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Assessment of polyphenolic content and *in vitro* antiradical characteristics of apple pomace

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Abstract

Apple pomaces, a by-product in the apple juice processing, were subjected to evaluation as potential sources of antioxidant phytochemicals on the basis of their total content of phenolics (from 4.22 to 8.67 mg/g), total flavonoids (from 0.45 to 1.19 mg/g) and total flavan-3-ols (from 2.27 to 9.51 mg/g), and *in vitro* antiradical activities. Some individual phenolic compounds including caffeic and chlorogenic acids, (+)-catechin and (−)-epicatechin, rutin, quercetin glycosides and phloridzin were identified and quantified by HPLC. The antiradical activity of apple pomaces was tested by measuring their ability to scavenge DPPH and hydroxyl radicals by ESR spectroscopy. The highest DPPH ($EC_{50}^{DPPH} = 6.33$ mg/ml) and hydroxyl ($EC_{50}^{OH} = 26.11$ mg/ml) radical scavenging activities were obtained in the case of Reinders pomace. The regression analysis produced moderate to high correlation coefficients between the antiradical activities ($1/EC_{50}^{DPPH}$ and $1/EC_{50}^{OH}$), and total phenolics, total flavonoids, total flavan-3-ols, and some individual phenolic compounds.

Keywords: Apple pomace; Phenolic compounds; HPLC; Antiradical activity; DPPH radical; Hydroxyl radical; ESR

1. Introduction

The diet plays an important role in the morbidity and mortality associated with the chronic diseases such as cardiovascular disease, cancer, hypertension and obesity. Several investigations have estimated that one-third of all cancer cases and one-half of cardiovascular diseases and hypertension can be attributed to diet (Lee & Smith, 2000; Willet, 1994; Wolfe, Wu, & Liu, 2003). The possible beneficial health effects of diets including fruits, vegetables and their products have been attributed to their phytochemicals (Block, Patterson, & Subar, 1992; Lampe, 1999; Liu, 2003). Fruits and vegetables contain many different dietary phytonutrients which contribute to the prevention of degenerative diseases caused by oxidative stress (Kaur & Kapoor, 2001). Polyphenols are one of the phytochemical groups whose "protective" properties include antioxidant, antimicrobial, anticancer and cardiovascular-protective activities (Bendini et al., 2006; Chu, Chang, & Hsu, 2000; Hertog et al., 1995; Liu, 2002; Süzgeç, Meriçli, Houghton, & Çubukçu, 2005).

Apples are well-known and widespread fruit of the genus Malus (about 25 species) belonging to the family Rosaceae. In numerous diets, apples are a very significant part and represent an important source of bioavailable polyphenolic compounds such as flavonols (with quercetin glycosides as the main representative), monomeric and oligomeric flavanols, dihydrochalcones (e.g., phloridzin), anthocyanidins, *p*-hydroxycinnamic and *p*-hydroxybenzoic acids (Escarpa & Gonzalez, 1998). The contents of phenolic compounds vary greatly among different varieties of apples, and between the peel and the flesh; apple peels contain a higher concentration of phenolic compounds (Escarpa & Gonzalez, 1998; Vrhosek, Rigo, Tonan, & Mattivi, 2004).

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The antioxidant compounds from waste product of food industry could be used for increasing the stability of foods by preventing lipid peroxidation and also for protecting oxidative damage in living systems by scavenging oxygen free radicals (Makris, Boskou, & Andrikopoulos, 2007). Apple pomace is a by-product in the apple juice processing, which is a rich source of polyphenols, minerals and dietary fibre (Boyer & Liu, 2004; Figuerola, Hurtado, Estevez, Chiffelle, & Asenjo, 2005; Schieber, Keller, & Carle, 2001; Sudha, Baskaran, & Leelavathi, 2007). Processing apples into juice has been found to affect to phenolic content. Conventional apple juice production (straight pressing of apple pulp or pressing after pulp enzyming) resulted in a juice poor in phenolics and with only 3–10% of the antioxidant activity of the fruit they were produced from (van der Sluis, Dekker, Skrede, & Jongen, 2002).

In view of the fact that most of the phenolic compounds remained in the apple pomace, our interest is focused on the apple pomaces (obtained from apple varieties – Pinova, Reinders, Jonagold, Iduna, Braeburn and sample obtained from factory Nectar) as a potential source of bioactive phenolics, which can be used for various purposes in the food, pharmaceutical and cosmetic industry. The objective of this study was to examine phenolic composition using the spectrophotometrical determination of total phenolics (TP), total flavonoids (TFd) and total flavan-3-ols (TFl), and individual phenolic compounds by HPLC. In effort to establish the antioxidative activity of apple pomace extracts against stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) and reactive hydroxyl radicals, a very sensitive analytical method, electron spin resonance (ESR) spectroscopy, was evaluated. The total phenolic, flavanoid and flavan-3-ol contents, and also a content of individual phenolic compounds of investigated apple pomaces, were correlated to their antioxidant activities.

2. Materials and methods

2.1. Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), Folin–Ciocalteu reagent, vanillin, caffeic acid, chlorogenic acid, (+)-catechin and (−)-epicatechin, quercetin, rutin and phloridzin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). These chemicals were of analytical reagent grade. Other used chemicals and solvents were of the highest analytical grade and obtained from "Zorka" Šabac (Serbia).

2.2. Pomace preparation

Apple varieties (Pinova, Reinders, Jonagold, Iduna, Braeburn) harvested in Serbia in the 2005 season, were collected from the Department for Fruit Growing and Viticulture, Faculty of Agriculture, University of Novi Sad.

Apples (1 kg) of each variety were cleaned by washing, stalks were removed, and the fruits were cut in four pieces and apple pulp was prepared by quick slicing in a domestic food processor (Bosch, Compact Kitchen Machine 4420, Gerlingen-Stuttgart, Germany). Straight pressed apple juice was prepared by immediate pressing of apple pulp. Apple pulps were pressed in a manual cider juice press to separate apple juice. The samples of the obtained apple pomace were taken. One sample of apple pomace was procured from a fruit juice industry (D.O.O. "Nectar", Bačka Palanka, Serbia). Moisture of each sample of apple pomace was determined using drying oven method, by drying a representative 10 g sample in a forced air oven (Sterimatic ST-11, Instrumentaria, Zagreb, Croatia) at 60 °C until the constant mass.

The yields of apple pomace such as moisture contents are shown in Table 1.

2.3. Extraction procedure

Samples of apple pomace (20 g) were extracted at room temperature using an ultrasonic bath, Heidolph DIAX 900 (Heidolph Instruments GmbH, Kelheim, Germany). The extraction was performed three times with different amounts of 80% methanol: 160 ml in 60 min, 80 ml in 60 min, 80 ml in 30 min at room temperature. The total extraction time was 150 min. The obtained three extracts were combined and evaporated to dryness under reduced pressure.

The yields, average of triplicate analysis, of extracts were: Pinova, $m = 2.87 \pm 0.14$ g; Reinders, $m = 2.68 \pm 0.13$ g; Jonagold, $m = 2.99 \pm 0.14$ g; Iduna, $m = 2.88 \pm 0.14$ g; Braeburn, $m = 2.52 \pm 0.13$ g; Nectar, $m = 1.65 \pm 0.08$ g.

2.4. Spectrophotometrical determination

2.4.1. Total phenolics

Total phenolics in apple pomace extracts were determined using the Folin–Ciocalteu reagent (Singleton, Orthofer, & Lamuela-Raventos, 1999). The reaction mixture was prepared by mixing 0.1 ml of methanolic solution (concentration 50 mg/ml) of extract, 7.9 ml of distilled water, 0.5 ml of the Folin–Ciocalteu's reagent and 1.5 ml of 20% sodium carbonate. After 2 h, the absorbance at 750 nm (spectrophotometer Camspec M105, Cambridge, UK) was obtained against blank that had been prepared in a similar manner, by replacing the extract with distilled water. The total phenolic content, expressed as mg chloro-

Table 1
The yields and moisture contents of apple pomaces

Apple varieties	Yield (g)	Moisture content (%)
Pinova	288.8	77.2
Reinders	216.1	79.8
Jonagold	342.6	80.1
Iduna	306.5	76.5
Braeburn	295.7	78.8
Nectar[a]	–	74.9

[a] Industrial apple pomace obtained from factory Nectar.

genic acid equivalents per g dry weight of apple pomace, was determined using calibration curve of chlorogenic acid standard.

2.4.2. Total flavonoids

Total flavonoids in apple pomace extracts were estimated according to Markham (1989). Flavonoids from apple pomace extracts (0.2 g) were extracted in 2 ml of extraction medium (70% [v/v] methanol, 5% [v/v] acetic acid and 25% [v/v] distilled water) at room temperature for 60 min. The resulting solution was filtered trough Whatman paper No. 4 and filtrate volume adjusted to 10 ml. The probes were prepared by mixing: 5 ml of extract, 1 ml of distilled water and 2.5 ml of $AlCl_3$ solution (26.6 mg $AlCl_3 \cdot 6H_2O$ and 80 mg CH_3COONa dissolved in 20 ml distilled water). A blank probe was prepared by replacing $AlCl_3$ solution with distilled water. The absorbance of probes and blank probe were measured immediately at 430 nm. Total flavonoid content, expressed as mg rutin per g dry weight of apple pomace, was calculated from a calibration curve using rutin as standard.

2.4.3. Total flavan-3-ols

Content of total flavan-3-ols in apple pomace extracts was determined using the vanillin assay described by Sun, Ricardo-da-Silva, and Spranger (1998). The probes were prepared by mixing: 1 ml of methanol (probe b) or 100 mg/ml of apple pomace extracts in methanol (probe s), 2.5 ml of 1% vanillin in methanol and 2.5 ml of 10% H_2SO_4. The blank probes were prepared by mixing: 1 ml of methanol (probe o) or 100 mg/ml of apple pomace extracts in methanol (probe c), 2.5 ml of methanol and 2.5 ml of 10% H_2SO_4. Reaction mixture was incubated for 20 min at 30 °C and absorbance at 500 nm was measured. The absorbance (A) of each apple pomace extracts was calculated as follows:

$$A = (A_s - A_b) - (A_c - A_o)$$

A_s – absorbance of probe s; A_b – absorbance of probe b; A_c – absorbance of blank probe c and A_o – absorbance of blank probe o.

Total flavan-3-ol content was calculated from calibration curve using (+)-catechin as a standard, and expressed as mg (+)-catechin equivalents per g dry weight of apple pomace.

2.5. HPLC analysis

2.5.1. Instrumentation and chromatographic conditions

HPLC analysis was performed using a liquid chromatograph HP1090 (Hewlett-Packard, Avondale, PA, USA) equipped with a diode array detector 79880A DAD (Hewlett-Packard, Avondale, PA, USA). A reversed-phase column, Zorbax SB-C18 (250 × 3.0 mm) with a 5 μm particle size (Agilent, Palo Alto, CA, USA), equipped with a pre-column, Zorbax SB-C18 (12.5 × 4.6 mm), was used at a flow-rate of 0.400 ml/min. Solvent gradient was performed by varying the proportion of solvent A (0.1% acetic acid in water) to solvent B (0.1% acetic acid in acetonitrile) as follows: initial 10% B; linear gradient to 20% B in 10 min; linear gradient to 50% B in 30 min; linear gradient to 10% B in 15 min. The set time of recording chromatograms and spectra was 30 min, while the total running time and post-running time were 55 and 5 min, respectively.

The injected volume of samples and standards was 10 μl and it were performed manually. All samples were prepared as methanolic solutions with a final concentration of 100 mg/ml. Solutions were filtered prior to injection through 0.45 μm membrane filter (Millipore, Bedford, MA, USA). The column temperature was 23 °C. The spectra were acquired in the range 210–400 nm and chromatograms plotted at 280/4 nm with reference wavelength 550/100 nm.

2.5.2. Identification and quantification

Phenolic compounds in samples were identified by matching the retention time and their spectral characteristics against those of standards. The purity of the peaks was determined to ensure the identification. The external standard method was the technique used for quantification. For each compound, a stock solution (concentration of 1 mg/ml) was made by accurately weighing out commercial standard of phenolic compounds followed by dissolution in methanol. Solutions used for calibration were prepared by dilution of the stock solutions. Peak areas from chromatograms were plotted against known concentrations of standards. Equations generated via linear regression were used to establish concentrations of phenolic compounds in samples.

2.6. ESR measurements

2.6.1. DPPH radical scavenging activity

The potential antioxidant activity of apple pomace extracts was assessed on the basis of the scavenging activity of the stable DPPH free radical. Blank probe was obtained by mixing 400 μl 0.4 mM methanolic solution of DPPH and 200 μl of water. The probe contained × μl of 100 mg/ml water solution of extract, (200 − x) μl water and 400 μl of 0.4 mM methanolic solution of DPPH radical. The range of the investigated extract concentrations was 2.5–10 mg/ml. DPPH radical exhibits a characteristic ESR signal (Fig. 1A).

After that the mixture was stirred for 2 min and transferred to a quartz flat cell ER-160FT. The ESR spectra were recorded on an ESR spectrometer Bruker 300E (Rheinstetten, Germany) under the following conditions: field modulation 100 kHz, modulation amplitude 0.256 G, receiver gain 2×10^4, time constant 40.96 ms, conversion time 327.68 ms, center field 3440.00 G, sweep width 100.00 G, x-band frequency 9.65 GHz, power 7.96 mW, temperature 23 °C.

The SA_{DPPH} value of the extract was defined as

$$SA_{DPPH} = 100(h_o - h_x)/h_o \ [\%]$$

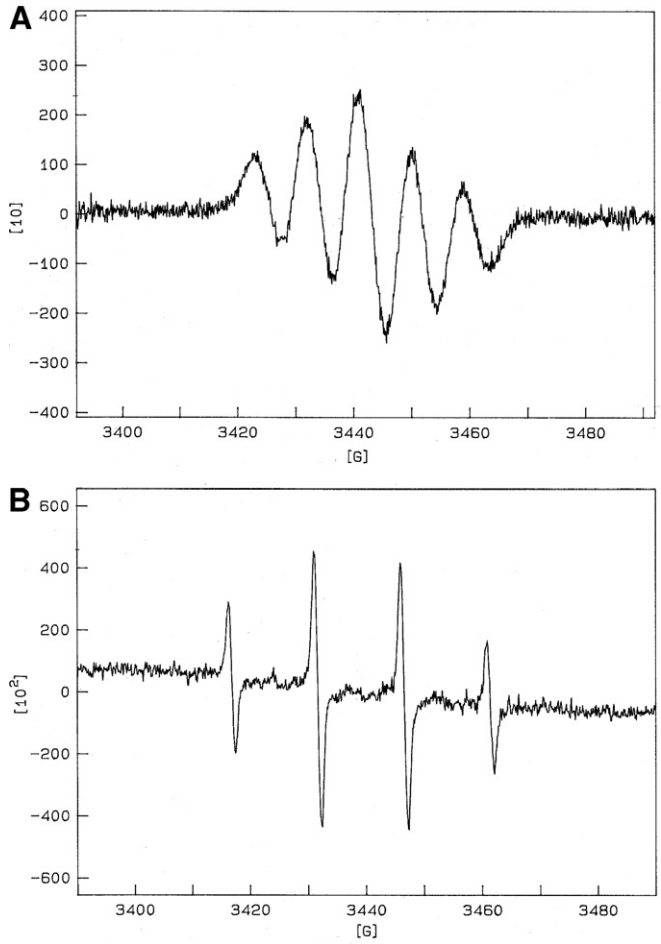

Fig. 1. ESR spectra of (A) DPPH radicals and (B) DMPO-OH spin adducts.

where h_o and h_x are the height of the second peak in the ESR spectrum of DPPH radicals of the blank and the probe, respectively.

2.6.2. Hydroxyl radical scavenging activity

The hydroxyl radicals was generated by Fenton reaction and detected because of their ability to form nitroxide adducts from the commonly used DMPO spin trap. The adduct DMPO–OH radical exhibits a characteristic ESR response (Fig. 1B). Hydroxyl radicals were obtained in the system: 0.2 ml 10 mM H_2O_2, 0.2 ml 10 mM $FeCl_2 \times 4H_2O$ and 0.2 ml 80 mM DMPO as spin trap (blank). The influence of extracts on the formation and transformation of hydroxyl radicals was investigated by adding the extract to the Fenton reaction system in the range of concentrations 10–30 mg/ml. ESR spectra were recorded after 5 min, with the following spectrometer settings: field modulation 100 kHz, modulation amplitude 0.512 G, receiver gain 5×10^5, time constant 81.92 ms, conversion time 163.84 ms, center field 3440.00 G, sweep width 100.00 G, x-band frequency 9.64 GHz, power 20 mW, temperature 23 °C.

The SA_{OH} value of the extract was defined as

$$SA_{OH} = 100(h_o - h_x)/h_o \ [\%]$$

where h_o and h_x are the height of the second peak in the ESR spectrum of DMPO-OH spin adduct of the blank and the probe, respectively.

2.7. Statistical analysis

All measurements were carried out in triplicate, and presented as mean values ± SEM (standard error of the mean). Regression analysis and significance of differences were carried out using a SPSS Statistical Software package (SPSS for Windows, 8.0, 1997, SPSS Inc., Chicago, IL, USA). Statistical significance level was fixed at $p < 0.05$.

3. Results and discussion

The various varieties, Pinova, Reinders, Jonagold, Iduna and Braeburn, which are usually consumed as fresh fruits in Serbian diet, were used to prepare apple pomaces. Also, a sample of industrial apple pomace was obtained from factory Nectar, Bačka Palanka.

The comparative evaluation of the polyphenolic composition of apple pomaces was based on three representative indices: the total phenolics (TP), the total flavonoids (TFd) and the total flavan-3-ols (TFl) contents (Table 2).

Table 2
Polyphenolic composition of apple pomaces

Apple pomace	TP (mg/g)	TFd (mg/g)	TFl (mg/g)	TFd/TP	TFl/TP
Pinova	7.96 ± 0.37	0.83 ± 0.032	9.51 ± 0.47	10.43	–
Reinders	8.67 ± 0.39	1.04 ± 0.050	7.89 ± 0.37	12.00	91.00
Jonagold	8.53 ± 0.39	1.19 ± 0.058	7.83 ± 0.31	13.95	91.79
Iduna	6.47 ± 0.31	0.62 ± 0.021	5.62 ± 0.27	9.58	86.86
Braeburn	5.59 ± 0.25	0.62 ± 0.015	3.86 ± 0.15	11.09	69.05
Nectar[a]	4.22 ± 0.18	0.45 ± 0.020	2.27 ± 0.08	10.66	53.79

[a] Industrial apple pomace obtained from factory Nectar; TP – total phenolics, TFd – total flavonoids, TFl – total flavan-3-ols.

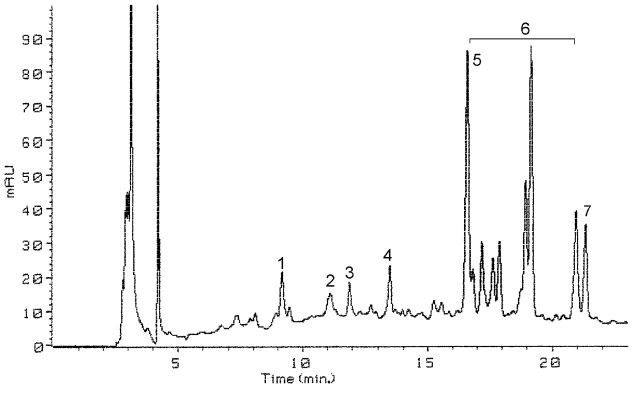

Fig. 2. HPLC chromatogram of phenolic compounds in Reinders pomace extract. Detection was performed at 280 nm. Peaks: 1 – chlorogenic acid; 2 – caffeic acid; 3 – (+)-catechin; 4 – (−)- epicatechin; 5 – rutin; 6 – quercetin-glycosides; 7 – phloridzin.

Table 3
The content of individual phenolic compounds in apple pomaces

Phenolic compound	Content of individual phenolic compounds in apple pomaces (mg/g)					
	Pinova	Reinders	Jonagold	Iduna	Braeburn	Nectar[a]
Chlorogenic acid	0.082 ± 0.004	0.100 ± 0.005	0.176 ± 0.009	0.130 ± 0.006	0.083 ± 0.004	0.030 ± 0.001
Caffeic acid	0.072 ± 0.004	0.067 ± 0.003	0.060 ± 0.003	0.087 ± 0.004	0.055 ± 0.003	0.025 ± 0.001
(+)-Catechin	0.065 ± 0.003	0.067 ± 0.003	0.078 ± 0.004	0.089 ± 0.004	0.127 ± 0.006	0.017 ± 0.001
(−)-Epicatechin	0.051 ± 0.002	0.114 ± 0.006	0.112 ± 0.005	0.125 ± 0.006	0.173 ± 0.008	0.024 ± 0.001
Rutin	0.364 ± 0.018	0.477 ± 0.024	0.316 ± 0.016	0.387 ± 0.019	0.345 ± 0.017	0.211 ± 0.010
Quercetin-glycosides	0.344 ± 0.017	0.610 ± 0.030	0.440 ± 0.022	0.336 ± 0.017	0.286 ± 0.014	0.378 ± 0.019
Phloridzin	0.018 ± 0.001	0.039 ± 0.002	0.008 ± 0.000	0.085 ± 0.004	0.062 ± 0.003	0.007 ± 0.000
Total[b]	0.996 ± 0.049	1.474 ± 0.074	1.190 ± 0.056	1.239 ± 0.062	1.131 ± 0.057	0.692 ± 0.035

[a] Industrial apple pomace obtained from factory Nectar.
[b] Mean of sum of the identified phenolics.

The Folin–Cioacalteu method is a rapid and widely-used assay to investigate the total phenolic content. This method is based on reducing power of phenolic hydroxyl groups, but is known that different phenolic compounds have different responses to the Folin–Cioacalteu reagent (Singleton et al., 1999). The contents of total soluble polyphenols of apple pomaces are expressed as chlorogenic acid equivalent and varied from 4.22 mg/g to 8.67 mg/g. Comparing the results obtained in methanol and water extracts of apple pomace reported by Sudha et al. (2007), the contents of phenolic compounds in our research were very similar or somewhat higher than those.

The total flavonoid contents of apple pomaces were measured according to the Markham methods. The contents of flavonoids were expressed as rutin equivalent and these ranged from 0.45 mg/g for Nectar pomace to 1.19 mg/g for Jonagold pomace.

In order to determine the flavan-3-ols content in apple pomaces, the vanillin assay was conducted and the (+)-catechin was used as a standard for calibration. The content of flavan-3-ols of apple pomaces varied from to 2.27 mg/g for Nectar pomace to 9.51 mg/g for Pinova pomace. According to the literature, the differences in the sensitivities to the vanillin assay were observed (Nakamura, Tsuji, & Tonogai, 2003). Namely, the higher reactivity was observed in the procyanidins which are highly polymerized, and the lower reactivity was observed in catechin which is highly esterified by gallic acid. In addition, epicatechin was more reactive than catechin.

Based on spectrophotometric determination, it can be concluded that in apple pomaces, the percentage of TFl in relation to TP ranged from 53.79% (Nectar pomace) to 91.79% (Jonagold pomace), while flavonoid levels did not exceed 13.95% of TP content (Table 2). The similar percentage distribution of these classes of polyphenols was found in different apple varieties (Vrhosek et al., 2004).

The chromatographic analysis was employed to identify and quantify major polyphenols present in apple pomaces. Fig. 2 shows a typical HPLC chromatogram of one of the samples (Reinders pomace extract). Phenolic acids (caffeic and chlorogenic acid), flavan-3-ols ((+)-catechin and (−)-epicatechin), flavonol (rutin) and dihydrochalcone (phloridzin) were identified in these extracts by matching their retention times (RT) and on-line ultraviolet (UV) spectra with those of standards. In addition, several other peaks (assign under the number 6), with retention times ranging from 15.8 to 21.2 min, had similar spectra to quercetin and were tentatively identified as quercetin glycosides (Escarpa & Gonzalez, 1999).

The content of individual phenolic compounds, expressed as mg per g dry weight of apple pomace, is listed in Table 3.

The highest concentrations of flavan-3-ols, (+)-catechin (0.127 mg/g) and (−)-epicatechin (0.173 mg/g), were found in Braeburn pomace. The Reinders pomace had the highest

Table 4
EC_{50}^{DPPH} and EC_{50}^{OH} values of apple pomaces

Apple pomace	EC_{50}^{DPPH} (mg/ml)	EC_{50}^{OH} (mg/ml)
Pinova	9.22 ± 0.45	33.75 ± 1.68
Reinders	6.33 ± 0.31	26.11 ± 1.30
Jonagold	9.50 ± 0.47	31.17 ± 1.55
Iduna	12.22 ± 0.61	32.01 ± 1.60
Braeburn	7.95 ± 0.39	34.47 ± 1.82
Nectar[a]	15.72 ± 0.78	52.83 ± 2.63

[a] Industrial apple pomace obtained from factory Nectar.

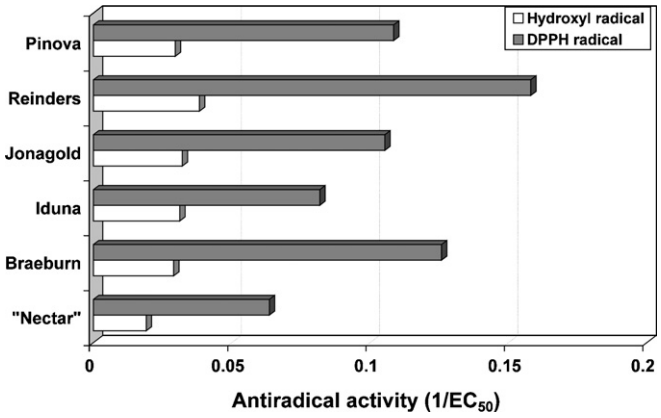

Fig. 3. Antiradical activities, defined as $1/EC_{50}^{DPPH}$ and $1/EC_{50}^{OH}$, of apple pomace.

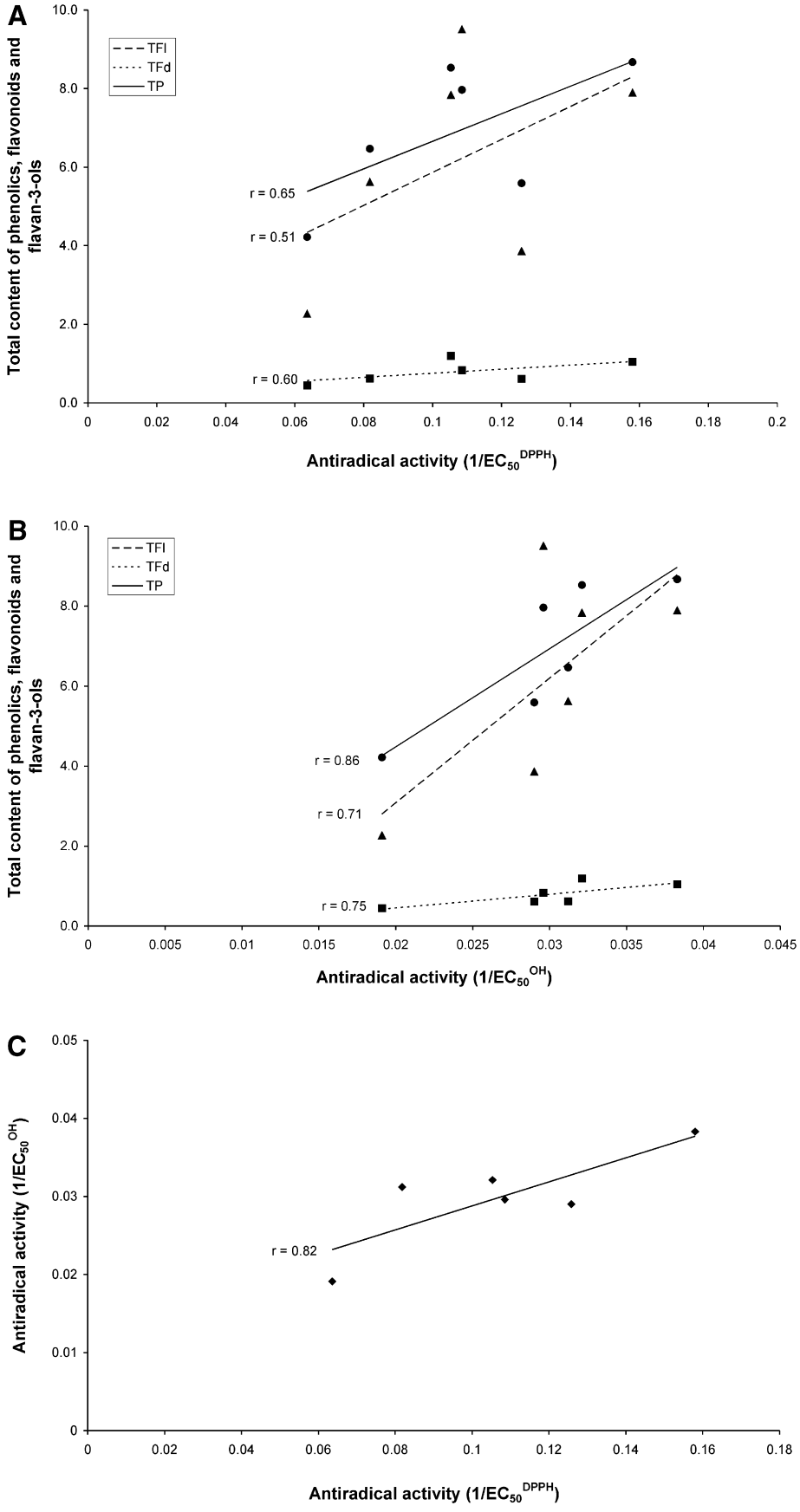

Fig. 4. Correlation between total phenolics, total flavonoids and total flavan-3-ols in apple pomace and antiradical activities – $1/EC_{50}^{DPPH}$ (A); and $1/EC_{50}^{OH}$ (B); and DPPH and hydroxyl antiradical activities (C).

polyphenols concentration (1.474 mg/g) among the six investigated pomaces and contained flavonols as the major phenolic compounds; rutin was present at 0.477 and quercetin glycosides at 0.610 mg/g (expressed as quercetin equivalent).

The total phenolic contents determined according to Folin–Cioacalteu method (Table 2) were higher than the sums of the individual phenolics identified by HPLC (Table 3). This difference can be explained by fact that Folin–Ciocalteau method is not an absolute measurement of the amount of phenolics because some other substances such as organic acids, residual sugars, amino acids, proteins and other hydrophilic compounds interfere with this assay. In addition, various phenolic compounds have different responses in Folin–Ciocalteau assay (Rigo et al., 2000; Singleton et al., 1999). The statistical analysis showed a good relationship between total phenolic contents obtained by Folin–Ciocalteau method and HPLC results ($r = 0.72$).

As one part of our investigation on antioxidant activity of apple pomace extracts, the scavenging activities on DPPH and hydroxyl radicals were measured by ESR method. The investigated apple pomace extracts showed dose-dependent radical scavenging activities (data was not shown). The EC_{50} value, defined as the concentration of extract required for 50% scavenging of DPPH or hydroxyl radicals under experimental condition employed, is a parameter widely used to measure the free radical scavenging activity (Cuvelier, Richard, & Berset, 1992); a smaller EC_{50} value corresponds to a higher antioxidant activity. The EC_{50}^{DPPH} and EC_{50}^{OH} values of apple pomaces, determined based on radical scavenging activities (SA_{DPPH} and SA_{OH}) of apple pomace extracts, are presented in Table 4.

It was observed that all investigated apple pomaces were less effective on hydroxyl radical scavenging than in DPPH test. Namely, the EC_{50}^{OH} values varied from 26.11 mg/ml to 52.83 mg/ml, while the EC_{50}^{DPPH} values ranged from 6.33 mg/ml to 15.72 mg/ml. The capacity of apple pomace to inhibit hydroxyl radical generated by the Fenton reaction could be due to direct scavenging effect and/or to inhibit of hydroxyl generation. The second mechanism occurs by ion chelation (Calliste, Trouillas, Allais, & Duroux, 2005). Among the six apple pomaces, the Reinders pomace revealed as the most effective at scavenging both DPPH and hydroxyl free radicals.

It is interesting to consider the correlation between phenolic composition and antioxidant activities of apple pomace extracts, as phenolic compounds contribute directly to antioxidant activity (Duh, 1999). $1/EC_{50}$ is representative of the antioxidant activity because the more increases, the more efficient is the apple pomace (Fig. 3).

In this study, there was a distinct correlation between studied parameters (TP, TFd, TFl and $1/EC_{50}$) in selected apple pomaces. The antiradical activities (EC_{50}^{DPPH} and $1/EC_{50}^{OH}$), as a function of total phenolics content, total flavonoids content and total flavan-3-ols content is shown in Fig. 4A, and B, respectively.

In the case of hydroxyl radical scavenging activity, the correlation coefficients, calculated from linear regression analysis, were high for total phenolics, total flavonoids and total flavan-3-ols. The correlation of $1/EC_{50}^{DPPH}$ values were moderately associated with the total phenolics, total flavonoids and total flavan-3-ols. When the relationship between two antiradical activities ($1/EC_{50}^{DPPH}$ and $1/EC_{50}^{OH}$) is plotted as shown at Fig. 4C, the correlation coefficient indicated that there is a significant positive relationship between DPPH and hydroxyl radical scavenging activity.

In order to determine the relative importance of individual phenolic compounds in radical scavenging activities, correlation analysis were made between the both $1/EC_{50}$ values and the results of the chromatography. A high degree of correlation existed between the content of rutin and radical scavenging activities on DPPH and hydroxyl radicals ($r = 0.80$ and $r = 0.93$, respectively). On the other hand, phloridzin seemed to influence the DPPH and hydroxyl radical scavenging activities weakly ($r = 0.13$ and $r = 0.33$, respectively).

4. Conclusions

This study indicated that selected apple pomaces possess considerable amounts of phenolic compounds and significant radical scavenging activity on stable DPPH and highly reactive OH radicals. The correlation coefficients exhibited a positive relationship between antiradical activities of apple pomaces and contents of total phenolic, flavanoid, flavan-3-ol and some individual phenolic compounds. Our results showed that, in addition to the grape seeds, the apple pomace, an inedible waste product of juice manufacture, might be another potent source of antioxidants. In view of the significant radical scavenging activity of selected apple pomaces, apple pomace could afford health benefits by preventing unwanted free-radical-induced oxidative reactions. Apple pomace should be regarded as a valuable product and has potential as a value-added ingredient for functional foods.

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