ANTHOCYANINS IN THE FRUITS OF WILD DOGWOOD (*CORNUS MAS* L.) OF CENTRAL BOHEMIAN ORIGIN

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Cornelian cherries – fruits of dogwood tree (*Cornus mas* L.), could be one of non-traditional sources for preparing of jams and other food products. This report describes the isolation and identification of polyphenolic compounds, esp. anthocyanins present in cornelian cherries of Bohemian origin and compares the composition with cornelian cherries of other provenances. On the basis of TLC, spectral data and acid hydrolysis there 5 anthocyanins were identified. The most manifested was pelargonidin-3-galactoside and cyanidin-3-galactoside. On average level orange coloured pelargonidin-3-rhamnosyl-galactoside was present. At low concentrations was only empetrin – delphinidin-3-galactoside present and only in traces orange pelargonidin-3-trisaccharide. The composition of anthocyanin complex of Bohemian cornelian cherries is very similar to that of North America (Du, Francis, 1973a, b) showing the predominance of glycosides of pelargonidine. We could not identify cyanidin-3-rhamnosylgalactoside, but cyanidin-3-galactoside was present at fairly good concentration. Only on very low level empetrin is present as a representative of delphinidin-3-monosides. No evidence was found for the presence of glycosides of other anthocyanidins such as petunidin as it was reported previously by Tamas and Stoleriu (1976).

cornelian cherries; composition; anthocyanin glycosides

INTRODUCTION

Recently, different non-traditional sources of ascorbic acid useful for food industry have been investigated. They are usually combined with rich content of polyphenolic compounds, esp. anthocyanins and flavonoids. One of such indigenous plant is the dogwood tree, *Cornus mas* L., fruits of this tree are also known as cornelian cherries. The fruits of average weight 1.1–5.6 g contain on average (Mikeladze, Vashakidze, 1968; Maisuradze, 1969) 0.33–0.46% tannins and colourants and are very rich in ascorbic acid – 303.3–559.0 mg.kg^{-1}, as it is shown in Tabs. I and II. The colourant complex is formed by anthocyanins and flavonoids.

I. Chemical characteristics of the cornelian cherries

Compound	Wild content (%)	Cultivated content (%)
Dry matter	15.70–17.62	14.48–19.33
Acids as malic acid	2.95–3.10	1.50–3.35
Total saccharides	9.46–10.60	7.97–12.41
Tannins and colourants	0.33–0.46	0.18–0.48
Pectines	0.73–1.23	0.97–1.44
Ascorbic acid ($mg.kg^{-1}$)	269.5–358.0	303.3–559.0

II. Content of carbohydrates and protein in the fruits of dogwood

Compound	Content (%)
Reducing saccharides	11.1
Saccharose	1.4
Soluble pectin	0.7
Insoluble pectin	0.2
Protopectin	0.22
Proteines	0.587

Du and Francis (1973a, b) have isolated by prolonged chromatography five anthocyanins and have indicated them as cyanidin-3-rhamnosylgalactoside and cyanidin-3-galactoside, pelargonidin-3-galactoside and pelargonidin-3-rhamnosylgalactoside and delphinidin-3-galactoside. In comparison with other *Cornus* species only small differences were observed among them, especially in the pattern of glycosylation. Du et al. (1974b, 1975) have found in the fruits of *C. kousa* cyanidin-3-glucoside, delphinidin-3-glucoside, pelargonidin-3-glucoside, in *C. florida* cyanidin-3-glucoside and galactoside with traces of 3-arabinoside, delphinidin-3-glucoside and galactoside and pelargonidin-3-galactoside, in *C. alba* as main cyanidin-3-galactoside and cyanidin-3-arabinoside and delphinidin-3-glucoside. Greater differences showed fruits of *Cornus alternifolia* – they have indicated the presence of delphinidin-3-glucoside and-3-rutinoside as main glycosides and also in lesser amount petunidin-3--rutinoside, in petiols cyanidin-3-galactoside and delphinidin-3-galactoside were present. Also in ripe fruits of *Cornus canadensis* found Du et al. (1974a) as main pelargonidin-3-glucoside, galactoside, rutinoside and robinobioside and in lesser amounts cyanidin-3-glucoside, galactoside, rutinoside and robinobioside were found. In respect to the amounts of anthocyanins repre-

sented in *C. mas* cornelian cherries, the most represented was pelargonidin-3-galactoside and then in descending order cyanidin-3-galactoside, pelargonidin-3-rhamnosylgalactoside, cyanidin-3-rhamnosylgalactoside and delphinidin-3-galactoside. Additionally to these three aglycons referred to in *C. mas* fruits, Tamas and Stoleriu (1976) after acid hydrolysis could identify four anthocyanidins, namely delphinidin, cyanidin, pelargonidin and petunidin. Sivcev and Abramovicz (1979) suggest that the maximum content of anthocyanins is a good criterion for the determination of the best date of harvest. As Lazar et al. (1971) confirmed, the anthocyanin complex was isolated from fresh fruits of *C. sanguinea* identical with compounds obtained after acidification of leucoanthocyanidin complex of dried fruits.

The structure of flavonoid compounds contained in fruits of *C. mas* was not previously studied, they are probably manifested in lesser amounts because they are converted during ripening into anthocyanins.

In this report we tried to isolate and identify anthocyanins contained in the fruits of *C. mas* of Central Bohemian origin and to elucidate their structure and composition of the complex.

MATERIAL AND METHODS

Plant material. 615 g cornelian cherries were collected in October 1994 in the area Prague-Suchdol.

Maceration. Fresh fruits were macerated with 400 ml 96% ethanol for five days. This extract was then decanted and a little part (20 ml) was evaporated under vacuum to dryness.

Acid hydrolysis. Dry anthocyanin concentrate was redissolved in 2M hydrochloric acid (20 ml) and heated in water bath for 1 hour. After acid hydrolysis was anthocyanidin hydrolysate shaked with ethyl acetate to remove flavonoids and then investigated by TLC, as well as original extract of anthocyanin glycosides. For detection of saccharides the hydrolysate after cooling was applied to polyamide column (1 x 10 cm) and eluted with 50 ml water and for anthocyanidins with 50 ml ethanol acidified with 0.01% HCl.

TLC. Polygram Cel 300 pre-coated plastic sheets Macherey-Nagel Co. with 0.1 mm cellulose MN 300 layer were used.

Solvent systems: S_1: conc. HCl-HCOOH-H_2O (2 : 5 : 3 V/V/V)
S_2: HCOOH-conc. HCl-H_2O (10 : 1 : 3 V/V/V)
S_3: conc. HCl-H_2O (3 : 97 V/V)
S_4: conc. HCl-H_2O (1 : 99 V/V)
S_5: AcOH-H_2O (15 : 85 V/V).

Chromatograms were sprayed with 1% $AlCl_3$ solution for the determination of colour shift of anthocyanidins and anthocyanins.

Spectral analyses. Separated compounds on chromatograms were eluted in EtOH (0.01% HCl) or MeOH (0.01% HCl), resp. in concentration cca 2 x 10^{-5} mol. Spectra were measured on the SPECORD UV VIS spectrophotometer (Carl Zeiss Jena) and their bathochromic and hypsochromic shifts after addition of aluminium chloride solution.

Authentic markers. As authentic preparations for co-chromatography and comparison of spectral data pelargonidin, cyanidin and delphinidin chlorides fy. FLUKA Chemie AG, Switzerland, were used. Authentic samples of D-galactose and L-rhamnose were produced by LACHEMA Brno.

Detection of saccharides. Saccharides in the thickened water eluate were identified by co-chromatography with authentic markers using descending PC on Whatman chromatographic paper No. 1 with the solvent system 1-butanol--acetic acid-water (4 : 1 : 5 V/V/V) and detection was made by benzidin and anthrone agents, anilinium hydrogen sulphate, aniline diphenylamine-trihydrogenphosphoric acid and neotetrazolium blue agent (LACHEMA Brno).

RESULTS AND DISCUSSION

After acidic hydrolysis of anthocyanidins the chromatography has been carried out on cellulose plates in conc. HCl – formic acid – water (2 : 5 : 3 V/V/V – S_1 and 1 : 10 : 3 V/V/V – S_2).

There were three anthocyanidins present on chromatograms. Results of TLC of anthocyanidins are given in Tab. III. Thus it was confirmed that in the cornelian cherries of Czech provenance three anthocyanidin aglycons identified as pelargonidin (I), cyanidin (II) and delphinidin (III) are present. The most manifested aglycon is pelargonidin and only in traces was present delphinidin. This is in accordance with the results of Du and Francis (1973a, b) who found in the berries of *Cornus mas* L. of North America origin glycosides derived from pelargonidin, cyanidin and delphinidin. On the other hand, we could not find the other aglycon – petunidin, mentioned by Tamas and Stoleriu (1976). The colour of I rested after spraying with

III. Chromatographic and spectral data of anthocyanidins found

Anthocyanidin	R_F (x 100) in		Visible colour	Visible max. in MeOH-HCl/EtOH-HCl (nm)	Colour shift with $AlCl_3$
	S1	S2			
Pelargonidin I	54	65	red-or.	520/536	– red-or.
Cyanidin II	38	55	magenta	535/551	+ violet
Delphinidin III	22	41	purple	546/562	+ blue

1% $AlCl_3$ orange without change of colour meanwhile the colour of II has changed from red to violet and III from red to blue.

The extract of anthocyanins was after thickening under vacuum chromatographied in solvent systems S_3 and S_4. We have found five anthocyanins on the chromatograms as it is given in Tab. IV.

IV. Chromatographic and spectral data of anthocyanins found

Anthocyanidin No.	R_F (x 100) in		Visible colour	Visible max. in MeOH-HCl/EtOH-HCl (nm)	Colour shift with $AlCl_3$
	S4	S5			
1	3	41	purple	538/542	+ blue
2	8	58	magenta	525/528	+ violet
3	23	–	red-or.	504/511	– red-or.
4	14	–	red-or.	508/512	– red-or.
5	33	–	red-or.	505/511	– red-or.

The red coloured anthocyanin 1 changed its colour after spraying with 1% $AlCl_3$ to blue and showed the basic spectrum and shifts after addition $AlCl_3$ characteristic for delphinidin. Acid hydrolysis yielded delphinidin and D--galactose. This compound was characterized as delphinidin-3-galactoside (empetrin). It was manifested only in little amounts.

The glycoside 2 was red coloured and after spraying with the solution of $AlCl_3$ the spot of this compound has changed to violet. On the basis of spectral analysis and after acid hydrolysis we could 2 glycoside identify as cyanidin-3-galactoside.

The most manifested was anthocyanin 3, orange coloured that has given no colour change after spraying with 1% $AlCl_3$. After measuring its spectra in ethanolic solution and after addition of $AlCl_3$ and after acid hydrolysis we could identify this glycoside as pelargonidin-3-galactoside.

The anthocyanin glycoside 4 was orange coloured and after spraying with 1% $AlCl_3$ there was no colour change. The spectral data confirmed that it is a glycoside derived from pelargonidin. After acid hydrolysis we could determine D-galactose and L-rhamnose. After comparison of R_F data we could identify the compound 4 as pelargonidin-3-rhamnosylgalactoside.

Only in small traces was present compound 5 slightly orange coloured that when sprayed with 1% $AlCl_3$ showed no colour change. Spectral data confirmed pelargonidin and R_F values are typical for pelargonidin trisaccharide.

Because this glycoside was present only in negligible amounts, we did not succeed in further identification.

From the obtained results it is possible to state that the composition of the anthocyanin complex of cornelian cherries of Bohemian origin is very similar to that obtained from the berries of dogwood growing in North America (Du, Francis, 1973a, b). Especially the manifestations of major glycosides are the same – in the highest concentration was present pelargonidin-3-galactoside followed by cyanidin-3-galactoside and pelargonidin-3-rhamnosyl-galactoside. In relatively very small amounts are represented delphinidin-3--galactoside and pelargonidin-3-rhamnosylgalactoside and only in traces unidentified pelargonidin trisaccharide. In cornelian cherries of our provenance we have not found cyanidin-3-rhamnosylgalactoside as it was reported previously in the berries of dogwood of North America origin (Du, Francis, 1973a, b).

The pattern of glycosylation of anthocyanidins is very simple – as for monoglycosides there were 3-O-galactosides and as for diglycosides there were 3-O-rhamnosylgalactosides only found. For the anthocyanin complex of the berries of dogwood typical glycosides of pelargonidin are represented in high concentrations, in medium concentrations are present glycosides derived from cyanidin and only in small concentrations are contained glycosides of delphinidin.

The anthocyanin complex of the fruits of dogwood tree could be conveniently used as red colourant in the processing of cornelian cherries in food industry for preparing stewed fruits, jams, marmalades and other food products and preserves.

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LACHMAN, J. – PIVEC, V. – ŠUBRTOVÁ, D. (Česká zemědělská universita, Agronomická fakulta, Praha, Česká republika):

Anthokyany v plodech dřínu obecného (*Cornus mas* L.) z lokality středních Čech.

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Plody dřínu obecného (*Cornus mas* L.) jsou bohaté na vitamin C, volné organické kyseliny, sacharidy, pektiny a anthokyanová barviva a mohou jak svým složením, tak i použitím připomínat třešně, např. pro přípravu džemů, marmelád, kompotů, a být tak netradičním a cenným doplňkem běžného sortimentu ovoce. Z tohoto důvodu bylo sledováno složení anthokyanového komplexu plodů dřínu obecného středočeského původu a provedeno srovnání se složením plodů dřínu severoamerické provenience (Du, Francis, 1973a, b).

Ethanolický extrakt plodů byl podroben TLC na celulózových deskách Macherey-Nagel v řadě vyvíjecích systémů a dále kyselé hydrolýze. Produkty hydrolýzy po separaci na koloně polyamidu byly podrobeny rovněž TLC a PC a kochromatografovány s autentickými preparáty (anthokyanidiny FLUKA Chemie AG Buchs, monosacharidy LACHEMA Brno). Anthokyanové glykosidy i anthokyanidiny získané hydrolýzou byly podrobeny spektrální analýze v methanolu a ethanolu okyseleném 0,01 % HCl na spektrofotometru SPECORD UV VIS Zeiss Jena.

Na chromatogramech bylo nalezeno celkem pět glykosidů anthokyanové povahy. Po kyselé hydrolýze ve 2M kyselině chlorovodíkové byly nalezeny tři různé anthokyanidiny, které byly identifikovány jako pelargonidin, kyanidin a delfinidin, jejichž zastoupení v této řadě klesalo.

Z pěti anthokyanů zastoupených na chromatogramech se v největší koncentraci vyskytoval oranžově zbarvený 3-galaktosid pelargonidinu a v menší koncentraci 3-rhamnosylgalaktosid pelargonidinu. Pouze ve stopách se na chromatogramech vyskytoval blíže neurčený triglykosid pelargonidinu. Ve střední koncentraci byl zastoupen 3-galaktosid kyanidinu a ve slabší koncentraci 3-galaktosid delfinidinu, tj. empetrin.

Složení plodů dřínu obecného naší provenience je podobné složení plodů dřínu severoamerického (Du, Francis, 1973a, b), a to jak po stránce kvalitativní, tak i po stránce kvantitativní. Neprokázali jsme 3-rhamnosylgalaktosid kyanidinu, nýbrž jeho možný hydrolytický produkt 3-galaktosid kyanidinu. Na rozdíl od zjištění auto-

rů Tamas a Stoleriu (1976) nebyl v plodech dřínu obecného naší provenience prokázán petunidin ani žádný jiný anthokyanidin.

Anthokyanový komplex se může vhodně uplatnit jako červené barvivo při zpracování plodů dřínu obecného v potravinářském průmyslu při přípravě kompotů, džemů, marmelád a jiných produktů.

plody dřínu obecného; složení; glykosidy anthokyanové povahy

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