

SUPPLEMENTARY MATERIAL

Chemical composition and antioxidant capacity of the fruits of European plum cultivar “Čačanska Lepotica” influenced by different rootstocks

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Table S1. Fertilization regime and plant protection of the plum trees.

Fertilize regime		Plant protection	
Autumn fertilization November	Nitrogen/NH ₄ NO ₃ / active substance 12 kg/ha	Autumn	Copper hydroxide /Cu(OH) ₂ / active substance 4 kg/ha
	Potassium/K ₂ SO ₄ / active substance 30 kg/ha	Spring	Copper hydroxide /Cu(OH) ₂ / active substance 4 kg/ha
	Phosphorus/P ₂ O ₅ / active substance 50 kg/ha	Vegetation	<i>Bacillus subtilis</i> QST 713 active substance 10 L/ha
Spring fertilization late February	Nitrogen/NH ₄ NO ₃ / active substance 45 kg/ha		Flonicamid, g/kg 500 active substance 140 g/ha
	Potassium/K ₂ SO ₄ / active substance 50 kg/ha		Spinetoram, g/kg 250 active substance 500 g/ha
	Phosphorus/P ₂ O ₅ / active substance 20kg/ha		Chlorantraniliprole 200 g/L active substance 600 mL/ha
			Abamectin g/L 18 active substance 1 L/ha

Isolation, purification, and characterization of anthocyanins in the methanol extract of plum fruit skin by ¹H NMR

The isolation and purification of anthocyanins in the methanol extract of plum-fruit skin by ¹H NMR was performed by using the procedure described by Rodriguez-Saona and Wrolstad [1]. Briefly, the Discovery®DCS-18 cartridge (500 mg, 3 mL, Supelco, USA) was conditioned by the passing of two column volumes of MeOH and three volumes of acidified deionized H₂O (0.01 % HCl, *v/v*). Then a solution of dry methanol extract (50 mg) of plum-fruit skins in deionized H₂O (1 mL) was applied on the cartridge and washed with two column volumes of acidified deionized H₂O to remove compounds not absorbed (e.g., acids and sugars). The cartridge was further washed with two volumes of ethyl acetate to remove phenolic acids and flavonoids. The anthocyanins were eluted with acidified MeOH (0.01 % HCl, *v/v*). The methanol was removed in a rotary evaporator under vacuum and protected from light. The

resulting anthocyanin-enriched fraction was further dissolved in CD₃OD. The ¹H NMR, COSY, HSQC, and HMBC spectra were recorded on a Bruker Avance NEO 600 spectrometer (Biospin GmbH, Rheinstetten, Germany). The individual compounds were identified through the comparison of the ¹H NMR data (Figure S1) with those published in the literature [2,3]. The chemical shifts, multiplicity, and the coupling constants of the aglycone and anomeric protons are given in Table S2.

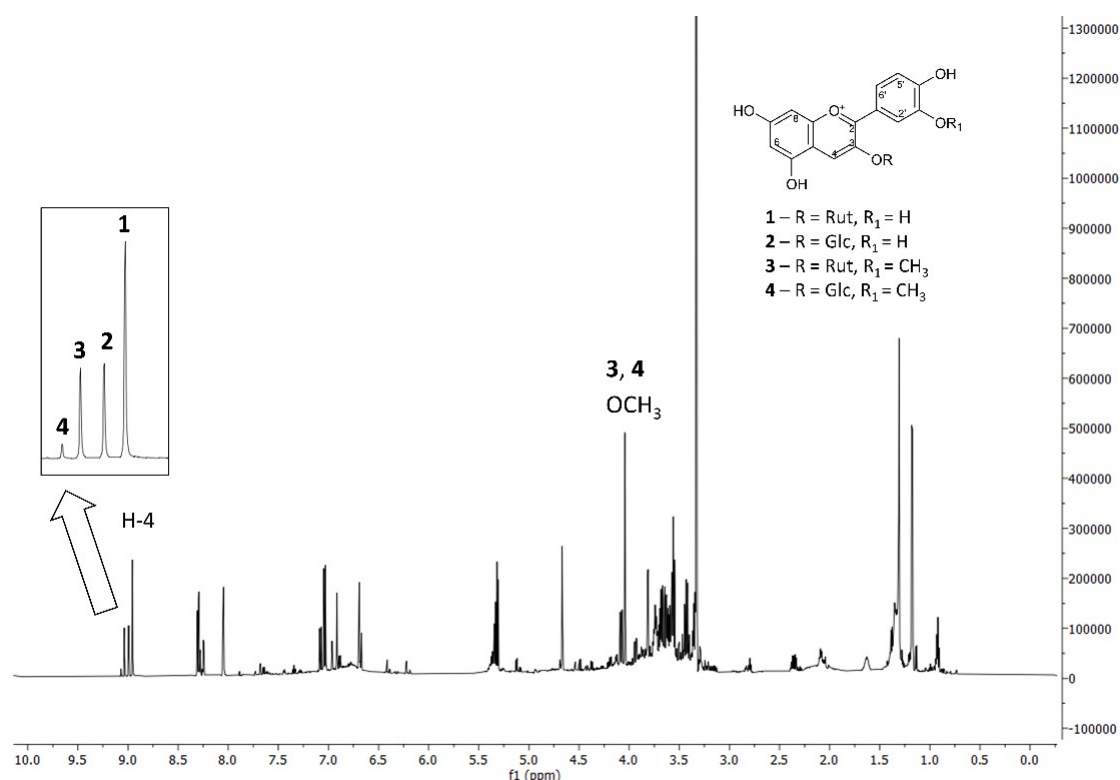


Figure S1. ¹H NMR spectrum of the anthocyanin-enriched fraction obtained from plum-fruit skins from plums grafted on “GXN-15” rootstock in CD₃OD.

Table S2. ¹H NMR spectral data of the anthocyanins in CD₃OD (600 MHz).

H	1	2	3	4
4	8.94 s	8.97 s	9.02 s	9.05 s
6	6.69 d (2.0)	6.67 d (2.0)	6.70 d (2.0)	6.70 d (2.0)
8	6.92 d (2.0)	6.92 d (2.0)	6.96 d (2.0)	6.96 d (2.0)
2'	8.03 d (2.2)	8.04 d (2.2)	8.22 d (2.2)	8.23 d (2.2)
3'	7.02 d (8.8)	7.02 d (8.8)	7.06 d (8.8)	7.06 d (8.8)
6'	8.30 dd (2.2, 8.8)	8.30 dd (2.2, 8.8)	8.28 dd (2.2, 8.8)	8.28 dd (2.2, 8.8)
OCH ₃	-	-	3.99 s	3.98 s
H-1'' (Glc)	5.30 d (7.6)	5.30 d (7.6)	5.32 d (8.0)	5.32 d (8.0)
H-1''' (Rha)	4.65 d (1.5)	-	4.65 d (1.5)	-
H-6''' (Rha)	1.16 d (6.5)	-	1.16 d (6.5)	-

References:

- Rodriguez-Saona, L.E.; Wrolstad, R.E. Extraction, Isolation, and Purification of Anthocyanins. *Curr. Protoc. Food Anal. Chem.* **2001**, *1*, F1.1.1-F1.1.11, doi:10.1002/0471142913.faf0101s00.

2. Andersen, Ø.M.; Fossen, T. Characterization of Anthocyanins by NMR. *Curr. Protoc. Food Anal. Chem.* **2003**, *9*, F1.4.1-F1.4.23, doi:10.1002/0471142913.faf0104s09.
3. Goulas, V.; Minas, I.S.; Kourdoulas, P.M.; Lazaridou, A.; Molassiotis, A.N.; Gerothanassis, I.P.; Manganaris, G.A. ¹H NMR Metabolic Fingerprinting to Probe Temporal Postharvest Changes on Qualitative Attributes and Phytochemical Profile of Sweet Cherry Fruit. *Front. Plant Sci.* **2015**, *6*, 959, doi:10.3389/fpls.2015.00959.