

Isolation of Anthocyanins by High-Speed Countercurrent Chromatography and Application of the Color Activity Concept to Different Varieties of Red Grape Pomace from Macedonia

Sanja Kostadinović Veličkovska^{1*}, Hamed Mirhosseini² and Elena Bogeva³¹Faculty of Agriculture, University "Goce Delčev", Krste Misirkov bb, 2000 Štip, Macedonia²Department of Food Technology, Faculty of Food Science and Technology, University Putra Malaysia, Sri Serdang, 43400 UPM Serdang, Selangor, Malaysia³Experimental laboratory of wines and wine-making, Elenov winery, Demir Kapija, Macedonia

Abstract

Anthocyanins of Macedonian grape pomace from three varieties "Pinot noir", "Merlot" and "Vranec" were isolated by high speed countercurrent chromatography. After purification of the fractions by means of preparative high performance liquid chromatography the structures of isolated pigments were elucidated by electrospray ionization multiple mass spectrometry (ESI-MSⁿ) and nuclear magnetic resonance (NMR) spectroscopy. The major anthocyanin malvidin-3-glucoside and the minor pigments delphinidin-3-glucoside, cyaniding-3-glucoside, petunidin-3-glucoside, and malvidin-3-*p*-coumaroyl-glucoside were isolated.

The "Color activity concept" was applied and visual detection thresholds of isolated anthocyanins were determined. The results of the "color activity value" of the isolated pigments and their detection thresholds were in good agreement with the color shade of the different varieties of red grape pomace.

Keywords: Red grape pomace; HSCCC; NMR; Anthocyanins; Color activity concept

Introduction

Anthocyanins are natural pigments responsible for orange, pink, red, violet and blue colours in the flowers and fruits of many plants which are using as colorants in food and beverages industry. Anthocyanins (of the Greek anthos=flower and kianos=blue) are under huge investigation due to the growing interest of substitution the potential cancerogenic synthetic colors which are still using in food industry [1].

Anthocyanins exhibit a huge range of biological activities as antioxidant activity and prevention of neuronal and cardiovascular diseases, cancer and diabetes. The role of natural pigments in the coloring of different plants is under current investigations of many studies [2].

The structure of anthocyanins and their properties as colorants in plants were subject of investigations of Brouillard et al. [3]. Radical scavenger activity of anthocyanins and their glycols were also studied in the work of Khknen and Heinonen [4].

Grape skin contains a great number of anthocyanins, the concentration varies greatly according to the variety and is influenced by cultivar, season and environmental factors. The most abundant anthocyanins in red grapes are 3-glycosides, 3-acetylglycosides and 3-*p*-coumaroyl-glycosides of malvidin (Mv), peonidin (Pn), delphinidin (Dp), petunidin (Pt) and cyanidin (Cy) [5,6]. Changes of anthocyanins profile in *Vitis vinifera* grapes grown in the Douro Valley was studied in the work of Mateus, as well as their concentration in produced wines [7,8].

In the work of Schwarz, several hundred milligrams of anthocyanins were isolated with single run using large-scale countercurrent chromatography [8]. Isolation of the anthocyanins from wine grapes and different wine fractions with step gradient countercurrent chromatography was evaluated [9,10].

The elucidation of tentative structure of isolated anthocyanins by

using NMR spectroscopy confirmed the structure of the most abundant pigments in plants [11-20].

The contribution of particular anthocyanins in the overall color of red wines was object of the study of Degenhardt et al. [21].

To the best of our knowledge there are no published results for the polyphenolic profile of grape pomace from the region of Macedonia. In the present study, the isolation of anthocyanins from the Macedonian pomace from "Vranec", "Merlot", and "Pinot Noir" grape varieties was performed by application of chromatographic methods, in particular high-speed countercurrent chromatography. NMR structure elucidation confirmed the most abundant anthocyanins responsible for the color of the grape pomace. Furthermore, the color activity concept of isolated anthocyanins was performed for investigation of particular contribution of isolated pigments to the color of three different varieties of grape pomace.

Materials and Methods

Raw material

The winery Elenov from Macedonia kindly provided all varieties of grape pomace. The monovarietal samples of grape pomace were obtained from 2009 vintage year after wine-making including 20 days of maceration time. From each variety, 1.5 kg of grape pomace

***Corresponding author:** Sanja Kostadinović Veličkovska, Faculty of Agriculture, University "Goce Delčev", Krste Misirkov bb, 2000 Štip, Macedonia, Tel: +38972579133; E-mail: sanja.kostadinovik@ugd.edu.mk

Received October 28, 2013; **Accepted** November 18, 2013; **Published** November 21, 2013

Citation: Veličkovska SK, Mirhosseini H, Bogeva E (2013) Isolation of Anthocyanins by High-Speed Countercurrent Chromatography and Application of the Color Activity Concept to Different Varieties of Red Grape Pomace from Macedonia. J Nutr Food Sci 3: 243. doi: [10.4172/2155-9600.1000243](https://doi.org/10.4172/2155-9600.1000243)

Copyright: © 2013 Veličkovska SK, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

was washed with nanopure water for removing polar impurities and sugar. In order to remove water the samples were lyophilized. After lyophilization, the dry grape pomace was crushed in powder. 500 g of powdered grape pomace from each variety was used for the extraction of anthocyanins.

Extraction

The samples were defatted with (3×200 ml) hexan. For extraction of anthocyanins, mixture of methanol/formic acid (19:1 v/v) was used. For complete extraction, the suspension was stored overnight at room temperature.

Solid phase extraction

After evaporation of solvents, the extracts obtained from all varieties of grape pomace were viscous. To remove sugars, organic acids, proteins, and salts XAD-7 column (45×5 cm) was used. The extracts were washed with nanopure water and after that the anthocyanins were eluted with mixture of methanol/formic acid (19:1 v/v). After evaporation the samples were freeze dried to give the anthocyanin/enriched XAD-7-extract.

Countercurrent chromatography

CCC liquid-liquid chromatography is a technique for separation and isolation of compounds from complex mixtures without solid stationary phase. Separation is achieved by a two-phase solvent system. A CCC method with higher separation power is known as high speed countercurrent chromatography (HSCCC). In the HSCCC separations the solvents are filled into a teflon tube which is wound around a coil. These coils are usually connected in series. A planetary rotation of the coils creates an alternating force field in the tube and mixing and settling zones are generated. Thus, the sample is continuously distributed between the two phases during separation [22].

For the selection of suitable solvent mixtures, anthocyanins should be distributed between the two phases. This can be observed visually by mixing an aliquot of the sample with the two phases in a vial.

A CCC-1000 high-speed countercurrent chromatograph (triple coil, ID 2.6 mm, total volume 850 mL, 800 rpm, Pharma-Tech, USA) was used. MTBE/n-butanol/ acetonitrile/water 2:2:1:5 v/v/v/v acidified with 0.1% trifluoroacetic acid was used as solvent system. The lower layer was the mobile phase and elution mode was head to tail. Flow rate was set at 3 mL/min. Detection was carried out at $\lambda=520$ nm (Knauer Variable Wavelength Monitor). 1.0 g of the respective freeze-dried XAD-7 extract from each variety of grape pomace was separated in each run.

Analytical HPLC-ESI-MSⁿ and HPLC-DAD

Separations of CCC fractions were carried out on a Luna 5 μ C18 column (250×4.6 mm) with guard column. A binary gradient of a mixture of water/acetonitrile/formic acid was used. Linear gradient was as follows: A 83/7/10; B: 40/50/10 (v/v/v); % A: 0 min. 94%, 20 min. 80%, 35 min. 60%, 35 min. 40%, 40 min. 90%, 45 min and 55 min 94%. Flow rate was set at 0.5 mL/min. Detection was either by DAD (Jasco MD-1510) and ESI-MSⁿ (Bruker, Esquire) in the positive ion mode. Data about the purity of anthocyanin fractions is based on the DAD chromatogram using detection wavelength of 520 nm.

Preparative HPLC

Purification of anthocyanin fractions was made by using preparative HPLC with HPLC pump 64 and UV/VIS detector. The purification of

CCC fractions was carrying out on a Luna RP-18 column (Phenomenex, Germany) at a flow rate of 0.5 mL/min. The two solvent systems A and B were used. Solvent A was mixture of water/acetonitrile/formic acid 87:3:10 (v/v/v), and solvent B was mixture of water/acetonitrile/formic acid 40:50:10 (v/v/v). The linear gradient was as follows: 0 min. 6% B; 20 min. 20% B; 35 min. 40% B; 40 min. 60% B; 45 min. 90% B; 55 min. 6% B. The obtained chromatograms were interpreted by using Borwin PDA chromatography software (Jasco, Germany) and the purified picks were detected on $\lambda=520$ nm as detection wavelength.

Proton and Carbon NMR

¹H and ¹³C NMR spectra were measured on a Bruker AMX 300 spectrometer (Bruker Biospin, Germany) at 300.13 and 75.49 MHz, respectively. Anthocyanins were dissolved in a mixture of methanol-*d*4/TFA-*d*1 (19:1, v/v). Data were processed by WIN NMR software version 6.1.0.0.

Color activity concept

The “color activity concept” was applied in order to find the impact of isolated pigments responsible for the color of grape pomace. The calculation of color activity values is determined as a ratio of concentration of the pigment and its visual detection threshold [21]. For that purpose, pure anthocyanins were dissolved in buffer solution at pH 3.0 and two solutions A and B were prepared.

The solution A was prepared by dissolving of 21 g C₆H₈O₇·xH₂O per liter nanopure water. The solution B was prepared by dissolving of 36.5 g NaHPO₄·2H₂O per liter water with nanopure grade. The buffer solution with pH 3 was prepared by mixing of 79.45 ml of solvent A and filled to 100 ml with solvent B.

The usual pH value of soft drinks is 3.0. That was the reason of testing the color activity index in buffer solution of pH 3.0.

Results and Discussion

The extract from grape pomace from variety “Vranec” had the darkest violet-blue color. The extract from “Merlot” variety had red-violet color and “Pinot Noir” grape pomace had dark red color.

The yield of anthocyanin-enriched extract from three extractions of grape pomace from “Vranec” variety was 3.7 g, from “Merlot” variety was 3.1 g and “Pinot noir” variety was 1.5 g only per 500 g of dry powdered grape pomace.

1.0 g of anthocyanin extract from each variety of grape pomace was injected in HSCCC system. The HSCCC chromatograms from the isolation of the anthocyanin-enriched grape pomace extract of “Vranec” are shown on Figure 1. In the fraction F1 dephindin-3-glucoside and cyanidin-3-glucoside were characterized. The second fraction F2 was the main fraction with the highest amount of isolated pigment. After purification by preparative HPLC, the predominant pigment in this fraction was malvidin-3-glucoside. Furthermore, in very small quantity the anthocyanins petunidin-3-glucoside and peonidin-3-glucoside were isolated from fraction F3. In the coil fraction malvidin-3-*p*-coumaroyl-glucoside was found (Figure 1).

Mass spectrometric data of the isolated pigments are presented in Table 1. The table showed the amount of pure pigments (in mg) isolated and purified from 1.0 g of crude anthocyanin extract from “Pinot noir”, “Merlot” and “Vranec” variety of grape pomace. According to the presented results, the skin and seeds of grapes after 20 days maceration time still contained significant amount of malvidin-3-glucosides and

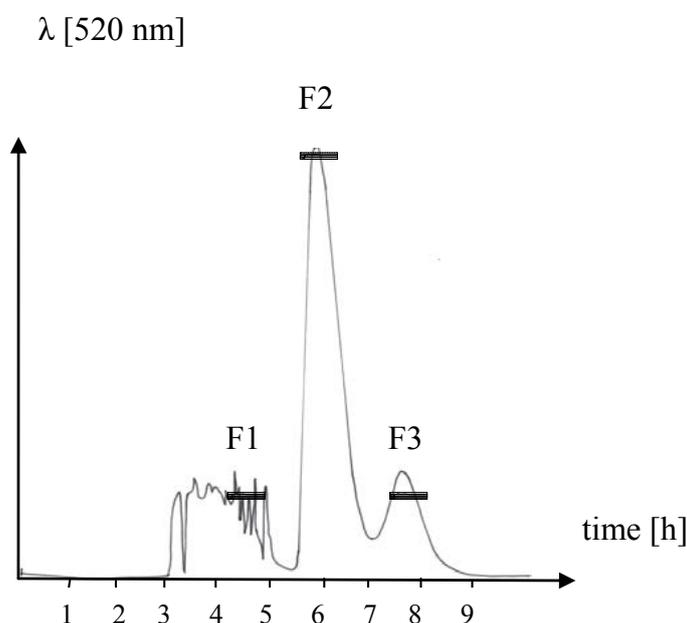


Figure 1: HSCCC separation of anthocyanins from "Vranec" grape pomace at 520 nm.

Variety	Fraction	Rt (min.)	Molecular ion M ⁺ (m/z)	Fragment ion (MS ²)	Structure	Amount of pure compound (mg)
Pinot noir	1	15.0	465.3	303.0	Delphinidin-3-glucoside	0.2
	1	19.5	449.4	287.0	Cyanidin-3-glucoside	0.7
	2	28.0	493.4	331.0	Malvidin-3-glucoside	10.0
	3	22.0	479.2	317.0	Petunidin-3-glucoside	0.8
	3	25.9	463.3	301.0	Peonidin-3-glucoside	0.6
	Coil fraction	44.7	655.6	329.0	Malvidin-3-p-coumaroyl-glucoside	0.9
Merlot	1	15.0	465.3	303.0	Delphinidin-3-glucoside	0.3
	1	19.5	449.4	287.0	Cyanidin-3-glucoside	2.1
	2	28.0	493.4	331.0	Malvidin-3-glucoside	14.2
	3	22.0	479.2	317.0	Petunidin-3-glucoside	0.9
	3	25.9	463.3	301.0	Peonidin-3-glucoside	2.7
	Coil fraction	44.7	655.6	329.0	Malvidin-3-p-coumaroyl-glucoside	4.2
Vranec	1	15.0	465.3	303.0	Delphinidin-3-glucoside	0.4
	1	19.5	449.4	287.0	Cyanidin-3-glucoside	3.3
	2	28.0	493.4	331.0	Malvidin-3-glucoside	19.0
	3	22.0	479.2	317.0	Petunidin-3-glucoside	0.8
	3	25.9	463.3	301.0	Peonidin-3-glucoside	1.4
	Coil fraction	44.7	655.6	329.0	Malvidin-3-p-coumaroyl-glucoside	5.5

Table 1: Isolated anthocyanins from "Pinot Noir", "Merlot" and "Vranec" grape pomace.

malvidin-3-*p*-coumaroyl-glucoside. The other isolated anthocyanins were present in very small quantities (Table 1).

A comparison of the results showed that the highest amounts of anthocyanins was obtained from "Vranec" grape pomace. Furthermore, this grape pomace contained the highest quantities of malvidin-3-glucoside and malvidin-3-*p*-coumaroyl-glucoside which explain the violet-blue color of this variety.

The experiments of separation of anthocyanins-3-*O*-glucoside from grape pomaces by using three coil high speed CCC system confirmed that the order of separation mainly depends on the degree of hydroxyl substitution in the B ring (Figure 2). Furthermore, the order of the elution of the pigments with the same number of substituted groups depends on their polarity. The findings in this study are in accordance

with the findings of Salas who confirmed the same order of separation in the "head-to-tail" mode of CCC separation [10].

Proton and carbon magnetic resonance spectroscopy

The chemical structure of anthocyanin is presented on Figure 2 and Table 2. After purification by using preparative HPLC and detection of purified pigments on HPLC-DAD, NMR spectroscopy was applied in order to confirm the tentative structure previously obtained from ESI-MS spectrometry.

Delphinidin-3-*O*-β-D-glucopyranoside: ¹H NMR Singlets from the aglycone moiety at δ 8.95 (1H, s, H-4), δ 8.05 (1H, d, H-2' H-6'), δ 6.67 (1H, s, H-8), δ 6.80 (1H, d, H-6), doublet and multiplets from the glucopyranoside moiety δ 5.30 (1H, d, G-1), δ 3.95 (1H, m, G-6),

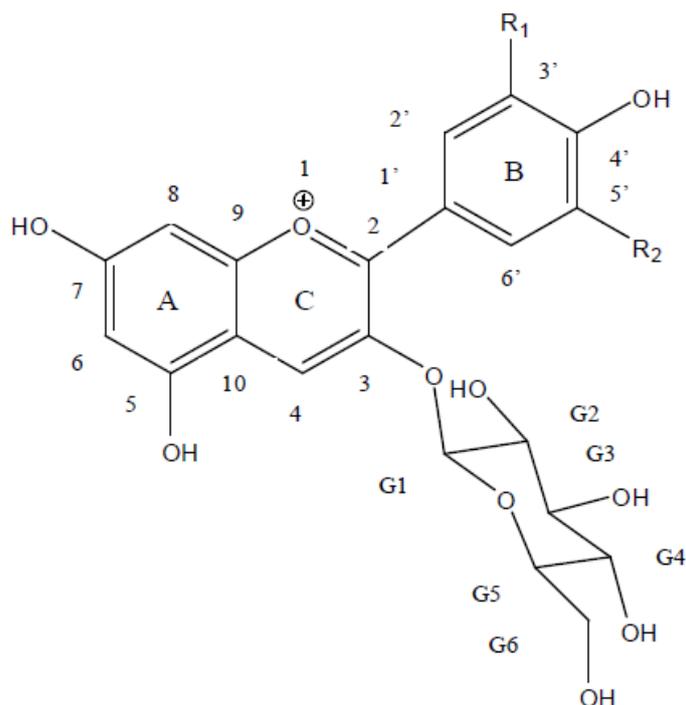


Figure 2: Chemical structure of anthocyanin-3-O-glucoside.

	<i>Delphinidin-3-glucoside</i>	<i>Cyanidin-3-glucoside</i>	<i>Petunidin-3-glucoside</i>	<i>Peonidin-3-glucoside</i>	<i>Malvidin-3-glucoside</i>	<i>Malvidin-3-p-coumaroyl-glucoside</i>
R1	-OH	-OH	-OCH ₃	-OCH ₃	-OCH ₃	-OCH ₃
R2	-OH	-H	-OH	-H	-OCH ₃	-OCH ₃

Table 2: Structure of anthocyanin-3-O-glucoside.

δ 3.70 (1H, d, G-6), δ 3,65 (4H, m, G-2, G-3, G-4, G-5) were in good accordance with the results from the ¹H NMR spectra of delphinidin-3-glucoside in the work of Bjorøy et al. [11,12, 14,15,23,].

¹³C NMR Signals from the aglycone moiety δ 158.68 (C5), δ 147.55 (C3'), δ 145.86 (C3), δ 120.05 (C1'), δ 117.93 (C2', C6'), δ 113.23 (C10) and from the glucopyranoside moiety δ 78.84 (G5), δ 74.79 (G2), δ 71.08 (G4) and δ 62.36 (G6) were in good agreement with the published results from ¹³C NMR spectra of delphinidin-3-O-glucoside [11].

Petunidin-3-O- β -D-glucopyranoside: ¹H NMR Singlets from aglycone moiety δ 9.0 (1H, s, H-4), δ 4.00 (3H, s, H₃-OCH₃) doublets from aglycone moiety δ 7.95 (1H, d, H-2'), δ 7.80 (1H, d, H-6'), doublets from glucopyranoside moiety δ 5.30 (1H, d, G-1) and multiplets from glucopyranoside moiety δ 3.90-3.40 (4H, m, G-2, G-3, G-4, G-6) were in good accordance with the results from the ¹H NMR spectra of petunidin-3-glucoside [12,15].

¹³C NMR Shifts from δ 164.25 (C2), δ 158.72 (C5), δ 149.85 (C3'), δ 147.51 (C4'), δ 145.8 (C5'), δ 145.80 (C4'), δ 113.49 (C6'), δ 78.9 (G3), δ 78.2 (G5), δ 74.96 (G2), δ 71.19 (G1), δ 62.42 (G6) confirmed the structure of the isolated anthocyanin. The signals from ¹³C NMR of the pigment were in correspondence to the signals for petunidin-3-O-glucoside isolated from *Liriope platyphylla* fruits [24-26].

Malvidin-3-O- β -D-glucopyranoside: ¹H NMR Singlets from the aglycone moiety δ 9.05 (1H, s, H-4), δ 8.05 (2H, s, H-2'), δ 3.95 (6H, s, OCH₃-3', OCH₃-5'), doublets from the aglycone moiety δ 6.90 (1H,

d, H-8), δ 6.64 (1H, d, H-6) and multiplets from the glucopyranoside moiety δ 5.30 (1H, d, G1) and δ 3.45-3.90 (4H, m, G2, G3, G4, G5) were in good accordance with the results from the ¹H NMR spectra of malvidin-3-glucoside [12,14,15,17-19].

¹³C NMR Shifts δ 170.10 (C7), δ 163.56 (C2), δ 159.32 (C5), δ 157.79 (C9), δ 149.75 (C3' C5'), δ 146.28 (C-4'), δ 145.69 (C3), δ 137.03 (C4), δ 119.79 (C1'), δ 113.61 (C10), δ 110.40 (C6') δ 103.92 (G1), δ 103.49 (C6) δ 78.28 (G3), δ 75.05 (G2), δ 71.20 (G4), δ 62.39 (G6), δ 57.32 (C3'C5'2 \times OCH₃) were in good accordance with the results reported for malvidin-3-glucoside [17-19].

Cyanidin-3-O- β -D-glucopyranoside: ¹H NMR Singlets from aglycone moiety δ 9.0 (1H, s, H-4), doublets from the aglycone moiety δ 8.25 (1H, dd, H-6'), δ 8.05 (1H, d, H-2'), δ 7.05 (1H, d, H-5'), δ 6.70 (1H, d, H-6) and multiplets from the glucopyranoside moiety δ 3.9 (1H, m, G-6), δ 3.55 (2H, m, G-3, G-5), were in good accordance with the results for cyanidin-3-O-glucoside by Jordheim [11,13,15,16,20].

¹³C NMR Shifts from the aglycon moiety δ 159.60 (C9), δ 159.30 (C5), δ 121.70 (C1'), δ 118.40 (C2'), δ 117.93 (C5') and δ 114.16 (C10) were in good accordance with the results from the ¹³C NMR spectra of cyanidin-3-O-glucoside [11,13,15,16,20].

Peonidin-3-O- β -D-glucopyranoside: ¹H NMR Singlets from the aglycone moiety δ 9.00 (1H, s, H-4), δ 8.05 (1H, s, H-4), δ 7.05 (1H, d, H-5'), δ 6.95 (1H, d, H-8), δ 6.70 (1H, s, H-6), doublets from the glucopyranoside moiety δ 8.3 (1H, dd, H-6'), δ 5.35 (1H, d, G-1), and multiplets from the glucopyranoside moiety δ 3.40-3.75 (4H, m, G-2,

G-3, G-4, G-5) were in good accordance with the results obtained by Yawadio et al. [20].

Since very small quantity of the pigment was isolated the ^{13}C NMR spectra was not recorded.

Malvidin-3-p-coumaroyl-O- β -D-glucopyranoside: ^1H NMR Singlets from δ 9.0 (1H, s, H-4), δ 8.1 (1H, s, H-2', H-6'), δ 7.9 (2H, s, H-2', H-6'), δ 7.4 (d, $\text{CH}_\beta=\text{CHCO}_2\text{R}$), δ 6.9 (1H, d, H-8), δ 7.25 (d, G-2, G-6), δ 6.6 (1H, d, G3, G5), δ 5.4 (1H, d, G1), δ 3.7-3.3 (H5, m, G2, G3, G4, G5, G6) were in accordance to the results [12,14,15,17-19]. The distance between the signals of 15 ppm confirmed that the double bond from the coumaroyl group is "trans" configured.

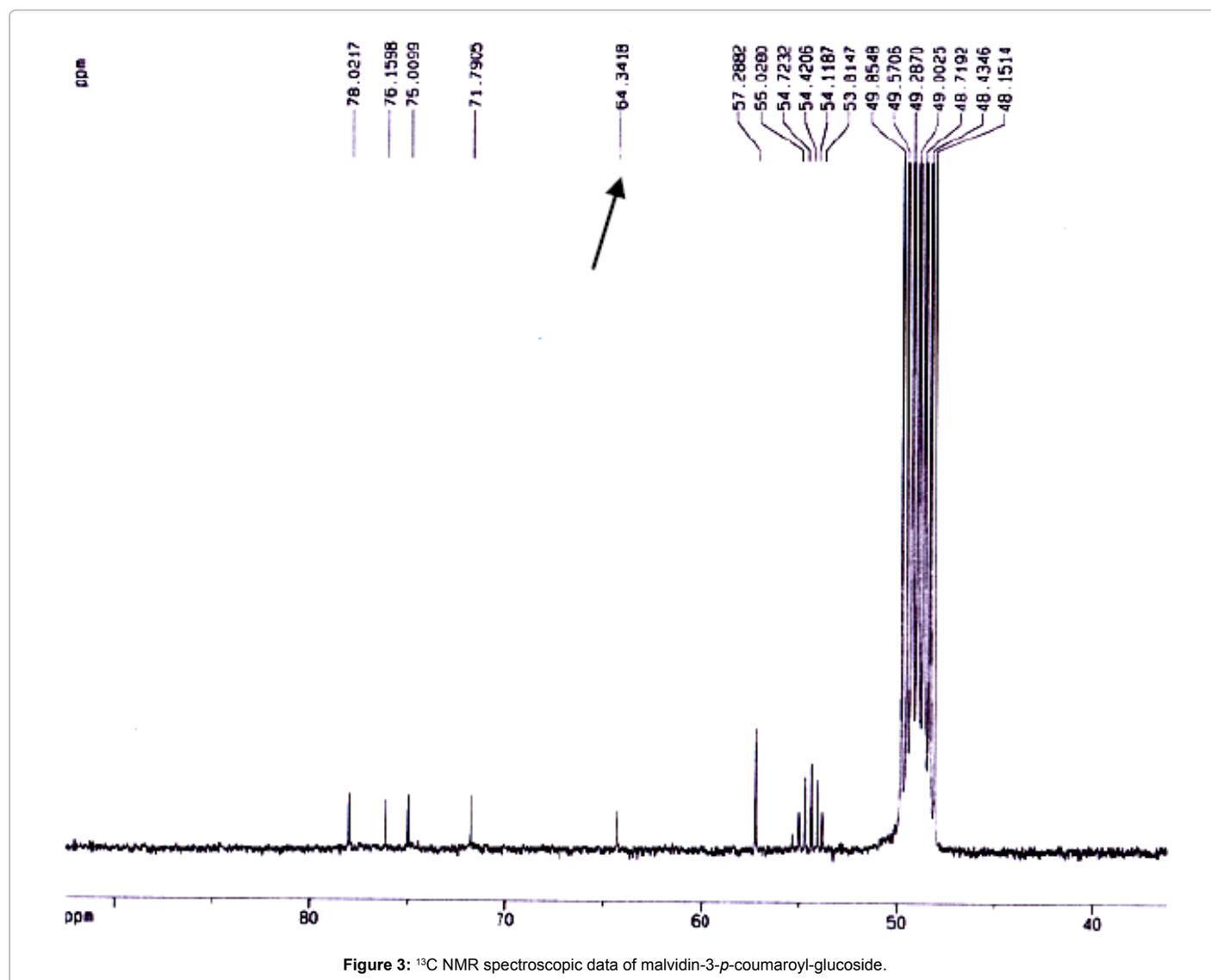
^{13}C NMR The chemical shifts obtained from ^{13}C NMR from coumaroyl group δ 168.83 (R1CO₂R2), δ 114.17 (CH=CH₂CO₂R), aglycon moiety δ 159.84 (C9), δ 158.73 (C2), δ 158.18 (C5), δ 149.77 (C5'), δ 145.20 (C3), δ 119.78 (C1'), δ 113.54 (C10), δ 110.72 (C2') δ 57.28 (OCH₃) and glucopyranoside moiety δ 131.15 (G1, G6), δ 126.98 (G1), δ 116.86 (G3, G5), δ 76.16 (G5), δ 75.01 (G2), δ 71.79 (G4) were in good accordance with the ^{13}C NMR spectra of malvidin-3-glucoside [17-19]. The most significant shift of signal from 62 ppm for G6 to 64.34

ppm was the proof of the attached *p*-coumaroyl group on position 6 of the glucose (Figures 2 and 3).

Visual detection thresholds and "color activity concept" of isolated anthocyanins

The color contribution of the isolated anthocyanins to the overall color of red wines was object of studies of Degenhardt et al. [21]. According to the results, the most abundant anthocyanin was malvidin-3-glucoside (500 mg/L of pure malvidin-3-glucoside per liter red wine) which contributes predominantly to the color of red wines.

Results obtained from isolation of anthocyanins from grape pomace are in agreement with those obtained for wines [21]. Although the process of polymerisation is very important for the color of the grapes, malvidin-3-glucoside is the most dominant monomer which contribute to the color of the grapes. On the other hand, the contribution of other pigments to the overall color of pomace was different for each grape variety. Table 3 presented the visual detection thresholds of isolated anthocyanins and the corresponding color activity values (CAVs). It is notable that delphindin-3-glucoside did not contribute to the overall color of grape pomace. However, petunidin-



Isolated anthocyanins	Visual detection thresholds (mg/L)	Color activity value (CAV) for Pinot Noir grape pomace	Color activity value (CAV) for Merlot grape pomace	Color activity value (CAV) for Vranec grape pomace
Delphinidin-3-glucoside	2	<1	<1	<1
Cyanidin-3-glucoside	1	<1	2.1	3.3
Petunidin-3-glucoside	0.2	4.0	4.5	4.0
Peonidin-3-glucoside	0.25	2.4	10.8	5.6
Malvidin-3-glucoside	1.25	8.0	11.3	15.2
Malvidin-3-p-coumaroyl-glucoside	0.75	1.2	5.6	7.3

Table 3: Visual detection thresholds and color activity values of isolated anthocyanins.

3-glucoside contributed more significant to the color of “Pinot Noir” grape variety in comparison with its contribution to the other varieties of grape pomace. Also, the color activity value of malvidin-3-*p*-coumaroyl-glucoside, responsible for blue color, is very small in “Pinot Noir” grape pomace in comparison to “Merlot” and “Vranec” grape pomace. This might be the reason for deeply red color of “Pinot Noir” grape variety in comparison of red violet and violet-blue color of “Merlot” and “Vranec” grape pomace, respectively. Contribution of peonidin-3-glucoside in “Merlot” grape pomace is significant for red shade of this grape variety. Higher color activity value of malvidin-3-*p*-coumaroyl-glucoside was responsible for violet-blue color of “Vranec” grape pomace in comparison with red-violet color of “Merlot” grape pomace (Table 3).

Conclusion

The study described the isolation of high valuable natural pigments (anthocyanins-3-glucosides) from grape pomace. Countercurrent chromatography was applied for the isolation of the pigments and high performance liquid chromatography, mass spectrometry and NMR spectroscopy for structure confirmation of isolated anthocyanins-3-*O*-glucosides. Quantities of isolated pigments indicated that the pomace from “Vranec” grape variety was the richest source for malvidin-3-*O*-glucoside and malvidin-3-*O*-*p*-coumaroylglucoside. The experiments showed that from 500 g of grape pomace obtained after 20 days of maceration time 19 mg of pure malvidin-3-*O*-glucoside and 5.5 mg of malvidin-3-*O*-*p*-coumaroyl-glucoside can be isolated. Delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, petunidin-3-*O*-glucoside and peonidin-3-*O*-glucoside were isolated in lower quantities.

The “color activity concept” explained the contribution of the most abundant anthocyanins-3-*O*-glucosides to the color of the three varieties of grape pomaces (Vranec, Merlot and Pinot Noir). Petunidin-3-*O*-glucoside contributed more significant to “Pinot Noir” grape pomace in comparison with the other two varieties. The violet color of “Vranec” grape variety was attributed to the significant quantity of malvidin-3-glucoside and the blue shadow was attributed to malvidin-3-*p*-coumaroylglucoside.

In conclusion, this work showed that countercurrent chromatography can be useful technique for isolation of high valuable natural pigments from low valuable sources as red grape pomace.

References

- Bakker J, Timberlake KF (1997) Isolation, Identification, and Characterization of New Color-Stable Anthocyanins Occurring in Some Red Wines. J Agric Food Chem 45: 35-43.
- Casta'eda-Ovando A, De Lourdes Pacheco-Hernandez M, Elena Paez-Hernandez M, Rodriguez JA, Andres Galan-Vidal C (2009) Chemical studies of anthocyanins: A review. Food Chem 113: 859-871.
- Brouillard R, Chassaing S, Fougerousse A (2003) Why are grape/fresh wine anthocyanins so simple and why is it that red wine color lasts so long? Phytochemistry 64: 1179-1186.
- Kähkönen MP, Heinonen M (2003) Antioxidant activity of anthocyanins and their aglycons. J Agric Food Chem 51: 628-633.
- Cantos E, Espín JC, Tomás-Barberán FA (2002) Varietal differences among the polyphenol profiles of seven table grape cultivars studied by LC-DAD-MS-MS. J Agric Food Chem 50: 5691-5696.
- Luque-Rodríguez JM, Luque de Castro MD, Pérez-Juan P (2007) Dynamic superheated liquid extraction of anthocyanins and other phenolics from red grape skins of winemaking residues. Bioresour Technol 98: 2705-2713.
- Mateus N, Machado JM, De Freitas V (2002) Development changes of anthocyanins in *Vitis vinifera* grapes grown in the Douro Valley and concentration in respective wines. J Agric Food Chem 50: 1689-1695.
- Schwarz M, Hillebrand S, Habben S, Degenhardt A, Winterhalter P (2003) Application of high-speed countercurrent chromatography to the large-scale isolation of anthocyanins. Biochemical Engineering Journal 14: 179-189.
- Vidal S, Hayasaka Y, Meudec E, Cheyrier V, Skouroumounis G (2004) Fractionation of grape anthocyanin classes using multilayer coil countercurrent chromatography with step gradient elution. J Agric Food Chem 52: 713-719.
- Salas E, Dueñas M, Schwarz M, Winterhalter P, Cheyrier V, et al. (2005) Characterization of pigments from different high speed countercurrent chromatography wine fractions. J Agric Food Chem 53: 4536-4546.
- Ha TJ, Lee MH, Jeong YN, Lee JH, Han SI, et al. (2010) Anthocyanins in Cowpea [*Vigna unguiculata* (L.) Walp. ssp. *unguiculata*]. Food Sci Biotechnol 19: 821-826.
- Nickavar B., Amin G (2004) Anthocyanins from *Vaccinium arctostaphylos* Berries. Pharmaceutical Biology 42: 289-291.
- Tsuda T, Ohshima K, Kawakishi S, Osawa T (1994) Antioxidative Pigments Isolated from the Seeds of *Phaseolus vulgaris* L. J Agric Food Chem 42: 248-251.
- Takeoka GR, Dao LT, Full GH, Wong RY, Harden LA, et al. (1997) Characterization of Black Bean (*Phaseolus vulgaris* L.) Anthocyanins. J Agric Food Chem 45: 3395-3400.
- Kuskoski EM, Vega JM, Rios JJ, Fett R, Troncoso AM, et al. (2003) Characterization of anthocyanins from the fruits of baguaçu (*Eugenia umbelliflora* Berg). J Agric Food Chem 51: 5450-5454.
- Jordheim M, Giske NH, Andersen ØM (2007) Anthocyanins in Caprifoliaceae. Biochemical Systematics and Ecology 35: 153-159.
- Andersen ØM, Viksund RI, Pedersen AT (1995) Malvidin 3- (6-acetylglucoside)-5-glucoside and other anthocyanins from flowers of *Geranium sylvaticum*. Phytochemistry 38: 1513-1517.
- Atanasova V, Fulcrand H, Le Guernevé C, Cheyrier V, Michel M (2002) Structure of a new dimeric acetaldehyde malvidin 3-glucoside condensation product. Tetrahedron Letters 43: 6151-6153.
- Aguirre MJ, Chen YY, Isaacs M, Matsuhira B, Mendoza L, et al. (2010) Electrochemical behaviour and antioxidant capacity of anthocyanins from Chilean red wine, grape and raspberry. Food Chem 121: 44-48.
- Yawadio R, Tanimori S, Morita N (2007) Identification of phenolic compounds isolated from pigmented rices and their aldose reductase inhibitory activities. Food Chem 101: 1616-1625.
- Degenhardt A, Hofmann S, Knapp H, Winterhalter P (2000) Preparative isolation of anthocyanins by high-speed countercurrent chromatography and application of the color activity concept to red wine. J Agric Food Chem 48: 5812-5818.
- Winterhalter P (2007) Application of countercurrent chromatography (CCC) to

- the analysis of natural pigments. Trends in Food Science and Technology 18: 507-513.
23. Bjørøy O, Fossen T, Andersen OM (2007) Anthocyanin 3-galactosides from *Cornus alba* 'Sibirica' with glucosidation of the B-ring. Phytochemistry 68: 640-645.
24. Lee JH, Chung MG (2011) Identification and characterisation of anthocyanins in the antioxidant activity-containing fraction of *Liriope platyphylla* fruits. Food Chem 127: 1686-1693.
25. Degenhardt A, Knapp H, Winterhalter P (2000) Separation and Purification of Anthocyanins by High-Speed Countercurrent Chromatography and Screening for antioxidant activity. J Agric Food Chem 48: 338-343.
26. Gutzeit D, Winterhalter P, Jerz G (2007) Application of preparative high-speed counter-current chromatography/electrospray ionization mass spectrometry for a fast screening and fractionation of polyphenols. J Chromatogr A 1172: 40-46.

Citation: Veličkovska SK, Mirhosseini H, Bogeve E (2013) Isolation of Anthocyanins by High-Speed Countercurrent Chromatography and Application of the Color Activity Concept to Different Varieties of Red Grape Pomace from Macedonia. J Nutr Food Sci 3: 243. doi: [10.4172/2155-9600.1000243](https://doi.org/10.4172/2155-9600.1000243)

Submit your next manuscript and get advantages of OMICS Group submissions

Unique features:

- User friendly/feasible website-translation of your paper to 50 world's leading languages
- Audio Version of published paper
- Digital articles to share and explore

Special features:

- 300 Open Access Journals
- 25,000 editorial team
- 21 days rapid review process
- Quality and quick editorial, review and publication processing
- Indexing at PubMed (partial), Scopus, EBSCO, Index Copernicus and Google Scholar etc
- Sharing Option: Social Networking Enabled
- Authors, Reviewers and Editors rewarded with online Scientific Credits
- Better discount for your subsequent articles

Submit your manuscript at: <http://www.editorialmanager.com/lifesciences>

