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ScienceDirect
Food Chemistry 106 (2008) 1234–1245
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

LC–DAD–MS (ESI+) analysis of the phenolic content of *Sorbus domestica* fruits in relation to their maturity stage

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Received 22 June 2007; received in revised form 4 July 2007; accepted 9 July 2007

Abstract

The aim of this study was the detailed qualitative and quantitative analysis of the phenolic content of several extracts and fractions of *Sorbus domestica* fruits. The analysis was realized by LC–DAD–MS (ESI+). Twenty-four different extracts and fractions of five different maturity stages of the fruit were analyzed for comparison reasons in order to determine the most beneficial for health type of consuming. Sixty-two different phenolics were identified. There were significant qualitative and quantitative differentiations in the phenolic content among the different types of the fruits. All categories were rich in benzoic, phenylpropanoic and cinnamoylquinic acids and derivatives. Unripe fruit categories were also rich in flavonoids, while well matured fruit categories had a low content of flavonoids. Fruit pulp, which was proved to be a strong antioxidant according to previous research work, contained very low amounts of both acids and flavonoids, but its phenolic content was highly qualitatively differentiated from the other categories.

Keywords: *Sorbus domestica* fruits; Five maturity stages; LC–DAD–MS (ESI+) analysis; Benzoic acids; Phenylpropanoic acids; Flavonoids

1. Introduction

Sorbus domestica (Rosaceae) fruits are shelf shown at the mountainous regions of the northern Greece and consumed by the local population, not only as a nutritious food, but also traditionally as an antidiabetic agent, as it is surmised that it impugns diabetic symptoms. There is up to now literature dealing with the antidiabetic activity of many natural products such as Ginseng, Bitter Melon and phenolics generally (Anila & Vijayalakahmi, 2000). People traditionally collect it unripe and mature fruits at room temperature for one until three weeks, or consume it after boiling, in pulp form. After we have established the good antioxidant activity of various fractions and types of the fruit and calculated the total phenolic content (Termentzi, Kefalas, & Kokkalou, 2006), we proceeded to the detailed phytochemical analysis of the phenolic content of all the types and fractions of *Sorbus domestica* fruits. The purpose of the study at five different maturity stages and types of consuming was to analyze the phenolic content of a fruit for which extremely few things are known, as well as to compare the content between the different maturity stages in order to come up to the best type of consuming.

Very little is by far known about the phenolic content of the fruits. A previous research (Olschlager, Milde, Schempp, & Treutter, 2004) refers to the presence of procyanidis, cinamic acids and quercetin. However, there are plenty reports about the phenolic content of the related species *S. aucuparia*, that contents quercetin and kaempherol aglycons and glycosides (Gil-Izquierdo & Mellenthin, 2001; Hakkinen & Auriola, 1998; Hakkinen, Karenlampi,

Abbreviations: M, molecular ion; A, aglycon; R_t, retention time.

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doi:10.1016/j.foodchem.2007.07.021

Heinonen, Mykkanen, & Torronen, 1999), cinamic and benzoic acids chlorogenic and neochlorogenic acid (Hakkinen et al., 1999; Kahkonen, Hopia, & Heinonen, 2001; Maata-Riihinen, Kamal-Eldin, Mattila, Gonzalez-Paramas, & Torronen, 2004).

For the qualitative and quantitative analysis of the phenolic content LC–DAD–MS (ESI+) analysis was used.

2. Materials and methods

2.1. Plant material

All fruit samples were collected in September 2003. Five fruit categories were tested: (A) Unripe fruits (yellow color). (B) Well matured on tree (brown color). (C) Collected unripe and matured for one week in dark, at room temperature. (D) As in C, but prolonged maturation at three weeks (dark brown color), form consumed by the local population. (E) Sterilized pulp from well matured fruits (disposed at local drugstores). Fruits of category A, C and D were harvested on the 10th of September, while that of B 10 days later. All plant material was directly extracted with methanol.

2.2. Extraction procedure

The procedure followed is previously described (Termentzi et al., 2006). Four different solvents were used for the partition of the initial extracts in order to simplify the analysis. The procedure resulted to 24 different samples (Table 1): (A) dichloromethane fraction (A1): 500 mg. Diethyl ether fraction (A2): 166.2 mg. Ethyl acetate fraction (A3): 700 mg. Butanol fraction (A4): 11 gr. Water fraction (A5): 90 gr. (B) Dichloromethane fraction (B1): 703.2 mg. Diethyl ether fraction (B2): 400 mg. Ethyl acetate fraction (B3): 720 mg. Butanol fraction (B4): 20.5 gr. Water fraction (B5): 126.7 g. (C) Dichloromethane fraction (C1): 201.6 mg. Diethyl ether fraction (C2): 115.6 mg. Ethyl acetate fraction (C3): 1.38 gr. Butanol fraction (C4): 49.2 gr. Water fraction (C5): 144.32 gr. (D) Dichloromethane fraction (D1): 383.1 mg. Diethyl ether fraction (D2): 11.7 mg. Ethyl acetate fraction (D3): 723.8 mg. Butanol fraction (D4): 16.34 gr. Water fraction (D5): 144.38 gr. (E) Diethyl ether fraction (E2): 117.8 mg. Ethyl acetate fraction (E3): 90.7 mg. Butanol fraction (E4): 8.2 gr. Water fraction (E5): 23.9 gr. All extracts, fractions and initial residues were kept at 0 °C under nitrogen atmosphere.

2.3. Establishment of the phenolic compound using LC–DAD–MS (ESI+)

This analysis was performed using an LC/DAD/MS system with a Finnigan MAT Spectra System P4000pump coupled with a UV6000LP diode array detector and a Finnigan AQA mass spectrometer. The separation was performed on a 125 × 2 mm Superspher 100-4 RP-18 column (Macherey-Nagel, 4 μm particle size) at a flow rate of 0.33 ml/min. The detection was monitored at 290, 340 and 365 nm. The MS-ESI(+) spectroscopy at a probe temperature of 450 °C, probe voltage of 4.9 kV and at 20 and 80 eV in the mass analyzer. The following gradient program was used: (A) AcOH (2%) and (B) MeOH, 90% A for 2 min, 0% A at 37 min, 0% A at 42 min, 90% A at 45 min and 90% A at 50 min. The concentrations of the 24 samples varied from 1.5 until 24 mg/ml; injection volume: 1 μl. The data were processed using the Xcalibur 1.2 software.

2.4. Preparation of standards for qualitative and quantitative determinations

Nine standards were prepared for quantitative and qualitative determination: Vanillic acid (R_t 2.24 min), *trans-p*-coumaric acid (R_t 4.28 min), Chlorogenic acid (R_t 6.63 min), Caffeic acid (R_t 6.82 min), Ferulic acid (R_t 7.42 min), Rutin (R_t 15.30 min), 7-methoxy flavone (R_t 18.81 min), Naringenin (R_t 18.94 min) and quercetin (R_t 19.45 min). All standards were from SIGMA (Germany). Vanillic acid (78 ppm, 1 μl injection volume) was used for the quantization of benzoic acids and derivatives, while *trans-p*-comumaric acid (92 ppm, 1 μl injection volume) and caffeic acid (105 ppm, 1 μl injection volume) for the corresponding phenylpropanoic acids and derivatives at 290 nm. Chlorogenic acid (147 ppm, 1 μl injection volume) was used for the quantization of cinnamoylquinic acid esters and 7-methoxy flavone

Table 1
Coding number of the different samples

	Type of partitioning				
	Dichloromethane	Diethyl ether	Ethyl acetate	*n*-Butanol	Water
Maturity stage/type of plant material					
(A) Unripe	A1	A2	A3	A4	A5
(B) Well matured on tree	B1	B2	B3	B4	B5
(C) Matured for 1 week at room temperature	C1	C2	C3	C4	C5
(D) Matured for 3 weeks at room temperature	D1	D2	D3	D4	D5
(E) Fruit pulp	–	E2	E3	E4	E5

Horizontally, the stages of partitioning are appeared together with all the organic solvents. Squarely, the maturity stage and the type of all the initial plant materials.

(1047 ppm, 1 μl injection volume) was used for the quantitative determination of flavones and flavonol esters at 340 nm. Quercetin (170 ppm, 1 μl injection volume) and rutin (175 ppm, 1 μl injection volume) were used for the determination of flavonols and their glycosides at 365 and 340 nm, respectively. The injection procedures are described above.

3. Results

3.1. Qualitative analysis of the phenolic content by LC–DAD–MS (ESI+)

3.1.1. General profile

The combination of diode array detection (DAD) and positive electrospray ionization mass spectrometry (ESI+), coupled to the HPLC with a reverse phase silica column, provided an accurate method for the analysis of the phenolic profile of *Sorbus domestica* fruits. Under the conditions used, most of the compounds detected had intensive signals corresponding to the pseudomolecular ion $[M+H]^+$. Formation of $[M+Na]^+$ was observed as well, specially at the high voltage 80 eV. Adducts are expressed in positive electronspray ionization (ESI+). 62 different phenolic compounds were traced in our samples and their identification was achieved by comparison to authentic samples, by comparison to the literature and by studying their retention times, UV–Vis absorption spectrum in correlation to proposed MS fragmentation mechanisms.

At the low voltage of 20 eV we observe the molecular ions and main fractions. At 80 eV further fragmentations of the molecules are noticed.

Throughout the results it appears that concerning the phenolic content there are large differences between the fractions of different polarity and smaller, but characteristic, between the fractions of same polarity but of different maturity stages. All the substances found in the chromatograms are given in Tables 2 and 3 by their retention time sequence in each case together with their UV and MS characteristics. At the last column the code numbers of the samples in which every substance was found are recorded (in correlation to Table 1).

3.1.2. LC–DAD–MS analysis of the phenolic acids and derivatives (Table 2)

3.1.2.1. LC–DAD–MS analysis of the benzoic acids and derivatives (Table 2). All the benzoyl derivatives were found in the dichloromethane and diethyl ether fractions, and only two in some ethyl acetate fractions.

Gallic alcohol (no. 2) comes out at R_t 2.26 min, giving the characteristic molecular ion at 157 amu and the UV absorption at 278 nm. It was found in the dichloromethane fractions of fruit categories A and C and in the diethyl ether fractions of fruit categories B and D.

Four simple benzoic acids (*p*-hydroxy benzoic acid, no. 3, vanillic acid, no. 4, protocatechuic acid, no. 5 and syringic acid, no. 8) were identified by their retention times, their characteristic UV absorbance and their mass fragmentations in comparison to the literature (Guasch-Jane, Ibern-Gomez, Andres-Lacueva, Jauregui, & Lamuela-Raventos, 2004; Tian, Nakamura, Cui, & Kayahara, 2005; Tsimidou, Papadopoulos, & Boskou, 1992; Waldron, Parr, Ng, & Ralph, 1996). Vanillic acid was also compared to the authentic sample. *p*-Hydroxy benzoic acid was found in the dichloromethane and ethyl acetate fractions of fruit categories B and D, while the three others in the diethyl ether fractions of all fruit categories.

At R_t 9.54 min, a common peak (no. 19) for the dichloromethane and the diethyl ether fractions of all fruit categories, tentatively identified as vanillic acid derivative is detected. The existence of at least one vanillic acid unit at the molecule represented by this peak is ensured by the UV spectra, giving two distinct peaks at 260 and 294 nm, which is a typical vanillic acid absorption (Waldron et al., 1996). Fragment at 169 amu = [vanillic acid + H]$^+$.

3.1.2.2. LC–DAD–MS analysis of phenylpropanoid acids, esters and derivatives (Table 2). The *n*-butanolic and the water fractions of all fruit categories contain the most phenylpropanoic acids and derivatives. However, substances of this category are also contained in the more lipophilic fruit fractions.

All phenylpropanoic acids detected with ESI+ give characteristic fragment ions [A−OH]+ that correspond to the detachment of the hydroxyl group from their carboxyl group. That is confirmed by the fragmentation of the authentic samples and by the literature (Fang, Yu, & Prior, 2002).

Esters of phenylpropanoic acids with quinic acids were dominant in our samples. They give a fragment ion [phenylpropanoic acid-OH]$^+$. In most cases the quinic acid moiety does not give fragment ion in ESI+ analysis (Fang et al., 2002), as it is also clear from the fragment ions of chlorogenic acid authentic sample. Its presence is concluded by the mass defect from the molecular ion.

3.1.2.2.1. Phenylpropanoid acids and derivatives in dichloromethane fractions. All four dichloromethane fractions have in their chromatograms two peaks at R_t 13.71 and 14.71 min that correspond to a caffeoyl-ferulic acid (no. 25) and a caffeoyl-ferulic acid derivative (no. 26), respectively. Fragments 163 = [caffeic acid-OH]$^+$ and 195 = [ferulic acid + H]$^+$. 137 = [caffeic acid-COOH+H+H]$^+$, detachment pre mentioned in the literature for cinnamic acids (Maffei Facino, Carini, Aldini, & De Angelis, 1997). First peak has a $[M+H]^+$ 357 and a fragment at 339 amu which corresponds to an homolytic fragmentation $[M-OH]^+$. Second peak has $[M+H]^+$ 411, and 433 = $[M+Na]^+$. Loss of the carboxylic group from the caffeoyl unit (*m*/*z* 137) is observed as well (Sanchez-Rabaneda et al., 2003; Sanchez-Rabaneda et al., 2004).

Unripe fruits contain three other substances that come up as intense peaks at 18.14 (no. 29), 18.80 (no. 30) and 19.57 (no. 33) min. All three have an intense fragment ion at 179 = [trihydroxy cinnamic acid-OH]$^+$. However their

Table 2
Retention times (R.T.), molecular ions ($[M+H]^+$), sodium adducts ($[M+Na]^+$), fragment ions (20 eV and 80 eV), relative intensities % (R.I.%) and UV absorptions (λ_{max}) of all phenolic acids and derivatives

No	R.T. (min)	$[M+H]^+$ (*m*/*z*)	$[M+Na]^+$ (*m*/*z*)	Fragment ion (*m*/*z*) at 20 eV (R.I.%)	Fragment ion (*m*/*z*) at 80 eV (R.I.)	UV λ_{max} (nm)	Identification	Samples (Table 1)
1	2.10	517	539	163(75), 347(75), 355(85), 377(100), 539(40)	163(100), 377(25), 539(35)	290sh, 320, 328	Caffeoylquinic acid hexoside[b]	A4, C4
2	2.26	157	–	157(100)	157(100)	278	Gallic alcohol[c]	A1, C1, B2, D2
3	2.29	139	161	139(60), 161(100)	161(100)	282	*p*-Hydroxy benzoic acid[b]	B1, D1, B3, D3
4	2.34	169	–	169(100)	151(100)	258, 294	Vanillic acid[a,b]	A2, B2, C2, D2, E2
5	2.91	155	–	155(100)	137(40), 155(100)	258, 294	Protocatehuic acid[b]	A2, B2, C2, D2, E2
6	2.99	517	539	163(100), 347(20), 355(40), 377(70), 539(15)	163(100), 377(40), 539(40)	290sh, 320, 328	Caffeoylquinic acid hexoside[b]	A4, C4
7	3.00	355	377	163(100), 355(40), 377(60)	163(100)	290sh, 320, 328	Caffeoylquinic acid[b]	A5, B5, C5, D5, E4, E5
8	4.12	199	–	159(40), 179(55), 199(100)	199(100)	280	Syringic acid[b]	A2, B2, C2, D2, E2
9	4.33	259	–	163(20), 259(100)	163(100),187(50), 259(50)	290sh, 310	Caffeic acid derivative[c]	A4, B4, C4, D4, E4
10	4.57	–	–	165(30), 183(70), 187(55), 243(95), 269(100), 351(20), 433(20), 515(30), 597(20)	187(20), 269(100), 433(20), 515(20), 597(10)	298	Dihydrocaffeic acid derivative[c]	D2
11	5.70	355	377	163(100), 355(50), 377(25)	163(100), 377(15)	290, 320, 328	Caffeoylquinic acid[b]	A4, B4, C4, D4, E4
12	6.48	355	377	163(100), 355(50), 377(25)	163(100), 377(20)	295sh, 322, 328	Chlorogenic acid[a]	A3,4,5, B3,4,5, C3,4,5, D3,4,5, E3,4,5
13	6.71	–	–	157(100), 203(50), 243(70)	163(38), 203(50), 243(100)	244, 300sh, 320, 328sh	Caffeoyl- (dimethyl ether quinic acid) derivative[b]	B2
14	7.12	369	391	161(55), 163(40), 369(60), 391(100)	163(80), 179(60), 391(100)	242, 295sh, 320, 328	Feruloylquinic acid[b]	A3, B3, C3, D3, E3
15	7.21	501	–	133(100), 163(35), 271(45), 301(40), 391(50), 501(20)	161(35), 179(100), 205(50), 281(75), 295(55), 501(85)	244, 298sh, 318	Bi (*p*-methoxy cinnamoyl)- caffeic acid biester[c]	A2, C2, E2
16	7.71	355	377	163(100), 355(35), 377(20)	163(100), 377(20)	290sh, 320, 328	Caffeoylquinic acid[b]	E4
17	8.35	291	313	291(100), 313(50)	133(100), 163(50), 203(40), 313(40)	244, 295sh, 322, 328	Caffeic acid derivative[c]	A3
18	9.35	501	523	157(100), 179(65), 181(15), 523(15)	163(100), 285(50), 523(20)	295sh, 322, 328	*p*-Coumaroyl-, caffeoyl-quinic acid biester[b]	A2, C2, E2
19	9.54	–	–	169(100), 213(30), 257(25), 277(40)	169(90), 235(100)	260, 294	Vanillic acid derivative[c]	A1, A2, B1, B2, C1, C2, D1, D2, E2
20	10.08	369	391	163(40), 369(30), 391(100)	163(30), 177(100), 179(30)	242, 298sh, 320, 328	Feruloylquinic acid[b]	A2,3,4, B3, C3, D3, E3
21	10.35	–	–	147(30), 187(95), 227(100)	147(35), 187(95), 227(100), 329(40)	288	*cis*-*p*-Coumaroyl, hydrosinapic acid derivative[c]	B2
22	10.45	–	–	133(30), 179(20), 227(100)	133(100), 147(30), 179(25), 227(30)	290sh, 310	*trans*-*p*-Coumaroyl, hydrosinapic acid derivative[c]	C2, D2
23	11.03	433	–	133(40), 165(50), 183(60), 187(100), 269(90), 351(30), 433(30)	133(70), 165(90), 187(50), 269(100), 433(30)	290	Hydrocaffeic acid derivative[c]	D2
24	12.18	369	391	194(40), 369(100), 391(65)	177(100), 179(40), 194(30), 369(30), 391(40)	242, 298sh, 320	Feruloylquinic acid[b]	A2
25	13.71	357	–	137(10), 163(45), 195(100), 357(10)	137(85), 163(100), 195(73), 339(20)	290sh, 314	Caffeoyl-ferulic acid[c]	A1, B1, C1, D1
26	14.71	411	433	163(45), 195(100), 411(15), 433(10)	137(80), 163(100), 195(80), 411(20)	295sh, 320, 328	Caffeoyl-ferulic acid derivative[c]	A1, B1, C1, D1

(*continued on next page*)

Table 2 (*continued*)

No	R.T. (min)	$[M+H]^+$ (*m/z*)	$[M+Na]^+$ (*m/z*)	Fragment ion (*m/z*) at 20 eV (R.I.%)	Fragment ion (*m/z*) at 80 eV (R.I.)	UV λ_{max} (nm)	Identification	Samples (Table 1)
27	14.83	581	603	163(20), 194(50), 195(100), 271(15), 339(35), 357(15), 581(60), 603(25)	581(80), 603(100)	246, 295sh, 322, 328	Caffeoyl-ferulic acid derivative[c]	A2, C2, D2
28	17.32	411	433	163(75), 195(50), 249(15), 305(15), 411(100), 433(90)	163(100), 287(45), 433(65)	290, 322, 328	Caffeoyl-, (methyl ether-, acetyl-) quinic acid[b]	A4, B4, C4, D4
29	18.14	567	589	179(100), 567(10), 589(20)	133(100), 179(40), 589(35)	290sh, 310	Trihydroxycinnamic acid derivative[c]	A1
30	18.80	609	–	179(90), 243(120), 359(50), 609(100)	359(10), 609(100)	295sh, 322, 328	Trihydroxycinnamic acid derivative[c]	A1
31	18.87	411	433	163(88), 195(45), 411(100), 433(95)	163(100), 433(50)	290sh, 322, 328	Caffeoyl-, (methyl ether-, acetyl-) quinic acid[b]	A4, B4, C4, D4, E4
32	19.47	411	433	163(75), 195(50), 411(95), 433(100)	163(100), 433(80)	290sh, 322, 328	Caffeoyl-, (methyl ether-, acetyl-) quinic acid[b]	A4
33	19.57	497	519	157(100), 179(20), 497(20), 519(40)	157(40), 179(100), 519(50)	280, 332	Trihydroxycinnamoyl, gallic alcohol derivative[c]	A1
34	33.02	–	–	147(30), 361(40), 435(20), 453(60), 617(20), 635(100)	147(100), 203(80), 435(40)	295sh, 310	*trans-p*-Coumaric acid derivative[c]	B1, D1
35	33.12	–	–	277(50), 295(100), 409(50), 635(50)	147(50), 197(70), 409(100)	295sh, 312	*trans-p*-Coumaric acid derivative[c]	B1, D1
36	35.50	619	641	437(100), 455(40), 619(20), 641(10)	147(10), 641(100)	290sh, 308	*trans-p*-Coumaric acid derivative[c]	B1, D1
37	36.04	619	641	437(50), 601(20), 619(100)	147(50), 203(80), 437(20), 641(100)	290sh, 312	*trans-p*-Coumaric acid derivative[c]	B1, D1
38	36.27	619	641	147(10), 355(40), 377(40), 437(30), 601(10), 619(100)	147(60), 215(60), 641(100)	290sh, 312	*trans-p*-Coumaric acid derivative[c]	B1, D1

[a] Identified by comparison to authentic samples.
[b] Identified by the literature.
[c] Identified by the retention times, UV spectra, MS and proposed fragmentation mechanisms.

differences in their UV spectra, their molecular weights and their retention times, testify different substitutions.

Finally, well matured fruits of categories B and D, at the lipophilic area of 33.02–36.27 min R_t, have several intense peaks (nos. 34–38) that correspond to five substances of the same UV absorbance (295 sh, 310 nm). All substances have a main fragment ion of 147 amu = [*p*-coumaric acid-OH]$^+$. UV spectra show that the substances are *trans-p*-coumaric acid derivatives (Sakakibara, Honda, Nakagawa, Ashida, & Kanazawa, 2003; Waldron et al., 1996) with lipophilic substitutions.

3.1.2.2.2. Phenylpropanoid acids and derivatives in diethyl ether fractions. Category D contains two tentatively identified hydrocaffeoyl derivatives at R_t 4.57 (no. 10), and 11.03 (no. 23). They both give an intense fragment at 165 amu = [hydrocaffeic acid-OH]$^+$, as well as an intense fragment ion at *m/z* 183, which corresponds to the dihydrocaffeic acid molecular ion.

Fruit categories A, C and E contain a substance (no. 15) with retention time 7.21 min, UV absorption of a typical phenylpropanoid acid and a molecular ion $[M+H]^+$ 501. The units of this complexional substance seem to be two *p*-methoxy cinnamic acids that esterify a caffeoyl unit. Fragment 161 = [*p*-methoxy cinnamic-OH]$^+$ and 133 = [*p*-methoxy cinnamic-COOH]$^+$, while fragment at *m/z* 163 is a typical caffeoyl fragmentation.

Two *p*-coumaroyl, hydrosinapic acid ester derivatives (nos. 21, 22) were tentatively identified in fruit categories A, B and C. The first category contains the *cis-p*-coumaric acid isomer and the other two the *trans*-, according to the UV spectrum and the slight differences at the retention times (Waldron et al., 1996). Fragment at 227 amu corresponds to [hydrosinapic acid + H]$^+$ and 147 amu to [*p*-coumaric acid-OH]$^+$ (Maffei Facino et al., 1997).

Fruit categories A, C and D also contain a substance (no. 27) at R_t 14.83 and with $[M+H]^+$ 581, $[M+Na]^+$ 603. UV spectrum, $[M+H]^+$ and MS fragmentation show that the substance is a more lipophilic derivative of caffeoyl-ferulic acid, compound no. 25.

Diethyl ether fractions of all fruit categories contain several esters of cinnamoylquinic acids, some of which are dominant in the samples. The main substance of the diethyl ether fraction of category B is a caffeoyl-, dimethyl ether quinic acid derivative (no. 13) (Fang et al., 2002; Kim,

Table 3
Retention times (R.T.), molecular ions ($[M+H]^+$), sodium adducts ($[M+Na]^+$), fragment ions (20 eV and 80 eV), relative intensities % (R.I.%) and UV absorptions (λ_{max}) of all flavonoids and derivatives

No	R.T. (min)	$[M+H]^+$ (*m/z*)	$[M+Na]^+$ (*m/z*)	Fragment ion (*m/z*) at 20 eV (R.I.%)	Fragment ion (*m/z*) at 80 eV (R.I.)	UV λ_{max} (nm)	Identification	Samples (Table 1)
39	13.65	465	–	465(100)	153(20), 229(20), 303(100)	254, 269sh, 368	Quercetin 7-*O*-hexoside[c]	E3
40	14.58	627	–	331(100), 463(10), 545(20), 627(10)	331(100), 463(10), 545(30)	284, 335sh	3′-,4′-,7-Trimethoxy,5-hydroxy flavanone (sorbyl-) pentoside[c]	B4, D4
41	14.67	581	603	581(100), 603(60)	137(30), 229(20), 303(100), 603(20)	256, 265sh, 302sh, 354	Quercetin (3,7)-*O*-biglycoside (rhamnose, pentose)[c]	A3, C3, E3
42	14.95	627	649	181(10), 303(40), 331(100), 465(40), 487(80), 627(20)	137(45), 257(30), 303(65), 331(100), 487(25), 649(35)	256, 265sh, 316, 365sh	Quercetin 3-*O*-(hexosyl-) caffeoyl ester[c]	D2, B3
43	15.00	629	651	303(50), 465(100), 487(45), 641(5)	153(15), 229(20), 257(18), 303(100), 465(15), 487(95), 629(20)	256, 265sh, 305sh, 354	Quercetin 3-*O*-(sorbyl-) hexoside[c]	A2,3,4, B2,3,4, C2,3,4, D2,3,4, E2,3,4
44	15.24	569	–	569(100)	137(25), 285(40), 303(30), 569(100)	252, 354	Bis(-*O*-) quercetin dimmer ($-2H_2O$)[d]	D2
45	15.32	611	633	303(40), 465(7), 611(100), 633(25)	153(5), 229(10), 257(10), 303(100), 465(5), 611(5), 633(10)	256, 265sh, 302sh, 356	Rutin[a]	A4, B4, C4, D4, E4
46	15.42	611	633	303(50), 465(45), 487(20), 569(100), 611(20), 633(15)	153(10), 303(100), 487(65), 569(55), 611(15), 633(20)	254, 265sh, 305sh, 356	Quercetin 3-*O*-(benzoyl-, acetyl-) hexoside[c]	A2,3, B3, C2,3, D3, E3
47	15.93	595	617	303(30), 595(65), 617(100)	257(30), 303(100), 617(30)	256, 265sh, 300sh, 356	Quercetin 3-*O*-birhamnoside[c]	C3
48	16.01	551	573	287(100), 419(20), 551(10)	287(100), 419(80), 573(50)	256, 262, 312, 360	Kaempherol 7-*O*-dipentoside[c]	A3, B3
49	16.03	595	617	133(100), 161(85), 179(65), 303(25), 435(55), 457(35), 595(45), 617(50)	133(100), 161(50), 179(20), 303(70), 457(20), 617(50)	254, 256sh, 300sh, 358	Qurcetin 3-*O*-(*p*-methoxy cinnamoyl-) pentoside[c]	A2, C2, E3
50	16.67	449	471	287(85), 449(95), 471(100)	287(80), 449(25), 471(100)	252sh, 264, 300sh, 322sh, 348	Kaempherol 3-*O*-hexoside[c]	B3, C3, D3, E3
51	16.80	595	617	147(20), 165(15), 595(70), 617(100)	147(45), 164(30), 303(100), 617(30)	256, 262sh, 285sh, 362	Quercetin 7-*O*-(*cis*-*p*-coumaroyl) ester, 4″-*O*-rhamnoside[c]	A3
52	17.32	595	617	287(65), 449(100), 595(40), 617(40)	165(35), 287(100), 617(20)	252sh, 264, 304, 322sh, 346	Kaempherol 3-*O*-(*trans*-*p*-coumaroyl-) hexoside[c]	B3, C3, E3
53	17.40	595	617	449(80), 595(100), 617(50)	163(80), 287(100), 617(10)	254, 266, 300sh, 318sh, 332	Kaempherol 7-*O*-(rhamnosyl-) caffeoyl ester[c]	D3
54	17.90	625	–	147(20), 317(30), 479(45), 625(100)	163(50), 259(25), 301(45), 317(100)	256, 265sh, 302sh, 358	Isorhamnetin 3-*O*-(*p*-coumaroyl-) hexoside[c]	A4, C4, E4
55	18.42	569	591	245(25), 285(100), 307(10), 569(10)	245(40), 285(100), 307(80), 591(60)	254, 370	Bis(-*O*-)quercetin dimmer ($-2H_2O$)[d]	B2
56	18.47	461	–	285(100), 307(20), 461(30)	217(30), 285(50), 307(100), 461(30)	246, 270, 320	Acacetin 7-*O*-feruloyl ester[c]	E3
57	18.61	569	591	285(100), 307(30), 569(10)	307(100), 591(50)	264, 292sh. 334, 348sh	Bis(-*O*-)quercetin dimmer ($-2H_2O$)[d]	D2
58	19.42	303	–	303(100)	229(40), 257(50), 303(100)	254, 265sh, 300sh, 370	Quercetin[a]	A2,3, C2,3, E2,3
59	19.60	569	591	285(100), 307(40), 569(10), 591(15)	307(100), 591(40)	254, 265sh, 370	Bis(-*O*-)quercetin dimmer ($-2H_2O$)[d]	C2
60	22.56	537	559	269(100), 559(20)	269(100)	260, 300sh, 354	Bis(-*O*-)kaempherol dimmer($-2H_2O$)[d]	E2
61	25.34	583	–	283(100), 583(20)	283(100)	246, 256, 300sh, 350	7-*O*-Methyl kaempherol dimmer ($-H_2O$)[d]	E2
62	24.88	603	–	271(40), 311(35), 603(100)	273(100), 303(80), 313(15)	266, 302, 364	Quercetin 2-4′(-*O*-), 3,5′(-*O*-) dimmer[b]	C5

[a] Identified by comparison to authentic samples.
[b] Identified by the literature.
[c] Identified by the retention times, UV spectra, MS and proposed fragmentation mechanisms.
[d] Proposed structures tentatively identified.

Iwai, & Matsue, 2005), R_t 6.71 min. Caffeic acid gives a typical fragment at 163 amu, while fragment ion at 203 amu is [dimethyl ether quinic acid-OH]$^+$. Fruit categories A, C and E contain a biester of Quinic acid with a *p*-coumaric and a caffeic acid (no. 18) with R_t 9.35 min (Clifford, Knight, Surucu, & Kuhnert, 2006; Del Rio et al., 2004). $[M+H]^+$ is 501. The caffeoylquinic acid mass defect from the molecular ion is 146 U, which corresponds to [*p*-coumaric acid-OH]$^+$.

Unripe fruits also contain two phenylpropanoic esters at R_t 10.08 (no. 20) and 12.18 (no. 24) min. The UV and MS characteristics show two feruloylquinic acid esters (isomers) (Fang et al., 2002; Sanchez-Rabaneda et al., 2004). $[M+H]^+$ is 369 and $[M+Na]^+$ 391. Fragment at *m/z* 194 corresponds to the molecular weight of ferulic acid (homolytic mechanism), *m/z* 177 [Ferulic acid-OH]$^+$ and *m/z* 179 [ferulic acid-CH_3]$^+$, loss pre mentioned in the literature (Maffei Facino et al., 1997).

3.1.2.2.3. Cinnamic acids and derivatives in ethyl acetate fractions. All ethyl acetate, as well as all butanol and water fractions contain chlorogenic acid identified by comparison to the authentic sample (no. 12).

At 7.12 and 10.08 min two isomer compounds come out in all fruit categories (no. 14 and no. 20). The two compounds correspond to two feruloylquinic acid isomers, as described above. It is worth mentioned that isomers of quinic acid esters with ferulic acid have been mentioned in the literature for several natural products' extracts (Clifford et al., 2006). For unripe fruits, one of the main substances is a caffeic acid derivative (no. 17), tentatively identified from the R_t 8.35 min, $[M+1]^+$ 291 and $[M+Na]^+$ 313 and from the typical fragment of the [caffeic acid-OH]$^+$ at 163 amu at 80 eV.

3.1.2.2.4. Cinnamic acids and derivatives in butanol and water fractions. Butanol fractions of all fruit categories are dominant in quinic acid esters with cinnamic acids. Two hexosilated caffeoylquinic acids come at 2.10 (no. 1) and 2.99 (no. 6) min, with $[M+1]^+$ 517. At 6.48 min, and compared to our standard, all fruit categories contain chlorogenic acid (no. 12) and at 5.70 min a caffeoylquinic acid (no. 11). Two more caffeoylquinic acids come out at R_t 3.00 min (no. 7) and R_t 7.71 min (no. 16) (Clifford et al., 2006; Del Rio et al., 2004; Kim et al., 2005; Sanchez-Rabaneda et al., 2004). Substances no. 12 and no. 7 are also present in the water fractions.

A common substance for all *n*-butanolic extracts (no. 9) comes out at 4.33 min with the same UV spectrum and the characteristic fragment *m/z* 163. $[M+1]^+$ is 259 and the molecule is tentatively characterised as caffeic acid derivative.

At 17.32 min and 18.87 all *n*-butanolic fractions contain two isomer substances (nos. 28 and 31), tentatively identified as caffeoyl-, (methyl ether-, acetyl-) quinic acids. *m/z* 163 corresponds to [caffeic acid-OH]$^+$. $[M+1]^+$ is 411 and it is stabilized giving the sodium adduct ion at *m/z* 433. Unripe fruits and fruits matured for one week at room temperature also contain a third isomer of the molecule at R_t 19.47 min (no. 32).

3.1.3. LC–DAD–MS analysis of flavonoids and derivatives (Table 3)

Most of the flavonoids detected were flavonols (especially quercetin), glycosides and dimmers. The greatest varieties of the flavonoids were detected in the diethyl ether and ethyl acetate fractions. Butanol fractions also contain some flavonol derivatives. It is worth mentioned that unripe fruits (A) and fruits matured for one week at room temperature (C), categories that had very good antioxidant capacity (Termentzi et al., 2006), in most cases share the same flavonol constituents. Fruit pulp (E) also contains great variety of flavonoids.

3.1.3.1. Quercetin aglycon, glycosides and esters. Quercetin aglycon (no. 58), identified in comparison to the standard, is present in the diethyl ether and ethyl acetate fractions of fruit categories A, C and E. A proposed fragmentation of the molecule is given in Fig. 1 (Fabre, Rustan, Hoffmann, & Quetin-Leclercq, 2001; Hakkinen & Auriola, 1998; Ma, Li, Van de Heuvel, & Claeys, 1997).

Fruit categories A, C and E except for quercetin aglycon also contain two more common quercetin glycosides. The first one (no. 41) at R_t 14.67 min is found in the ethyl acetate fractions and is tentatively identified as a biglycoside of quercetin (3,7)-*O*-(rhamnose, pentose). According to the UV spectrum, the bonded hydroxyl groups are 3,7-OH (Mabry, Markham, & Thomas, 1970). The existence of a rhamnose and a pentose on the molecule is explained from the molecular ion (*m/z* 581), without exact localization of the two sugar moieties. The second common molecule (no. 49) is a quercetin 3-*O*-(*p*-methoxy cinnamoyl-) pentoside ($[M+H]^+ = 595$). Band I at 358 nm shows that the pentose is bonded on the 3-OH of quercetin. It also proves that the acid esterifies the sugar and not the aglycon. $435 = [\text{Quercetin pentoside} + H]^+$ and $457 = [\text{Quercetin pentoside} + Na]^+$. $179 = [p\text{-methoxy cinnamic acid} + H]^+$, $161 = [p\text{-methoxy cinnamic acid-OH}]^+$ and $133 = [p\text{-methoxy cinnamic acid-COOH}]^+$.

Diethyl ether, ethyl acetate and butanol fractions of all fruit categories contain a quercetin 3-*O*-(sorbyl-) hexoside, at R_t 15.00 (no. 43). Comparing the UV absorption, the R_t and the MS fragmentation to rutin (authentic sample), we conclude that the molecule is a quercetin biglycoside with a glucose as the sugar bonded on the aglycon (*m/z* 465 and $487 = [465 + Na]^+$). The fact that $[M+H]^+$ is 629 amu and of a very low intensity is explained by the presence of a sorbitol moiety attached to the glucose. Sorbitol is a polyol of 182 molecular weight, found in large quantities in the relative species' *Sorbus aucuparia* fruits (Brunetton, 1993). It is less stable in comparison to sugars, due to its linear structure, giving a weak molecular ion. At 80 eV fragments at *m/z* 153, 229 and 257 are fragments of quercetin (Fig. 1). It is also worth mentioned that in all fruit categories this substance is a main constituent, except from fruits well matured on tree (B) where it was found in traces.

All butanol fractions contain rutin (no. 45), identified by comparison to the authentic sample.

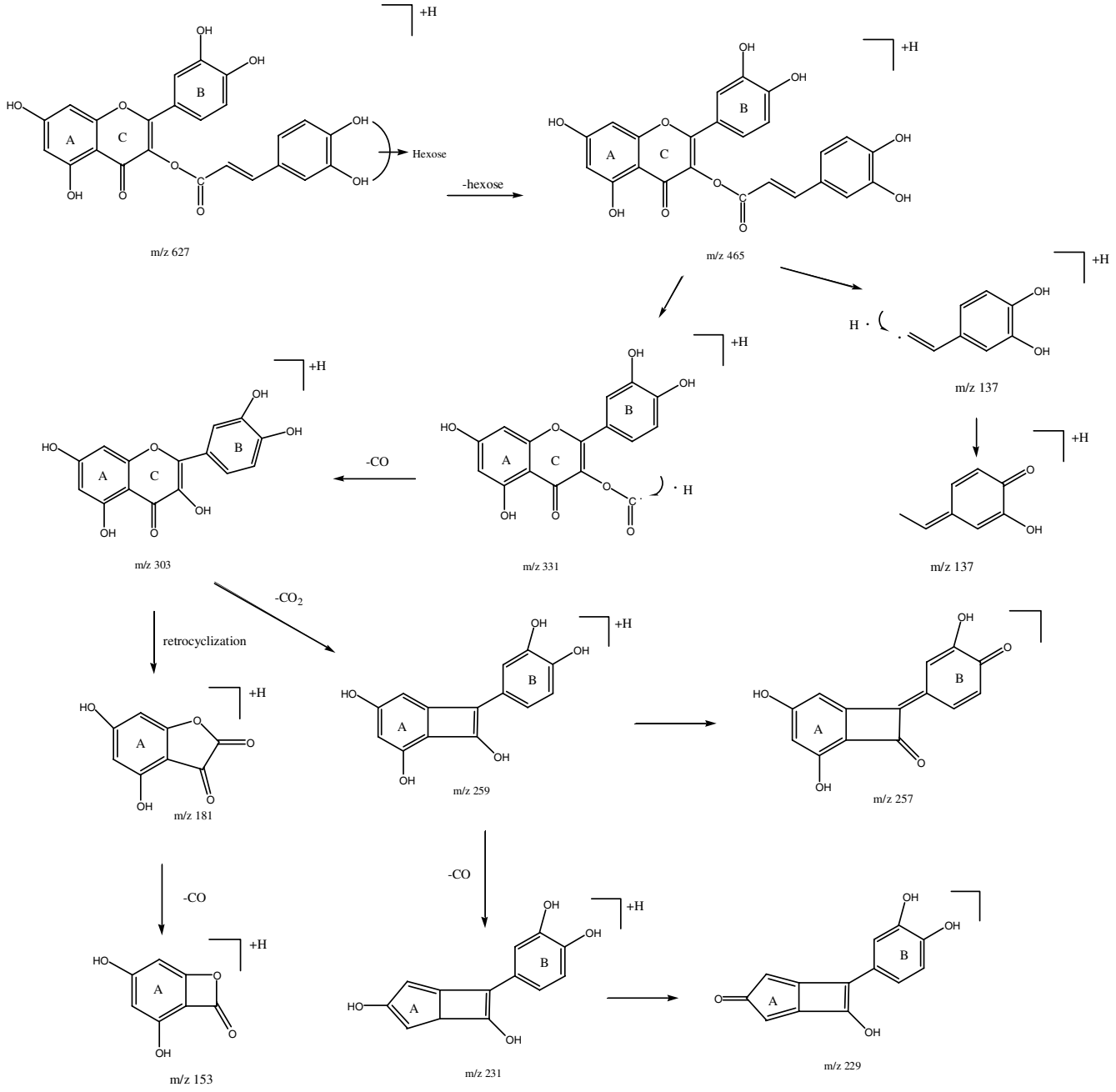

Fig. 1. Proposed fragmentation mechanism of quercetin 3-*O*-(hexosyl-) caffeoyl ester and quercetin aglycon for ESI+.

Diethyl ether and ethyl acetate fractions of fruit categories A, B and C and ethyl acetate fractions of D and E contain a quercetin derivative (no. 46), R_t 15.42 min, with $[M+H]^+$ 611. Fragments $569 = [M-CH_3CO+H+H]^+$, $465 = [M-CH_3CO-\text{Benzoic acid}+OH+H+H]^+$, and $465 = [\text{quercetin hexoside}+H]^+$. The molecule is tentatively identified as quercetin 3-*O*-(benzoyl-, acetyl-) hexoside.

Ethyl acetate fraction of fruits matured for one week at room temperature (C) contains a quercetin 3-*O*-birhamnoside (no. 47) with $[M+H]^+$ 595 and $[M+Na]^+$ 617. The shift of the first band of the UV spectrum indicates that the sugars are bonded on the 3-OH of the aglycon.

Ethyl acetate fraction of unripe fruits (A) contains a quercetin 7-*O*-(rhamnosyl-) *cis*-*p*-coumaroyl ester (no. 51), (tentatively identified). The substance comes out at 16.80 min, almost 1 min later than quercetin birhamnoside. The two substances have the same molecular ion. The decrease at the intensity of band I together with the 8 nm hypsochromic shift compared to a quercetin 7-*O* glycoside (Mabry et al., 1970) lead to the conclusion that a *p*-coumaric acid esterifies the 7-OH group of the aglycon ([coumaric acid+H]$^+$ 165 and [coumaric acid-OH]$^+$ 147 amu). According to the UV spectra of this compound the shoulder at 285 nm indicates that the *p*-coumaric acid is the *cis*-isomer

(Waldron et al., 1996). Thus, the rhamnose is attached to the phenolic group of the *cis-p*-coumaric acid.

Ethyl acetate fractions of fruit pulp (E) contain a quercetin-*O*-hexoside (no. 39), at R_t 13.65 min, UV spectrum 254, 269sh, 368nm and $[M+H]^+$ 465. The UV spectrum shows that the glucose is on 7-OH group and that 3-OH is uncommitted.

Finally, fruit categories B and D, which both are well matured contain at R_t 14.95 min, (no. 42) an hexosilated caffeoyl ester of quercetin $[M+H]^+ = 627$. The UV absorption (316 nm) has a great hypsochromic shift (Gabrieli & Kokkalou, 1990; Harborne, 1999) compared to that of quercetin aglycon, indicative for flavonol 3-*O*-esters, 331 = [quercetin+CO+H]$^+$, after the fragmentation of the acid and 181 = [caffeic acid+H]$^+$. A proposed fragmentation mechanism is given in Fig. 1.

3.1.3.2. Quercetin dimmers. All quercetin dimmers were found in diethyl ether fractions and one in a water fraction. Unripe fruits do not contain dimmers.

Fruit categories B, C and D contain four similar quercetin dimmers (nos. 44, 55, 57, 59), but with significant differences at their R_t (15.24, 18.42, 18.61 and 19.60 min). They all have $[M+H]^+$ 569 and $[M+Na]^+$ 591. They all also have two characteristic fragments at 285 and $[285+Na]^+ = 307$. 285 = [quercetin-OH]$^+$. The dimmer is produced by two quercetin molecules after the loss of two H_2O molecules. The UV absorption shows that for the second and the fourth molecule the two monomers are bonded on non active hydroxyl groups, while the first and the third have active hydroxyl groups committed, as there are significant hypsochromical shifts at the UV spectra.

Fruits matured for one week at room temperature contain in their aqueous fraction a quercetin dimmer (no. 62, R_t 24.88 min), pre mentioned in the literature as a natural (Ly et al., 2005) and as a synthetic product (Krishnamachari, Levine, & Pare, 2002). In this case the substance is a probable artefact since, although is it quite lipophilic, it was found in the water fraction.

3.1.3.3. Kaempherol glycosides, ester. Three kaempherol glycosides and one kaempherol glycosilated ester with cinnamic acid were found, all in the ethyl acetate fractions.

Fruit categories A and B contain a kaempherol 7-*O*-dipentoside at R_t 16.01 min (no. 48) with $[M+1]^+$ 551 and $[M+Na]^+$ 573. The position of glycosilation comes up from the UV absorbance (Mabry et al., 1970). 287 = [M−pentose−pentose+H]$^+$ corresponds to the molecular weight of kaempherol.

All fruit categories except for unripe fruits contain a kaempherol 3-*O*-glucoside (no. 50) at R_t 16.67 min. The identification was achieved by the UV (Mabry et al., 1970) and the MS spectrum, $[M+H]^+$ 449 and $[A+H]^+$ 287.

Fruit categories B, C and E contain a kaempherol 3-*O*-(*trans-p*-coumaroyl) hexoside (no. 52). UV spectrum is typical of kaempherol 3-*O*-hexoside but $[M+H]^+ = 595$. Fragment 165 = [coumaric acid+H]$^+$. The *trans*-isomer of the acid results from a distinct absorbance at 304 nm (Waldron et al., 1996).

Category D contains a kaempherol 7-*O*-(rhamnosyl-) caffeoyl ester (no. 53). The position of esterification is concluded by the hypsochromic shift of band I (332 nm) (Gabrieli & Kokkalou, 1990) and the identity of the acid by the characteristic fragment *m*/*z* 163. The mass defect from the molecular ion *m*/*z* 595 shows that the sugar is rhamnose.

3.1.3.4. Kaempherol dimmers. The diethyl ether fractions of fruit pulp contains two kaempherol dimmers (tentatively identified), expressed as two intense peaks at 22.56 (no. 60) and 25.34 min (no. 61). The first substance is tentatively identified as kaempherol dimmer (–$2H_2O$) with $[M+H]^+$ 537. The position of the union is not possible to be allocated. Intense fragment at 269 is [Kaempherol-OH]$^+$. The two kaempherol molecules are bonded on two –OH groups. The second substance is tentatively identified as a dimmer of 7-*O*-methyl kaempherol with $[M+H]^+$ 583. The dimmer is formatted after the loss of one H_2O molecule. Intense peak at *m*/*z* 283 is [7-*O*-methyl kaempherol-OH]$^+$.

3.1.3.5. Other flavonoids. The ethyl acetate fraction of fruit pulp (E), which generally is differentiated in content from the other fruit categories, also contains an acacetin derivative with $[M+H]^+$ 461, $[A+H]^+$ 285 and $[A+Na]^+$ 307 (no. 56). There is an 8 nm hypsochromic shift of Band I compared to the 7-*O*-glycoside of acacetin (Mabry et al., 1970). [M−A] = 176. All these lead to the conclusion that a ferulic acid esterifies the 7-OH group of the aglycon.

The *n*-butanolic fractions of categories A, C and E contain an Isorhamnetin 3-*O*-(*p*-coumaroyl-) hexoside (no. 54), at R_t 17.90 min. UV spectrum is identical of an isorhamnetin 3-*O*-glycoside (Mabry et al., 1970) and $[M+H]^+$ is 625. 479 = [M−coumaric acid+OH+H]$^+$. 147 = [coumaric acid-OH]$^+$ and is esterified on the sugar. Fragment at 301 amu corresponds to the –CH_3 detachment of the methoxy group of isorhamnetin aglycon ([A−CH_3]$^+$).

Finally *n*-butanol fractions of the most matured fruits B and D contain at R_t 14.58 a 3′-,4′-,7-trimethoxy,5-hydroxy flavanone (sorbyl-) pentoside (no. 40) with a typical flavanone UV (Mabry et al., 1970) and $[M+H]^+$ 627. $[A+1]^+$ is 331. Fragment ion at 463 amu is a pentose adduct and 627 an addition of a sorbitol molecule.

3.2. Quantitative analysis

Benzoic acids and benzoic acid derivatives are expressed as vanillic acid equivalents, cinnamic acids as caffeic acid equivalents and phenylpropanoylquinic esters as chlorogenic acid equivalents. Flavonols are expressed as quercetin

Table 4
Quantitative determination of the phenolic content (phenolic acids and flavonoids) of the five maturity stages

	Maturity stages				
	Unripe	Well matured on tree	Matured for 1 week (room temperature)	Matured for 3 weeks (room temperature)	Fruit pulp
Benzoic acids and derivatives[a]	4.17	8.94	4.57	7.09	2.31
Cinnamic acids and derivatives[b]	10.55	9.91	14.24	12.19	2.55
Total phenolic acids	14.72	18.85	18.18	19.28	4.86
Flavonoid glycosides/esters[c]	7.46	2.72	8.83	1.99	7.17
Flavonoid aglycons/dimmers[d]	1.22	0.36	1.46	0.46	0.73
Total flavonoids	8.68	3.08	10.59	2.45	7.9

[a] Benzoic acids and derivatives are expressed as vanillic acid equivalents.
[b] Cinnamic acids and derivatives are expressed as *trans-p*-coumaric acid equivalents, caffeic acid equivalents and chlorogenic acid equivalents in cases of esters with quinic acid.
[c] Flavonoid glucosides are expressed as rutin equivalents and flavonoid esters as 7-methoxy flavone equivalents.
[d] Flavonoid aglycons and dimmers are expressed as quercetin derivatives.

equivalents and flavonol glycosides as rutin equivalents. In all fruit categories, apart from fruit pulp, phenolic acids are dominant compared to flavonoids (Table 4).

Fruit pulp contains the lowest quantities of acids. Almost half of the acids are benzoic acids and derivatives and the other half phenylpropanoic acids and derivatives. Compared to the rest fruit categories, unripe fruits contain the lowest quantities of acids with the cinnamic acids dominant compared to the benzoics. As maturation proceeds concentration of acids increases, especially when fruits mature at room temperature. Though, while maturation on tree gives rise to benzoic acids and phenylpropanoic acid concentration remains at the same levels with unripe fruits, maturation at room temperature stimulates the increase of cinnamic acids, preserving the benzoic levels at almost the same concentration with the unripe fruits.

On the other hand, well maturated fruits, both on tree and at room temperature have low concentrations of flavonoids compared to the unripe fruits. However maturation at room temperature favours the formation of dimmer aglycons. Flavonoids to increase during the first stages of maturation at room temperature, while fruit pulp conserves almost the same amounts with unripe fruits. At all fruit types flavonoid aglycons and dimmers were at least four times less than the corresponding glycosides.

4. Discussion

It was proved that *Sorbus domestica* fruits are rich in phenolics. The comparison between the different maturity stages showed that there is small, but significant qualitative differentiation between the phenolic content of different maturity stages, and clear quantitative differentiation.

All fruits contain the same benzoic acids and derivatives. However fruit categories A and C, which are the Unripe categories, contain, compared to the Well matured categories B and D, almost half benzoic constituents. As maturation proceeds the generation of benzoic acids is favoured. Fruit pulp contains by far the less benzoyl compounds.

Concerning the phenylpropanoids, all fruit categories in common almost half of the cinnamoylquinic acids mentioned. The Unripe categories A and C contain glycosilated caffeoylquinic acids, substances not present in the Well matured categories B and D and in fruit pulp. Well matured categories B and D contain all the dihydrocinnamic acid derivatives (nos. 10, 21, 22, 23), fact that is somehow expected through the maturation processes. The same types of fruits (B and D) contain very lipophilic coumaroyl derivatives, substances that must get formed with maturation. Category B has caffeoyl dimethyl ether quinic acid as main constituent. The quinic acid methylation follows the maturation process. Unripe fruit categories (A and C) and Fruit pulp (E) share some common phenylpropanoic substances, such as nos. 15 and 18, both main peaks in our samples (Fig. 2). Unripe fruits contain several phenylpropanoids that are absent in all other categories (nos. 17, 24, 32 and the three trihydroxycinnamoyl derivatives). These substances disrupt with maturation. Finally, fruit pulp, lacks in some phenylpropanoids (ferulic acid derivatives, nos. 25, 26, 28), which are present in all other categories. The quantification of the phenylpropanoic acids showed that maturation at room temperature favours their formation (Table 4).

Concerning flavonoids, all fruit categories contain three common quercetin glycosides (nos. 43, 45, 46). Not mature categories A and C, together with fruit pulp (E), categories proved to have good antioxidant power, contain some common flavonoids (nos. 41, 49, 54, 58), that are absent in the well matured categories B and D, categories that generally did not have strong antioxidant capacity. It is also obvious from the samples' content that prolonged maturation favours the formation of some esters on the aglycons (no. 42) and on the flavonoid sugars (nos. 52, 53), probably due to enzymic action during the maturation. Flavonol dimmers were found in all fruit categories except the

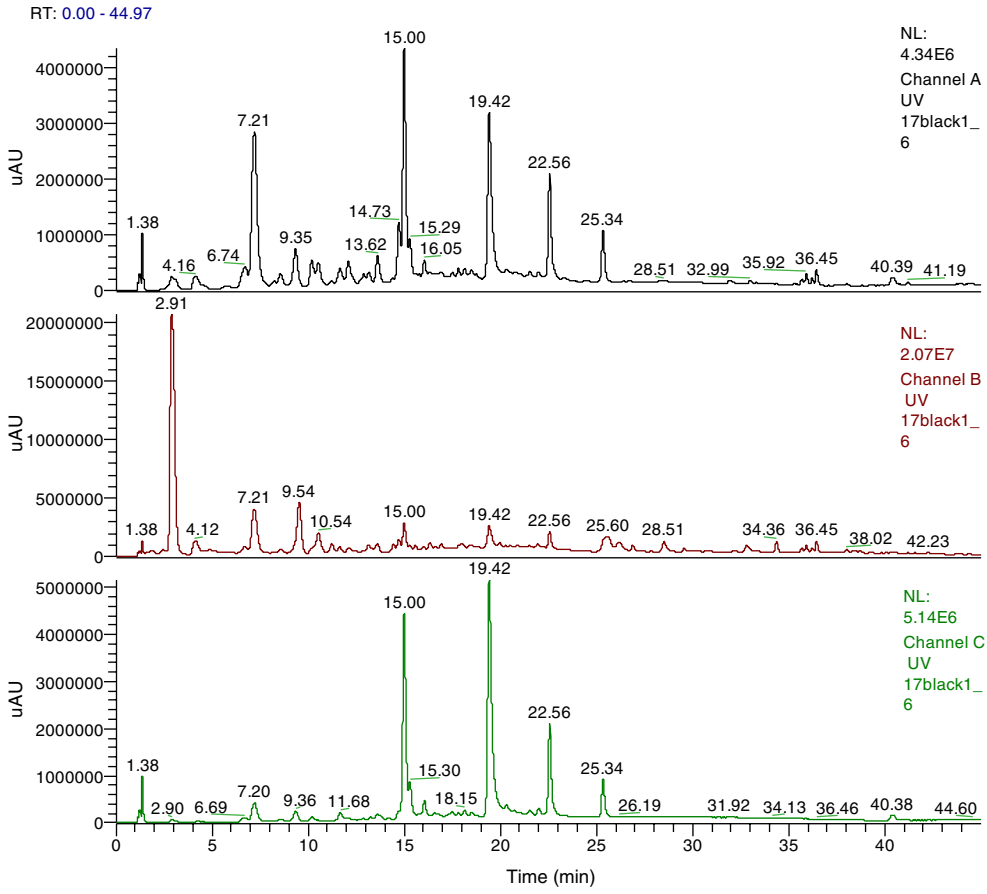

Fig. 2. HPLC chromatogram of the diethyl ether fraction of the fruit pulp (E) of *Sorbus domestica* fruits at 340, 290 and 365 nm, respectively.

Unripe fruits. That observation suggests that maturation provokes their formation. Further investigation could show whether these dimmers are artefacts due to the conditions of maturation (dehydration or condensation). Quantitative analysis showed that flavonoid concentration diminishes with maturation.

Procyanidins were not present in samples, with the exception of some traces detected during the HPLC analysis and only by their UV spectrum.

This is the first detailed phytochemical analysis of many types of *Sorbus domestica* fruits. It was proved that the fruits are rich in phenolic constituents, that justify the good in vitro antioxidant capacity, especially for unripe fruits and fruits matured for one week at room temperature, as mentioned in our previous research work. These categories can be used as a rich source of natural antioxidants in the daily diet. However it is worth mentioned that fruit pulp, that was one of the best antioxidants, has a very low conciseness in both flavonoids and phenolic acids. This category has a differentiation in the content, especially for flavonoids, from the other fruit categories, and possibly substances like nos. 39, 56, 60, 61 and 58 (which is present in this category in large quantities, Fig. 2) are responsible for this antioxidant power.

Finally, it is worth mentioned that fruit category D, which is the form that people traditionally consume *Sorbus domestica* as hypoglycaemic agent, is the type of fruits that contains the most phenolic acids and the less flavonoids. A conjecture that could explain the use of this particular form as a traditional medicine against type-2 diabetes is that quercetin, which is the dominant flavonoid in our samples, could act as a prooxidant, to the opposite direction (Choi, Chee, & Lee, 2003). Phenolic acids on the other hand are proved to be beneficial as hypoglycaemic agents (Adisakwattana, Roengsamran, Hsu, & Yibchok-anun, 2005; Shearer et al., 2003).

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