]^+$, were detected at m/z 303. In Fig. 5 are shown fragmentations of flavonols peaks. F1, F3 (same pseudomolecular ion $[M - H]^+$ at m/z 627) corresponding to quercetin-3,7-*O*-digalactoside, quercetin-3,7-*O*-diglucoside. Peaks F4 and F5 also with identical molecular ions at m/z 449, thus, these peaks were tentatively assigned respectively as quercetin-3-*O*-galactoside and quercetin-3-*O*-glucoside based on their fragmentation pattern and relative fragment ion abundances. Similar reasoning was applied for the assignment of peaks F7 and F8 ($[M - H]^+$ at m/z 465), releasing typical MS^2 fragments ions, but with different characteristics UV and different retention time, these peaks were associated with kaempherol-3-*O*-galactoside and kaempherol-3-*O*-glucoside.

3.6. Identification of flavones

The *C*-glycosylated flavones were also found in *Crataegus* fruits species. Peaks F2 and F6 showed a pseudomolecular ion $[M - H]^+$ respectively at m/z 595 and 433 (Fig.5). Their identities were assigned based on their pseudomolecular ions and MS^2 spectra, releasing fragments corresponding to the losses of rhamnosylhexosyl (−146 to 162 uma) to obtain the Luteolin aglycon at m/z 287. This disaccharide should be either a rutinoside = Glc-Rha (m/z 308) or a Gal-Rha (m/z 308) linked to the luteolin aglycone by either Glc or Rha sugar. The fragment corresponding to the losses of a hexosyl (glucose = −162 uma) giving an apeginin aglycon at m/z 271 (Ferreres et al., 2003). Therefore, these peaks were assigned to luteolin-7-*O*-rutinoside and apigenin-7-*O*-glucoside.

3.7. Quantification of antioxidants in Crataegus fruits species

The quantification of Phenolic compounds present in the fruit extracts was performed by comparison with the standard curves appropriate in each case. The individual phenolic compounds were divided into five subclasses such as phenolic acids, anthocyanins, proanthocyanidins, flavonols and flavones. Cyanidin-3-*O*-glucoside was used as the standard for anthocyanin quantification, quercetin-3-*O*-glucoside for flavonols, (−)-epicatechin and (+)-catechin for proanthocyanidins. The phenolic contents in the different parts of each *Crataegus* fruits species are summarized in Table 3.

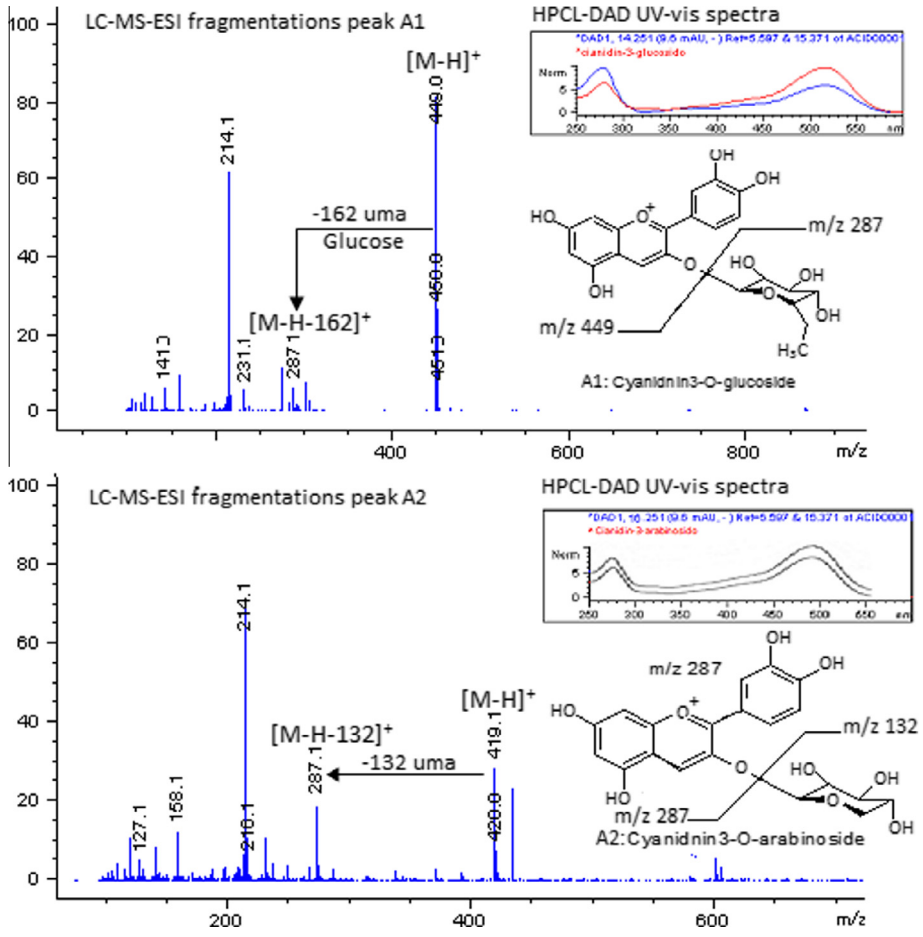


Figure 4 MS spectra, ion nomenclature and major fragments from red *Crataegus* peel extract.

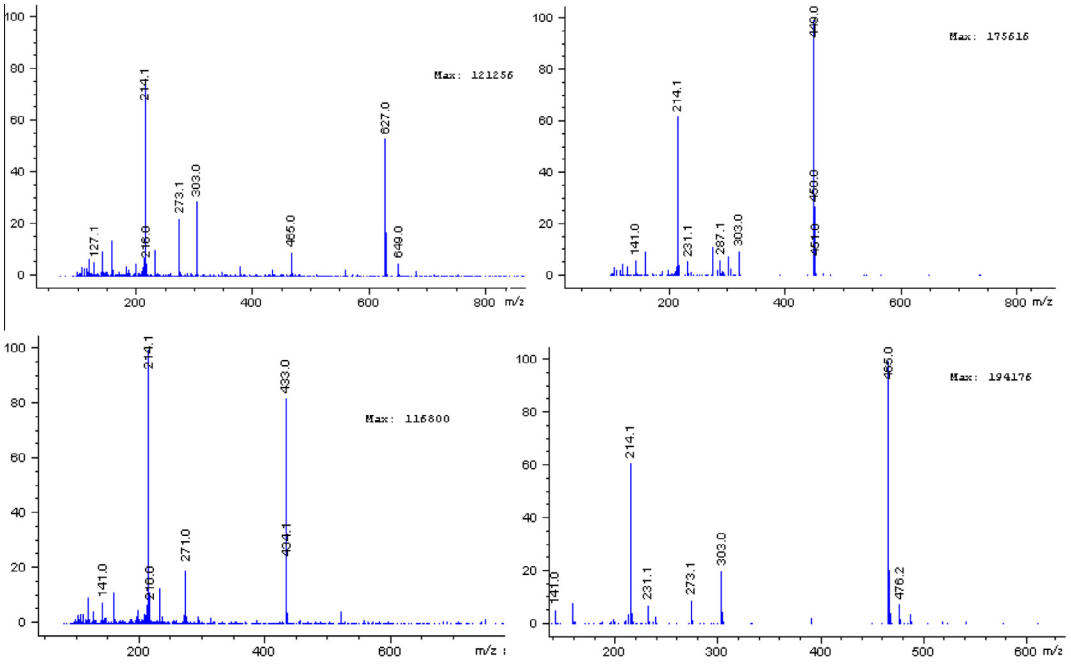


Figure 5 MS spectra and fragmentation pattern of the identified flavonols and flavones in *Crataegus* fruits extract.

Table 3 Antioxidants content in dried parts of red and yellow *Crataegus* fruit (mg. 100 g^{-1}).

	C. monogyna			*C. azarolus*		
	Peel	Pulp	Seed	Peel	Pulp	Seed
Phenolic acids						
5-*O*-caffeoylquinic acid	12.78	17.84	19.80	nd	nd	8.59
Chlorogenic acid	30.85	14.80	18.49	8.61	3.10	12.24
p-Coumaric acid	nd	nd	nd	nd	nd	6.48
Procatechuic acid	nd	nd	8.25	19.02	8.61	nd
Total phenolics acid	43.63	32.64	46.54	27.63	11.71	27.31
Antocyanins						
Cyanidin-3-*O*-glucoside	49.00	nd	nd	nd	nd	nd
Cyanidin-3-*O*-arabinoside	15.50	nd	nd	nd	nd	nd
Total anthocyanins	64.50	0	0	0	0	0
Procyanidins						
CA-(4α → 8)-CA-(4α → 8)-CA	32.31	6.18	122.1	nd	nd	nd
EC-(4β → 8)-EC-(4β → 8)-EC	64.08	nd	36.67	nd	nd	nd
EC-(4β → 8)-EC	63.50	37.89	110.8	nd	nd	nd
(−)-Epicatechin	124.9	20.56	56.32	38.45	8.21	18.36
Total procyanidins	293.93	64.63	325.89	38.45	8.21	18.36
Flavonols						
Quercitin-3,5-*O*-digalactoside	88.29	nd	nd	58.48	nd	nd
Quercitin-3,5-*O*-diglucoside	98.46	nd	nd	28.91	nd	nd
Kaempherol-3-*O*-galactoside	142.9	nd	nd	44.41	nd	nd
Kaempherol-3-*O*-glucoside	149.1	nd	nd	21.42	nd	nd
Quercitin-3-*O*-galactoside	312.3	nd	nd	nd	nd	nd
Quercitin-3-*O*-glucoside	221.1	7.58	nd	45.79	0.83	nd
Total flavonols	1011.85	7.58	0	199.1	0.83	0
Flavones						
Apigenin-7-*O*-glucoside	3.86	nd	nd	nd	nd	nd
Luteolin-7-*O*-rutinoside	nd	1.41	nd	21.39	0.83	nd
Total flavones	3.86	1.41	0	21.39	0.83	0

nd: Not detected.

Hawthorn fruit extracts are a good source of antioxidant. Being flavonols the most abundant group they represent 73.38% and 67.59% followed by tannins with a percentage 21.31 and 13.05 of the total composition, respectively in red and yellow fruits. The lower phenols content correspond to the flavones family (0.27% *C. monogyna* and 7.16% *C. azarolus*). Anthocyanin pigmentations were present only in red fruits.

Chlorogenic acid and (−)-epicatechin are the most abundant phenolic compounds identified in all fruit parts of both *Crataegus* species with the highest levels are localized in peel fraction. Chlorogenic acid and its isomer 5-CQA, are widely recognized to be an antioxidant for human LDL (Nardini et al., 1995). It is also known as a scavenger for reactive species of oxygen and nitrogen (Kono et al., 1997).

Other compounds are specific to each fruit parts of the two species such as hyperoside which is identified only in red peel fruit, while *p*-coumaric acid is specific for the yellow seed fruits.

4. Conclusions

The knowledge of the phenolic compounds in two species of hawthorn fruit, including flavonoids, tannins, anthocyanin, phenolic acids and their derivatives, will help to define their potential as a source of antioxidants. The phenolic profiles by HPLC–DAD–MS of the different parts revealed high predominance of flavonols and tanins, which are compounds that modulate a variety of biological events. The quantification data allowed us to compare the three parts selected from the two species. Most phenolic compounds could be detected in all varieties, although their quantities differed considerably. The highest levels of phenolics were detected in the red peel fruits.

Therefore, it can be concluded that *Crataegus* fruits can be used to enhance the bioactive compounds into food products and to reduce oxidative stress, to retard or prevent various human diseases. However, hawthorn may be explored for pharmaceutical applications.

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