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Selected Polyphenols in Fruits of Different Cultivars of Genus *Prunus*

By

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K e y w o r d s : Chlorogenic acid, epicatechin, rutin, peach, apricot, cherry, peel, pulp.

S u m m a r y

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Chlorogenic acid, epicatechin and rutin were analyzed in different cultivars of peach, apricot and sweet cherries. In peach and apricot the phenolics were analyzed in peel and pulp separately, while in sweet cherries the phenolics were analyzed in the whole fruit only. The analyses were performed using high performance liquid chromatography with a diode-array detector. Differences in the contents of all phenolic compounds between the cultivars were distinguished. At peach and apricot varieties we noticed that the contents of chlorogenic acid, epicatechin and rutin were higher in peel compared to pulp. Therefore it can be suggested to the consumers that, regarding health promoting properties of fruit, unpeeled fruits should be eaten or used for processing. The highest values of chlorogenic acid, epicatechin and rutin were detected in apricot peel (on average 705.2, 86.3, 347.4 mg kg⁻¹ respectively). The highest values of analyzed phenolics in pulp were as follows: chlorogenic acid (125.43 mg kg⁻¹) in peach, epicatechin in apricots (43.46 mg kg⁻¹). Both apricots and peaches exhibited similar values for the content of rutin (4.84 and 4.60 mg kg⁻¹ respectively). The average content of phenolics in the whole cherry fruit was similar to or higher than the content of phenolics in apricot and peach pulp (103.6 mg kg⁻¹ for chlorogenic acid, 66.3 mg kg⁻¹ for epicatechin and 17.42 mg kg⁻¹ for rutin). The content of analyzed phenolics is comparable to the content of phenolics in apple and therefore the analyzed species can be considered as important source of antioxidants.

I n t r o d u c t i o n

Different stone-fruit species like peach (*Prunus persica*), cherry (*Prunus avium*) and apricot (*Prunus armeniaca*) which include several cultivars, significantly differ in the appearance as well as in chemical composition. In addition to

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this the chemical composition of fruits can vary a lot also due to other factors, such as climatic conditions, ecological factors; and due to different horticultural practices, maturity, post harvest condition in fruit production (VEBERIC & al. 2005, in press).

In the recent years fruits species have been investigated in order to gain better knowledge of the biochemical composition of different tissues or organs and the changes of the composition due to different ecological or technological measures applied. The research focused also on the group of polyphenols. These are naturally occurring compounds in all fruit species as the result of the secondary metabolism. They have a lot of different functions in plants. For a fruit grower the involvement of phenolics into the resistance/susceptibility of plants in the relation to infection of different pathogens is very important (RÜHMANN & al. 2002, USENIK & al. 2004). The phenolics are also known for their influence of the auxin metabolism and are therefore involved in the root formation process of cuttings in the nurseries (OSTERC & al. 2004, TROBEC & al. 2005, in press). Phenolics are also indicators of incompatibility between scion and rootstock (USENIK & STAMPAR 2000, 2002). Between other numerous functions, perhaps the most important for the fruit-grower and the consumer is the influence of phenolics on color, flavor and taste (astringency) of the fruits (KIM & al. 2003). Therefore, there is a growing interest in analytical data on natural phenols in fruits, other plant tissue as well as in fruit processed products (STAMPAR & al. 2005, in press) due to health promoting properties as well as commercial benefits (TREUTTER 2001). Many phenolic phytochemicals are supposed to have antioxidative, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and antiinflammatory activities (KIM & al. 2003). In addition, they may reduce cardiovascular diseases. Increasing evidence shows that flavanoids can be absorbed into the human body in amounts that should be sufficient to exert antioxidant or other biological activities *in vivo* (PROTEGENTE & al. 2002). Fruits present complex mixtures of polyphenols, which are often poorly characterized. The phenolics substances in fruits are mainly phenolic acids and flavonoids, which are divided into several subclasses. The main flavonoid subclasses found in stone- fruits are flavonols, anthocyanidins and flavanols (catechins and proanthocyanidins). In addition to this diversity, polyphenols may be associated with various carbohydrates and organic acids and with one another (MANARCH & al. 2004). In general more phenolic acids and flavonoids are found in outer tissues (fruit peel) due to their role in the defense against ultraviolet radiation, aggression by pathogens and other ecological roles (MANARCH & al. 2004).

Due to the important role of polyphenols in plant mechanisms as well as due to their increasing importance in human nutrition, we have studied selected polyphenols (chlorogenic acid (phenolic acids), epicatechin (flavanols) and rutin (flavonols)) in different cultivars of stone fruits (apricots, cherries and peaches). In the case of apricot and peaches the comparison between the contents of selected polyphenols in peel and pulp was carried out.

Material and Methods

Plant material

The cherries were obtained from the trial orchard of the Slovenian national agricultural institute in Ljubljana, Slovenia. The peach and apricot cultivars were obtained from the research station Bilje at Nova Gorica, Slovenia. All the fruits were picked at commercial maturity stage, which was determined according to color, taste and inner quality parameters (sugars, organic acids and firmness).

Cherry cultivars:

'Büttners Späte Knorpelkirsche', 'Elisa' ('Durone Tardivo di Valstaffora'), 'Giorgia', 'Lapins', 'Napoleon' ('Bigarreau Napoleon'), 'Nordwunder', 'Sunburst', 'Van', 'Vega', 'Vigred'

Apricot cultivars:

'Cafona', 'Harcot', 'Orange Red', 'Perla', 'Sabbatani', 'San Castrese', 'Sungiant'

Peach cultivars:

'Anita', 'Maria Laura', 'Maria Marta', 'Norman', 'Redhaven', 'Royal Gem', 'Suncrest', 'Veteran'

The fruits were harvested at optimal ripening time in the year 2004. Phenolic compounds were analyzed in the pulp and peel of apricots and peaches and in the whole fruit (stoned) of cherries. For every cultivar 4 replications were done ($n=4$), each repetition included 10 fruits sampled from 5 trees. The fruits were stored at -20°C until the preparation of the samples.

Extraction and the HPLC analysis

The samples were prepared according to the method described by ESCARPA & GONZALEZ 1998. The samples of 10 g pulp and 5 g of peel (in the case of cherries 5 g of whole fruits without the stone) were extracted with methanol containing 1% 2,6-di-tert-butyl-4-methylphenol (BHT) using an ultrasonic bath. The samples were extracted with 10 ml of solvent for 1 h, 10 ml for 30 min, and finally 5 ml for 30 min. The tree extraction fractions were combined to final volume of 25 ml and filtered through 0,25 μm membrane filter (Mecherey-Nagel) prior to the injection to HPLC. BHT was added to samples to prevent the oxidation during the extraction. It did not interfere with extracted phenols during the subsequent HPLC analysis, because it was eluted on the end of the gradient or in the equilibration delay between the two analyses.

The samples were analyzed on Thermo Finnigan Surveyor HPLC system with diode array detector at 280 nm. Also the spectra of compounds were recorded between 210 and 350 nm. The elution solvents were aqueous 0.01 M phosphoric acid (A) and 100% methanol (B). The samples were eluted according to a linear gradient described by ESCARPA & GONZALEZ 1998. The injection amount was 20 μl and the flow rate was 1 ml/min. The column used was a Phenomenex Synergi 4u MAX - RP 80 A, operated at 25°C .

The following phenolic compounds were identified: chlorogenic acid (5-*O*-caffeoylquinic acid), (-)-epicatechin and rutin (quercetin 3-*O*-rutinoside). Identification of compounds was achieved by comparing the retention times, spectra as well as with addition of the internal standard. The concentrations of phenolic compounds were calculated with the help of a corresponding external standard.

Statistical analysis

The analysis of data was performed as the analysis of variance (ANOVA) using the program Statgraphics plus 4.0. The differences between the treatments were estimated with a multiple range test using the Tukey HSD test at $\alpha < 0.05$.

Results

Polyphenols in cherries

The highest values of analyzed phenolics were in the concentration of chlorogenic acid (average 103.62 mg kg⁻¹ FW), followed by epicatechin (66.3 mg kg⁻¹ FW) and rutin (17.42 mg kg⁻¹ FW) (Table 1). The highest values of chlorogenic acid were attained by the cultivar 'Van', whose content was 3.18-fold higher than the content in the cultivar 'Vigred'. The cultivar 'Van' had also high amounts of epicatechin and rutin. The highest amounts of epicatechin were attained by the cultivar 'Napoleon' and the lowest by the cultivar 'Sunburst' (4.4- fold lower than in the cultivar 'Napoleon'). This cultivar had also the lowest values of rutin, which were 2.6-fold lower than in the cultivar 'Vega', which exhibited the highest contents. The cultivars high in concentration of all three analyzed phenolics were 'Napoleon' and 'Van'. The cultivars low in the concentrations of all 3 phenolics were 'Elisa' and 'Büttners'.

Table 1. The content of chlorogenic acid, epicatechin and rutin in different cultivars of sweet cherries expressed in mg kg⁻¹ of fresh weight. Average and standard error are presented. Different letters indicate significant differences ($\alpha < 0,05$).

Cultivar	Chlorogenic acid			Epicatechin			Rutin		
Büttners	76.79 ± 6.60	a		40.53 ± 4.25	ab		16.99 ± 0.41	abc	
Elisa	82.19 ± 4.20	a		50.05 ± 4.38	abc		13.18 ± 1.06	abc	
Giorgia	93.06 ± 6.93	ab		71.02 ± 3.83	abc		18.17 ± 0.90	bcd	
Lapins	132.19 ± 14.91	bc		78.11 ± 10.65	bc		11.66 ± 0.85	ab	
Napoleon	136.51 ± 9.93	bc		126.50 ± 9.15	d		18.51 ± 2.23	bcde	
Nordwunder	89.06 ± 12.40	ab		54.34 ± 12.02	abc		19.49 ± 1.43	cde	
Sunburst	134.81 ± 9.09	bc		28.76 ± 7.34	a		9.69 ± 0.21	a	
Van	160.70 ± 3.39	c		90.38 ± 4.05	cd		25.24 ± 1.90	de	
Vega	81.91 ± 15.94	a		54.80 ± 14.67	abc		26.08 ± 0.10	e	
Vigred	48.96 ± 4.83	a		68.48 ± 5.87	abc		15.18 ± 3.10	abc	

Polyphenols in peach

The polyphenols in peach were separately analyzed in peel and in pulp (Table 2). Similarly to the results attained in apricots also the peach peel exhibited higher values compared to pulp. This difference was the least pronounced in the case of chlorogenic acid, where the values were 2-fold higher in peel than in pulp. The ratio between the content of epicatechin and rutin was 11 and 16.5-fold higher (respectively) in peel than in pulp. The cultivar 'Anita' exhibited low values of analyzed phenolics in peel and in pulp. On the other hand the cultivar 'Veteran' had high values. The average values of chlorogenic acid, epicatechin and rutin in peel were 266.45, 29.98 and 76.1 g kg⁻¹ FW and in pulp were 125.43, 2.55 and 4.60 g kg⁻¹ FW respectively. We noticed that the cultivars rich in chlorogenic acid in peel had also high amount of this phenol in pulp and vice versa. This did not hold true

for the content of epicatechin and rutin. The cultivars did not significantly differ in the contents of epicatechin in the peach pulp.

Table 2. The contents of chlorogenic acid, epicatechin and rutin in different peach cultivars expressed in mg kg^{-1} of fresh weight. Average and standard errors are presented. Different letters indicate significant differences ($\alpha < 0,05$).

Cultivar	Tissue	Chlorogenic acid		Epicatechin		Rutin	
Anita	Peel	111.38 $\pm$ 6.82	ab	28.01 $\pm$ 1.31	abc	22.44 $\pm$ 1.46	a
Maria Laura	Peel	521.63 $\pm$ 67.59	e	25.63 $\pm$ 1.87	abc	167.51 $\pm$ 12.91	c
Maria Marta	Peel	65.24 $\pm$ 4.75	a	29.88 $\pm$ 1.2	bc	46.07 $\pm$ 3.92	ab
Norman	Peel	208.78 $\pm$ 27.55	bc	34.63 $\pm$ 3.47	c	84.94 $\pm$ 8.75	b
Redhaven	Peel	187.57 $\pm$ 23.00	abc	22.14 $\pm$ 2.12	ab	65.51 $\pm$ 11.87	b
Royal gem	Peel	321.86 $\pm$ 18.47	cd	33.13 $\pm$ 1.24	c	63.50 $\pm$ 4.8	b
Suncrest	Peel	453.37 $\pm$ 46.96	de	18.61 $\pm$ 1.24	a	83.49 $\pm$ 3.42	b
Veteran	Peel	361.74 $\pm$ 39.20	d	47.82 $\pm$ 3.6	d	75.34 $\pm$ 12.42	b
Anita	Pulp	44.00 $\pm$ 6.16	a	2.74 $\pm$ 0.081	a	2.75 $\pm$ 0.05	a
Maria Laura	Pulp	222.64 $\pm$ 14.52	bc	3.30 $\pm$ 0.412	a	4.76 $\pm$ 0.16	b
Maria Marta	Pulp	25.95 $\pm$ 2.18	a	2.67 $\pm$ 0.152	a	6.62 $\pm$ 0.32	c
Norman	Pulp	42.76 $\pm$ 5.84	a	2.21 $\pm$ 0.176	a	3.11 $\pm$ 0.33	a
Redhaven	Pulp	57.98 $\pm$ 1.44	a	2.71 $\pm$ 0.256	a	5.16 $\pm$ 0.23	b
royal gem	Pulp	181.73 $\pm$ 8.15	b	2.09 $\pm$ 0.473	a	4.59 $\pm$ 0.19	b
Suncrest	Pulp	170.02 $\pm$ 17.47	b	1.79 $\pm$ 0.018	a	4.47 $\pm$ 0.32	b
Veteran	Pulp	258.32 $\pm$ 25.27	c	2.91 $\pm$ 0.606	a	5.33 $\pm$ 0.18	b

Polyphenolics in apricot

The polyphenols in apricot were separately analyzed in peel and in pulp (Table 3). In general, the values in peel were sometimes higher compared to the values in pulp. This was especially noted for rutin (about 70-fold higher concentrations in peel). Chlorogenic acid concentrations were 8.3-fold higher and epicatechin concentration was about 2-fold higher in peel compared to pulp. Not all cultivars, which exhibited high values in peel, had also high values in pulp. In general 'Perla' had quite low values of analyzed phenols in both peel and pulp; while 'Orange red' had high values of analyzed phenolics in peel, but only moderate values in pulp. The average values of chlorogenic acid, epicatechin and rutin in peel were 705.2, 86.3 and 347.4 g kg^{-1} FW and in pulp were 85.25, 43.46, 4.48 g kg^{-1} FW respectively.

Discussion

Chlorogenic acid was reported to be the main phenolic acid in peaches (TOMAS-BARBERAN & al. 2001) in contrast to cherries, where neochlorogenic and p-cumaroylquinic acids are in larger amounts (GONCALVES & al. 2004). In apricots the amount of chlorogenic and neochlorogenic acids were reported to be at the same level (HERRMANN 2001). From the group of flavanols, epicatechin is the main

form in cherries (GONCALVES & al. 2004), it has somewhat higher content than catechin reported for apricots (DRAGOVIC-UZELAC & al. 2005), while catechin was stated to be the main flavanol in peaches (TOMAS-BARBERAN & al. 2001). Rutin as an important quercetin-glucoside was reported to be present in lower contents in all three species (HERRMANN 2001). All tree species showed similar chromatograms, but the differences in the content of individual phenolics were considerable.

Table 3. The contents of chlorogenic acid, epicatechin and rutin in different apricot cultivars expressed in mg kg^{-1} of fresh weight. Average and standard errors are presented. Different letters indicate significant differences ($\alpha < 0,05$).

Cultivar	Tissue	Chlorogenic acid		Epicatechin		Rutin	
Cafona	peel	799.55 $\pm$ 47.88	cd	64.80 $\pm$ 9.26	a	361.91 $\pm$ 52.82	ab
Harcot	peel	470.66 $\pm$ 16.91	ab	66.70 $\pm$ 1.04	a	214.81 $\pm$ 8.47	a
Orange red	peel	1158.99 $\pm$ 45.17	e	107.37 $\pm$ 1.51	b	441.01 $\pm$ 31.28	b
Perla	peel	299.82 $\pm$ 24.13	a	41.38 $\pm$ 3.49	a	383.58 $\pm$ 20.19	b
sabbatani	peel	987.46 $\pm$ 126.52	de	69.49 $\pm$ 7.71	ab	410.03 $\pm$ 61.07	b
San Cas-trese	peel	657.26 $\pm$ 32.27	bc	74.18 $\pm$ 6.11	ab	316.81 $\pm$ 18.47	ab
Sungiant	peel	562.49 $\pm$ 21.17	bc	179.84 $\pm$ 18.23	c	303.64 $\pm$ 15.06	ab
Cafona	pulp	102.85 $\pm$ 2.57	c	80.21 $\pm$ 4.65	d	5.36 $\pm$ 0.94	ab
Harcot	pulp	56.66 $\pm$ 1.27	a	30.86 $\pm$ 2.41	ab	6.77 $\pm$ 1.85	b
Orange red	pulp	80.94 $\pm$ 12.96	abc	45.61 $\pm$ 4.29	bc	6.69 $\pm$ 1.53	b
Perla	pulp	62.32 $\pm$ 2.03	ab	27.33 $\pm$ 2.81	ab	2.31 $\pm$ 0.35	a
Sabbatani	pulp	100.14 $\pm$ 7.53	c	22.16 $\pm$ 6.53	a	5.95 $\pm$ 0.19	ab
San Cas-trese	pulp	99.47 $\pm$ 6.96	bc	46.16 $\pm$ 2.38	bc	4.33 $\pm$ 0.99	ab
Sungiant	pulp	94.39 $\pm$ 9.77	bc	51.87 $\pm$ 4.18	c	2.48 $\pm$ 0.11	a

The study on phenolics performed by GONCALVES & al. 2004 on 4 cherry cultivars showed somewhat lower amount of chlorogenic acid (31 - 97 mg kg^{-1} FW) than in our study. In our experiment the cultivars with high contents were over this range. For example, the cultivar 'Van', which was analyzed in both studies, had in our trial 2.5-fold higher values. Also in the study by GONCALVES & al. 2004 performed over the period of two years, the content of chlorogenic acid in ripe fruits of 'Van' varied about 2-times between the years. The authors explain the differences to be the consequence of different weather conditions between the years. We would like to add that probably other factors like growing the same cultivar on different locations influenced the phenolic content as well. The differences could also appear due to different extraction and analyses procedures. The trial performed by GONCALVES & al. 2004 and our trial showed the same range in concentrations of epicatechin (about 30-126 mg kg^{-1} FW), while the concentration of rutin was higher in their study (about 28-137 mg kg^{-1} FW). Again differences in the content of epicatechin and rutin appeared in both studies in the cultivar 'Van'. The

wider range of content of different phenolics can be explained also by higher number of cultivars included in our study.

The highest contents of the analyzed phenolics were in the peach peel compared to pulp. For The chlorogenic acid content was on average 2.1-fold higher in peel compared to pulp. Similar results were reported by TOMAS-BARBERAN & al. 2001. They reported the range of the content of chlorogenic acids in peel of ripe peaches to be from 107.2 and up to 434.7 mg kg⁻¹ and in the pulp from 26.4 up to 242.2 mg kg⁻¹ FW. This agrees with our results, except for the cultivar 'Maria Laura' which is a nectarine and exhibited values around 521,6 mg kg⁻¹ FW of peel. The nectarine cultivar 'Brite Pearl' showed similar concentrations in the study by TOMAS-BARBERAN & al. 2001. However, the authors did not notice an important difference in the contents of chlorogenic acid in the peel and pulp between peaches and nectarines. In our study the content of epicatechin in peel and in pulp is lower than in the previously mentioned study. This may be because in the study by TOMAS-BARBERAN & al. 2001, epicatechin was expressed as catechin, which may have led to the overestimation of epicatechin. Our results agree more with results brought forward by HERRMANN 2001. The values for rutin are again in agreement with TOMAS-BARBERAN & al. 2001. The lowest values of rutin were in our study achieved by the cultivar 'Anita' both in peel and pulp. This is an early ripening white flesh peach cultivar (25 days before 'Redhaven'). Similar findings were reported also by TOMAS-BARBERAN & al. 2001, who noticed that the yellow flesh peaches and nectarines showed more pronounced concentrations of rutin compared with white flesh peaches.

Also apricots, similar to peaches, had higher values of analyzed phenolics (in general) in peel compared with pulp. The values for phenolics were somewhat higher compared with results achieved by DRAGOVIC-UZELAC & al. 2005. In their study, however, whole fruits were used for the analysis and the contents of phenolics were not investigated separately in peel and pulp. Therefore their results are more in agreement with our results obtained from the pulp, since the peel presents a minor part of the edible portion of the fruit. It is interesting to see that apricots contained higher average amounts of all investigated phenolics in the peel compared to those analyzed in peach. In the pulp, the content of chlorogenic acid was higher in peach pulp, the content of epicatechin in apricot pulp and rutin was about the same in pulp of both species. The peach fruit regarding the content of analyzed phenolics in this study could be well compared with results obtained on apple (VEBERIC & al. 2005, in press); while the apricot peel has in general somewhat higher concentrations of chlorogenic acid and epicatechin. Also the study by DRAGOVIC-UZELAC & al. 2005 showed that the analyzed apricot cultivars exhibited higher concentrations of epicatechin and rutin and about the same level of chlorogenic acid compared to apple cultivar 'Idared'. The study by LEONTOWICZ & al. 2002 noted that the content of caffeic, p-coumaric and ferulic acids, which were not analyzed in our study, were higher in apple compared to peaches. This resulted in higher total radical trapping antioxidative potential of apples compared to peaches. The authors noticed that diet supplemented with apples or peaches improved lipid metabolism and increased the plasma antioxidant potential.

Also cherries exhibited high contents of phenolics. Because of the presence of quercetin-glucoside derivates, epicatechin and other phenolics in sweet cherry, may contribute to making sweet cherries a beneficial source of health protective antioxidants GONCALVES & al. 2004. This is certainly true for peaches and apricots as well.

Conclusions

We noticed that at peach and apricot varieties the contents of chlorogenic acid, epicatechin and rutin were higher in peel compared to the pulp. Therefore it can be suggested to the consumers that, regarding health promoting properties of fruit, unpeeled fruits should be eaten or used for processing. The content of analyzed phenolics is comparable to the contents of phenolics in apple and therefore the analyzed species can be considered as important source of antioxidants. For further investigation of the content of phenolics in individual cultivars, data collected over several years and from different locations should be used for a detailed analysis. In this study, due to a high number of cultivars, general contents of analyzed phenolics for species were investigated, what is an important contribution toward better understanding of inner quality of fruits and provides useful data for studying the nutritional values of this species.

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