Influence of different vinification techniques on stilbenes and total polyphenolic content in Teran wines

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Abstract
The aim of this work was to study the influence of different vinification techniques on stilbenes and total polyphenolic content in red wines made from cv. Teran (*Vitis vinifera* L.). Four different vinification techniques were carried out: control (7 days maceration), prolonged maceration (10 and 21 days), and thermovinification. Four stilbenes were determined by high-performance liquid chromatography (HPLC), and total polyphenols were determined by spectrophotometric methods. The results showed the treatment of extended maceration (21 days) had a significant effect on the increase of total (35.74 mg/L) and individual stilbenes (*trans*-piceid, *cis*-piceid, *trans*-piceatannol, *trans*-resveratrol), as well as total polyphenols (2702.88 mg GAE/L). Treatment of thermovinification resulted in significant increase of the total polyphenolic content by nearly 20% in relation to control.

Keywords: extended maceration, thermovinification, polyphenols, resveratrol, piceid

Introduction
Grapes contain a large amount of different polyphenolic compounds in skins, pulp, and seeds (Jackson, 2008). The polyphenolic composition of wines is affected by the grape variety, geographical location, soil, weather conditions and terroir components, and content depends on extraction from grapes during winemaking.
Polyphenols are directly related to wine color, astringency, bitterness and oxidative level. Their health beneficial features, particularly of stilbenes, include regulating cholesterol level, exhibiting antioxidant, anti-obesity, anti-diabetic, anti-atherosclerosis, and anti-inflammatory activities, and showing cardio and cancer-protective effects (Castillo-Sánchez et al., 2008; Gambacorta et al., 2011; Hossain et al., 2016).
Stilbenes are natural compounds occurring in many plant families including Vitaceae and *Vitis vinifera* L. cultivars (Bavaresco et al., 1999). The synthesis of these phytoalexins in grapevine is stimulated by stresses (Ribeiro de Lima et al., 1999). Resveratrol (3,5,4'-trihydroxystilbene) is considered to be the most important stilbene in wine. Red wines usually contain higher stilbene concentrations than white wines, which is because stilbenes are present in solid parts of the grapes, mainly in the grape skin, and are extracted during maceration (Atanacković et al., 2012). *Trans*-resveratrol is present in a concentration range of 0.2–13 mg/L in red and 0.1–0.8 mg/L in white wines. Concentrations of piceid (resveratrol-3-β-D-glucopyranoside) isomers were reported to be in a range of 0.3–9 and even up to 68 mg/L in red wines, and 0.1–2.2 mg/L in white wines (Moreno-Arribas and Polo, 2009). *Trans*-piceatannol (3,3',4,5'-tetrahydroxystilbene) was found in concentrations up to 6.4 mg/L in red and up to 0.2 mg/L in white wines (Moss, 2014).
Winemaking technique plays an important role in the extraction of polyphenols and their further stability in wines (Boulton, 2001). According to several authors, duration and

temperature of skin maceration have the largest impact on wine polyphenols (Auw et al., 1996; Koyama et al., 2007).
Post-fermentation extended maceration usually lasts 4 days to 4 weeks and is achieved at temperatures between 15ºC and 35°C. During this time water-soluble and alcohol-soluble compounds diffuse from the skin and seeds of the grapes into the fermented juice, resulting in a richer wine with greater capability of aging (Joscelyne, 2009).
Thermovinification is a pre-fermentative process of heating whole or crushed grapes to stimulate the rapid extraction of polyphenols from grape skins. The efficiency of extraction is temperature-dependent. The heat, within the range 60-80°C, destroys hypodermal cell membranes, releasing anthocyanins, and denatures polyphenol oxidase, preventing browning (Atanacković et al., 2012).
Teran (*Vitis vinifera* L.) is a traditional Croatian grapevine variety, mostly found on the Istrian peninsula. Teran wine is mainly produced by 5-10 days of maceration (Plavša et al., 2012). Its total polyphenolic content increases significantly with the length of maceration (Plavša et al., 2012), while no detailed investigation of its stilbene content has been carried out. The aim of this study was to investigate the influence of different maceration times and temperatures on stilbenes and total polyphenolic content of Teran wine.

Materials and methods

The grapes of cv. Teran (*Vitis vinifera* L.) were harvested at technological maturity (21.6˚ Brix, 8.3 g/L of total acidity expressed as tartaric acid, and pH 3.2) in 2018 in Western Istria (Croatia). The experiment was performed in the experimental cellar of the Institute of Agriculture and Tourism in Poreč. Grapes were destemmed and crushed and then homogenously transferred into 110 L stainless steel vats with the addition of 30 g/hL of selected dry yeast Fermol Mediterranee (*Saccharomyces cerevisiae*, AEB). Four different vinification techniques were carried out. Three maceration periods were applied: 7 days (TM7, control treatment), 10 days (TM10), and 21 days (TM21) at the temperature of 24°C, and 10 days of maceration with heating on 45°C for the first 48 hours (thermovinification, TTV). The heating was followed by cooling down before yeast inoculation. Three replications of each vinification technique were done.
Standard physico-chemical parameters were determined according to the OIV methods. Average values with standard deviation were: relative density 0.9974±0.0008, alcoholic strength by volume (%) 12.78±0.50, reducing sugars (g/L) 3.13±0.13, pH 3.19±0.06, free SO_2 (mg/L) 8.33±2.60.
Total polyphenols were determined by the Folin-Ciocalteu colorimetric method (Singleton et al., 1999) using a Cary 50 UV/Vis spectrophotometer (Varian Inc., Harbour City, CA, USA). The absorbance was measured against blank at the wavelength of 765 nm. Results were expressed as gallic acid equivalents in mg/L of wine (mg GAE/L).
Analysis of stilbenes was carried out by high-performance liquid chromatography (HPLC), according to the method proposed by Mark et al. (2005). The HPLC system used was an Agilent Infinity 1260 equipped with a G1311B quaternary pump, a G1329B auto sampler, a G1316A column oven, a G4212B DAD detector, and a G7121B FLD detector. Wine samples were filtered through 0.45 μm PTFE filters and 10 μL of wine were injected on a Zorbax SB-C18 column (4.6 x 250 mm, 5 μm). Two solvents were used for the separation: methanol-water-acetic acid (10:90:1, v/v) as solvent A and methanol-water-acetic acid (90:10:1, v/v) as solvent B. The flow rate was 1.5 mL/min. The solvent gradient system was as follows: 0.0-18.0 min from 0% to 40% B, 18.0-25.0 min from 40% to 100% B, and 25.0-27.0 min to 100% B. Chromatographic separations were monitored at 306 nm. Identification was performed by comparing retention times and UV/Vis spectra of wine

samples with those of pure standards. Standard calibration curve was constructed for each stilbene compound and used for quantification.
The data were processed using one-way analysis of variance (ANOVA). Fischer's least significant difference test (LSD) was used to compare mean values ($p < 0.01$ and $p < 0.05$). Statistical analyses of results of one year of research were performed using Statistica 10 software (StatSoft, Inc. 1984-2008).

Results and discussion

The total polyphenolic content in Teran wines varied from 2448.33 mg to 2916.67 mg GAE/L (Figure 1). The obtained results showed that total polyphenolic content was influenced by maceration duration and that the increase was proportional according to maceration time. Similar results were reported by Budić-Leto et al. (2008), who observed an increase in the polyphenolic content in wines made by extended maceration, as extraction of monomeric and polymeric polyphenolics increases with maceration time (Kantz and Singleton, 1991).

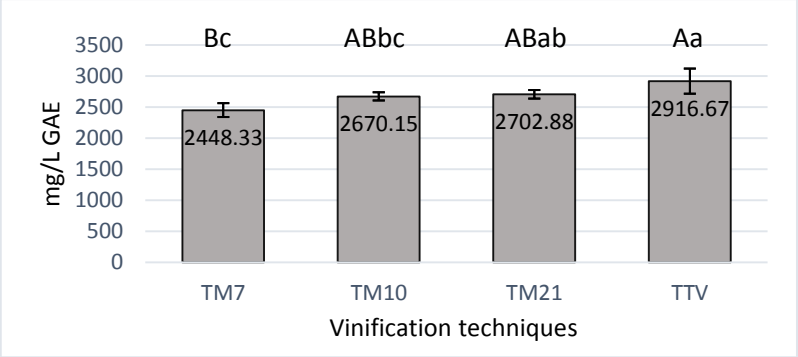

Figure 1. Influence of different vinification techniques on total phenols in Teran wines
Capital letters represent significant differences at $p < 0.01$ level, and lower-case letters represent significant differences at $p < 0.05$ level according to the LSD test.

Results showed that wines produced by TTV and extended maceration technique TM21 had significantly higher amounts of polyphenolic compounds than those from the control wine. It is important to note that there was no significant difference between TTV and TM21 treatments with respect to total polyphenolic content ($p<0.05$). The increase of approximately 20% noted for TTV treatment, with respect to the control, similar to relatively higher amounts of polyphenolic compounds in wines produced by thermovinification than those from the control group reported by Wang et al. (2016), is due heat destroyed skin cell membranes, releasing the pigments, tannins and different polyphenolic substances into the must (Atanacković et al., 2012).

Table 1. Effect of different vinification techniques on stilbenes in Teran wines (mg/L)

	TM7 (control)	TM10	TM21	TTV
trans-piceid	18.16 ± 1.54^{Bb}	17.09 ± 0.17^{Bb}	21.54 ± 0.50^{Aa}	17.69 ± 0.60^{Bb}
cis-piceid	8.95 ± 0.40^{Bb}	8.84 ± 0.07^{Bb}	10.58 ± 0.08^{Aa}	8.67 ± 0.36^{Bb}
trans-piceatannol	0.58 ± 0.05^{Bc}	0.64 ± 0.02^{Bb}	0.78 ± 0.02^{Aa}	0.63 ± 0.03^{Bbc}
trans-resveratrol	2.84 ± 0.06^{Aa}	2.41 ± 0.07^{Bb}	2.85 ± 0.08^{Aa}	2.48 ± 0.13^{Bb}
total stilbenes	30.52 ± 2.01^{Bb}	28.98 ± 0.25^{Bb}	35.74 ± 0.38^{Aa}	29.47 ± 0.96^{Bb}

Each value is the mean ± standard deviation, n=3. Capital letters represent significant differences at $p<0.01$ level, and lower-case letters represent significant differences at $p<0.05$ level according to the LSD test.

Prolonged maceration resulted in a significant increase in total and individual stilbenes (Table 1). The prolonged maceration TM21 had significantly different (p<0.01) and the highest concentration of both *trans*- and *cis*-piceid (21.54±0.50 mg/L and 10.58±0.08 mg/L, respectively) and piceatannol (0.78±0.02 mg/L). However, there was no difference between TM7 and TM21 wines in concentration of *trans*-resveratrol (2.84±0.06, 2.85±0.08, respectively). Similar results were reported by Kocabey et al. (2016) on red wines made from the *Vitis vinifera* L. Karaoglan, who observed no difference in *trans*-resveratrol content between 5, 10 and 15 days of maceration duration (2.55±0.03, 2.19±0.26, and 2.68±0.16 mg/L, respectively), suggesting that the extraction of resveratrol was complete during or at the end of alcoholic fermentation.

Conclusions

Treatment of extended maceration TM21 increased the concentration of total and individual stilbenes, as well as total polyphenols, while treatment of thermovinification TTV significantly increased concentration of total polyphenols. No significant difference in concentration of total polyphenols was found between TTV and TM21 treatment. These results suggest that the use of extended maceration and thermovinification in Teran wine production increase the polyphenolic concentration and consistently enhance the health benefits and sensory properties of these wines.

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Utjecaj različitih vinifikacijskih tehnologija na sadržaj stilbena i ukupnih polifenola u vinima sorte ‘Teran’

Sažetak

Cilj ovog istraživanja bio je utvrditi utjecaj različitih vinifikacijskih tehnologija na sadržaj stilbena i ukupnih polifenola u crnom vinu sorte 'Teran' (*Vitis vinifera* L.). Provedene su četiri različite tehnologije proizvodnje: kontrola (7 dana maceracije), produljena maceracija (10 i 21 dan) i termovinifikacija. Koncentracija četiri stilbena određena je korištenjem tekućinske kromatografije visoke djelotvornosti (HPLC), a analize ukupnih polifenola provedene su spektrofotometrijskim metodama. Iz dobivenih rezultata vidljiv je značajan porast ukupnih (35.74 mg/L) i pojedinačnih stilbena (*trans*-piceid, *cis*-piceid, *trans*-piceatanol, *trans*-resveratrol), kao i ukupnih polifenola (2702.88 mg GAE/L) u tretmanu produljene maceracije (21 dan). Tretman termovinifikacije rezultirao je značajnim porastom koncentracije ukupnih polifenola za gotovo 20% u odnosu na kontrolu.

Ključne riječi: produljena maceracija, termovinifikacija, ukupni fenoli, resveratrol, piceid