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Total phenolic content and antioxidant capacity of Teran red wine: influence of pre-fermentative mash procedures

Fumica Orbanić[1], Sara Rossi[1], Ena Bestulić[1], Karin Kovačević Ganić[2], Natka Ćurko[2], Marina Tomašević[2], Tomislav Plavša[1], Ana Jeromel[3], Sanja Radeka[1]

[1]*Institute of Agriculture and Tourism, Karla Huguesa 8, Poreč, Croatia (fumica@iptpo.hr)*
[2]*University of Zagreb, Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10000 Zagreb, Croatia*
[3]*University of Zagreb Faculty of Agriculture, Svetošimunska cesta 25, 10000 Zagreb, Croatia*

Abstract

Six vinification treatments, including control treatment (K7) were carried out to investigate: the impact of 48-h pre-fermentative mash cooling (8 °C) followed by 13-days maceration (C15), 28-days maceration (C30) and *saignée* technique (CS15); and the impact of 48-h heating (50 °C) followed by 13-days maceration (H15) and 28 days (H30) on total phenolic content (TPC) and antioxidant capacity (AC) in Teran wines. TPC was analysed by a method based on Folin–Ciocalteu reagent and AC was determined by FRAP and ORAC assay. The results showed higher TPC and AC in all treatments compared to the control wine (K7). The highest TPC was found after *saignée* technique applied (CS15). Pre-fermentative heating and 30-day maceration treatment (H30) showed the strongest antioxidant capacity, with both FRAP and ORAC assay.

Keywords: Teran red wine, pre-fermentative procedures, prolonged maceration, total phenolic content, antioxidant capacity

Introduction

Phenolic compounds are the main class of secondary metabolites in plants. Wine is one of the beverages with the highest phenolic content (Drosou et al., 2015) which also plays a major role in some organoleptic characteristics of wine, such as colour and astringency (Bai et al., 2013). The final phenolic content in wine depends on the type of soil, climate, grape, harvest, and pre- and post-harvest treatments (Bai et al., 2013; Muñoz-Bernal et al., 2021). The health-promoting properties, including antimicrobial, anticarcinogenic, and antioxidant properties (Büyüktuncel et al., 2014), of phenolic compounds are influenced by their structure, solubility, conjugation with other polyphenols or other compounds (Singh et al., 2018; Zeb, 2020). The antioxidant activity of phenolic compounds is attributed to the capacity of scavenging free radicals, donating hydrogen atoms, electrons, or chelate metal cations. Many studies have shown a strong and positive correlation ($p \leq 0.05$) between the phenolic compound contents and the antioxidant potential (Plavša et al., 2012; Minatel et al., 2017). The interest in natural antioxidants has increased in recent years because of their presumed safety and potential nutritional and therapeutical effects. Among natural antioxidants, red wine has attracted particular interest due to a high content of biologically active compounds (Büyüktuncel et al., 2014). This implies that the antioxidant capacity of wine, an important property closely related to the total amount of phenols, varies with grape varieties, vintages, weather, and wine-making procedures (Lissi et al., 2014), pre-fermentative procedures, fermentation/maceration, and maturation. Maceration is a critical step to obtain the best characteristics of wine since a large number of phenolic compounds come from seeds and skins (Aleixandre-Tudo and du Toit, 2018; Muñoz-Bernal et al., 2020). Also, extended (prolonged) maceration is a widely used winemaking technique, based on extended the contact of grape solids and wine after the end of fermentation (Sacchi et al., 2005). This technique has been used to alter the mouthfeel of the wines, possibly by facilitating proanthocyanidin extraction and the formation of polymeric pigments (Casassa et al., 2013). Additionally, different pre-fermentative enological techniques have been used and studied to understand their effects on the color and phenolic compound composition of the wine (Heredia and Guzman-Chozas, 1993; Cejudo-Bastante et al., 2014).

One example is cold pre-fermentative maceration, also known as cold soaking or cryomaceration, which could be adopted to extract water-soluble compounds in the absence of alcohol at low temperatures (5 - 10 °C), mainly phenolic and volatile compounds (Heredia et al., 2010; Barros et al., 2022). Another non-conventional technique, called thermovinification, entails short heating of the skins from 50 to 80 °C extracting them with the juice, pressing, and then cooling before fermentation. If heating at the same temperature before fermentation is extended for a longer period (for instance, up to 24 h), the process is called pre-fermentative heat treatment (Escudier et al., 2008; Rossi et al., 2022). The heat damages the hypodermal cell membranes, releasing anthocyanins, and it also denatures polyphenol oxidase, preventing browning. Since there is no alcohol present at the time of heating, it would not be expected to increase tannin extraction (Sacchi et al., 2005). Furthermore, frequently in use is the *saignée* technique, also known as pre-fermentation juice runoff. The juice is removed before fermentation, thus increasing the skin-to-juice ratio, thereby enhancing the extraction of phenolic compounds and stabilizing the apparent color of the wine (Sacchi et al., 2005). Teran (*Vitis vinifera* L.) is the most widespread red autochthonous cultivar on the Istrian peninsula, in Croatia (Plavša et al., 2012; Rossi et al., 2022). This study aimed to investigate how pre-fermentative cooling or heating procedure, *saignée* technique, and various maceration durations affect the total phenolic content (TPC), and antioxidant capacity (AC) in Teran red wines.

Material and methods

The grapes of *cv*. Teran (*Vitis vinifera* L.) was grown in wine-growing hill Western Istria, the town of Poreč, in a typical Istrian terroir. The harvest was held in 2020 when the sugar content was measured at 18.9 °Brix, 8.0 g L^{-1} of total acidity expressed as tartaric acid, and pH 3.2. On the same day, manually harvested grapes were destemmed and crushed with standard equipment and homogenized. Red grape mash was equally divided according to the plan of the experiment (Table 1).

Table 1: Overview of the experiment: pre-fermentative procedures, winemaking techniques and maceration duration in Teran wine treatments

Treatment*	Pre-fermentative procedure (48 hours)	Fermentation and maceration			Maceration duration + pre-fermentative procedure
		Winemaking technique	**Maceration/ fermentation temperature**	**Maceration duration**	
CS15	Cooling - at 8 °C	*Saignée*	24 °C	13 days	15 days
C15		Prolonged maceration		13 days	15 days
C30				28 days	30 days
H15	Heating - at 50 °C			15 days	15 days
H30				28 days	30 days
K7	/	Standard		7 days	/

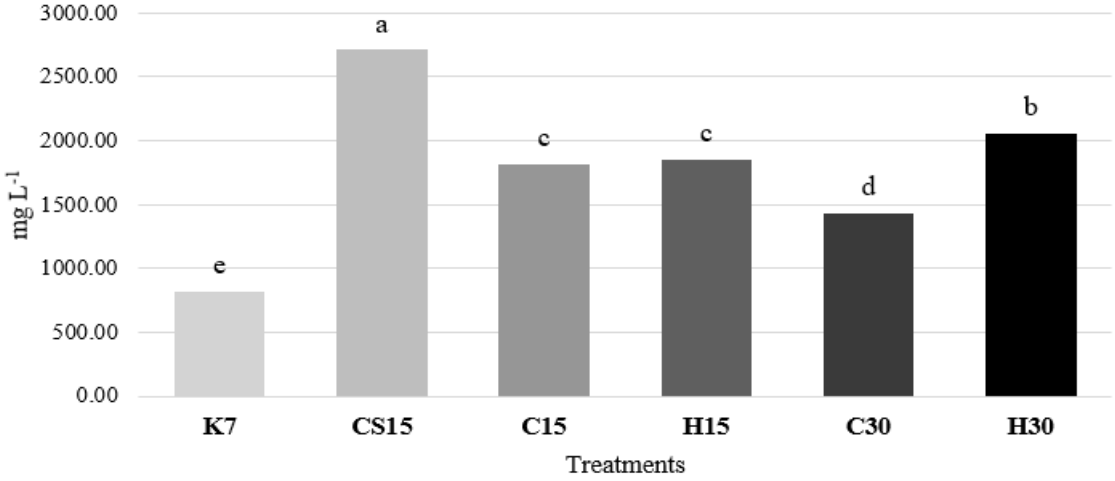
**C-cooling; H-heating; S-saignée; K-contol; 7,15,30 – days of maceration duration in total*

Five vinification treatments were submitted to pre-fermentative mash cooling at 8 °C (cryomaceration) or mash heating at 50 °C for 48 hours, proceeded with fermentation at 24 °C and followed by prolonged maceration in two periods of duration: 13 days, respectively 15 days in total including pre-fermentative procedure (C15; H15) and 28 days, in total 30 days (C30; H30). In one of the treatments with pre-fermentative mash cooling, the *saignée* technique was performed before fermentation. A proportion (33 %) of the total juice quantity was racked and concentrated mash proceeded with fermentation (24 °C) and prolonged 13-days maceration, 15 days in total (CS15). This experiment has also included a control treatment (K7), with a standard 7-day maceration and a maceration/ fermentation at temperature of 24 °C. Every treatment was performed in three replicats in 220 L-stainless steel tanks. All grape mashes were inoculated with 30 g hL^{-1} of selected *Saccharomyces cerevisiae* dry yeast (Fermol Mediterranee, AEB), and chaptalized with 3 kg hL^{-1} of saccharose. After the end of maceration, mashes were pressed and wine was racked in clean tanks. After six months the wine were bottled and stored prior to analysis. The total phenolic content of each wine was determined by the Folin–Ciocalteu colorimetric method (Singleton and Rossi, 1965) using a Cary 50 UV/Vis spectrophotometer (Varian Inc., Harbour City, CA, USA), at the Institute of Agriculture and Tourism in Poreč. The absorbance was measured against a blank at a wavelength of 765 nm, and the results were expressed as

gallic acid equivalents in mg L^{-1} of wine (mg GAE L^{-1}). While analysis of the antioxidant capacity in the wines were performed at the Faculty of Food Technology and Biotechnology in Zagreb by the ferric reducing/antioxidant power (FRAP) assay, and the oxygen radical absorbance capacity (ORAC) assay. The FRAP assay was conducted according to the method of Benzie and Strain (1996) and the results are expressed in mmol L^{-1} $FeSO_4 \times 7H_2O$. The ORAC assay was performed according to Ninfali et al. (2005), as briefly described in Mazor Jolić et al. (2011). Fluorescence was measured by a Varian Cary Eclipse Spectrofluorometer (Palo Alto, CA, USA). The results were calculated as ORAC values using the difference in the area under the fluorescein decay curve between the blank and the sample. The results are expressed as mmol L^{-1} of Trolox equivalents (TE). Analyses of antioxidant capacity by FRAP and ORAC assays were conducted in triplicate. One-way analysis of variance (ANOVA) and Fisher's least significance difference (LSD) test were used to compare mean values ($p < 0.05$). Statistics were performed using Statistica 10.0. software (Sta-Soft Inc. Tulsa, OK).

Results and discussion

Obtained results showed that total phenolic content (TPC) in all treatments was significantly higher in comparison to the control treatment (K7), where 821.52 mg L^{-1} was measured ($p < 0.05$) (Figure 1).



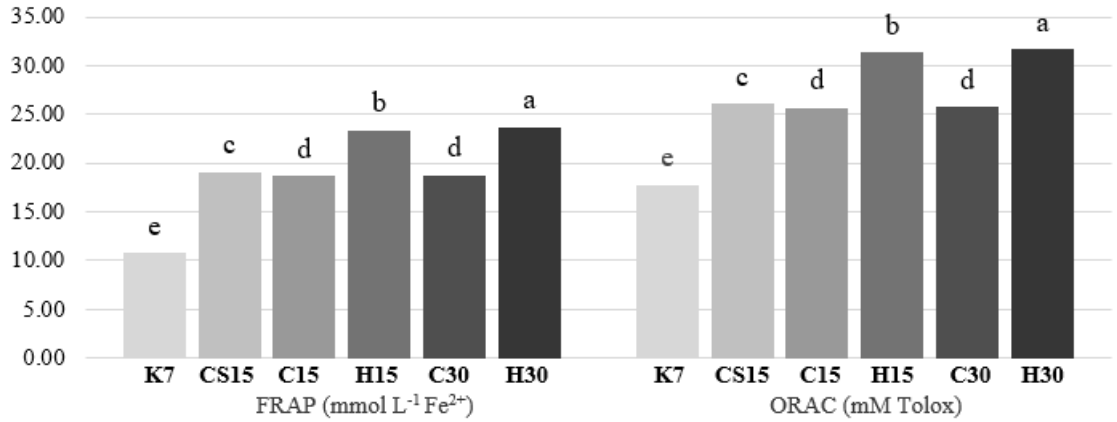
Lower-case letters above column represent significant differences at p < 0.05 level according to the LSD test.

Figure 1: Total phenolic content (mg L^{-1}) in Teran wines, 2020.

These results are in accordance with Rossi et al. (2020), who investigate similar vinification procedures and prolonged macerations on Teran wine of grapes from the same vineyard. In our investigation, the highest content of phenols, 2710.61 mg L^{-1}, was found in treatment submitted to pre-fermentative mash cooling, *saignée* technique, and prolonged post-fermentative 15-day maceration (CS15). This is confirmed by the fact that removing the juice with a certain level or ratio would theoretically increase the concentration of water-soluble compounds in the juice (Wu et al., 2017; Cheng and Watrelot, 2022). Regarding TPC among treatments with pre-fermentative mash cooling or heating and prolonged post-fermentative 15-day maceration (C15 and H15), a significant difference was not evident. Possibly, because the extraction of phenols did not reach the maximum within 15 days of maceration. This is in correspondence with results published by Plavša et al. (2012), where TPC increases significantly with the length of maceration and 20-day-long maceration shows higher values than 15-day-long maceration. But the difference was seen in respect of our treatments submitted to prolonged post-fermentative 30-day maceration (C30 and H30), where the heating procedure in H30 exhibited a greater effect on TPC in comparison to the cooling procedure in C30. In our previous study, dealing with total and free anthocyanins, C30 also showed lower values in comparison to other treatments (Orbanić et al., 2022).

According to the FRAP assay, values in all treatments were statistically higher compared to the control wine (K7). Treatment that underwent pre-fermentative heating and prolonged 30-day maceration (H30) provided significantly the highest antioxidant capacity, 23.67 mmol L^{-1} Fe^{2+}, with respect to the control treatment where 10.77 mmol L^{-1}

Fe^{2+} was measured. An identical situation was obtained with ORAC assay, values ranged from 17.76 mM Trolox found in K7 to 31.67 mM Trolox in H30 treatment (Figure 2).



Lower-case letters above column represent significant differences at $p < 0.05$ level according to the LSD test.

Figure 2: Antioxidant capacity in Teran wines (2020) analysed by FRAP and ORAC assay

This could be explained by the fact that the heating process is not effective in extracting tannins, which are responsible for phenolic stability (Sacchi et al., 2005). Therefore, providing direct condensation and co-pigmentation reactions of tannins with flavanol-3-ol monomers, anthocyanins, anthocyanin-derived pigments, and oligomers is decreased (Smith et al., 2015). Consequently, some phenolic compounds stay unbound thus enhancing antioxidant activity, with the hydrogen atoms of the adjacent hydroxyl groups (o-diphenol), located in various positions of the rings A, B, and C, with the double bonds of the benzene ring, and with the double bond of the oxo functional group (-C=O) of some flavonoids (Minatel et al., 2017). When correlation coefficients (r) were examined, a very strong correlation between FRAP and ORAC at 0.998 ($p < 0.05$) was noted. The results of correlation analysis showed that these methods are almost comparable in characterizing antioxidant capacities. Other researches using antioxidant capacity measurement methods have also shown a high correlation (Radeka et al., 2022; Tahmaz and Söylemezoğlu, 2022). Moreover, the positive linear correlation of moderate strength among TPC and antioxidant capacity by FRAP assay was determined, exhibiting an r value of 0.657 ($p < 0.05$) (Figure 3), providing evidence that the predominant source of antioxidant activity derives from phenolic compounds in wine (Paixao et al., 2007). A similar correlation was obtained in the case of ORAC assay and TPC, with an r value of 0.627 ($p < 0.05$) (Figure 4). These results could be explained with consideration that phenolic subgroups, possible synergy, and antagonism among them, degree of polymerization, and radical molecules contained in wine have different influences on antioxidant capacity (Di Majo et al. 2008). Additionally, Arnous et al. (2002) reported that polymeric and other types of pigments may not have similar antioxidant characteristics in comparison with monomeric anthocyanins.

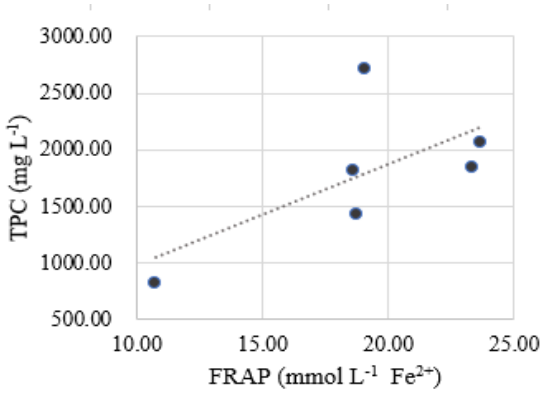


Figure 3: Correlation among TPC and FRAP values in Teran wines (r = 0.657)

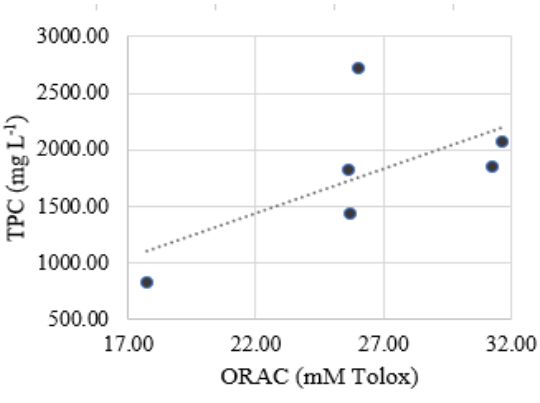


Figure 4: Correlation among TPC and ORAC values in Teran wines (r = 0.627)

Conclusions

Applied pre-fermentative procedures demonstrated a significant impact on total phenolic content as well as on the antioxidant capacity of Teran red wines when compared with wine produced by standard technique. Obtained evidence could be employed in applying investigated vinification procedures in the winemaking industry to produce Teran and other red wines of the desired style, thus providing diversification of the wine market. Furthermore, with considerable bioactive content, which acts as added value, the beneficial properties of moderate consumption of wine are enhanced.

Acknowledgement

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Sadržaj ukupnih fenola i antioksidativni kapacitet vina sorte ´Teran´: utjecaj predfermentativnih tretmana masulja

Sažetak

Provedeno je šest vinifikacijskih tretmana, uključujući kontrolu (K7) kako bi se istražio: utjecaj predfermentacijskog hlađenja masulja (48 h; 8 °C) koji se nastavio maceracijom od 13 dana (C15), 28 dana (C30), te *saignée* tehnologijom (CS15); i utjecaj tretmana grijanja (48 h; 50 °C), koji se nastavio maceracijom od 13 (H15) i 28 dana (H30) na koncentraciju ukupnih fenola (TPC) i antioksidativni kapacitet (AC) vina ´Teran´. TPC određeni su metodom koja se temelji na reakciji s Folin–Ciocalteu reagensom, a antioksidativni kapacitet metodama FRAP i ORAC. Prema dobivenim rezultatima, vrijednosti TPC i AC u vinima svih tretmana više su u odnosu na kontrolni tretman (K7). Najviše TPC dobiveno je u tretmanu sa *saignée* tehnologijom (CS15). Tretman koji uključuje predfermentativno zagrijavanje i produženu maceraciju od 30 dana (H30) pokazao je najveći antioksidativni kapacitet, koristeći obje metode (FRAP i ORAC).

Ključne riječi: Teran, predfermentacijski tretmani, produljena maceracija, ukupni fenoli, antioksidativni kapacitet