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Determination and Quantitation of Anthocyanins and Hydroxycinnamic Acids in Different Cultivars of Sweet Cherries (*Prunus avium* L.) from Nova Gorica Region (Slovenia)

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Summary

The anthocyanins and hydroxycinnamic acids in 5 cultivars of dark coloured sweet cherries were characterised and quantified by means of HPLC and UV-VIS spectrophotometry. Phenolic components were extracted with pure methanol, without addition of acid and water. The samples were diluted in the mixture of methanol and formic acid just before the injection on the column and separated on Hypersil PEP 300 C18 chromatographic column using gradient solvent system consisting of formic acid, water and methanol. DAD detector was employed and two wavelengths were chosen for determination of different components; 320 nm for hydroxycinnamates and 520 nm for anthocyanins. We detected the presence of cyanidin-3-glucoside and cyanidin-3-rutinoside as major anthocyanins, while pelargonidin-3-rutinoside was identified among minor pigments. The major hydroxycinnamic acids were characterised as neochlorogenic acid and 3'-*p*-coumaroylquinic acid. Total anthocyanin content (expressed as cyanidin-3-glucoside) ranged from 29 to 62 mg/100 g of pitted cherry fresh weight (FW), with the highest content observed in Petrovka, a local cultivar. Concentrations of neochlorogenic acid and 3'-*p*-coumaroylquinic acid ranged from 19.5 to 53.0 mg/100 g FW and from 7.5 to 50.6 mg/100 g FW, respectively. The relative amounts of these two phenolic acids varied widely between the cherry cultivars examined in this study.

Key words: sweet cherries (*Prunus avium* L.), anthocyanins, hydroxycinnamic acids, HPLC

Introduction

Phenolic compounds are important components of many fruits, vegetables, and beverages and contribute to their colour and sensory properties such as bitterness and astringency (*1*). The interest in anthocyanins, whose main qualities are attractive bright colour, water solubility, and their easy incorporation into aqueous systems, has recently increased due to their beneficial health effects (*2*).

Epidemiological studies have shown that consumption of food rich in phenolic content is correlated with

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reduced incidence of heart diseases (*3*). Phenolic compounds retard the progression of arteriosclerosis by acting as antioxidants toward low-density lipoproteins (LDL) (*4*) and inhibit the *in vitro* oxidation of LDL (*5,6*). It was also confirmed that phenolic components from sweet cherries show antioxidant activity toward liposomes and LDL (*7*).

Major phenolics (Fig. 1) in sweet cherries are anthocyanins, especially in dark-coloured sweet cherries (*8,9*). Dark coloured genotypes have been found to contain 3-rutinoside and 3-glucoside of cyanidin as major anthocyanins and the same glycosides of peonidin as minor anthocyanins (*8–10*).

Sweet cherries are also rich in phenolic acids. The most abundant phenolic acids are caffeic acid derivatives of the hydroxycinnamic acid (such as neochlorogenic acid), which represent 35–87 %, and *p*-coumaric acid derivatives (such as 3'-*p*-coumaroylquinic acid), which represent 10–65 % of all hydroxycinnamic acid's derivatives in sweet cherries, respectively (*8,9,11*). These components are important for their potential contribution to the colour of sweet cherries through copigmentation (*12*).

Phenolic components of sweet cherries are normally extracted with methanol containing formic acid or even hydrochloric acid (*8,9*) to increase the yield of extraction of anthocyanins in red flavylium form, which are stable in highly acid medium (*8*). But the disadvantage of low pH is in the chemical transformation of some pigments. It was already proven that phenolic components readily decompose in acid environment (*13*) and the profile of phenolic compounds is changed.

For the same reason acylated anthocyanins undergo the decomposition processes by losing the acyl group (*8*). The use of non-acidified solvents to prevent degradation of the least stable anthocyanins and the formation of artefacts has been recommended (*8*), but not verified in practice for the case of sweet cherries.

In our study we describe a method of extraction, sample preparation and separation of anthocyanins and hydroxycinnamic acids in sweet cherries, which represents a combination and modification of previously reported methods (*9,14*). By using pure methanol as extraction solvent we aimed to avoid the decomposition of phenolic components, and to make the sample preparation step faster, since at low temperatures pure methanol easily evaporates compared to water-methanol solution. HPLC was used for separation, identification and quantification of hydroxycinnamic acids and anthocyanins in five sweet cherry cultivars that are most common in Nova Gorica Region, Slovenia.

Materials and Methods

Plant material

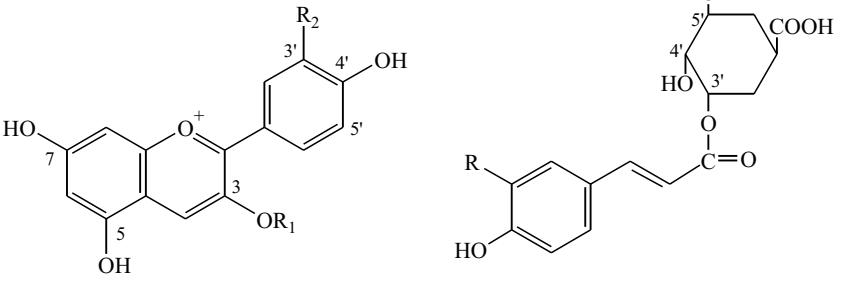
Three samples (1–1.5 kg) for each of five sweet cherry (*Prunus avium* L.) cultivars, Bing, Napoleon, Petrovka (local cultivar), Lambert and Stella compacta were harvested at commercial maturity at Bilje Agricultural Centre, Nova Gorica, Slovenia. Within 1 h of harvest all samples were packed in PE-aluminium bags in N_2 atmosphere, and kept at –25 °C for 6–9 weeks before analyses were carried out.

Standards and reagents

Cyanidin-3-glucoside, cyanidin-3-rutinoside, peonidin chloride and pelargonidin chloride were purchased from Extrasynthèse (Genay, France), peonidin-3-galactoside, petunidin-3-glucoside, delphinidin-3-glucoside, malvidin-3-glucoside, from Polyphenol co. (Finland), while chlorogenic, caffeic and *p*-coumaric acids were purchased as standards from Sigma. Pelargonidin-3-rutinoside and pelargonidin-3-glucoside were extracted from strawberries by the same protocol as sweet cherries in our study (*15*). Methanol (HPLC gradient grade) was purchased from J. T. Baker and formic acid (98–100 %) from Riedel-de Haën.

Extraction and preparation of sample for HPLC and spectrophotometric analysis

The mass of 40 g of pitted and accurately weighted dark coloured sweet cherries (random selection) were homogenised in 80 mL of methanol (min. 99.9 %). After 15 min of extraction at room temperature (while agitating) the homogenate was centrifuged (3000 min^{-1}, 15 min). The supernatant was removed and then evaporated under reduced pressure at 30 °C to yield a syrup-like resi-



cyanidin-3-glucoside: R_1 = glucose, R_2 = OH;
cyanidin-3-rutinoside: R_1 = rutinose, R_2 = OH;
peonidin-3-glucoside: R_1 = glucose, R_2 = OCH_3;
peonidin-3-rutinoside: R_1 = rutinose, R_2 = OCH_3;
pelargonidin-3-rutinoside: R_1 = rutinose, R_2 = H;

3'-*p*-coumaroylquinic acid: R = H
3'-caffeoylquinic acid = neochlorogenic acid: R = OH

Fig. 1. Chemical structures of anthocyanins (left) and most common phenolic acids (right) generally present in sweet cherries (*Prunus avium* L.)

due. The residue was in turn diluted in 25 mL of methanol and stored in freezer prior to analysis. All extracts were prepared in two replicates.

Spectrophotometric assay of total phenols and total anthocyanins

Total anthocyanin content of appropriately diluted extracts was determined by pH-differential method (*16*) and expressed as cyanidin-3-glucoside, using a molar extinction coefficient of 26 900 L/(mol cm) and molar mass (449 g/mol) (*17*). Total phenols were assayed in methanolic extracts using a modified Folin-Ciocalteu method (*18*) and were expressed as gallic acid equivalents.

HPLC analysis of phenolic components in sweet cherries

Just before the HPLC analysis, a 400 μL aliquot of sweet cherry methanolic extract was evaporated near dryness. The residue was diluted back to 400 μL with the HPLC eluent (8 % CH_3OH in $HCOOH/H_2O$ at pH=2.1) and filtered through 0.45 μm PTFE filters (Millipore). The filtrate was analysed by means of high performance liquid chromatography (HPLC) using the equipment and chromatographic conditions, specified in Table 1.

Detection was carried out at 320 nm for hydroxycinnamates and at 520 nm for anthocyanins. A 10-minute re-equilibration period was used between individual runs. The chromatograms were recorded at room temperature (20 °C).

Characterisation and quantification of phenolic compounds by HPLC

Compounds were characterised on the basis of retention times and UV-VIS spectra of previously described standards and by using data of already published UV-VIS spectra (*8–11,13,15,19*). The concentrations of anthocyanins and hydroxycinnamic acids were determined from calibration curves, prepared by injecting 20 μL of appropriately diluted standard stock solutions, which corresponded to 0.1 to 4.75 μg of cyanidin-3-glucoside and 1 to 10 μg of chlorogenic acid, respectively. The ratio of the average absorbance in the 400–440 nm range versus the absorbance maximum in the visible range of spectrum ($A_{400–440}/A_{vis\ max}$) was calculated from the recorded UV-VIS spectra and used as an indicator of 3-glycoside structure of anthocyanins (*17*).

Results and Discussion

Comparison between methanol and methanol-formic acid extraction

To confirm the expected advantages (reduced loss of the less stable anthocyanins) and possible disadvantages (lower yield of phenolic components) of extraction with pure methanol, samples of sweet cherries (cultivar Bing) were analysed following extraction with CH_3OH: HCOOH (98:2 volume fraction) mixture or extraction with pure CH_3OH. The anthocyanins remaining in the sample were extracted in the second extraction to obtain the extraction yield of a single extraction step. This was found to be 94 %, and the calculated concentrations were corrected accordingly. HPLC analysis of acid extracts revealed no improvement of extraction yield in the case of acid extraction. A complete loss of two anthocyanins (peaks 3 and 4 in Fig. 2, chromatogram C) was observed with acid extraction and substantially

Table 1. HPLC conditions

Instrument	Hewlett Packard 1100 liquid chromatograph with DAD detector (190–600 nm)
Column	Hypersil PEP 300 C18, 250 x 4.6 mm, 5 μm particle size
Guard column	Alltech, 10 x 4.1 mm
Injection volume	20 μL
Flow rate	1.0 mL/min
Mobile phase	A = HCOOH / H_2O, pH = 2.1, B = CH_3OH (HPLC grade)
Gradient	8 % B (0 min), 12 % B (11 min), 30 % B (17 min), 33 % B (28 min), 100 % B (30–35 min), 8 % B (36 min)

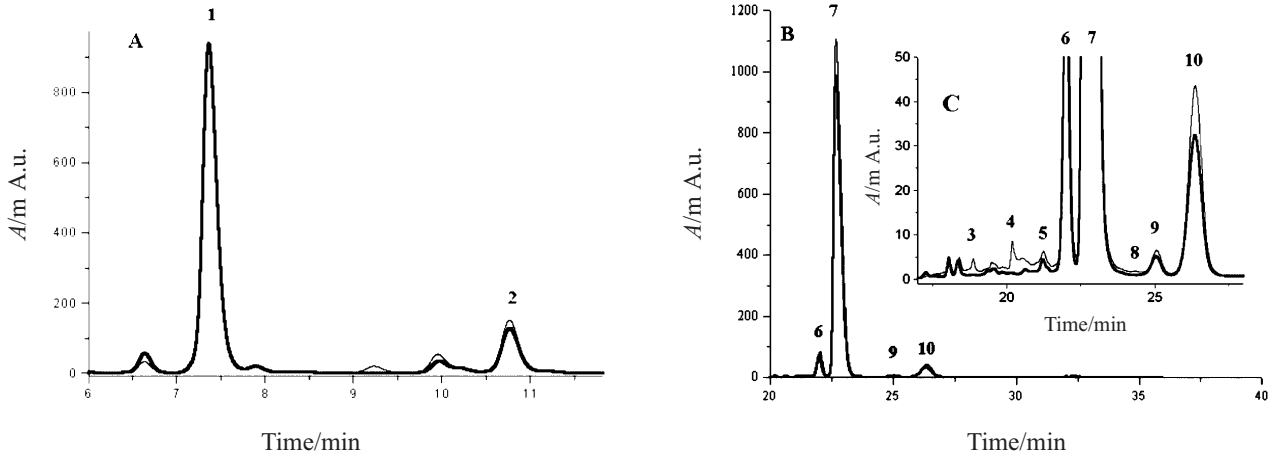


Fig. 2. Comparison of CH_3OH and CH_3OH: HCOOH extraction of sweet cherries: chromatogram A, major peaks of hydroxycinnamic acids recorded at 320 nm; chromatogram B, major peaks of anthocyanins recorded at 520 nm; chromatogram C, the loss of peaks 3 and 4 after methanol-acid extraction; (— **CH_3OH,** — CH_3OH: HCOOH = 98:2 (v/v))

lower peak areas compared to extracts in methanol (Fig. 2, chromatogram B) were obtained for peaks 6, 7 and 10 (by 19, 9 and 25 %, respectively). Only slight differences were observed in peak areas of major hydroxycinnamic acids (Fig. 2, chromatogram A). This confirms that phenolic compounds are efficiently extracted into pure methanol without the addition of formic acid, which on the other hand results in degradation of anthocyanins as indicated by lower peak areas of major pigments and complete loss of peaks 3 and 4. Therefore, compared to commonly used acid extraction of phenolic components from cherries (*8–10*), the methanolic extraction provides a more reliable profile of anthocyanins.

Phenolic composition of sweet cherries

Hydroxycinnamic acids

The obtained HPLC profile of hydroxycinnamic acids (Fig. 3 left) showed two major peaks in the 0–15 min region. They were identified based on the comparison of recorded UV-VIS spectra of peaks 1 and 2 to UV-VIS spectra published in the literature (*9*), where major peaks from sweet cherry extracts were reported as neochlorogenic acid and 3'-*p*-coumaroylquinic acid. The recorded spectra of peaks 1 and 2 showed distinctive features in the 320 nm region and no absorbance at 520 nm. Therefore, we concluded that peak 1 (λ_{max}=242 and 322 nm, shoulder at 305 nm) corresponds to neochlorogenic acid and peak 2 (λ_{max}=312 nm) to 3'-*p*-coumaroylquinic acid, which showed strong variability between different cultivars of sweet cherries.

Anthocyanins

HPLC analyses of methanol extract of Bing cherries (Fig. 3 right) revealed the presence of 8 anthocyanins (peaks 3–10). Other cultivars showed similar anthocyanin profile with only small variation in relative intensity of individual anthocyanin peaks.

Based on well-known spectral characteristics (ratio $A_{400–440}/A_{vis\ max}$) of anthocyanin-3-glycosides (*17*) as well as on comparison between UV-VIS spectra of eluted anthocyanins and anthocyanin standards, we assigned eight compounds detected at 520 nm as 3-glycosides of different anthocyanins and identified the peaks as follows: peak 6 matched the retention time and spectral characteristics of cyanidin-3-glucoside, while peak 7

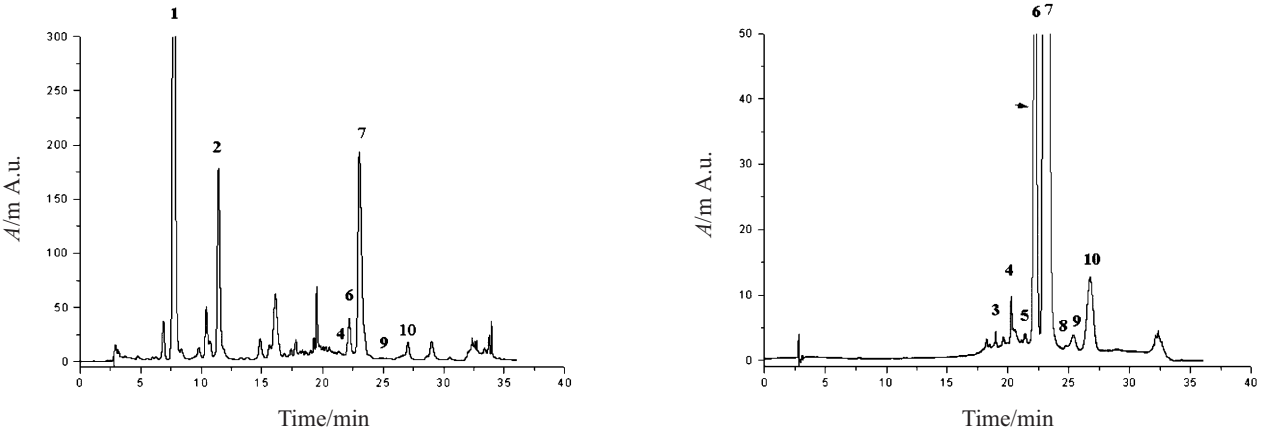


Fig. 3. HPLC separation of phenolic components in sweet cherries of cultivar Bing, monitored at 320 nm (left) and 520 nm (right). Peaks: 1, neochlorogenic acid; 2, 3'-*p*-coumaroylquinic acid; 3–5, unknown anthocyanins; 6, cyanidin-3-glucoside; 7, cyanidin-3-rutinoside; 8, peonidin-3-glucoside; 9, pelargonidin-3-rutinoside and 10, peonidin-3-rutinoside.

Table 2. Mean values and standard deviations[a] of hydroxycinnamic acids content, total anthocyanin content and total phenolic content

	Mass fraction (mg/100 g of sweet cherry FW)				
HPLC assay	**Lambert**	**Bing**	**Stella Compact**	**Napoleon**	**Petrovka**
neochlorogenic acid (as chlorogenic acid)	35.5 ± 4	27.12 ± 3	30.24 ± 3	19.46 ± 2	53.05± 6
3'-p-coumarylquinnic acid (as chlorogenic acid)	8.54 ± 1	8.04 ± 1	7.52 ± 1	50.65 ± 6	16.42 ± 2
Total anthocyanic content (as cyanidin-3-glucoside)	29.28 ± 3	28.19± 1	38.91± 6	33.71± 2	62.13± 2
Spectrophotometric assay					
Total anthocyanic content (as cyanidin-3-glucoside)	28.23 ± 2	28.12 ± 1	35.24 ± 5	30.94 ± 1.5	63.42 ± 2.5
Total phenol content (as gallic acid equivalents)	117.22± 7	97.38± 5	120.98± 5	144.02± 14	196.98± 26

[a] Mean values and standard deviations for all determinations based on n = 3

matched the chromatographic and spectral characteristics of cyanidin-3-rutinoside, which is in accordance with published data (*9,10*). Similarly, by using the methanolic extract from strawberries, peak 9 was identified as pelargonidin-3-rutinoside, where this compound is found as most common anthocyanin (*15*). UV-VIS spectra ($\lambda_{vis\ max}$ 518–520 nm) and elution order of peaks 8 and 10 indicated that they were most probably peonidin-3-glucoside and peonidin-3-rutionoside, respectively, which were also previously reported in sweet cherries (*9,10*).

The anthocyanins corresponding to chromatographic peaks 3, 4 and 5 could not be identified against available standards. Their UV-VIS spectra, however, showed increased absorption in 300–330 nm region (Fig. 4), which indicates possible acylation of glycoside with *p*-coumaric and malonic acids (*17*). Malonic groups are also known as the most labile acyl groups, and such compounds are readily decomposed in presence of acids (*20*). Therefore, the loss of peaks 3 and 4 during acid extraction (Fig. 2, chromatogram C) supports their assignment as acylated anthocyanins. However, due to the lack of apropriate standards, this could not be absolutely confirmed by the elution order of acylated and non-acylated anthocyanins from literature. Acylated anthocyanins with succinic acid (*21*) and malonic acid (*22,23*) from banana bracts and callus tissue of *Rudbeckia hirta* L. were, for example, reported to elute earlier than non-acylated compounds, while an earlier study reports that acetates, *p*-coumarates and caffeoates of anthocyanins in red grapes elute after non-acylated anthocyanins on RP-HPLC (*24*).

In all 5 analysed cultivars, peaks 6 and 7 together represented from 95 to 98 % of total peak area attributed to anthocyanins, peak 10 varied from 1 to 4 % among different cultivars, while other five peaks together represented only 0.6 to 1.1 % of the total peak area.

Quantification of hydroxycinnamic acids and anthocyanins in sweet cherries

The contents of two hydroxycinnamic acids varied among different cultivars, as previously reported (*9*). The ratio of neochlorogenic to 3'-*p*-coumaroylquinic acid was the highest in Lambert cherries (4.4), which represents an 11-fold increase compared to Napoleon cultivars, where the ratio was only 0.38. The highest values for the sum of the two hydroxycinnamic acids were found in Petrovka and Napoleon cultivars (70 mg/100 g of fresh weight, FW), while the lowest values were measured in Bing cultivar (35 mg/100 g). These values are in general agreement with the data from literature (*7,8,25*).

Total anthocyanin content in sweet cherries, examined by means of HPLC by summation of contributions from peaks 3–10, varied from 29 to 62 mg / 100 g of FW. The exception was Petrovka variety, where we observed twice the amount of total anthocyanins, 62 mg/100 g of FW (as cyanidin-3-glucoside), compared to other cultivars.

The results of spectrophotometric pH differential assay, reported in Table 2, showed similar variations in total anthocyanin content among investigated cultivars

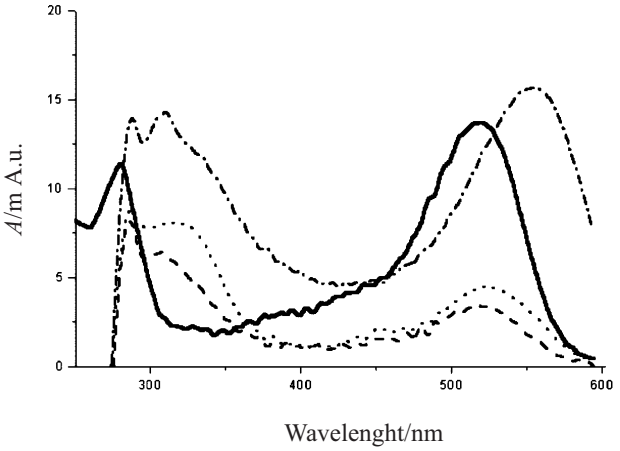


Fig. 4. UV-VIS spectra of peaks 3, 4 and 5 in comparison with cyanidin-3-glucoside UV-VIS spectra

and consistence with HPLC results. Our values of total anthocyanin content are in general lower, compared to some earlier reports in the literature, but show good agreement with some recent investigations relying on acidic extraction (*10*). At the same time, the values for total anthocyanins are higher compared to the report where extraction to methanol/water was used (*7*).

Regarding the total phenolic content (*18*) Petrovka cultivar with 197 mg / 100 g of FW was outstanding compared to other cultivars. Total anthocyanin content in Petrovka is in agreement with recently reported values for anthocyanins in cultivar of comparable colour intensity (*10*). Higher concentration of total phenolics has been observed also in Napoleon cultivar (144 mg / 100 g of FW), which arises mainly from its high hydroxycinnamic acid content. At the same time, the lowest total phenolic content (97 mg / 100 g of FW) was determined in Bing cultivar, which is characterised by low hydroxycinnamic acid content.

Conclusion

In our study the modified combined method of extraction and separation of different phenolic compounds in sweet cherries has been successfully used for determination of anthocyanins, hydroxicinnamic acids, and total phenolics in five sweet cherry cultivars. The results of comparison of acidic and non-acidic extraction have confirmed that using pure methanol for extraction reduces the loss of anthocyanins, compared to previously reported and commonly used acid extractions of phenolic components from cherries (*8–10*).

This enabled the detection of eight anthocyanins including two unidentified compounds, which could not be detected using the acidic extraction. Regarding the presence of major anthocyanins and hydroxycinnamic acids the results are generally consistent with those reported in literature (*8–10*) and show that the dark coloured sweet cherries have an anthocyanin composition of 3-glucoside and 3-rutinoside of cyanidin as the major anthocyanins, and pelargonidin-3-rutinoside as minor anthocyanin. The presence of neochlorogenic acid and 3'-*p*-coumaroylquinic acid as hydroxycinnamic acids is also in accordance with published data (*7–9,25*).

Our results showed high content of anthocyanins in Petrovka cultivar, which exhibits HPLC profile of phenolic components similar to other world spread cultivars. Because of its high anthocyanin content this local variety should not be neglected. Other traditional cultivars, grown in Slovenia, should also be assayed for their phenolic content in future studies, especially because no data on traditional Slovenian varieties are available in the literature.

References

1. R. A. Arnold, A. C. Noble, V. L. Singleton, *J. Agric. Food Chem. 28* (1980) 675–680.
2. H. Wang, G. Cao, R. L. Prior, *J. Agric. Food Chem. 45* (1997) 304–309.
3. M. H. Criqui, B. L. Ringel, *Lancet*, *344* (1994) 1719–1723.
4. E. N. Frankel, J. Kanner, J. B. German, E. Parks, J. E. Kinsella, *Lancet*, *341* (1993) 454–457.
5. E. N. Frankel, A. L. Waterhouse, P. L. Teissedre, *J. Agric. Food Chem. 43* (1995) 890–894.
6. S. Meyer, O. Yi, D. A. Pearson, A. L. Waterhouse, E. N. Frankel, *J. Agric. Food Chem. 45* (1997) 1638–1643.
7. M. Heinonen, A. S. Meyer, E. N. Frenkel, *J. Agric. Food Chem. 46* (1998) 4107–4112.
8. J.-J. Macheix, A. Fleuriet, J. Billot: *Fruit Phenolics*, CRC Press, Boca Raton, FL (1990).
9. L. Gao, G. Mazza, *J. Agric. Food Chem. 43* (1995) 343–346.
10. M. Esti, L. Cinquanta, F. Sinesio, E. Moneta, M. Di Matteo, *Food Chem. 76* (2002) 399–405.
11. G. Mazza, E. Miniati: *Anthocyanins in Fruits, Vegetables, and Grains*, CRC Press, Boca Raton, Florida (1993) p 57.
12. G. Mazza, R. Broouillard, *Phytochemistry, 29* (1990) 1097.
13. B. Harborne: *Comparative Biochemistry of the Flavonoids*, Academic Press, New York (1967).
14. J. D. Donovan, A. S. Meyer, A. L. Waterhouse, *J. Agric. Food Chem. 46* (1998) 1247–1252.
15. J. Bakker, P. Bridle, S. J. Bellworthy, *J. Sci. Food Agric. 64* (1994) 31–37.
16. R. E. Wrolstad, J. D. Culbertson, C. J. Cornwell, L. R. Mattick, *J. Assoc. Off. Anal. Chem. 65* (1982) 1417–1432.
17. M. M. Giusti, L. E. Rodríguez-Saona, R. E. Wrolstad, *J. Agric. Food Chem. 47* (1999) 4631–4637.
18. M. A. Amerine, C. S. Ough: *Methods for Analysis Musts and Wines*, New York, John Wiley & Sons (1988) pp. 204–206.
19. V. Hong, R. E. Wrolstad, *J. Agric. Food Chem. 38* (1990) 708–715.
20. M. Teusch, G. Forkmann, *Phytochemistry, 26* (1987) 2181–2183.
21. E. A. Pazmino-Duran, M. M. Giusti, R. E. Wrolstad, M. B. A. Gloria, *Food Chem. 73* (2001) 327–332.
22. M. £uczkiewicz, W. Cisowski, *Chromatographia*, *48* (1998) 360–364.
23. M. £uczkiewicz, W. Cisowski, *Tisue and Organ Culture*, *65* (2001) 57–68.
24. L. W. Wulf, C. W. Nagel, *Am. J. Enol. Vitic. 29* (1978) 42–49.
25. J. E. Friedrich, C. Y. Lee, *Polyphenols Communications 98, XIXth International Conference of Polyphenols*, Lille (1998) pp. 527–528.

Određivanje antocijanina i hidroksicinamske kiseline te njihova količina u različitim vrstama trešnje *Prunus avium* L. iz Nove Gorice (Slovenija)

Sažetak

Antocijanini i hidroksicinamske kiseline određeni su i utvrđena je njihova količina HPLC- i UV-VIS-spektrofotometrijom u pet vrsta tamnocrvenih trešanja. Fenolni spojevi ekstrahirani su čistim etanolom bez dodatka kiseline i vode. Uzorci su bili razrijeđeni smjesom metanola i mravlje kiseline neposredno prije injekcije. Na kromatografskoj koloni Hypersil PEP 300 C18 razdvojeni su uzorci korištenjem gradijenta sustava otapala od mravlje kiseline, vode i metanola. Da bi se odredili pojedini sastojci, primijenjen je DAD-detektor i dvije valne duljine, i to: 320 nm za hidroksicinamate i 520 nm za antocijanine. Kao glavni sastojak antocijanina utvrđena je prisutnost cijanidin-3-glukozida i cijanidin-3-rutinozida, dok je utvrđena manja količina pelargonidina-3-rutinozida. Glavne hidroksicinamske kiseline bile su neoklorogenska i 3´-*p*-kumaroil-kinska kiselina. Ukupni udjel antocijanina (izražen kao cijanidin-3-glukozid) iznosio je od 29 do 62 mg / 100 g mase svježih plodova bez koštica pri čemu je najveću vrijednost imala vrsta Petrovka. Koncentracija neoklorogenske kiseline iznosila je od 19,5 do 53,0 mg / 100 g svježih trešanja bez koštica, a 3´-*p*-kumaroil-kinske kiseline 7,5–50,6 mg / 100 g. Relativne količine tih dviju fenolnih kiselina znatno su se razlikovale među pojedinim ispitivanim vrstama trešanja.