

## Comparative Study of Aromatic and Polyphenolic Profiles of Croatian White Wines Produced by Cold Maceration

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### Abstract

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Aroma profiles and phenolic components of white wines made from cv. Pošip and Škrlet (*Vitis vinifera* L.) both native Croatian, non-aromatic grape varieties enhances the knowledge about these varieties, prepared without maceration (control) and subjected to the pre-fermentative cold maceration (CM). Individual phenolic compounds in wines were determined by high-performance liquid chromatography (HPLC) system, while total phenolics and flavan-3-ols in wines were determined by spectrophotometric methods. CM had the significant impact on the total phenolic and flavan-3-ols composition of 'Škrlet' wine. The significant increase of some individual phenolic compounds was observed in 'Pošip' wine. Volatile aroma compounds in wines were determined by gas chromatography (GC) with the previous extraction on the solid phase micro extraction (SPME). The primary aroma compounds, i.e. terpenes were under the significant influence of CM process in 'Škrlet' wine. Aroma profiles, based on the content of 24 specific compounds, grouped in eight aroma series that contribute to wine odor were developed. CM significantly increased five aroma series in 'Škrlet' wine and only three in 'Pošip' wine.

**Keywords:** GC; HPLC; OAV; Pošip; pre-fermentation; Škrlet

Polyphenolic and aromatic compounds are essential for wine composition and their sensorial attributes. Polyphenol compounds have been shown to provide an antioxidant, antibacterial and antimicrobial activity that can have a potential health benefit for consumers (XIA *et al.* 2014). While white wines are usually characterized by lower polyphenolic content, it can be useful to increase their concentration (OLEJAR *et al.* 2015).

Aromatic compounds identified in wines, around 1000, belonging to various chemical families (MORENO & PEINADO 2012) have a major role in the final

product quality. However, all of them do not contribute to the same extent to wine aroma. In order to determine the sensory importance of a compound, in addition to its concentration, it is important to know its odour perception threshold. Based on the ratio of the concentration to its perception threshold, the 'odour activity value' (OAV) can be defined, allowing one to estimate the contribution of all compounds quantified to wine aroma profile (PEINADO *et al.* 2004). Grouping the OAVs of aromatic compounds with similar descriptors into aroma series makes up the aromatic profile of wine. This method has

recently been used for distinguishing wine grape varieties (NOGUEROL-PATO *et al.* 2012a; GENOVESE *et al.* 2013) and wines (NOGUEROL-PATO *et al.* 2012b).

Since both group of compounds are mainly present in berry skin, pre-fermentative cold maceration (CM) is a technological practice aimed to enhance and optimize their extraction and improve fruity and floral characters of wine (PEINADO *et al.* 2004; ESTI *et al.* 2006; SELLI *et al.* 2006; ZINNAI *et al.* 2006; HERNANZ *et al.* 2007; OLEJAR *et al.* 2015). The CM takes place from the crushing of grapes till the beginning of the alcoholic fermentation (HEREDIA *et al.* 2010). During CM, the skins of crushed and destemmed white grapes are macerated in their own juice in a non-alcoholic and low-temperature environment (HERNANZ *et al.* 2007). Delay in the start point of fermentation is ensured by keeping the must at low temperatures (5–10°C), for a different time period, typically from 3 h up to few days (RAMEY *et al.* 1986; ORTEGA-HERAS *et al.* 2012). This process usually provides good results depending on the grape cultivar employed and experimental conditions (DARIAS-MARTÍN *et al.* 2000; SELLI *et al.* 2006). There are only a few researches found in the literature regarding the effect of cold maceration on the chemical composition of white wines (DARIAS-MARTÍN *et al.* 2000; PEINADO *et al.* 2004; ESTI *et al.* 2006; HERNANZ *et al.* 2007; OLEJAR *et al.* 2015; SOKOLOWSKY *et al.* 2015). Results reported shown significant increases in phenolic and antioxidant activity (BAIANO *et al.* 2012; OLEJAR *et al.* 2015). Since the increasing of some polyphenolics can cause more astringent and bitter taste that is not desirable in white wines, the temperature of maceration lower than 10°C can limit their extraction (RAMEY *et al.* 1986). Contradictory results were presented regarding the aromatic profiles of different varieties. Studies obtained with some aromatic varieties ('Gewürtztraminer' and 'Muscat' together with 'Narince'), showed benefits from skin contact due to a high amount of aroma precursors that can be extracted from their skins, while some others varieties ('Sauvignon blanc', 'Chardonnay' or 'Airen') had similar sensory profile to control wines or even less varietal characters, lower fruitiness and negative spicy attributes (TEST *et al.* 1986; SELLI *et al.* 2006; CEJUDO-BASTANTE *et al.* 2011; OLEJAR *et al.* 2015).

Among the 130 indigenous varieties of Croatia, 'Pošip' and 'Škrlet' are those of least concern because they are grown in more than one wine-growing subregion and they are commercially important

(MALETIĆ *et al.* 2015). Individual clonal selection for 'Škrlet' variety is completed, and for 'Pošip' is still underway. This study reveals the enological potential of these varieties by determining the physicochemical properties and aroma profiles of 'Škrlet' and 'Pošip' wines, and it evaluates the effect of pre-fermentative cold maceration as the alternative to standard wine-making procedure.

## MATERIAL AND METHODS

**Wines.** Healthy grapes of cv. 'Pošip' (200 kg) and 'Škrlet' (200 kg) were handpicked to 20 kg boxes at optimum maturity. 'Pošip' grape was harvested in September 2011, in wine subregion Central and southern Dalmatia and 'Škrlet' grape in August 2011, in subregion Moslavina and transported to experimental cellar at the Department of Viticulture and Enology, Faculty of Agriculture in Zagreb. Each grape variety was processed separately to obtain two batches of must, one by direct pressing of the destemmed and crushed grapes (1 ml/l of 5% H<sub>2</sub>SO<sub>3</sub> was added) and other by pressing the pomace (mixed with 1 ml kg of 5% H<sub>2</sub>SO<sub>3</sub>) resulting from grapes subjected to pre-fermentative cold maceration at 10°C for 6 hours. The CM and the following alcoholic fermentation were done in 100 l stainless steel vats. The must obtain was settled at 15°C for 24 h and then racked. Then it was run straight to 10 l glass containers and inoculated with a pure culture of *Saccharomyces bayanus* yeast strain Lalvin EC 1118 (Lallemand, Canada). The wines were made in triplicate, so a total 12 wines were submitted to study. Fermentation was conducted at 18°C in a cellar with controlled temperature. After the alcoholic fermentation was complete, eg. the residual sugars were less than 4 g/l, the wines were removed from the yeast deposits, sulfited by 1 ml/l of 5% H<sub>2</sub>SO<sub>3</sub> and the samples for analyses were taken.

**Reagents and standards.** Acetonitrile and methanol were purchased from J.T. Baker (Derventer, Netherlands). Vanillin, gallic acid, *p*-hydroxybenzoic acid, vanillic acid, syringic acid, caftaric acid, caffeic acid, coumaric acid, *p*-coumaric acid, ferulic acid, (+)-catechin, (–)-epicatechin and *trans*-resveratrol were purchased from Sigma-Aldrich (St. Louis, USA). Procyanidin B1, procyanidin B3, quercetin-3-*O*-glucoside were obtained from Extrasynthese (Genay, France). Analytical standards linalool (≥ 99%), citronellol (≥ 99%), geraniol (≥ 97%), nerol (≥ 97%) and *p*-cresol (≥ 99%) were purchased

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from Sigma Aldrich (USA). Ethyl butanoate (99%), ethyl-2-methylbutanoate (98%), ethyl hexanoate (99%), ethyl benzoate (99%), ethyl octanoate (99%), ethyl decanoate (99%), ethyl dodecanoate (98%), 1-propanol, butan-1-ol, 3-methylbutan-1-ol, 1-octanol, 2-phenylethanol, ethyl acetate, hexyl acetate, 3-methyl-1-butyl acetate, 2-phenyl-ethyl acetate, propyl acetate and isobutyl acetate were obtained from Fluka (Germany). Folin-Ciocalteu reagent, ethanol, tartaric acid and 85% ortho-phosphoric acid were from Kemika (Croatia).

**Standard analysis.** Standard physicochemical wine parameters were determined according to the classical enological methods (OIV 2007).

**Analysis of phenolic compounds.** The total phenolics (TP) were determined by the Folin-Ciocalteu colourimetric method (SINGLETON & ROSSI 1965). TP was analyzed spectrophotometrically using an Analytik Jena Specord 400 at 765 nm (Analytik Jena AG, Germany). Results are given as gallic acid equivalents (GAE mg/l). The content of flavan-3-ols was determined by the vanillin assay using the daily prepared working solution of 4% vanillin in methanol (DI STEFANO *et al.* 1989). The results were expressed as catechin equivalents (CAT mg/l). Identification and quantification of individual polyphenolic compounds in wine were determined by high-performance liquid chromatography (HPLC) system (Agilent 1100 Series; Agilent Technologies, USA). A ChromSpher 5 C<sub>18</sub> column (250 mm × 4.6 mm I.D. and 5 µm particle size) was used. A gradient of solvents A (water-phosphoric acid, 99.5 : 0.5, v/v) and B (acetonitrile-water-phosphoric acid, 50 : 49.5 : 0.5, v/v/v) was used. The flow was 1 ml min and the temperature was 30°C. Chromatograms were recorded at 275 nm. Direct injection of 20 µl of wine samples previously filtered through PTFE 0.45 µm membrane filter was done. Identification was performed by comparing retention times with those of pure standards. Quantification was performed using standard calibration curves.

**Analysis of volatile aroma compounds.** Aroma compounds from wines were extracted by solid-phase microextraction (SPME) (ARTHUR & PAWLISZYN 1990). Ten milliliters of wine, 10 µl of internal standard *p*-cresol (concentration 1 g/l in methanol) and 2 g of NaCl were mixed for 30 min at 40°C in a 20 ml sample vial tightly capped with a PTFE-silicon septum with inserted SPME fiber (50/30-µm DVB/Carboxen/PDMS; Supelco, USA). After the extraction, fiber was desorbed in the GC injector for 10 minutes. Wine volatiles were analyzed us-

ing an Agilent Gas Chromatograph 6890 (USA) series system coupled with an Agilent 5973 Inert mass-selective detector and an automatic injector (7683B Series Injector). The capillary column used was Phenomenex Zebron 5ms with dimensions 30 m × 250 µm × 0.25 µm. The interface temperature of the detector was set at 250°C and the ion source working in EI mode at 70 eV was held at 280°C. Helium 5.0 was vector gas used (Messer Croatia, Croatia), at 1.9 ml/min constant flow rate. After SPME extraction the fiber was transferred to an injector port and desorbed at 250°C for 5 min and analyzed according to next temperature program: 30°C, 5 min/60°C, 4.4°C/min/65°C, 3.5°C/min, 140°C, 10°C/min, 250°C, 20°C/min, 2 minutes.

Volatile compounds were identified by GC/MS using the Enhanced Chemstation software (Agilent Technologies, USA). Aroma compounds were identified by comparing the peak retention times against those of referent standards and matching the mass spectra against Nist05 mass library (Wiley & Sons, USA). Calibration curves were done preparing range of concentrations of standards in a model wine solution (12% ethanol, 5 g/l tartaric acid and pH 3.3) with internal standards and conducting the analysis by the same extraction and chromatographic conditions as described previously.

**Aromatic profile analysis.** Odour activity values (OAV) of volatile aroma compounds were calculated as the quotients of their concentration (*c*) and the corresponding odour perception threshold (*t*) reported in the literature (FALQUE *et al.* 2001). Each volatile compound has been associated with odour descriptors reported in literature, and based on this assigned to one or several aromatic series: fruity, floral, herbaceous, caramel, chemical, fatty, balsamic and solvent.

**Statistical analysis.** Mean values of concentrations and their standard deviations were calculated from three replicates. One-way analysis of variance (ANOVA) was performed using the SAS System for Windows 9.0, 2004 (SAS Institute Inc., USA). The differences in the content levels were estimated with the t-test. Statistically significant were considered *P* < 0.05.

## RESULTS AND DISCUSSION

The average values of standard physicochemical parameters in 'Škrlet' wines were: alcoholic strength

13.24 ± 0.07% vol., titratable acidity 7.16 ± 0.60 g/l (as tartaric acid), volatile acidity 0.42 ± 0.04 g/l (as acetic acid), pH 3.07 ± 0.12 and sugar-free extract 19.55 ± 0.55 g/l. We found a significant influence of CM treatment on the titratable acidity and pH ( $P < 0.001$ ). The results in 'Pošip' wines were: alcoholic strength 14.82 ± 0.17% vol., titratable acidity 3.81 ± 0.23 g/l (as tartaric acid), volatile acidity 0.50 ± 0.07 g/l (as acetic acid), pH 3.81 ± 0.10 and sugar-free extract 24.32 ± 1.16 g/l where was found statistically significant difference amongst medians ( $P < 0.001$ ).

The average total phenolics and flavan-3-ols contents (Table 1) were significantly greater under the CM influence, in both 'Pošip' and 'Škrlet' wines, but with no enhanced bitterness and astringency, based on sensory evaluation (data not shown). Results for TP were similar to the results for different macerated white wines from Croatia (JAGATIĆ KORENIKA *et al.* 2014). The results of individual phenolic profiles of wines showed several significant differences, especially for the hydroxybenzoic acids and catechin in 'Pošip' wines and hydroxycinnamic acids in 'Škrlet' wines. These results are in accordance with those reported in other white wines studies (HERNANZ *et al.* 2007; OLEJAR *et al.* 2015). Regarding taste perception thresholds of individual phenols, only tartaric acid in 'Škrlet' wines and quercetin-3-glucoside had potential taste impact described as puckering and velvety astringent.

The means of volatile aromatic compound concentrations in 'Pošip' and 'Škrlet' wines are presented in Table 2 and their odour activity values (OAVs) in Table 3. OAVs > 1 are considered as active odorants, although some studies have reported the relevance of compounds present at OAV > 0.2 to the overall aroma (GÓMEZ-MÍGUEZ *et al.* 2007a). The concentrations of all individual monoterpenols differed statistically only among the control and CM 'Škrlet' wines. Reported results could be explained by varietal differences between two cultivars and the effect of CM which is normally used to enhance the varietal character of white wines (PEINADO *et al.* 2004). Among the monoterpenols of greatest significance to wine aroma, i.e. linalool and geraniol, only concentrations of linalool with his flowery character, were exceeded its sensory threshold in both CM wines. Very similar observation was reported by RADEKA *et al.* (2008) in 'Malvazija istarska'. The significantly higher concentration of C6 alcohols was found in CM wines, but below its perception threshold. The most abundant compounds in this

experiment were higher alcohols that contribute more to the intensity of the odor than to its quality (ETIÉVANT 1991). CM exhibited significantly higher concentration of butan-1-ol and 3-methylbutan-1-ol in 'Škrlet wine', but lower concentrations of 1-propanol and isobutanol in 'Pošip wine'. Among all higher alcohols, 1-propanol, 3-methylbutan-1-ol and 2-phenylethanol showed the concentration above their perception thresholds. The far most interesting alcohol analysed, with highest odour activity value (OAV) was 2-phenyl ethanol in 'Škrlet' which has floral, rose-like aroma that could have the highly potential impact on wine aroma (Table 3). Beside alcohols, esters are also the characteristic product of fermentation and well-known contributors to the aroma of flowers and ripe fruits (WATERHOUSE *et al.* 2016). Among thirteen ethyl and acetate esters, seven concentrations significantly differed under the CM influence, both in 'Pošip' and 'Škrlet' wines (Table 2). Esters detected above their perception thresholds in all wines were ethyl butanoate, ethyl-2-methyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl acetate, 3-methyl-1-buthyll acetate and only in 'Pošip' wines ethyl decanoate and 2-phenylethyl acetate. Compounds mentioned enhance banana, apple and pineapple flavours (PEINADO *et al.* 2004; TOMAŠEVIĆ *et al.* 2017). These results indicate that application of CM could contribute to the fresh and fruity character of the aromatic profile (PEINADO *et al.* 2004; ÁLVAREZ *et al.* 2006) and more distinctiveness of Croatian native wines in research.

In order to establish more objective aroma profiles and the effect of CM on wines, OAVs of the aroma compounds with similar principal odour descriptors were grouped into aroma series. This method is used to relate quantitative information derived by chemical analysis to sensory perceptions (PEINADO *et al.* 2004). Table 3 shows the perception thresholds for each compound as previously reported (ETIÉVANT 1991; GUTH 1997a; LOPEZ *et al.* 1999; FERREIRA *et al.* 2000; MOYANO *et al.* 2002; PEINADO *et al.* 2004; SMYTH *et al.* 2005; RIBERAU-GAYON *et al.* 2006; GÓMEZ-MÍGUEZ *et al.* 2007; TAO *et al.* 2010; WU *et al.* 2016) and OAVs combined in their own aroma series. Results in Figure 1 showed the highest contribution of the fruity series to the overall aroma in all wines, followed by the same sequence in CM wines. It can be seen that the fruity and floral series were higher in macerated wines. The global OAV for each series was obtained by subjecting the data to a factorial analysis of variance (Table 4). The effect of CM, regarding the



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Table 1. Mean values of phenolic components (mg/l) in 'Pošip' (Pos) and 'Škrlet' (Skr) white wines

Compounds	TPT	Taste descriptor	Variety	Control	Cold maceration	Significance
Hydroxybenzoic acids						
Gallic	50	puckering astringent	Pos	0.45 ± 0.11	1.26 ± 0.11	*
			Skr	2.57 ± 1.20	2.21 ± 0.24	n.s.
<i>p</i> -Hydroxybenzoic	n.a.	n.a.	Pos	0.87 ± 0.11	0.55 ± 0.03	*
			Skr	3.24 ± 0.38	2.88 ± 0.57	n.s.
Vanillic	53	puckering astringent	Pos	0.70 ± 0.03	0.50 ± 0.27	n.s.
			Skr	0.46 ± 0.19	2.33 ± 0.11	*
Syringic	52	puckering astringent	Pos	0.56 ± 0.09	0.85 ± 0.07	*
			Skr	0.09 ± 0.02	0.20 ± 0.10	n.s.
Hydroxycinnamic acids						
Caftaric	5	puckering astringent	Pos	2.97 ± 0.33	3.46 ± 0.26	n.s.
			Skr	8.99 ± 2.02	16.96 ± 0.85	*
Caffeic	13	puckering astringent	Pos	2.04 ± 0.88	1.10 ± 0.09	n.s.
			Skr	0.35 ± 0.02	0.78 ± 0.13	*
Coutaric	10	astringent	Pos	0.20 ± 0.07	0.36 ± 0.22	n.s.
			Skr	1.22 ± 0.26	3.04 ± 0.72	*
<i>p</i> -Coumaric	23	puckering astringent	Pos	0.30 ± 0.04	1.12 ± 0.61	n.s.
			Skr	0.26 ± 0.03	0.55 ± 0.09	*
Fertaric	10	puckering astringent	Pos	0.49 ± 0.07	0.59 ± 0.25	n.s.
			Skr	0.35 ± 0.12	0.24 ± 0.03	n.s.
Ferulic	13	puckering astringent	Pos	0.88 ± 0.22	1.01 ± 0.46	n.s.
			Skr	0.34 ± 0.06	0.34 ± 0.12	n.s.
Flavan-3-ols						
Catechin	119/290	puckering astrin- gent, rough/bitter	Pos	1.33 ± 0.14	2.03 ± 0.10	*
			Skr	1.80 ± 0.44	2.36 ± 0.11	n.s.
Epicatechin	270/270	puckering astrin- gent, rough/bitter	Pos	0.30 ± 0.18	0.23 ± 0.02	n.s.
			Skr	0.35 ± 0.03	0.39 ± 0.05	n.s.
Procyanidin B1	139/231	bitter, astringent	Pos	1.19 ± 0.25	1.07 ± 0.03	n.s.
			Skr	0.34 ± 0.08	0.50 ± 0.08	n.s.
Procyanidin B3	116/289	bitter, astringent	Pos	4.48 ± 1.08	5.15 ± 0.84	n.s.
			Skr	2.56 ± 0.08	2.88 ± 0.41	n.s.
Flavonols						
Quercetin-3-glucoside	0.1	velvety astringent, drying	Pos	0.28 ± 0.03	0.30 ± 0.06	n.s.
			Skr	0.25 ± 0.02	0.28 ± 0.04	n.s.
Stilbenes						
<i>trans</i> - Resveratrol	n.a.	n.a.	Pos	0.19 ± 0.03	0.20 ± 0.08	n.s.
			Skr	0.40 ± 0.14	0.44 ± 0.10	n.s.
Total phenolics <sup>a</sup>			Pos	265.21 ± 7.41	340.36 ± 26.39	*
			Skr	327.57 ± 6.93	480.67 ± 6.46	*
Total flavan-3-ols <sup>b</sup>			Pos	5.28 ± 0.71	7.73 ± 0.37	*
			Skr	9.42 ± 0.59	14.51 ± 0.59	*

n.s. – not significant; \* $P \leq 0.05$ ; n.a. – not available; <sup>a</sup>total phenolics expressed as mg/l gallic acid equivalents; <sup>b</sup>total flavan-3-ols expressed as mg/l catechin equivalents. Values are presented as averaged concentrations over 3 replicates. Taste perception thresholds (TPT; mg/l) and taste descriptors reported in the literature (OKAMURA *et al.* 1981; SCHARBERT *et al.* 2005; HUFNAGEL & HOFMANN 2008). For flavan-3-ols both thresholds for astringency/bitterness are presented

Table 2. Mean values of aroma compounds (mg/l) in 'Pošip' (Pos) and 'Škrlet' (Skr) white wines

Compounds	Variety	Control	Cold maceration	Significance	Threshold (mg/l)	Aroma description
<b>Terpenes (µg/l)</b>						
Linalool	Pos	25.54 ± 5.89	25.91 ± 6.46	n.s.	15 µg/l <sup>h</sup>	flowery, muscat <sup>a</sup>
	Skr	22.94 ± 2.74	34.73 ± 3.49	*		
Citronellol	Pos	0.75 ± 0.10	0.72 ± 0.08	n.s.	100 µg/l <sup>i</sup>	rose <sup>b</sup>
	Skr	1.59 ± 0.15	3.38 ± 0.44	*		
Geraniol	Pos	12.43 ± 2.39	17.19 ± 4.62	n.s.	30 µg/l <sup>i</sup>	citric, geranium <sup>a,c</sup>
	Skr	10.31 ± 1.10	19.22 ± 1.99	*		
Nerol	Pos	2.25 ± 0.96	2.44 ± 0.60	n.s.	300 µg/l <sup>m</sup>	violet, floral <sup>d</sup>
	Skr	4.41 ± 0.76	8.47 ± 1.12	*		
Total terpenes	Pos	40.97 ± 9.34	46.26 ± 11.76	n.		
	Skr	39.25 ± 4.75	65.8 ± 7.04	n.		
<b>C<sub>6</sub> alcohols</b>						
1-Hexanol	Pos	0.08 ± 0.00	0.15 ± 0.02	*	8.0 <sup>i</sup>	herbaceous, grass, woody <sup>b,e</sup>
	Skr	0.45 ± 0.03	0.64 ± 0.03	*		
<b>Higher alcohols</b>						
1-Propanol	Pos	22.64 ± 4.25	18.73 ± 6.92	n.s.	9.0 <sup>j</sup>	alcohol, ripe fruit <sup>e</sup>
	Skr	26.19 ± 6.56	11.88 ± 1.53	*		
Butan-1-ol	Pos	1.98 ± 0.39	3.59 ± 0.38	*	150 <sup>k</sup>	medicinal, phenolic <sup>b,e,f</sup>
	Skr	0.62 ± 0.14	0.92 ± 0.03	*		
Isobutanol	Pos	24.67 ± 3.01	16.98 ± 2.49	*	40 <sup>i</sup>	alcohol, solvent, green, bitter <sup>e</sup>
	Skr	8.58 ± 4.78	9.48 ± 2.42	n.s.		
3-methylbutan-1-ol	Pos	96.17 ± 3.48	87.00 ± 5.71	n.s.	60 <sup>j</sup>	solvent, sweet, alcohol, nail polish <sup>a,e</sup>
	Skr	87.05 ± 8.60	116.83 ± 2.87	*		
1-Octanol	Pos	0.01 ± 0.00	0.03 ± 0.01	n.s.	0.90 <sup>l</sup>	jasmine, lemon <sup>b</sup>
	Skr	0.01 ± 0.00	0.01 ± 0.00	n.s.		
2-Phenylethanol	Pos	15.84 ± 1.71	18.84 ± 2.39	n.s.	10 <sup>i</sup>	honey, rose <sup>a</sup>
	Skr	41.63 ± 3.15	40.77 ± 4.13	n.s.		
Total higher alcohols	Pos	161.31 ± 47.2	145.17 ± 17.9	n.		
	Skr	164.08 ± 23.23	179.89 ± 10.98	n.		
<b>Ethyl esters</b>						
Ethyl butanoate	Pos	0.30 ± 0.04	0.12 ± 0.10	*	0.02 <sup>i</sup>	banana, pineapple, strawberry <sup>a</sup>
	Skr	0.24 ± 0.02	0.31 ± 0.01	*		
Ethyl-2-methylbutanoate	Pos	0.01 ± 0.00	0.03 ± 0.01	*	0.001 <sup>i</sup>	apple <sup>g</sup>
	Skr	0.01 ± 0.00	0.45 ± 0.35	*		
Ethyl hexanoate	Pos	0.76 ± 0.05	1.03 ± 0.29	n.s.	0.08 <sup>h</sup>	banana, green apple <sup>a</sup>
	Skr	1.06 ± 0.27	1.35 ± 0.17	n.s.		
Ethyl benzoate	Pos	0.02 ± 0.01	0.09 ± 0.11	n.s.	0.575 <sup>n</sup>	heavy, floral, fruity <sup>g</sup>
	Skr	0.02 ± 0.01	0.02 ± 0.01	n.s.		
Ethyl octanoate	Pos	0.99 ± 0.03	1.19 ± 0.49	n.s.	0.014 <sup>k</sup>	fruity, sweet, floral, banana, pear <sup>c</sup>
	Skr	0.54 ± 0.18	0.68 ± 0.13	n.s.		
Ethyl decanoate	Pos	0.30 ± 0.01	0.35 ± 0.17	n.s.	0.20 <sup>n</sup>	fruity, fatty, pleasant <sup>a</sup>
	Skr	0.06 ± 0.45	0.07 ± 0.05	n.s.		

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Table 2. To be continued

Compounds	Variety	Control	Cold maceration	Significance	Threshold (mg/l)	Aroma description
Ethyl dodecanoate	Pos	0.14 ± 0.03	0.16 ± 0.05	n.s.	0.50 <sup>o</sup>	oily, fatty, fruity <sup>d</sup>
	Skr	0.10 ± 0.01	0.21 ± 0.04	*		
Total ethyl esters	Pos	2.52 ± 0.17	2.97 ± 1.22	n.		
	Skr	2.03 ± 0.94	3.09 ± 0.76	n.		
<b>Acetate esters</b>						
Ethyl acetate	Pos	32.02 ± 3.31	62.45 ± 6.84	*	7.5 <sup>i</sup>	pineapple, fruity, solvent, balsami <sup>c,f</sup>
	Skr	20.14 ± 3.61	36.76 ± 4.37	*		
Hexyl acetate	Pos	0.07 ± 0.01	0.07 ± 0.02	n.s.	1.5 <sup>h</sup>	apple, cherry, pear, floral <sup>e</sup>
	Skr	0.05 ± 0.02	0.07 ± 0.01	n.s.		
Isoamyl acetate	Pos	3.20 ± 0.09	4.14 ± 0.81	n.s.	0.16 <sup>k</sup>	banana <sup>a</sup>
	Skr	0.64 ± 0.21	1.03 ± 0.12	*		
2-Phenylethyl acetate	Pos	0.26 ± 0.01	0.27 ± 0.05	n.s.	0.25 <sup>p</sup>	fruity, rose <sup>b,e</sup>
	Skr	0.07 ± 0.04	0.08 ± 0.01	n.s.		
Propyl acetate	Pos	0.04 ± 0.00	0.09 ± 0.02	*	4.7 <sup>h</sup>	celery <sup>e</sup>
	Skr	0.09 ± 0.02	0.11 ± 0.02	n.s.		
Isobutyl acetate	Pos	0.01 ± 0.00	0.04 ± 0.01	n.s.	1.6 <sup>j</sup>	sweet, fruity, apple, banana <sup>g</sup>
	Skr	0.02 ± 0.01	0.07 ± 0.01	*		
Total acetate esters	Pos	35.6 ± 3.43	67.06 ± 7.75	n.		
	Skr	21.01 ± 3.91	38.12 ± 4.54	n.		

n.s. – not significant; \* $P \leq 0.05$ ; n. – missing data. Values are presented as averaged concentrations over 3 replicates. Aroma descriptions based on <sup>a</sup>NOGUEROL-PATO *et al.* 2012b; <sup>b</sup>PEINADO *et al.* 2006; <sup>c</sup>GÓMEZ-MÍGUEZ *et al.* 2007; <sup>d</sup>SMYTH 2005; <sup>e</sup>PEINADO *et al.* 2004; <sup>f</sup>FRANCO *et al.* 2004; <sup>g</sup>flavournet online databases; threshold references based on <sup>h</sup>ETIEVANT 1991; <sup>i</sup>GUTH 1997b; <sup>j</sup>PEINADO *et al.* 2004; <sup>k</sup>GÓMEZ-MÍGUEZ *et al.* (2007); <sup>l</sup>TAO *et al.* (2010); <sup>m</sup>RIBEREAU-GAYON *et al.* (2006); <sup>n</sup>FERREIRA *et al.* (2000); <sup>o</sup>MOYANO *et al.* (2002); <sup>p</sup>LOPEZ *et al.* (1999)

Table 3. Odour activity values (OAVs) for the aroma compounds in ‘Pošip’ (Pos) and ‘Škrlet’ (Skr) white wines

Compounds	Variety	Control	Cold maceration	Aroma series
Terpenes				
Linalool	Pos	1.63 ± 0.39*	1.73 ± 0.43*	2, 4 <sup>a</sup>
	Skr	1.53 ± 0.18*	2.31 ± 0.23*	
Citronellol	Pos	0.01 ± 0.00	0.01 ± 0.00	2 <sup>b</sup>
	Skr	0.02 ± 0.00	0.03 ± 0.00	
Geraniol	Pos	0.41 ± 0.08	0.57 ± 0.16	2 <sup>c,d</sup>
	Skr	0.34 ± 0.04	0.64 ± 0.07	
Nerol	Pos	0.01 ± 0.01	0.01 ± 0.00	2 <sup>e</sup>
	Skr	0.01 ± 0.00	0.03 ± 0.01	
C <sub>6</sub> alcohols				
1-Hexanol	Pos	0.01 ± 0.00	0.02 ± 0.00	3 <sup>a,b</sup>
	Skr	0.06 ± 0.00	0.08 ± 0.00	
Higher alcohols				
1-Propanol	Pos	2.51 ± 0.47*	2.08 ± 0.77*	1, 5 <sup>a</sup>
	Skr	2.91 ± 0.73*	1.32 ± 0.17*	

Table 3. To be continued

Compounds	Variety	Control	Cold maceration	Aroma series
Butan-1-ol	Pos	0.01 ± 0.00	0.02 ± 0.00	7 <sup>a,b,f</sup>
	Skr	0.00 ± 0.00	0.01 ± 0.01	
Isobutanol	Pos	0.62 ± 0.07	0.42 ± 0.06	3, 5 <sup>a</sup>
	Skr	0.21 ± 0.12	0.23 ± 0.06	
3-methylbutan-1-ol	Pos	1.60 ± 0.06*	1.45 ± 0.10*	5, 4, 6 <sup>a,g</sup>
	Skr	1.45 ± 0.05*	1.94 ± 0.05*	
1-Octanol	Pos	0.01 ± 0.01	0.03 ± 0.02	2 <sup>b</sup>
	Skr	0.01 ± 0.01	0.01 ± 0.01	
2-Phenylethanol	Pos	1.58 ± 0.17*	1.88 ± 0.24*	2 <sup>g</sup>
	Skr	4.16 ± 0.32*	4.07 ± 0.41*	
<b>Ethyl esters</b>				
Ethyl butanoate	Pos	14.83 ± 2.08*	5.83 ± 5.06**	1 <sup>g</sup>
	Skr	12.17 ± 1.26*	15.67 ± 0.76*	
Ethyl-2-methylbutanoate	Pos	11.67 ± 1.53*	33.33 ± 15.28*	1 <sup>c</sup>
	Skr	13.33 ± 5.77*	446.67 ± 355.72*	
Ethyl hexanoate	Pos	9.50 ± 0.63*	12.87 ± 3.58*	1 <sup>g</sup>
	Skr	9.12 ± 5.02*	16.87 ± 2.10*	
Ethyl benzoate	Pos	0.04 ± 0.01	0.15 ± 0.19	1
	Skr	0.03 ± 0.02	0.04 ± 0.03	
Ethyl octanoate	Pos	70.95 ± 2.30*	85.24 ± 34.78*	1, 2 <sup>b</sup>
	Skr	38.81 ± 12.88*	48.57 ± 9.37*	
Ethyl decanoate	Pos	1.50 ± 0.05*	1.75 ± 0.87*	1 <sup>c</sup>
	Skr	0.30 ± 0.23	0.33 ± 0.23	
Ethyl dodecanoate	Pos	0.29 ± 0.06	0.32 ± 0.10	1, 6 <sup>e</sup>
	Skr	0.20 ± 0.02	0.42 ± 0.07	
<b>Acetate esters</b>				
Ethyl acetate	Pos	4.27 ± 0.44*	8.33 ± 0.91*	1, 7, 8 <sup>d,h</sup>
	Skr	2.68 ± 0.48*	4.90 ± 0.58*	
Hexyl acetate	Pos	0.05 ± 0.01	0.05 ± 0.02	1, 2 <sup>a</sup>
	Skr	0.03 ± 0.01	0.05 ± 0.01	
Isoamyl acetate	Pos	20.00 ± 0.57*	25.89 ± 0.57*	1 <sup>a</sup>
	Skr	4.00 ± 1.32*	6.45 ± 1.32*	
2-Phenylethyl acetate	Pos	1.03 ± 0.05*	1.11 ± 0.22*	1, 2, 4 <sup>a,b</sup>
	Skr	0.29 ± 0.18	0.33 ± 0.06	
Propyl acetate	Pos	0.01 ± 0.00	0.02 ± 0.01	3 <sup>c</sup>
	Skr	0.02 ± 0.00	0.02 ± 0.00	
Isobutyl acetate	Pos	0.01 ± 0.00	0.02 ± 0.01	1 <sup>c</sup>
	Skr	0.07 ± 0.05	0.04 ± 0.01	

OAV – odor activity values (calculated by dividing the concentration by the odor threshold value of the compound); \*OAV > 1; Aroma series: 1 – fruity; 2 – floral; 3 – herbaceous; 4 – caramel; 5 – chemical; 6 – fatty; 7 – balsamic; 8 – solvent, based on <sup>a</sup>PEINADO *et al.* 2004; <sup>b</sup>PEINADO *et al.* 2006; <sup>c</sup>NOGUEROL-PATO *et al.* 2012; <sup>d</sup>GÓMEZ-MÍGUEZ *et al.* 2007; <sup>e</sup>SMYTH 2005; <sup>f</sup>FRANCO *et al.* 2004; <sup>g</sup>NOGUEROL-PATO *et al.* 2012; <sup>h</sup>WU *et al.* 2016



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Table 4. ANOVA of the aroma series in the control wines and in the wines under pre-fermentative cold maceration treatment of ‘Pošip’ and ‘Škrlet’ varieties

Series	Maceration effect		Variety effect	
	Pos C – Pos CM	Skr C – Skr M	Pos C – Skr C	Pos CM – Skr CM
Fruity	n.s.	*	*	n.s.
Floral	n.s.	n.s.	*	n.s.
Herbaceous	*	*	*	n.s.
Caramel	n.s.	*	*	n.s.
Chemical	n.s.	n.s.	n.s.	n.s.
Fatty	n.s.	*	n.s.	*
Balsamic	*	*	*	*
Solvent	*	*	*	*

n.s. – not significant; \*  $P \leq 0.05$ ; Pos C – ‘Pošip’ control; Pos CM – ‘Pošip’ cold maceration; Skr C – ‘Škrlet’ control; Skr CM – ‘Škrlet’ cold maceration

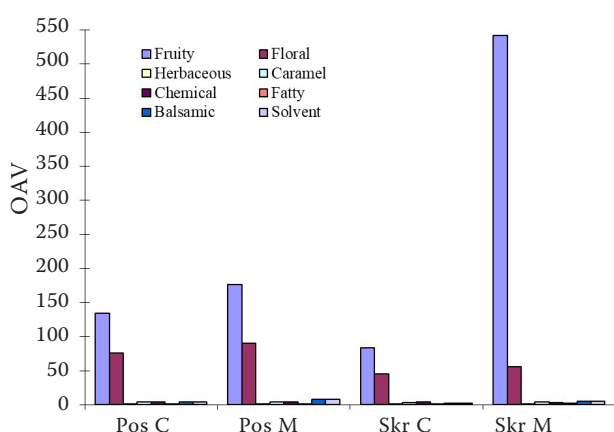


Figure 1. Odour activity values of odorant series of control and CM ‘Pošip’ and ‘Škrlet’ wines

aroma series, was more distinctly in ‘Škrlet’ wine. It had multiple significant effects on the OAV for the fatty, balsamic, solvent, caramel, herbaceous and fruity series. Significant effect of CM in ‘Pošip’ wine was noticed by increasing the solvent and balsamic series and a decrease of the herbaceous aroma. The ‘Pošip’ and ‘Škrlet’ control wines did not show significant only in the chemical and fatty series, while the macerated wines exhibited significant differences only in fatty, balsamic and solvent series.

## CONCLUSIONS

Besides the determination of phenolic and aroma compounds, this study demonstrates that short cold

pre-fermentative maceration applied to the ‘Pošip’ and ‘Škrlet’ grape (*Vitis vinifera* L.) affects the polyphenolic and aroma profiles of both wines. It had the significant impact on the total phenolics and flavan-3-ol composition of ‘Škrlet’, and some individual phenolic compounds in ‘Pošip’ wine. The primary aroma compounds, i.e. terpenes were under the significant influence of cold maceration process in ‘Škrlet’ wine. Cold maceration significantly increased five aroma series in the ‘Škrlet’ wine and only three in ‘Pošip’ wine. Regarding the positive changes in the aromatic profile of ‘Škrlet’ wine, at this stage, our results suggest that the cold maceration process can be used in practice for improving the varietal characteristics.

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