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Short communication

Fruit as a substrate for a wine: A case study of selected berry and drupe fruit wines

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for the production of the relevant fruit wines.

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ABSTRACT

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1. Introduction

Although dietary patterns differ a lot around the world, daily intake of foods with an enhanced glycemic index is now more a common thing. Indeed, such a habit has significantly contributed to the prevalence of chronic degenerative disease (Törrönen et al., 2010). The occurrence of "metabolic syndrome" – mix of health conditions responsible, inter alia, for development of type 2 diabetes and cardiovascular diseases – confirms such a trend (Bisbal et al., 2010). However, the prevention based on appropriate foodstuffs may successfully address this alarming situation at the global level (Herrera et al., 2009).

Fruits and vegetables along with their derived products represent a rich source of bioactive compounds. Their adequate intake (400–500 g/ day) may significantly contribute to homeostasis (Jaganath, 2008). Antioxidants including phenolics take part in the antioxidant protection (Shukitt-Hale et al., 2008). Prior studies focusing on antioxidant activity of fruit species gave an advantage to the strawberries vs. drupe fruits (Contessa et al., 2013). Variation of this particular bioactivity among different cultivars of the aforementioned berry fruit was also reported (Singh et al., 2011; Xu et al., 2014). Finally, the abundance of phenolic acids in the strawberries has been thoroughly documented

(Guerrero-Chavez et al., 2015; Mandave et al., 2014). However, drupe fruits was also claimed for their antioxidant potential (Khallouki et al., 2012; Khumalo et al., 2017; Liu et al., 2015a; Martini et al., 2017; Usenik et al., 2008). It is important to emphasize that the processing of fruits during the vinification procedure usually does not affect retain the content of phenolic compounds in the final product (Czyzowska and Pogorzelski, 2002).

The objective of this study was to estimate for the first time the potential of selected fruit cultivars belonging to the berry (strawberry) and drupe fruits (apricot, peach, plum and sweet cherry) as the substrates for the production of new fruit wine products enriched with phenolic compounds. All these cultivars are international except for the plums.

2. Material and methods

This study aimed to estimate the potential of the selected berry (strawberry) and drupe (apricot, plum and sweet

cherry) fruits as the substrates for the production of new fruit wines enriched with phenolic compounds. Sweet

cherry wine (cultivar Burlat) stood out both for the profound content of phenolics (followed by their chemical

profile) and potent anti-DPPH radical activity. The same fruit wine samples exhibited high redox potentials,

slightly lower than strawberry and plum wine samples. Therefore, sweet cherry cultivar Burlat may be well

recommended for development of novel fruit-based products endowed with naturally occurring phenolics. In any

case, the remaining four cultivars are also worth further research efforts, particularly apricot cultivar

Kečkemetska ruža, which is one of the representatives of greatly underestimated fruit type thus far as a substrate

2.1. Plant material

The fruits used in our vinification procedure were from well know Serbian regions for their cultivation. They were purchased from commercial producers (phytosanitary health, 100%). Strawberry (*Fragaria x*

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Table 1

The conditions for identification and quantification of selected phenolic compounds.

Phenolic compound	Molecular formula	Mass	Ionisation mode ESI	MRM transition	Cone voltage (V)	Collision energy (eV)	t _R (min)
Epicatechin	C ₁₅ H ₁₄ O ₆	290	+	291→139	26	16	20.30
Kaempferol	$C_{15}H_{10}O_{6}$	286	+	287→153	56	36	31.52
Gallic acid	$C_7H_6O_5$	170	-	169→125	30	20	4.74
Protocatechuic acid	C ₇ H ₆ O ₄	154	-	153→109	30	20	9.18
p-Hydroxybenzoic acid	$C_7H_6O_3$	138	-	137→93	30	20	14.30
Catechin	$C_{15}H_{14}O_{6}$	290	+	291→139	26	20	15.82
Chlorogenic acid	C16H18O9	354	+	355→163	20	12	15.62
Vanillic acid	$C_8H_8O_4$	168	+	169→93	26	14	17.16
Caffeic acid	$C_9H_8O_4$	180	-	179→135	30	20	18.04
p-Coumaric acid	C ₉ H ₈ O ₃	164	+	165→91	22	22	23.86
Rutin	C27H30O16	610	-	609→301	60	20	25.08
Ellagic acid	$C_{14}H_6O_8$	302	-	301→89	50	56	25.51
Naringenin	$C_{15}H_{12}O_5$	272	+	273→153	24	24	31.32
Quercertin	$C_{15}H_{10}O_7$	302	-	301→151	30	20	29.93

ESI - Electrospray Ionisation; MRM - Multiple Reaction Monitoring; tR - Retention time.

ananassa.) cultivar Asia and plum (*Prunus domestica*) cultivar Čačanska rodna were from Valjevo. Plum cultivar Čačanska rodna is an indigenous variety from Serbia. The fruit is blue, and the flesh is yellow, juicy, aromatic and firm. Peach (*Prunus persica*) cultivar Rita star, apricot (*Prunus armeniaca*) cultivar Kečkemetska ruža and sweet cherry (*Prunus avium*) cultivar Burlat originated from Grocka (Republic of Serbia). Fruit ripeness was determined by a refractometer PAL-87S (Atago, Tokyo, Japan). Ripe fruit (harvested in 2017) was immediately made into wine.

2.2. Wine making

The experiments conducted during microvinification were divided into two sets. In both cases, fruit was first disintegrated. Subsequently, 10 g of $K_2S_2O_5$ 100 kg⁻¹ was added to the obtained pomace. The first set included the control without added sugar. Total soluble solids (expressed in 'Brix) were measured in the fruit pomaces of both the first and second sets. Aiming to increase total soluble solids of must up to 20.5°Brix, sugar was added in the second set. Drupe fruits (apricot, peach, plum and sweet cherry) were processed by two methods. The pits were removed from the fruits before they were fragmented (I method) or the fruits were fragmented together with the pits but without cracking them (II method). The obtained fruit pomaces were inoculated with the pure strain Saccharomyces cerevisiae of the selected wine yeast Lievito Secco (Enartis, Italy) at the dose of $20 \text{ g} 100 \text{ kg}^{-1}$. The pits were actually expected to enhance the level of phenolics. All experiments in microvinification (done in triplicate) were conducted in watts with the pigeage system (Hromil, Kovilj-Serbia). More precisely, 15 kg of the fruits were fermented in the watts of 30 L. Alcohol fermentation was conducted at 20 °C over 7 to 10 days. During this process, the pomace was stirred twice a day. After fermentation, each fruit wine was separated from the pomace by sedimentation. Afterwards, they were racked off the lees and kept at 12 °C for the next six months, until further studies.

2.3. Physicochemical properties of fruit wines

The pH value was determined by a microprocessor-based pH/mV/°C pH 212 (Hanna Instruments, Woonsocket, RI, USA). Further, 25 mL was titrated with 0.25 M NaOH aiming to estimate Total Titratable Acids (TTA) of the fruit wine samples. The titration endpoint (pH 7.0 \pm 0.5) was indicated by pH meter. Total Soluble Solids (TSS, expressed in °Brix) were measured in the fruit juice using the refractometer PAL-87S (Atago, Tokyo, Japan). The alcohol concentration was determined by the alcohol density meter DMA 35 (Anton Paar, Graz, Austria) after samples distillation. The strength by volume (vol. %) was calculated

using 20 °C/20 °C tables (Organisation Internationale de la Vigne et du Vin (OIV), 2009).

2.4. Standards and reagents

All chemicals and reagents of analytical grade were purchased from Sigma Aldrich (Steinheim, Germany). The Premium Syringe Filters (Captiva) Regenerated Cellulose ($0.45 \mu m$, 15 mm) were obtained from Agilent Technologies (Santa Clara, CA, USA). Water HPLC grade was provided by Ultrapure Water System Arium pro UV Sartorius (Göttingen, Germany).

2.5. Solid phase extraction (SPE)

Aiming to decrease the influence of the matrix during phenolics identification, solid-phase extraction (SPE) was applied, Oasis HLB 6CC 200 mg cartridges (Waters, Milford, MA, USA) (Kaihkonen et al., 2001). While the fruit wine samples were filtered through syringe filter, SPE was performed as described by Ferreiro-González et al. (2014) with some modification. The conditioning of cartridges and equilibration were carried out with 5 mL of methanol and HPLC-grade water, respectively. Furthermore, 5 mL of each sample was loaded. The washing was conducted both with 5 mL of HPLC-grade water and 5% methanol. The eluation was carried out with 6×1 mL of methanol containing 0.1% formic acid. Finally, each sample was evaporated to dryness, reconstituted in 1 mL of solution like gradient at the start and used for the analysis.

2.6. UPLC/MS-MS analysis

UPLC/MS-MS analysis was performed using a Waters Acquity Ultra Performance H-Class System (Waters, Milford, MA, USA). UPLC separation was achieved on the column compartment with ZORBAX Eclipse XDB C18 column (150 mm \times 4.6 mm; 5 μ m). During analysis, the column was kept at 25 °C while mobile phase flow-rate at 0.7 mLmin^{-1} and injection volume was $10 \mu \text{L}$ (Gođevac et al., 2009). Phenolic compounds were identified by comparing their retention times (t_B) and mass spectra with the relevant standards. IntelliStart program (Waters, Milford, MA, USA; 2005) provided parameters that were used for quantification (Table 1). UPLC was coupled with a triple quadrupole mass spectrometer Acquity TQD (Waters, Milford, MA, USA) with the software MassLynx 4.1 (Waters, Milford, MA, USA; 2005) which was used for data acquisition and processing. Finally, the ionisation source conditions were as followed: capillary voltage of 3.5 kV, source temperature of 150 °C and desolvation temperature of 450 °C, with a flow rate of 900 L h⁻¹. Nitrogen and argon were used as cone and collision

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