



Isolation of anthocyanins by high-speed countercurrent chromatography and application of color activity concept to different red grape pomaces

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Introduction

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

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.

Results and discussion

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 delphinidin-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 (Table 1).

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.

Conclusion

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.

References:

Veličkovska SK, Mirhosseini H, Bogeveva 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: 10.4172/21559600.1000243.

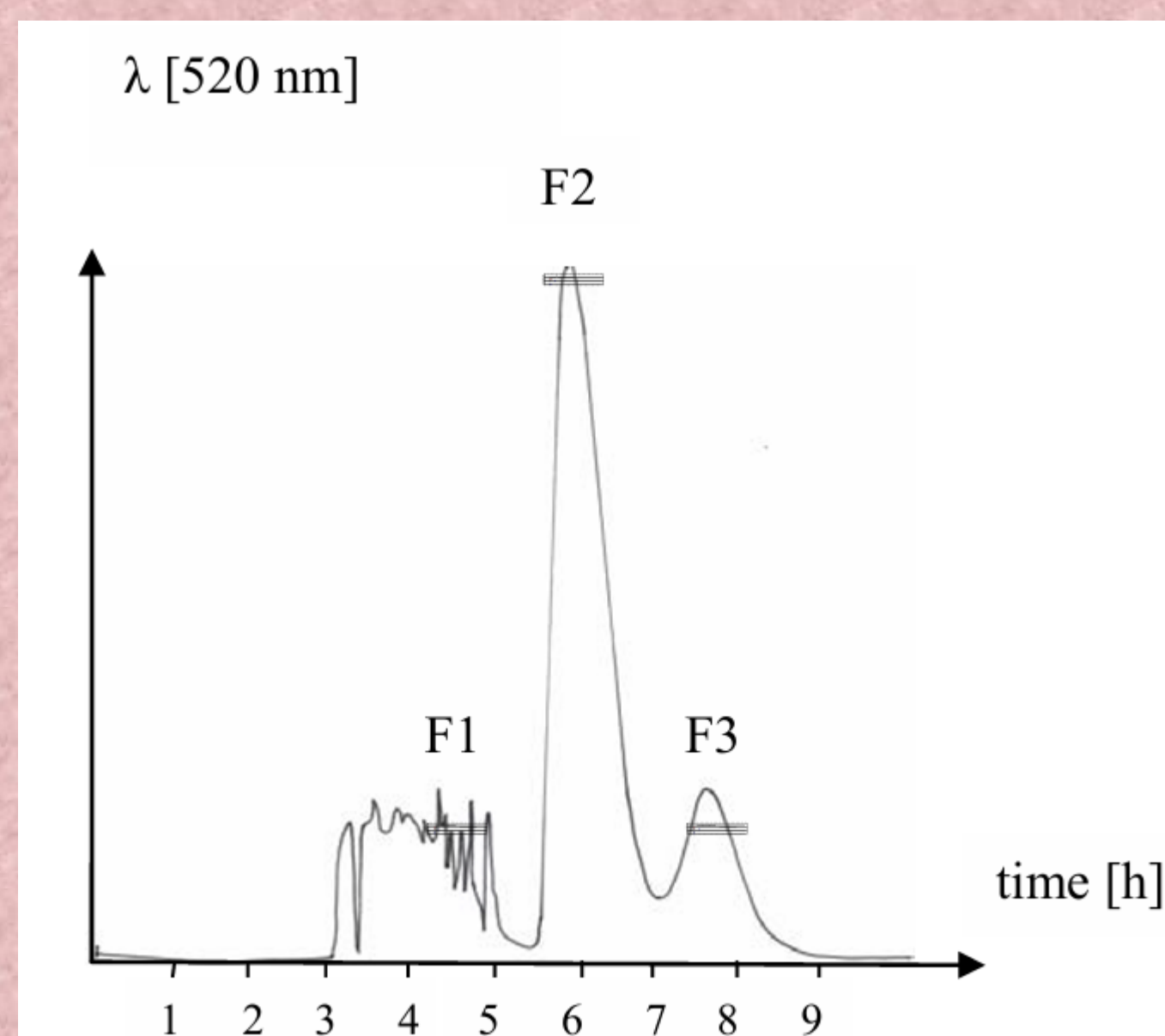


Figure 1: HSCCC separation of anthocyanins from "Vranec" grape pomace at 520 nm.