

INFLUENCE OF COLD MACERATION TREATMENT ON AROMATIC AND SENSORY PROPERTIES OF VUGAVA WINE (*Vitis vinifera* L.)

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ABSTRACT

In Croatian viticultural sub-region of Middle and South Dalmatia, several high-quality white wines, mainly using the native grape varieties, are being produced and one of them is Vugava from the island of Vis. The aim of this two-year study was to investigate the effect of cold maceration conditions on aromatic and sensory properties of Vugava wines in comparison to the control wine produced without maceration. The GC/MS method was used for the determination of higher alcohols, methanol, ethyl acetate, and acetaldehyde concentrations. Aromatic profile was obtained by odor activity values and sensory evaluation. The significant difference in concentrations of total higher alcohols and isoamyl alcohol was determined in both vintages unlike the differences in basic wine composition. There was no visible difference or significant increase of methanol and ethyl acetate concentration what is positive from the health and sensory point of view. Sensory evaluation of Vugava wines showed that treatment of cold maceration (20 h/10 °C) resulted in increased complexity and better rated wine.

Keywords: aroma, cold maceration, higher alcohols, methanol, Vugava, white wine

INTRODUCTION

The pleasant and detectable aroma of wine is one of the main characteristic that can enable the differences and recognition among the vast array of wine styles produced throughout the world, especially when native, less known grapevine varieties are processed. The complex pool of volatile compounds that arise from grapes and metabolic activities of yeast and bacteria display a specific odor often defined with the terms “vinous” or “fermented” (de-la-Fuente-Blanco *et al.*, 2016). Beside the fact that the major volatile fractions of wine like alcohol, higher alcohols and their acetates, ethyl and acetate esters or acetaldehyde can be affected by the numerous conditions through the alcoholic fermentation, there are a lot of procedures, both in viticulture and in winemaking that can be used for producing wines with more highlighted aromatic profile. Pre-fermentative practices can determine the aromatic characteristics, especially in white wines production (Ribéreau Gayon *et al.*, 2006). Pre-fermentative cold maceration (CM) is normally used in winemaking to enhance varietal character of white wines (Peinado *et al.*, 2004) with considerable variations depending on the grape cultivar employed and experimental conditions (Darias-Martín *et al.*, 2000; Selli *et al.*, 2006). According to the different studies of maceration process affecting the aromatic profiles of white wines, contradictory results were presented. Studies performed with some aromatic varieties, showed benefits from skin contact due to a high amount of aroma precursors that can be extracted from their skins, while some others varieties 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). Short cold pre-fermentation maceration applied to Croatian native varieties Pošip and Škrlet had the significant influence on increasing of primary aroma compounds, i.e. terpenes and results obtained by Jagatić Korenika *et al.* (2018) suggested that practice that was used enhanced varietal typicality. In wines made from neutral grape varieties, the detectable aroma arises from combination of different volatile organic compounds and it determines wine character and quality (Sanchez Palomo *et al.*, 2006). Higher (fusel) alcohols are the major constituents of the wine volatiles and according to de-la-Fuente-Blanco *et al.* (2016) the scientific literature is not unanimous about the role played by higher alcohols in wine. Some researchers noted negatively effect of higher alcohols in younger and less expensive wines but not in premium wines, at similar levels (San-Juan *et al.*, 2011). Research conducted by de-la-Fuente-

Blanco *et al.* (2016) suggested that the effect of higher alcohols extremely depends on the aromatic context i.e. when lacking specific aroma, they have buffering effect of wine aroma base. The same research confirms the sensory importance of isobutanol and isoamyl alcohol on wine aroma perception and a negative role of aliphatic higher alcohols on wine aroma quality regarding their ability to suppress fruity and woody notes. Some other authors noted high sensory impact of isoamyl alcohol and β - phenyl ethanol (Capone *et al.*, 2013; Gomez-Miguez *et al.*, 2007).

Among many different aromatic compounds, esters have a significant effect on the fruity flavors in wine. Higher ester content is found in white wines compared to red wines, especially when a lower temperature is used during vinification (Clarke and Bakker, 2004). One of the most abundant and significant acetate esters is ethyl acetate that is only formed in small quantities by the yeast metabolism in the fermentation process, and any large concentrations are due to the presence of acetic acid bacteria during storage and barrel aging (Moreno and Peinado, 2012). Ethyl acetate can seriously impair the smell of wine, even below its perception threshold and long before the concentration of acetic acid in the wine causes it to become acetic (Moreno and Peinado, 2012). Acetaldehyde is the major carbonyl compound and metabolic intermediates found in wine (Schreier, 1979). Aldehydes contribute to flavor with aroma descriptors such as ‘bruised apple’ and ‘nutty’ but can also be a sign of wine oxidation (Swiegers *et al.*, 2005). Numerous studies have shown that the administration of large concentrations of acetaldehyde can lead to a range of behavioral effects, notably those linked with symptoms of hangover and carcinogenic effect- the importance of screening acetaldehyde levels in alcoholic beverages has now been given special attention as a result of health concerns (Salaspuro, 2011). Methanol is formed by enzymatic hydrolysis of pectin present mostly in skins and other grape solids. More methanol is produced when must is fermented on grape skins (Radeka *et al.*, 2012) hence there are generally higher levels in red than in rosé or white wines. Methanol is a toxic chemical but even wine containing the maximum allowable methanol content by regulation never has a high enough concentration to give rise to public health concerns. It seems possible that at one time they were intended to serve as an index of appropriate fruit handling in harvest and subsequent processing (FIVS, 2016).

Vugava is a near threatened, native white variety from the Coastal region of Croatia with a high reputation during history. Once it was used as a table grape, and its dessert wines made from grapes dried in the sun were especially

appreciated. As a disadvantage with this variety, premature ripening may be counted in some years, leading to problems with harvesting and fermentation due to high temperatures. Vugava has extremely high qualitative potential, it regularly accumulates high sugar, and because of its intense distinctive and pleasant varietal aroma, it produces famous white wines with a pronounced fruity aroma (Maletić et al., 2015). In order to demonstrate its high potential, it is important to apply modern technological solutions in wine-making, above all in order to preserve the aroma and freshness of wine. Due to the deficiency of information about the effect of technology on the aroma profile of Vugava wine, the aim of this two-year study was to apply different lengths of cold maceration to pomace and analyze the volatile compounds and sensory properties of produced wines in comparison to standard wine.

MATERIALS AND METHOD

Grapes and winemaking

The experiment was performed during vintages 2006 and 2007 with grapes of Vugava variety (*Vitis vinifera* L.) grown in viticultural subregion of Middle and South Dalmatia, wine growing hill Island of Vis, with a typical Mediterranean climate. Specific vineyard was situated in wine growing locality Radovinka on the southwestern part of the island, owned by cooperative "Podšpilje". Healthy grapes were manually harvested and transported to experimental wine cellar of Institute for Adriatic Crops and Karst Reclamation in Split, in 20 kg-plastic cases. The degree of ripeness was monitored by standard chemical analysis (sugars, total acidity and pH). All the vinifications were done with randomly chosen 3 cases of grapes. Three treatments were studied: (i) A- control vinification without cold maceration, (ii) B- cold maceration 10 hours on 10 °C, alcoholic fermentation on 18 °C, (iii) C- cold maceration 20 hours on 10 °C, alcoholic fermentation on 18 °C. After cooling of pomace in refrigeration tanks, free-run juices of the individual treatments were sulphited with 5 g.hL⁻¹ 5%-H₂SO₃ and sedimented for 24 hours. Alcoholic fermentation of the precipitated musts of all treatments was carried out in triplicates with selected wine yeasts *Saccharomyces cerevisiae* Lalvin ICV D47 (Lallemand SA, Montreal, Canada). During the fermentation process, the breakdown of sugar was monitored daily using a refractometer together with measuring the must temperature. After the completion of alcoholic fermentation, wines were racked-off and sulphited with 15 g.hL⁻¹. Wines were stored for 3 months at temperatures not exceeding 20 °C, filtered and samples were taken for analyses and sensory evaluation.

Analyses of volatile aroma compounds

Aroma compounds from wines were extracted by solid phase micro extraction (SPME) (Arthur and Pawliszyn, 1990) and analyzed using an Agilent Gas Chromatograph 6890 (USA) series system coupled with an Agilent 5973 Inert mass-selective detector and an automatic injector (7683B Series Injector). Volatile compounds were identified by 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). All the analyzes were performed in the Laboratory for grape, must and wine at University of Zagreb Faculty of Agriculture, Department of Viticulture and Enology.

Odor activity values

Odor activity values (OAV) of volatile aroma compounds were calculated as the quotients of their concentration (c) and the corresponding odor perception threshold (OPT) reported in the literature (Sáenz-Navajas et al., 2015). It estimates the contribution of individual compound to the aroma of wine and yields aroma profile together with all quantified components (Peinado et al., 2004). Each volatile compound has been associated with odor descriptors reported in literature.

Sensory evaluation of wine

The sensory properties of the wines were evaluated using the 100 points method and the ranking method with the participation of 10 certificated wine evaluators. The evaluators had to rank the wines according to aroma and overall impression of quality by method proposed by Amerine and Roessler (1976).

Statistical analysis

One-way analysis of variance (ANOVA) was performed on the data collected using the Statistica, ver. 7.1. (StatSoft, Inc., Tulsa, USA). The differences between the mean values (n=3) of the dependent variables were determined by the Least Significant Difference (LSD) at the level of significance of p<0.05 and p<0.01. A multivariate analysis was performed by Principal Component Analysis (PCA) to identify the differences between wines and to identify differences in the maceration process.

RESULTS AND DISCUSSION

Alcoholic fermentation dynamics

Average data of must from vintage 2006 were 94 °Oe (206 g.L⁻¹), 6.9 g.L⁻¹ titratable acidity (as tartaric acid) and pH 3.47. In vintage 2007 grapes were more ripe with 106 °Oe (236 g.L⁻¹), 5.3 g.L⁻¹ titratable acidity and pH 3.39. The breakdown of sugars and the dynamics of fermentations are shown in Fig. 1a/b. Regarding the higher concentration of sugars in Vugava must 2007, the alcoholic fermentation lasted longer in all studied treatments in comparison to vintage 2006. Application of cold maceration affected fermentation in the same way in both vintages by decreasing the fermentation rate in the following order A > B > C. Application of CM at low temperature (10 °C) and with SO₂ addition influenced the start and duration of fermentation. The difference in completion of fermentation between A and C treatment was four days in 2006, and six days in 2007. There was no difference in CM effect on completion of fermentation to dryness in both years.

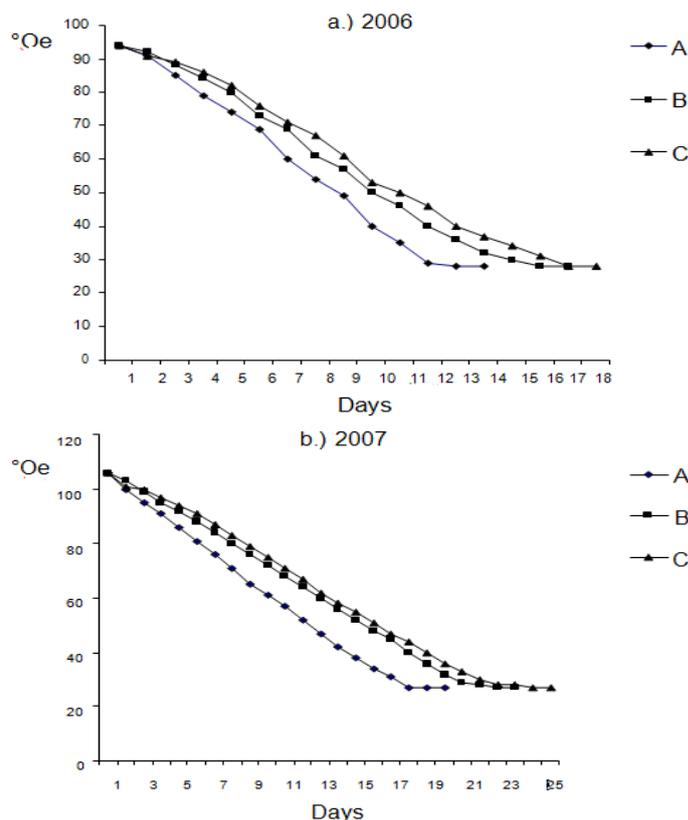


Figure 1a/b Effect of cold maceration on fermentation performance in Vugava wines, 2006 and 2007.

Wine composition

According to obtained results there was no significant difference for any basic quality parameter (Fig. 3) that is in accordance with research published by Peinado et al. (2004) and different from previous studies (Cabarroglu et al., 1997; Darias-Martin et al., 2000). Methanol deriving from the demethylation of pectin was influenced by CM only in Vugava 2006 where the concentration significantly decreased (Table 1) what is in opposite from results published by Darias-Martin et al. (2000) and similar to study by Bavčar et al., (2011). Methanol levels ranged from the highest found in control wine 2006, to the lowest in control wine 2007, both within range typical for white wines (40- 120 mg.L⁻¹) according to Sponholz (1989) and under the OIV limitation for methanol content in white wine (250 mg.L⁻¹). CM wines in both years did not exhibit an increased level of acetaldehyde. The range of values measured in all wines were under the detectable value of 80 g.L⁻¹ for white wines (McCloskey and Mahaney, 1981). At low level in wine acetaldehyde gives a pleasant, fruity aroma but at higher level, it nevertheless imparts an irritating odor that has been described as a green, grassy, nutty or rotten apple-like aroma (Waterhouse et al. 2016).

Table 1 Concentration, odor perception thresholds (OPT), odor descriptors and odor activity values (OAV) for methanol, acetaldehyde, ethyl-acetate and higher alcohols (mg.L⁻¹) in Vugava wines 2006 and 2007.

Compound	OPT ′	Odor descriptors [″]	Vintage	A	B	C
Methanol	668	cabbage	2006	103.6 ^a	76.4 ^c	82.6 ^b
			2007	58.5	59.7	64.1
Acetaldehyde	80	pungent, overripe apple	2006	50.2	51.5	53.4
			2007	43.8	43.1	42.8
Ethyl acetate	7.5	fruity, pineapple, nail polish remover	2006	20.5 (2.73)*	26.0 (3.47)*	27.7 (4.23)*
			2007	54.9 ^a (7.32)*	51.6 ^a (6.88)*	44.0 ^b (5.87)*
Higher alcohols (HA)						
1-Propanol	9	alcohol, ripe fruit, sweet	2006	37.74 ^b (4.19)*	47.31 ^a (5.26)*	48.22 ^a (5.36)*
			2007	41.52 (4.62)*	40.05 (4.45)*	39.00 (4.33)*
Isobutanol	40	alcohol, solvent green, bitter	2006	39.97 ^c	46.83 ^b (1.17)*	57.75 ^a (1.44)*
			2007	30.10	31.25	33.50
1-Butanol	150	medicinal, phenolic, whiskey	2006	0.97	1.20	n.d.
			2007	1.67	1.58	2.12
Isoamyl alcohol	60	fruity, banana, solvent	2006	292.00 ^a (4.86)*	182.72 ^b (3.05)*	190.72 ^b (3.18)*
			2007	268.41 ^a (4.47)*	285.53 ^b (4.76)*	282.31 ^{ab} (4.71)*
2-Butanol	n.a.	apricot, oily, sweet, wine	2006	n.d.	0.02 ^a	n.d.
			2007	6.38 ^a	n.d.	n.d.
Total HA			2006	374.00 ^a	278.12 ^c	296.82 ^b
			2007	348.12	358.44	356.94

Legend: All data present mean value (n=3); A=control, B= cold maceration 10 °C/10 h, C= cold maceration 10 °C/20 h; n.a. – not available; Different lower case superscript letters represent statistically significant differences between means at p≤0.05; OAV- odor activity values, * OAV > 1 (values in brackets); ′, ″ OPT and OD reported in: **Peinado et al. (2004)**, **Gómez-Míguez et al. (2007)**, **Güth (1997)**, **Swiegers et al., (2005)**.

The concentration of individual higher alcohols (HA) is shown in Table 1. The most abundant compound was isoamyl alcohol, what is in accordance with results for other Croatian white varieties (**Jagatić Korenika et al., 2018**). The results obtained show the significant effect of CM on 1-propanol, isoamyl alcohol, isobutanol and total HA concentration. In both years no repeatability was observed in CM effect on total or individual concentrations of HAs, though the fermentation was conducted under same conditions. An explanation can be hidden in the significant influence of must turbidity on the synthesis of higher alcohols (**Postel et al., 1972; Dittrich, 1987; Herjavec and Prusina, 2008**) since Vugava must 2007 was more turbid than the must from vintage 2006. The total amount of HAs after fermentation never exceeded 400 mg.L⁻¹ what can adversely affect the quality of the wine (**Rapp and Mandrey, 1986**). The significant influence of CM on the increase of total HAs in previous research was explained by enrichment of the amino acids involved in the formation of higher alcohols via the Ehrlich mechanism (**Ramey et al., 1986; Falque and Fernandez, 1996; Cabaroglu et al., 1997; Darias-Martin et al., 2000; Selli et al., 2006a**). There are also studies with conflicting results (**Palomo et al., 2006; Selli et al., 2006; Palomo et al., 2007**) where a decrease in the concentration of total HAs have been observed, what was explained by blocking the Ehrlich mechanism, the major biosynthetic pathway of higher alcohols, due to increased levels of nitrogen compounds in the must after the maceration process (**Rapp and Versini, 1995**). Compared to the total concentration of HAs, the content of isoamyl alcohol in Vugava wines accounted for 70% in 2006, and 77% in 2007. **Usseglio-Tomasset (1995)** pointed out that isoamyl alcohol is constantly present in wine, and that is most represented in relation to other higher alcohols with relative content about 73.6% of total higher alcohols. In 2007 wines, isoamyl alcohol concentrations were much closer to the upper limit of 300 mg.L⁻¹, which are reported as limiting for white wine quality. The concentration of 1- propanol in Vugava wines were within the ranges previous reported by **Amerine and Ough (1980)** and **Herjavec (1989)**. **Usseglio- Tomasset (1995)** stated that in wine compared to the total content of higher alcohols, about 5% is 1-propanol. In Vugava wines from both harvests, 1-propanol accounted for about 8% of the total concentration of higher alcohols.

Concentration of ethyl acetate differed regarding the vintage. According to **Ribéreau-Gayon et al. (2006)** this ester at low concentrations (50-80 mg.L⁻¹) has a significant positive effect on wine quality. More studies confirmed that concentrations less than 50 mg.L⁻¹, contribute to the complexity of the odor, while above 150 mg.L⁻¹ give negative aromas reminiscent of nail polish, glue and vinegar (**Ough and Amerine, 1980; Amerine and Roessler, 1983**). There were statistically significant differences among treatments during harvest 2007, where CM resulted in decreasing of ethyl acetate concentration what is in accordance with **Bavčar et al. (2011)** and partly with **Peinado et al. (2004)**.

Factors PC1 and PC2 described 99.58% interdependence of all observed parameters for Vugava wines 2006 and 2007 (Fig. 2). Parameters that differ significantly or are similar for the harvests were also visible. Thus, the four parameters observed differ significantly depending on the year of harvest: isoamyl alcohol, isobutanol, 1-propanol, and total higher alcohols. The red rectangle in Fig. 2(b) is highlighted and its magnified image represents Fig. 3

which points to the similarities of wines from two different harvests in alcohol, total extract, reducing sugars, total acidity, volatile acids, ash, pH, 2-butanol and 1-butanol.

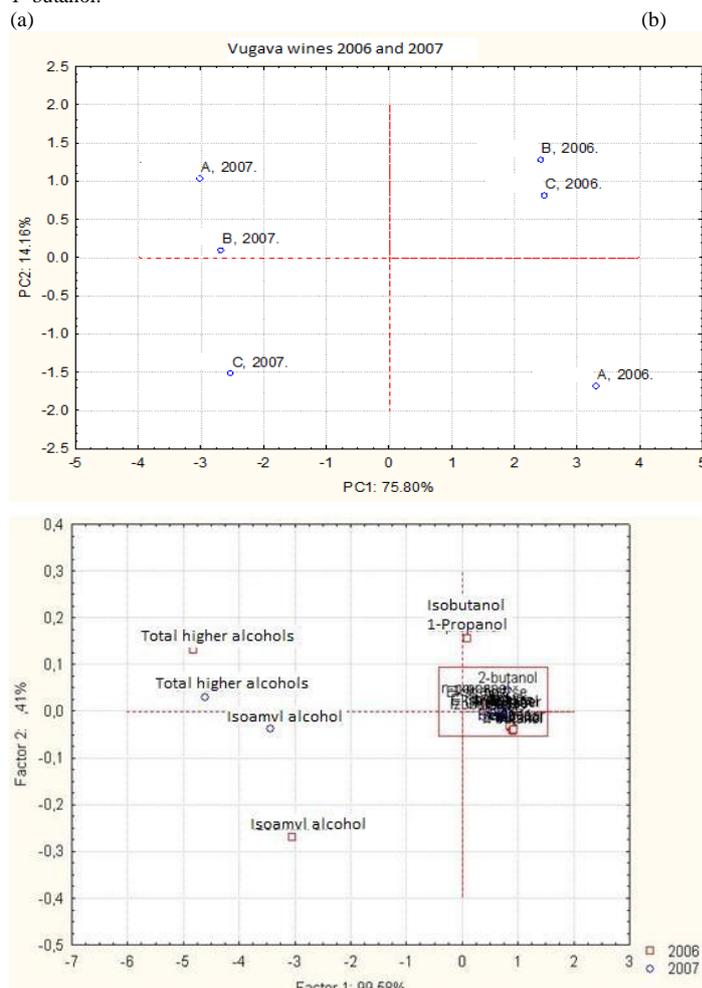


Figure 2 Score plot (a) and loading plot (b) of the first and second principal components after the PC analysis of chemical compounds in Vugava wines 2006 and 2007

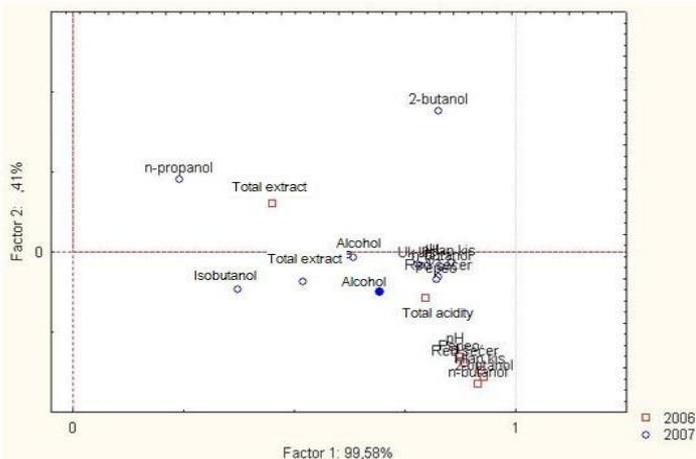


Figure 3 A separate section of the PCA diagram of the observed parameters for Vugava wine 2006 and 2007

Odor activity values

Table 1 shows odor descriptors and odor activity values (OAV) for each volatile compound of Vugava wines. 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). Among higher alcohols only 1- propanol, isoamyl alcohol, and isobutanol (B, C 2007) were detected above odor perception thresholds, with OAV > 1, together with ethyl acetate. The most abundant alcohol detected in wines, isoamyl alcohol at optimal concentrations brings the typical banana and pear-like aroma (Swiegers et al., 2005; Samappito and Butkhop, 2010) while excessive concentrations cause the nail polish odor of wines. One of the most significant aliphatic higher alcohols with the smell of ripe fruit is 1-propanol which had the highest OAV in CM wines from 2006. Cold maceration affected and OAV for isobutanol in the same vintage, known for alcohol, wine-like or solvent odor.

Sensory evaluation

In order to compare the chemical composition and odor activity values sensory evaluation was performed for vintage 2007. Control and CM wines were compared and best wine was selected by 10 certificated evaluators. Using the 100-points method the highest evaluated wine was Vugava wine (C) produced by CM 10 °C/20 h (Table 2). The ranking method was used to rank the wines according to the overall impression of quality and aroma quality of Vugava wines. Differences were found between wines of different treatments, both by the aroma and overall quality. The results presented here indicate that the evaluators rated the wine obtained by CM 10 °C/20 h (C) as the best due to the overall impression of quality. The wine C was characterized by a distinctive aroma and a more intense, full and rounded taste. The wine B and A were ranked as the second without statistical significant difference for the overall quality and aromatic characteristics. This results are similar to those for Žilavka wine (Herjavec and Prusina, 2008), where maceration period of 20 hours resulted with better quality compared to the wines obtained by maceration of 10 hours at the same temperature. Maceration process resulted in a more distinctive aroma of the Traminer variety (Herjavec and Majdak, 2002) and Malvasia Istriana (Radeka, 2008) as well.

Table 2 Sensory evaluation of Vugava wine 2007 by 100-points and ranking method

Sensory method	A	B	C	
100 points	79.0	80.0	81.7	
Ranking	Aroma quality	7 ^a	11 ^b	12 ^b
	Overall quality	6 ^{Aa}	11 ^{Bb}	13 ^{Bb}

Legend: Values for ranking method outside the range 8-16 are significant at p≤0.05, and outside the range 7-17 at p≤0.01.

CONCLUSION

The present two-year research demonstrated how pre-fermentative cold maceration (CM) technique significantly affected the aromatic and sensory properties of Vugava wine. Application of CM affected fermentation by decreasing the fermentation rate. Dry white wines obtained by treatments did not show significant difference in basic chemical composition in comparison to control wine and with respect to higher alcohols it resulted with counter effect. The most abundant volatile compound was isoamyl alcohol with concentration above its threshold together with 1-propanol and isobutanol. The CM showed an opposite effect on methanol and ethyl acetate concentration by no visible

difference or significant decrease what is positive from the health and sensory point of view. Concentrations of total higher alcohols, acetaldehyde and ethyl acetate were within the range of values with positive contribution to the complexity of the odor mostly through fruity aroma nuances. Results showed positive effect of CM technique in production of Vugava wine with distinctive aroma of the variety, with a pronounced fruity aroma, and more intense and rounded flavor. The results of this research should contribute to a better valorization of the aromatic potential of Vugava variety, which will assist in practical production of high quality autochthonous wine with highly pronounced cultivar characteristics.

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