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Antioxidant capacity and major phenol compounds of horticultural plant materials not usually used

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2,4,6-Tripyridyl-s-triazine (TPTZ) (PubChem CID: 77258)
6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (PubChem CID: 40634)
Acetic acid (PubChem CID: 176)
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ABSTRACT

Horticultural plant materials not usually used from onion, carrot, beetroot, sea buckthorn, black and red currants as well as a wastewater powder from olive oil production were analyzed for total phenols content (FC), ferric reducing ability of plasma (FRAP), radical scavenging capacity (ABTS), and for major phenolic compounds by HPLC-MS. Antioxidant capacity and phenol content varied significantly between species and cultivars, with extracts of sea buckthorn leaves being superior. In different species, different phenolic compounds were closely associated with FRAP, ABTS and FC. For instance, hydrolysable tannins were major antioxidants in sea buckthorn whereas quercetin was the major antioxidant in onion peel and skin. This study shows that horticultural plant materials usually left in the field or waste materials from processing may have high antioxidant properties, and that extracts of these materials therefore could be of potential interest for development of antioxidant food additives.

1. Introduction

Numerous fruits and vegetables are rich in antioxidants. However, material left behind at harvest and after food processing may contain even higher amounts of natural antioxidants than the part of the vegetable or berry usually consumed (Telesko & Wojdyło, 2015). Leaves of several berry species, such as sea buckthorn (*Hippophae rhamnoides*) (SBT) and black currant (*Ribes nigrum*), have e.g. been shown to be rich in antioxidant compounds (Morgenstern, Ekholm, Scheewe, & Rumpunen, 2014; Vagiri et al., 2015). Onion (*Allium cepa*) peel and outer layers (skin) are typically removed and discarded in industrial food production although these waste materials might have high antioxidant properties as well (Gawlik-Dziki et al., 2015). Leaves from carrots (*Daucus carota*) are a source of antioxidants (Yanishlieva-Maslarova & Heionen, 2001), as are several parts of the beetroot (*Beta vulgaris*), for instance the pomace (Vulić et al., 2014) and the juice (Wootton-Beard & Ryan, 2011). Olive (*Olea europaea*) mill wastewater, a rest product from olive oil production, has very high antioxidant

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capacity (Rubio-Senent et al., 2015) and is especially rich in the phenolic compound hydroxytyrosol (Frascari et al., 2016). However, very little information is available concerning the variation in content of total and specific major phenols in the plant materials of the mentioned species.

In this study, different horticultural plant materials not usually used were collected and analyzed for content of total phenols. Superior cultivars were then selected for analyses of antioxidant properties (radical scavenging capacity and ferric reducing ability) as well as for identification of major polyphenols that contribute to the antioxidant properties.

2. Material and methods

2.1. Plant material and sample preparation

In July 2015, leaves were collected from sea buckthorn (*Hippophae rhamnoides*), black currant (*Ribes nigrum*) and red currant (*Ribes rubrum*) plants grown at the Department of Plant Breeding, Balsgård, Swedish University of Agricultural Sciences, Sweden. For every species, 15 cultivars were sampled (Table 1 and Supplemental Table 1). For each cultivar, two biological replicates were collected, each consisting of ten fully mature leaves picked from the middle part of five shoots per plant. The leaves were air dried in a ventilated convection oven at 30 °C for 3 days, milled into powder with an analytical mill (Yellow line, A10, IKA-Werke, Staufen, Germany) and frozen in −20 °C until further analysis. The dry weight (DW) was defined as the weight of the thawed powder.

In September 2015, leaves of carrots (*Daucus carota*) and beetroots (*Beta vulgaris*), as well as whole yellow onions (*Allium cepa*), were collected from commercial fields in the south of Sweden. Carrot leaves were sampled at Nyskördade morötter AB, Fjälkinge, beetroot leaves at Alléns organic farm, Vittskövle, and onion bulbs at Åhus grönt, Horna gård, Åhus. For each species three cultivars were collected (Table 1). The plant material was washed in cold water post-collection. The carrot and beetroot leaves were then dried in a ventilated convection oven at 30 °C for 3 days. The onion bulbs were first put to dry in a sunny area for 2 days at ambient temperature to mimic a natural post-harvest drying in the field. The onion bulbs were then peeled, and the outer skin and outer peel layers were separated and dried at 30 °C for 7 days. Finally, the plant material was milled and stored frozen until analysis similarly as the berry leaves.

A commercially available olive (*Olea europaea*) polyphenol powder (Lundoliv P1100, Phenoliv AB) obtained from olive oil production wastewater was included in the study for comparison.

2.2. Chemicals

Folin-Ciocalteu's phenol reagent, gallic acid, sodium carbonate, ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), sodium carbonate monohydrate, sodium acetate, formic acid (pro analysis, 98–100%) and sodium persulfate were obtained from Sigma-Aldrich Inc. St. Louis, MO, USA. TPTZ (2,4,6-tripyridyl-s-triazine), ascorbic acid, iron (III) chloride hexahydrate, iron (II) sulfate and sodium phosphate were obtained from Fluka, Switzerland and hydrochloric acid (40 mmol/L), acetic acid, methanol (for liquid chromatography), acetonitrile (isocratic grade for liquid chromatography), 85% ortho-phosphoric acid were obtained from Merck KGaA, Darmstadt, Germany.

The HPLC standards neo-chlorogenic acid, cynarin, epigallocatechin, isorhamnetin-3-*O*-glucoside, isorhamnetin-3-*O*-rutinoside, kaempferol, kaempferol-3-*O*-glucoside, kaempferol-3-*O*-rutino-

Table 1
Total phenols (FC), ferric reducing ability of plasma (FRAP) and radical scavenging capacity (ABTS) for samples selected for further analyses of antioxidant capacity with standard deviation (SD) for two biological replicates (n = 2) for each sample (except for olive mill wastewater powder for which the standard deviation is presented for two technical replicates). GAE = gallic acid equivalents. TEAC = Trolox equivalent antioxidant capacity.

Species and plant material	Cultivar name	FC (mg GAE/g DW)	SD	FRAP (mmol Fe^{2+}/100 g DW)	SD	ABTS (mmol TEAC/100 g DW)	SD
Sea buckthorn leaves	'Finskaja'	153.6	32.4	231.8	2.6	191.8	33.2
	'Mary'	149.2	16.6	188.7	19.1	186.2	4.0
	'Prozrastjnaja'	142.5	35.8	152.6	7.4	191.1	32.5
	EIR 'BHi 727102'	140.9	16.7	206.0	26.0	181.6	24.7
	'Tatjana'	133.6	5.8	166.8	2.6	171.8	5.2
	'Ljublitelskaja'	133.3	18.5	147.4	6.9	162.9	28.2
Black currant leaves	'Ben Finlay'	97.3	0.6	118.1	8.5	108.9	0.3
	'Chernyi Zhemchug'	86.1	0.0	108.2	0.9	106.3	3.4
	'Ben Alder'	85.3	5.1	104.4	0.3	106.3	3.1
	'Ben Hope'	83.2	2.8	101.2	4.5	94.3	8.6
	'Ben Klibreck'	82.6	3.0	95.7	1.5	98.3	2.5
Red currant leaves	'Röda Versailler'	79.8	3.9	101.4	4.0	96.4	0.6
	'Vit jätte'	75.5	4.8	91.4	0.5	89.8	5.1
	'Rondom'	72.0	5.6	80.5	5.4	82.7	2.8
	'Red Lake'	68.5	10.9	79.1	10.8	76.4	1.4
	'Blanka'	67.1	1.2	77.6	1.1	74.0	12.8
Onion peel	'Donna'	51.1	15.2	18.7	5.4	29.5	9.3
	'Barito'	50.6	6.7	15.7	0.6	26.9	1.8
	'Hy Park'	27.8	2.6	9.1	2.3	12.6	1.7
Onion skin	'Donna'	68.2	8.4	43.6	4.4	63.4	8.3
	'Barito'	54.7	1.6	28.2	1.8	45.5	0.2
	'Hy Park'	57.7	10.9	28.8	6.8	43.4	10.8
Carrot leaves	'Nairobi'	17.6	0.9	15.0	1.7	21.4	13.2
	'Romans'	17.6	2.9	15.5	2.7	13.0	0.9
	'Napoli'	16.2	0.8	14.6	1.8	11.1	1.2
Beetroot leaves	'Action'	19.0	1.8	10.6	1.7	10.0	1.3
	'Storuman'	16.6	2.4	10.7	3.9	9.3	3.3
	'Forono'	16.2	0.4	8.2	1.5	8.4	2.4
Olive mill wastewater powder	Phenoliv™	68.2	0.2	102.3	4.9	33.7	0.0

side, quercetin-3-*O*-galactoside, and quercetin-4-*O*-glucoside were obtained from Extrasynthèse, Genay, France. Caffeic acid, p-hydroxybenzoic acid, protocatechuic acid, quercetin-3-*O*-glucoside, rutin and vanillic acid were obtained from Sigma-Aldrich Inc St. Louis, MO, USA. 2,4,5-trihydroxybensaldehyd, 3,4-dihydroxyphenylacetic acid, catechin, chlorogenic acid, epicatechin, ferulic acid, 3-hydroxytyrosol, p-coumaric acid, quercetin, quercetin-3-*O*-malonyl-glucoside, syringic acid and tyrosol were obtained from Sigma-Aldrich Inc. St. Louis, MO, USA.

2.3. Sample extraction

Samples were extracted by adding 1.5 mL 50% ethanol containing 0.05 M ortho-phosphoric acid to 50 mg of leaf powder in 2 mL Eppendorf tubes. The tubes were vortexed (Janke & Kankel, Staufen, Germany) for 10 s before being put in an ultrasonic bath (Bandelin Sonorex, Berlin, Germany) at ambient temperature for 15 min. The samples were then centrifuged for 10 min at 16 g and the supernatants were transferred into new tubes. A dry matter analysis was carried out for both extracts and dry powders where the samples were weighed before and after drying at 102 °C for 16 h.

2.4. Analysis of total phenols content - FC

The total phenols content of all samples was analyzed following the method of Morgenstern et al. (2014) based on the work of Singleton, Orthofer, and Lamuela-Raventós (1999). Thus the Folin-Ciocalteu (FC) reagent was used and results were calculated with gallic acid as standard. The six best performing cultivars of sea buckthorn and five best performing cultivars of red and black currants, respectively, were chosen for further detailed analyses. For vegetables samples, all cultivars available were further analyzed.

2.5. Analysis of radical scavenging ability - ABTS

The radical scavenging ability of the extracts was measured using the ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) method of Re et al. (1998). The radical scavenging ability was calculated as TEAC (Trolox equivalent antioxidant capacity).

2.6. Analysis of ferric reducing ability of plasma - FRAP

The Ferric Reducing Ability of Plasma (FRAP) method was conducted according to Morgenstern et al. (2014) based on the work of Benzie and Strain (1996). The absorbance of each sample was measured at 593 nm after 4 min in a plate reading spectrophotometer (Tecan Sunrise).

2.7. Analysis of ascorbic acid

Since the content of ascorbic acid could possibly bias the results of the total phenols content analysis by contributing to the antioxidant capacity of the extracts of sea buckthorn, red currant and black currant leaves, an analysis of ascorbic acid was also conducted. For the extraction of ascorbic acid, 15 mL of 2% metaphosphoric acid was added to 0.05 g leaf powder in a test tube. The extraction occurred in an ultrasonic bath (Bandelin Sonorex, Berlin, Germany) at ambient temperature for 10 min. The samples were then centrifuged at 4000 rpm for 10 min before 1 mL of the supernatants were transferred to HPLC-vials. HPLC analysis was performed according to the method of Morgenstern et al. (2014).

2.8. High performance liquid chromatography mass spectrometry analyses (HPLC-MS)

The HPLC-MS apparatus used for analyses of single phenolic compounds was a Sciex API 150EX Turbo Ionspray mass spectrometer with a Perkin-Elmer 200 autosampler and two Perkin Elmer LC-200 Micro pumps. The analysis was performed using a Phenomenex AQ C18 (4 × 3.0 mm) guard column. Separations were achieved using a Phenomenex Synergi 4 μm Hydro-RP 80 Å (250 × 4.6 mm) analysis column for the sea buckthorn, onion, black and red currant samples. For the olive, carrot and beetroot samples, a 5 μM Alltech C18 (7.5 × 4.6 mm) guard column and a 5 μM Alltech Alltima C18 (150 × 4.6 mm) analysis column were used. The injection volume was 4 μL for all samples and the flow rate was kept at 1 mL min^{-1}. Regardless of the eluent, it was split to 0.3 mL min^{-1} before the Ion Spray unit. The general conditions were: nebulizer gas (nitrogen), 9.0 min^{-1}; curtain gas, 12 min^{-1}; Ionspray needle voltage, −4000; temperature, 300 °C. The mass spectrometer was operated in a negative mode. All standard mixes were prepared in absolute ethanol to a concentration of 1 mg/mL. The amount of known polyphenols (μg/g DW) in samples was quantified by use of available standards. External standards were injected before and after each sample to be able to check for possible drifts during runs. Unknown peaks considered to be of specific interest were quantified using a closely related molecule as a standard.

2.8.1. Sea buckthorn leaves

For the sea buckthorn extracts, the HPLC-MS analysis was performed according to Morgenstern et al. (2014) with minor modifications. The binary mobile phase consisted of (A) 0.4% formic acid in H_2O (v/v) and (B) 100% acetonitrile. The gradient profile for elution of B was 0% for 3 min, during 3–30 min it was gradually increased to 30%, and from 30 to 35 min it was gradually increased to 40% and then kept stable until 38 min. Between 38 and 42.5 min the percentage of B was decreased from 40% to 0%. Full scan mass spectra were obtained by acquiring data at 125–2250 amu. Twelve [M−H] ions (*m/z*: 169.2, 285.1, 289.1, 301.2, 305.2, 447.3, 451.2, 463.4, 477.4, 609.5, 623.5, 935.5) were selected for ion specific HPLC-MS analysis of major phenols.

2.8.2. Black and red currant leaves

For the black and red currant extracts, the HPLC-MS method used was modified from Vagiri, Ekholm, Andersson, Johansson, and Rumpunen (2012). The binary mobile phase consisted of (A) 0.4% formic acid in H_2O (v/v), and (B) 90% acetonitrile, 5% methanol and 5% H_2O (v/v/v). The gradient profile for elution of B was 8% for 2 min, during 2–21.5 min it was gradually increased to 16%, from 21.5 to 51.5 min it was gradually increased to 23%, and between 51.5 and 56.5 min to 40% when it was decreased to 8% until 61.5 min was kept stable until the run ended at 67.5 min. Eighteen [M−H] ions (*m/z* 289.1, 353.3, 305.1, 565.2, 463.1, 609.4, 463.1, 301, 549.2, 447.3, 593.3, 169, 623.3, 477.2, 353.3, 533.2, 533.2, 341) were selected for ion specific HPLC-MS analysis of major phenols.

2.8.3. Onion peel and skin

For the onion extracts, the HPLC-MS analysis method was modified from Tsao, Yang, Young, and Zhu (2003). Both onion peel and skin samples were run with the same method. The binary mobile phase consisted of (A) 1% acetic acid and 5% acetonitrile in H_2O (v/v/v), and (B) 95% acetonitrile and 5% methanol (v/v). The gradient program was run as follows: 2% B for 2 min, 2% B to 15% B between 2 and 8 min, 15% B to 21% B between 8 and 28 min, 21% B to 80% B between 28 and 32 min where 80% of solution B was kept stable for 4 min before being decreased to 2% B during

1 min. Between 37 and 40 min, B was kept at 2%. Full scan mass spectra were obtained by acquiring data at 90–1500 amu. Twelve [M−H] ions (285.1, 193.1, 167.1, 137.1, 477.2, 447.3, 786.9, 625.3, 301.1, 463.1, 625.2, and 463.2) were selected for ion specific HPLC-MS analysis of major phenols.

2.8.4. Carrot leaves

For carrots the HPLC-MS analysis was performed according to Ma et al. (2013). Full scan mass spectra were obtained by acquiring data at 90–1000 amu. Six [M−H] ions (*m/z* 353.3, 353.3, 179.0, 516.1, 609.4, 549.2) were selected for ion specific HPLC-MS analysis of major phenols.

2.8.5. Beetroot leaves

For beetroot the HPLC-MS analysis was performed according to Gennari et al. (2011). Full scan mass spectra were obtained by acquiring data at 90–1500 amu. Five [M−H] ions (153.0, 609.5, 563.0, 609.3, 639.0) were selected for ion specific HPLC-MS analysis of major phenols.

2.8.6. Olive polyphenol powder

For the olive polyphenol powder the HPLC-MS analysis was performed according to the method described by Vagiri et al. (2012). Scans were performed in the range *m/z* 90–1500. 12 [M−H] ions (*m/z* 153.2, 609.4, 197.2, 137.2, 167.1, 137.1) were selected for ion specific HPLC-MS analysis of major phenols.

2.9. Statistical analyses

ANOVA was used to analyze differences in antioxidant capacity among species and cultivars (biological replicates, n = 2, except for olive polyphenol powder of which only one sample was available) with Tukey's post hoc test. Correlation analyses were conducted to reveal any relationship between FC, FRAP and ABTS data. PCA (principal component analyses) were made to reveal associations between FC, FRAP and ABTS data and contents of major phenols for each species. All the statistical analyses were conducted in IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, N.Y., USA). Differences between means resulting in $p < 0.05$ were considered significant. To ensure the reliability of the study, a coefficient of variation (CV) of maximum 10% was allowed between technical replicates. When this was not the case, the analysis was re-run.

3. Results and discussion

3.1. Total phenols

The total phenols content as analyzed by Folin-Ciocalteu varied largely among samples, from 16.2 to 153.6 mg GAE/g DW (Table 1). There were statistically significant differences between both cultivars ($P < 0.001$, Table 1) and species ($P < 0.001$, Table 2). Sea buckthorn leaf extracts had the highest total phenol content of all analyzed samples (89.2–153.6 mg GAE/g DW) whereas carrot and beetroot leaf extracts had the lowest content (Table 1 and Supplemental Table 1). Onion skin extracts had higher content of total phenols than extracts of onion peels, the difference was however not significant. The higher content in skin compared to peel is of interest since the skin typically is discarded before consumption. The superior content of phenols in sea buckthorn leaves makes this plant material the most interesting for further exploration.

3.2. FRAP and ABTS antioxidant capacity

Sea buckthorn leaf samples also had the highest FRAP and ABTS antioxidant capacity whereas carrot and beetroot samples had the lowest values (Tables 1 and 2). There was a great difference in antioxidant capacity between the onion skin and peel samples where the skin had double the ferric reducing ability and radical scavenging capacity ($p < 0.05$). There were also large differences between samples of red and black currant cultivars, where black currant leaves had higher FRAP and ABTS values ($p < 0.05$) compared to red currant leaves. There was a considerable variation between cultivars in antioxidant capacities for e.g. onion skin and peel, but also among sea buckthorn, red currant and beetroot cultivars which can be noticed from the high coefficient of variation (CV %) for both FRAP and ABTS. Hence, the selection of the proper cultivar may offer opportunities to increase the value of waste materials intended for extraction of antioxidants.

3.3. Correlation between antioxidant properties

There was an overall high correlation (Pearson) between the three methods measuring different antioxidant properties: FC-FRAP 0.958, FC-ABTS 0.980, FRAP-ABTS 0.957 ($p < 0.001$). The corresponding equations of the straight lines fitted to the data were for FC-FRAP $y = 2.46x - 24.44$ ($R^2 = 0.918$), for FC-ABTS $y = 2.39x - 22.10$ ($R^2 = 0.961$), and for FRAP-ABTS $y = 0.91x + 6.73$ ($R^2 = 0.916$). Thus, the different methods in general yielded similar ranking of samples. For instance, carrot leaves had low FC, FRAP and ABTS values whilst the SBT samples consistently showed high levels. For the olive polyphenols however, this was not the case. Both FC and ABTS showed average levels when compared to the other species, whereas FRAP was considerably higher. Our results indicate that hydroxytyrosol, the dominating phenolic compound in the olive wastewater powder, would be a much stronger ferric reducer (FRAP) than radical scavenger (ABTS).

3.4. Analysis of ascorbic acid

The amount of ascorbic acid in leaves has commonly to be corrected for in total phenol analysis using the FC-reagent (Vagiri et al., 2012; Vagiri et al., 2015) due to its proneness to interfere. In this study sea buckthorn cultivars had in average 0.86 mg/g DW ascorbic acid contributing with only 0.7% to the average total

Table 2
Average content of total phenols (FC) and antioxidant capacity (FRAP and ABTS) for selected plant materials (see Table 1) with two biological replicates (n = 2). FC is shown in mmol GAE/100 g DW, FRAP is shown in mmol Fe^{2+}/100 g DW and ABTS is shown in mmol TEAC/100 g DW. CV = coefficient of variation among cultivars. A different letter in a column indicates a significant difference ($p < 0.05$) among groups.

Plant material	Cultivars	FC $\bar{x}$		CV%	FRAP $\bar{x}$		CV%	ABTS $\bar{x}$		CV%
Sea buckthorn leaves	n = 6	83.6	a	13.1	182.2	a	17.8	180.9	a	11.5
Black currant leaves	n = 5	51.1	b	6.9	105.5	b	8.0	102.8	b	6.4
Red currant leaves	n = 5	42.7	bc	9.2	86.0	b	12.1	83.8	c	11.6
Onion peel	n = 3	25.4	cd	31.1	14.5	c	33.5	23.0	e	38.2
Onion skin	n = 3	35.4	d	14.2	33.5	c	24.6	50.8	d	21.9
Carrot leaves	n = 3	10.1	e	9.4	15.0	c	11.3	15.1	e	48.6
Beetroot leaves	n = 3	10.2	e	11.2	9.8	c	23.1	9.3	e	21.6
Olive mill waste water powder	n = 1	40.1	–	NA	102.3	–	NA	33.7		NA

phenol content. Similarly, red currant cultivars in average had 0.51 mg/g DW contributing with 0.8% and black currant samples had in average 0.06 mg/g DW contributing with 0.08% to the average FC-value. We thus considered the amount of ascorbic acid in the dry leaf extracts to be negligible in comparison to the total amount of phenols in our study.

3.5. Analysis of major phenolic compounds

The results of the analyses of major phenolic compounds are presented below for each species separately. Full chromatographic overviews and contents of the major phenolic compounds detected by HPLC-MS can be found in the supplementary material. The chromatograms are total scans of representative samples for each species. Therefore, occasional cases of peaks not being separated, which are otherwise separated can be seen (see eg. Table 4 and Supplemental Fig. 2). Furthermore, there are occasional cases where no peak for a specific phenol in the concerned sample can be seen (see e.g. Table 3 and Supplemental Fig. 1). Identification of phenolic compounds was made by comparison of retention time and absorbance spectra in available HPLC standards (see Section 2.2) and for other compounds by $[M-H]^-$ (m/z) and published literature data. To examine interrelations among major phenolic compounds and antioxidant properties for each species studied, a principal component analysis (PCA) was performed for each species separately (Fig. 1).

3.5.1. Sea buckthorn leaves

Fifteen phenolic compounds were identified in the sea buckthorn leaf extracts by HPLC-MS analysis (Table 3). The main compounds were hydrolysable tannins, rutin and catechin, which is in accordance with what Morgenstern et al. (2014) found, but in addition, high amount of isorhamnetin-3-*O*-rutinoside was also identified. The sea buckthorn tannins were identified based on the mass spectra together with the $[M-H]^-$ (m/z) obtained from the work of Morgenstern et al. (2014). As shown by the CV % (Table 3), there were large differences between cultivars especially with regards to contents of epigallocatechin, rutin, quercetin and quercetin-3-*O*-galactoside. The cultivar specific contents of phenols can be seen in Supplemental Table 2. The PCA showed that sea buckthorn tannin Ia (sbtan1a) and sea buckthorn tannin Ib (sbtan1b) were associated with the total phenol content (FC) and the radical scavenging activity (ABTS), whereas sea buckthorn tannin II (sbtan2) was associated with ferric reducing ability of plasma (FRAP) (Fig. 1A). This information might be of particular interest for future research in e.g. incorporation of antioxidant extracts into foods since these phenol compounds could reduce oxidation in different ways. The dry matter of the sea buckthorn leaf powder was 90.1% and the yield of the extract was 0.47 g/g DW powder.

3.5.2. Black currant leaves

In the black currant leaf extracts, 19 phenolic compounds were identified (Table 4). Quercetin-3-*O*-malonyl-glucoside A and B and quercetin-3-*O*-glucoside were identified in high amounts as previously reported by Vagiri et al. (2012). Additionally, we also identified rutin, quercetin and chlorogenic acid in high amounts. The cultivar specific contents of phenols can be seen in Supplemental Table 3. In the PCA plot (Fig. 1B), quercetin-3-*O*-galactoside (quegal) and kaempferol-3-*O*-glucoside B (kaegluB) were closely associated to the ABTS value, whereas kaempferol-3-*O*-rutinoside (kaerut), chlorogenic acid (chlac) and rutin (rut) were somewhat associated to the FC value. The dry matter of the black currant leaf powder was 91.5% and the yield of the extract was 0.38 g/g DW powder.

3.5.3. Red currant leaves

Since the red currant leaves had not been widely studied before, we used the same HPLC-MS method as for black currant leaves for the analysis. From the red currant leaf extracts, 21 phenolic compounds were identified (Table 5). The main compounds detected were rutin, quercetin-3-*O*-malonyl-glucoside and hydrolysable red currant tannins. The tannins were identified based on the mass spectra solely. There were great cultivar differences in concentrations of all the identified phenolic compounds in both red currant (particularly for quercetin-3-*O*-galactoside and neo-chlorogenic acid) and black currant extracts (particularly for isorhamnetin-3-*O*-glucoside and myricitin-malonyl-glucoside B). The cultivar specific contents of phenols can be seen in Supplemental Table 4. In the PCA analysis (Fig. 1C), isorhamnetin-3-*O*-rutinoside (isorharut), isorhamnetin-3-*O*-glucoside A (isorhagluA) and quercetin (que) were associated with FRAP, ABTS and FC. Since in this case, both FRAP, ABTS and FC values were associated with the same phenolic compounds, the choice of plant material for e.g. incorporation into food products is not as crucial as compared to for instance sea buckthorn where the phenols associated with FRAP and ABTS differ. The dry matter of the red currant leaf powder was 92.4% and the yield of the extract was 0.36 g/g DW powder.

Table 3
Major phenolic compounds in sea buckthorn leaves, average of 6 cultivars (biological replicate n = 1, technical replicate n = 3). CV = coefficient of variation among cultivars.

Phenolic compound	m/z $[M-H]^-$	Content (μg/g DW)	CV%	Detection limit (μg/g DW)	t_R (min)	Peak number
Kaempferol-3-*O*-glucoside	447.3	59.8	8.8	0.7	10.4	1
Gallic acid	169.2	182.9	9.6	4.4	13.0	2
Unknown peak	783.1	NA	NA	NA	16.8	3
Procyanidin monomer glucoside	451.2	9.1	18.0	0.4	19.3	4
Epigallocatechin	305.2	19.2	24.4	4.6	19.7	5
SBT tannin II	953.5	5368.1	5.6	27.1	20.9	7
SBT tannin Ia	935.5	4874.2	9.9	31.6	21.0	6
Catechin	289.1	335.0	15.6	10.4	21.5	–
SBT tannin Ib	935.5	6118.0	8.9	31.6	21.6	8
Unknown peak	609.5	NA	NA	NA	23.8	9
SBT tannin III	935.5	6794.4	6.5	31.6	25.4	10
Unknown peak	623.2	NA	NA	NA	25.6	11
Rutin	609.5	1309.0	23.0	30.9	26.7	12
Quercetin-3-*O*-galactoside	463.4	175.4	19.6	6.8	28.1	13
Isorhamnetin-3-*O*-rutinoside	623.5	1885.2	8.7	27.8	28.9	14
Kaempferol	285.1	3.0	27.1	0.7	30.1	15
Isorhamnetin-3-*O*-glucoside	477.4	63.9	12.8	4.6	30.6	16
Quercetin	301.2	5.0	19.3	3.0	37.5	19

Table 4
Major phenolic compounds in black currant leaves, average of 5 cultivars (biological replicate n = 1, technical replicate n = 3). CV = coefficient of variation among cultivars.

Phenolic compound	*m/z* [M−H]⁻	Content (μg/g DW)	CV%	Detection limit (μg/g DW)	t_R (min)	Peak number
Gallic acid	169.2	9.3	47.1	1.7	5.6	1
Neo-chlorogenic acid	353.3	1047.7	39.1	26.9	11.3	2
Epigallocatechin	305.1	1108.3	44.9	10.5	15.2	3
Catechin	289.1	376.8	25.4	25.9	17.8	4
Chlorogenic acid	353.3	1481.2	46.1	18.8	18.0	5
Epicatechin	289.1	285.7	46.4	8.0	23.5	6
Myricitin-malonyl-glucoside A	565.2	87.0	22.9	10.8	36.5	7
Myricitin-malonyl-glucoside B	565.2	11.6	62.7	10.8	38.0	8
Rutin	609.4	1816.4	34.0	22.8	38.2	9
Quercetin-3-*O*-galactoside	463.1	719.1	51.0	7.4	40.0	10
Quercetin-3-*O*-glucoside	463.1	1705.1	23.4	7.2	41.0	11
Kaempferol-3-*O*-rutinoside	593.3	585.0	34.7	1.5	45.8	12
Quercetin-3-*O*-malonyl-glucoside A	549.2	4458.9	16.8	7.7	46.4	13
Quercetin-3-*O*-malonyl-glucoside B	549.2	2553.0	59.6	7.7	47.0	14
Isorhamnetin-3-*O*-rutinoside	623.3	36.2	19.9	3.0	47.3	Not separated
Kaempferol-3-*O*-glucoside	447.3	447.5	21.1	3.0	48.9	15
Isorhamnetin-3-*O*-glucoside	477.2	5.1	74.7	2.6	50.2	16
Kaempferol-malonyl-glucoside A	533.2	102.4	51.9	3.0	53.6	17
Kaempferol-malonyl-glucoside B	533.2	5.8	50.6	3.0	56.0	18
Quercetin	301.2	BLD	NA	NA	60.7	–

When a PCA was carried out between all plant materials (results not shown), each species grouped together as expected. However, red and black currant leaves formed a clear group except for two cultivars of *Ribes rubrum* producing white berries. Anthocyanins were not determined in this study because of their known lack of stability. Nevertheless, the white currant cultivars separated from the larger red currant group indicating that the composition of phenols in the leaves differ depending on whether or not the cultivar has coloured berries.

3.5.4. Onion peel and skin

In onion peel and skin, 12 and 11 major phenol compounds were identified, respectively (Table 6). Quercetin-4-*O*-glucoside was a major compound in both peel and skin, which had considerably higher levels of quercetin than the peel. Interestingly, Mizuno, Tsuchida, Kozukue, and Mizuno (1992) showed that the first and second exterior onion layers contain 90% of the free quercetin, accordingly to our research, the first layer containing more than the second. Ferulic acid was identified in the onion peel only, suggesting that the compound is degraded in the outermost layers (Table 6). In onion peel, the variation between cultivars was the greatest for isorhamnetin-3-*O*-glucoside and kaempferol whilst in the onion skin the greatest variation was detected for quercetin-3-*O*-glucoside and quercetin-3,4-diglucoside. The cultivar specific contents of phenols can be seen in Supplemental Table 5. In the PCA plot (Fig. 1D), vanillic acid (vanac) and kaempferol (kae) were closely associated with all antioxidant variables. Additionally, quercetin (que) was closely associated with both FRAP and ABTS as well as p-hydroxybezoic acid (phydbenac). Although quercetin-4-*O*-glucoside (que4glu) was the main compound found in both peel and skin extracts, it was interestingly not closely associated with any of the antioxidant variables. This indicates that the amount of a phenolic compound is not the only important factor, but also the antioxidant property of a compound *per se*. The dry matter of the onion peel powder was 92.6% and for the onion skin powder 91.2%. The yields of the extracts were 0.44 g/g DW for the onion peel powder and 0.26 g/g DW for the onion skin powder.

3.5.5. Carrot leaves

Seven major phenolic compounds were found in the carrot leaf extracts where chlorogenic acid was the most prevalent one followed by an unknown peak with [M−H] at *m/z* 447.2 and kaempferol-malonyl-glucoside (Table 7). The caffeic acid and rutin content varied the most between cultivars. However, the rutin peak could not be separated from an unknown compound (in the given chromatogram) with a molecular ion [M−H] of *m/z* 593.3. Moreover, an erroneous peak at the retention time of 42.2 min (Supplemental Fig. 5) should be disregarded due to an erroneous input in the software. The cultivar specific contents of phenols can be seen in Supplemental Table 6. There is, to our knowledge, no previous study on the phenol content of carrot leaves. The dry matter of the carrot leaf powder was 94.3% and the yield of the extract was 0.35 g/g DW powder.

In the PCA plot (Fig. 1E), kaempferol-malonyl-glucoside (kaemalglu) and quercetin-3-*O*-malonyl-glucoside A (quemalgluA) were found to be closely associated with FRAP, whereas rutin (rut), cynarin (cyn), caffeic acid (cafac), neo-chlorogenic acid (neochlac) and carrot peak 77 (cap477) were associated with ABTS. As in the sea buckthorn leaf analysis, this indicates that the different carrot phenolic compounds may counteract oxidation in different ways.

3.5.6. Beetroot leaves

In the beetroot extracts, 5 main phenolic compounds were identified including xylosylvitexin, a compound previously noticed by Gennari et al. (2011) (Table 7). Contents of rutin and xylosylvitexin varied most among cultivars but there were large differences for all identified phenolic compounds. The cultivar specific contents of phenols can be seen in Supplemental Table 7. In the PCA (Fig. 1F), ABTS showed to be closely associated to xylosylvitexin (xylvit) whereas FC showed to be closely associated to rutin (rut). The dry matter of the beetroot leaf powder was 91.8% and the yield of the extract was 0.34 g/g DW powder.

3.5.7. Olive mill wastewater powder

In the olive mill wastewater powder, hydroxytyrosol was revealed as the dominating phenolic compound with an average of 40191.3 μg/g DW (standard deviation 2103.5) with only small amounts of five other compounds detected (Supplemental Table 8). Hydroxytyrosol seems to be more associated with FRAP than with ABTS (Table 2). The dry matter of the powder was 91.7%.

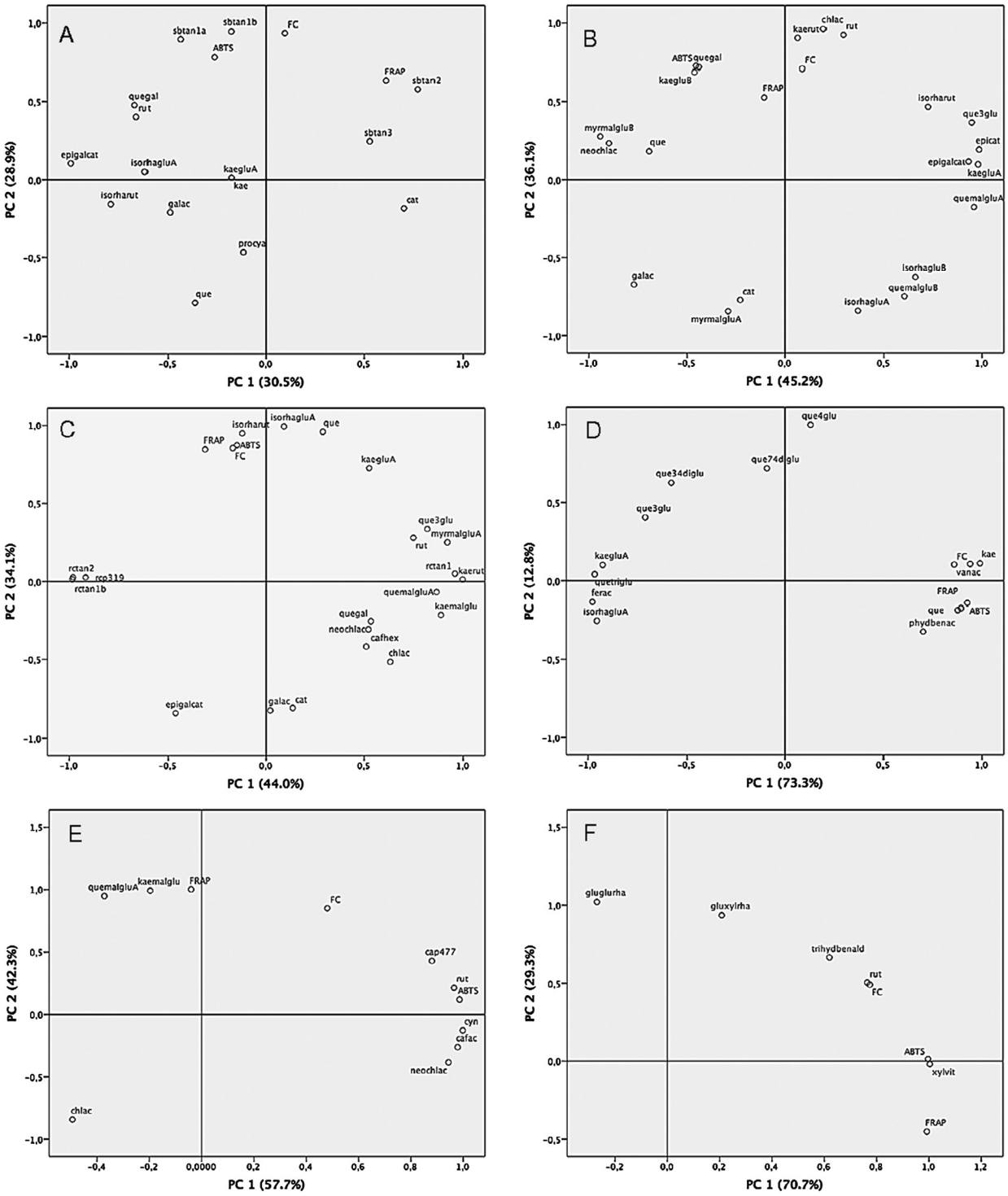

Fig. 1. Principal component analyses showing associations between HPLC-MS, FC, FRAP and ABTS data for (A) sea buckthorn leaves, (B) black currant leaves, (C) red currant leaves, (D) onion peel and skin, (E) carrot leaves, and (F) beetroot leaves. Abbreviations of phenolic compounds in this figure. *Abbreviation, HPLC standard/compound*: cafac, caffeic acid; cafhex, caffeoyl hexose; cap447, carrot p447; cat, catechin; chlac, chlorogenic acid; cyn, cynarin; dihydpheac, 3,4-dihydroxyphenylacetic acid; epicat, epicatechin; epigalcat, epigallocatechin; ferac, ferulic acid; galac, gallic acid; gluglurha, glucopyranosyl-glucopyrasyl-rhamnetin; gluxylrha, glucopyranosyl-xylosyl-rhamnetin; hydtyr, hydroxytyrosol; isorhagluA, isorhamnetin-3-*O*-glucoside A; isorhagluB, isorhamnetin-3-*O*-glucoside B; isorharut, isorhamnetin-3-*O*-rutinoside; kae, kaempferol; kaegluA, kaempferol-3-*O*-glucoside A; kaegluB, kaempferol-3-*O*-glucoside B; kaemalglu, kaempferol-malonyl-glucoside; kaerut, kaempferol-3-*O*-rutinoside; myrmalgluA, myricitin-malonyl-glucoside A; myrmalgluB, myricitin-malonyl-glucoside B; neochlac, neo-chlorogenic acid; onp533, onion p533; phydbenac, p-hydroxybenzoic acid; procya, procyanidin monomer glucoside; que, quercetin; que34diglu, quercetin-3,4-diglucoside; que3glu, quercetin-3-*O*-glucoside; que4glu, quercetin-4-*O*-glucoside; que74diglu, quercetin-7,4-diglucoside; quegal, quercetin-3-*O*-galactoside; quemalgluA, quercetin-3-*O*-malonyl-glucoside A; quemalgluB, quercetin-3-*O*-malonyl-glucoside B; quetriglu, quercetin-3,7,4-triglucoside; rcp319, red currant p319; rctan1, red currant tannin I; rctan1b, red currant tannin Ib; rctan2, red currant tannin II; rut, rutin; sbtan1a, sea buckthorn tannin Ia; sbtan1b, sea buckthorn tannin Ib; sbtan2, sea buckthorn tannin II; sbtan3, sea buckthorn tannin III; syrac, syringic acid; trihydbenald, 2,4,5-trihydroxybenzaldehyde; tyr, tyrosol; vanac, vanillic acid; xylvit, xylosylvitexin.

Table 5
Major phenolic compounds in red currant leaves, average of 5 cultivars (biological replicate n = 1, technical replicate n = 3). CV = coefficient of variation among cultivars.

Phenolic compound	*m/z* [M−H]$^-$	Content (μg/g DW)	CV%	Detection limit (μg/g DW)	t_R (min)	Peak number
Quercetin	301.2	BLD	NA	NA	NA	–
Gallic acid	169.2	12.0	51.4	7.2	5.6	1
Unknown peak	299.0	NA	NA	NA	9.9	2
Unknown peak	447.3	NA	NA	NA	11.4	3
Neo-chlorogenic acid	353.3	518.3	175.2	12.7	11.3	4
Epigallocatechin	305.1	90.7	51.0	0.8	15.2	5
Caffeoyl hexose	341.0	191.5	22.0	29.7	17.2	6
Catechin	289.1	621.2	49.7	20.4	17.8	7
Unknown peak	319.0	415.1	52.1	42.0	18.9	8
Chlorogenic acid	353.3	39.6	102.9	6.1	18.0	9
Red currant tannin I	755.3	137.1	52.8	7.1	23.7	10
Red currant tannin Ib	755.3	2146.0	118.9	7.1	30.8	11
Red currant tannin II	739.4	1917.9	119.9	2.7	36.0	12
Myricitin-malonyl-glucoside	565.2	39.1	44.4	3.4	36.5	13
Rutin	609.4	4900.7	43.6	17.8	38.2	14
Quercetin-3-*O*-galactoside	463.1	38.1	169.5	0.9	40.0	15
Unknown peak	592.8	NA	NA	NA	41.5	16
Quercetin-3-*O*-glucoside	463.1	1618.4	46.4	5.0	41.0	17
Kaempferol-3-*O*-rutinoside	593.3	1347.6	60.9	2.8	45.8	18
Quercetin-3-*O*-malonyl-glucoside	549.2	2665.5	59.2	27.1	46.4	19
Isorhamnetin-3-*O*-rutinoside	623.3	9.4	43.3	1.2	47.3	20
Kaempferol-3-*O*-glucoside	447.3	926.3	139.9	0.6	48.9	21
Isorhamnetin-3-*O*-glucoside	477.2	3.4	50.7	1.3	50.2	22
Kaempferol-malonyl-glucoside	533.2	261.0	85.2	4.1	53.6	23

Table 6
Major phenolic compounds in onion peel and skin, average of 3 cultivars (biological replicate n = 1, technical replicate n = 3). CV = coefficient of variation among cultivars.

Phenolic compound	*m/z* [M−H]$^-$	Peel content (μg/g DW)	CV%	Skin content (μg/g DW)	CV%	Detection limit (μg/g DW)	t_R (min)	Peak number
Quercetin-3,7,4-triglucoside	786.9	175.3	14.3	26.3	33.1	0.9	9.6	1
Unknown peak	625.3	NA	NA	NA	NA	NA	10.2	2
p-hydroxybenzoic acid	137.1	4.1	10.9	12.2	28.0	2.2	12.6	3
Quercetin-7,4-diglucoside	625.3	416.2	15.6	366.4	23.0	2.2	12.7	4
Vanillic acid	167.1	12.1	13.1	20.0	8.1	25.0	13.8	5
Quercetin-3,4-diglucoside	625.2	952.8	14.5	444.7	57.4	0.5	14.3	6
Unknown peak	639.2	NA	NA	NA	NA	NA	15.0	7
Quercetin-3-*O*-glucoside	463.1	729.5	19.8	301.8	79.2	24.5	20.1	8
Ferulic acid	193.1	30.7	21.5	BLD	NA	NA	20.9	9
Kaempferol-3-*O*-glucoside	447.3	129.7	24.2	32.2	69.0	1.5	25.0	10
Isorhamnetin-3-*O*-glucoside	477.2	113.7	66.6	10.7	40.5	0.4	25.6	11
Quercetin-4-*O*-glucoside	463.2	1936.6	10.2	1767.6	35.0	7.1	26.4	12
Unknown peak	447.2	NA	NA	NA	NA	NA	28.0	13
Unknown peak	477.2	NA	NA	NA	NA	NA	29.7	14
Quercetin	301.1	623.8	33.2	1779.8	18.3	3.5	32.7	15
Kaempferol	285.1	93.2	36.3	263.9	19.7	0.2	33.5	16

Table 7
Major phenolic compounds in carrot and beetroot leaves, average of 3 cultivars (biological replicate n = 1, technical replicate n = 3). BLD = below level of detection. CV = coefficient of variation among cultivars. Peak 8 for the carrot leaves was disregarded due to an erroneous input in the software.

Species	Phenolic compound	*m/z* [M−H]$^-$	Content (μg/g DW)	CV%	Detection limit (μg/g DW)	t_R (min)	Peak number
Carrot leaves	Cynarin	516.1	BLD	NA	NA	NA	–
	Neo-chlorogenic acid	353.3	100.0	28.5	24.2	8.4	1
	Chlorogenic acid	353.3	6322.2	5.1	6.6	13.9	2
	Caffeic acid	179.0	10.2	55.1	1.7	17.3	3
	Unknown peak 593.3	593.3	NA	NA	NA	19.7	4
	Rutin	609.4	15.6	38.2	5.8	31.2	5
	Peak 447.3	447.3	2874.6	7.4	1.4	33.1	6
	Quercetin-malonyl-glucoside	549.2	23.1	5.0	7.2	37.9	7
	Disregarded peak	NA	NA	NA	NA	42.5	8
	Kaempferol-malonyl-glucoside	533.2	1387.9	17.3	10.9	44.7	9
Beetroot leaves	2,4,5-trihydroxybenzaldehyde	153.0	33.7	26.2	5.1	5.8	1
	Unidentified peak 593.3	593.3	NA	NA	NA	6.9	2
	Xylosylvitexin	563.0	2596.1	73.6	7.6	8.2	3
	Rutin	609.5	18.9	84.1	2.6	8.9	4
	Glucopyranosyl-glucopyranosyl-rhamnetin	639.0	485.3	44.7	2.6	10.8	5
	Glucopyranosyl-xylosyl-rhamnetin	609.3	856.8	50.7	3.6	9.6	6
	Unknown peak 605.2	605.2	NA	NA	NA	15.5	7
	Unknown peak 577.2	577.2	NA	NA	NA	15.7	8

4. Conclusion

For many horticultural crops, the leaves of fruit trees, berry shrubs and root vegetables are not harvested or are at harvest separated and left in the field as a waste, although they may be valuable for different purposes. Post-harvest handling may further result in residues with high content of bioactive compounds that could be refined and used. In this study, the aim was to screen plant materials from different horticultural crops with regards to their antioxidant capacities and analyze the content of major phenolic compounds that may contribute to the antioxidant capacity. The fact that not commonly used plant materials from food production may have high antioxidant properties is interesting but not new to the research on this topic. However, the phenolic compounds that give rise to these antioxidant properties have also been revealed in this study. The large variation among and within species shows that by proper choice of cultivar, the content of specific phenolic compounds and the antioxidant capacity of the plant material can be largely increased and thereby also increase its value for further use.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jff.2017.09.003.

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