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Effects of Fruit Zone Leaf Removal on the Concentrations of Phenolic and Organic Acids in Istrian Malvasia Grape Juice and Wine

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Summary

Phenolic acids, represented by hydroxycinnamic and hydroxybenzoic acids and their derivatives, are the most abundant phenolic compounds in grape juice and wine of white grapevine cultivars. The effects of fruit zone leaf removal on the concentration of hydroxycinnamic, hydroxybenzoic and organic acids of cv. Istrian Malvasia grape juice and wine are investigated in this study. Fruit zone leaf removal was applied at three different phenological stages: before blooming, at berry set and at veraison. Control treatment without leaf removal was also included. At veraison two intensities of leaf removal were applied. Phenolic and organic acids were identified and quantified using high-performance liquid chromatography. Juice samples from the grapes treated at the stages before blooming and berry set had the lowest concentrations of tartaric acid and the highest concentrations of malic acid, while juices from veraison stage treatments had the lowest concentrations of malic acid and the highest concentrations of tartaric acid. Before blooming treatment significantly lowered the concentration of all hydroxycinnamic and hydroxybenzoic acids in grape juice in comparison with other investigated treatments, which did not affect the concentration of total hydroxycinnamic acids considerably. The concentration of hydroxybenzoic acids in grape juice was lower in veraison treatments in comparison with berry set and control treatments. The concentrations of hydroxycinnamic, hydroxybenzoic and organic acids in wine followed the same trends as observed in grape juice. It is concluded that the concentration of phenolic and organic acids in grape juice and wine of white grapevine cultivars can be managed with the timing of fruit zone leaf removal according to the desired wine style.

Key words: leaf removal, phenolic acids, organic acids, Istrian Malvasia, grape juice, wine

Introduction

Although present in low concentrations in grapes and wines, phenolic compounds considerably contribute to the sensory characteristics of wines, especially colour, astringency and bitterness (*1–3*), and are the major substrate for oxidation of grape juice and wine (*3,4*). Different factors affect the phenolic composition of grapes and wines, such as cultivar, ecological conditions, agro-

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technical and canopy management practices in vineyard and techniques of vinification (*5–7*).

Hydroxycinnamic acids are the most abundant class of phenolic compounds in free-run grape juice and consequently in white wines (*3,8*). The predominant hydroxycinnamic acids present in grape berries and wines are coutaric, caftaric and fertaric acids and their *trans*-isomers (*5,9–11*). Recent studies have shown that hydroxycinnamic acids may contribute to astringency of grape juice and wine (*1,2*). Hydroxybenzoic acids are present in grapes and wines at lower concentrations than hydroxycinnamic acids (*9*). Seven hydroxybenzoic acids have been identified in wines: *p*-hydroxybenzoic, protocatechuic, vanillic, gallic, syringic, salicylic and gentisic acids (*3*), the last two present only in trace amounts.

Organic acids make major contribution to the composition, stability and organoleptic characteristics of white wines, and their preservative properties also enhance wine microbiological and physicochemical stability (*3*). Tartaric, malic and citric acids account for the majority of the acidity in grapes. During alcoholic fermentation, succinic acid develops due to the action of yeasts in the average amount of 1 g/L, while during malolactic fermentation malic acid may be converted into lactic acid due to the action of malolactic bacteria (*3*). Several other organic acids are present in grape juice and wine at very low concentrations.

Previous research of the phenolic composition of grapes and wines of white cultivars was mostly based on the characterization of different cultivars (*12–15*), changes in the phenolic composition during ripening of grapes (*13,16*) and during wine storage (*17,18*), the impact of oenological treatments on the phenolic composition of wine (*9,14,19–22*) and the relationship of phenolic composition with the antioxidant capacity of wine (*8,23,24*).

Fruit zone leaf removal is a canopy management practice in viticulture performed in order to improve the exposure of clusters and the remaining leaves to sunlight and air circulation in the fruit zone to improve the composition of fruit (*25–28*).

The impact of leaf removal on the phenolic composition of grapes and the resultant wines has been widely studied on red grapevine cultivars (*28–33*), but the knowledge concerning white grapevine cultivars is still limited. Moreover, unlike with tannins and anthocyanins, little work has been done to determine how hydroxycinnamic acids vary with different viticultural practices (*5*). In a leaf removal trial conducted on Pinot noir cultivar, hydroxycinnamic acids were affected only slightly, mostly at the beginning of the maturation period (*29*).

Istrian Malvasia (*Vitis vinifera* L.) is considered an autochthonous white grapevine in the region of Istria (Croatia). It is the most widespread cultivar in Istria and the second most widespread in Croatia according to the Croatian Main Register of Grape, Wine and Fruit Wine Producers, provided by the Institute of Viticulture, Enology and Pomology, Zagreb.

The aim of this study is to investigate the impact of different timing and severity of fruit zone leaf removal on the concentration of hydroxycinnamic, hydroxybenzoic and organic acids of cv. Istrian Malvasia grape juice and wine.

Materials and Methods

Fruit zone leaf removal treatments

The experiment was performed in season 2007 on *Vitis vinifera* L. cv. Istrian Malvasia vines grafted on *Vitis berlandieri* × *Vitis riparia* SO4 rootstock, in a vineyard located at Kaštelir (Istria, Croatia), 220 m above sea level. Row and vine spacing were 2.7×1.2 m. Vines were trained to bilateral Guyot training system. Shoots were vertically positioned and sustained with two pairs of catching wires. First shoot trimming was applied two weeks after the end of blooming, and the second shoot trimming was applied three weeks after the first shoot trimming. Other viticultural practices were standard for the cultivar and region. The soil in the vineyard was typical, medium deep, anthropogenized red Mediterranean soil (*Terra rossa*).

A randomized block design was used in this experiment, with five canopy manipulation treatments. Three treatments consisted of the manual removal of basal leaves from the shoots, usually up to the first cluster (three to four leaves per shoot), at different grapevine phenological stages: before blooming, at berry set and at veraison. Another treatment was applied at veraison, with three more leaves per shoot removed (veraison+3). Control treatment without leaf removal was also included. Each treatment was done in three replications with five adjacent vines.

Grape juice sampling

Grapes were harvested on 7 September 2007, when Brix values were around 23 °Brix and titratable acidity was between 6 and 7 g/L. Samples for grape juice analysis were taken after crushing and destemming of grapes and stored in a freezer at –20 °C until analysis. From each replication in the vineyard, one sample of grape juice was obtained, leading to three samples per treatment.

Vinification

Grapes from three replications were assembled from the vineyard and processed as one single wine. Vinification was performed in a minivinification cellar of the Institute of Agriculture and Tourism in Poreč, Croatia. Grapes were destemmed, crushed and pressed, and the obtained must was treated with 20 g/hL of potassium metabisulphite. After 24 h of sedimentation at 10 °C, musts were racked and 20 g/hL of selected wine yeast *Saccharomyces bayanus* Lalvin EC 1118 were added. Alcoholic fermentation was carried out at 17 °C, in 50-litre stainless steel barrels. After fermentation has finished, wines were racked and kept at 4 °C. One month after the end of fermentation, the wines were taken for analysis.

Chemicals

Vanillic, syringic and *p*-coumaric acids were obtained from Fluka (Sigma-Aldrich, Buchs, Switzerland). Ferulic, gallic, protocatechuic, *p*-hydroxybenzoic, *trans*-

-caftaric, *trans*-coutaric and *trans*-fertaric acids were obtained from Sigma-Aldrich (Steinheim, Germany). Caffeic acid and HPLC grade methanol were obtained from Merck (Darmstadt, Germany). Formic acid was of analytical grade and was supplied by Kemika (Zagreb, Croatia).

HPLC analysis of phenolic compounds

Grape juice and wine samples were filtered through a 0.45-μm filter (Nylon Membranes, Supelco, Bellefonte, PA, USA) before high-performance liquid chromatography (HPLC) analysis. A volume of 20 μL of each sample was injected for HPLC analysis using a Varian Pro Star Solvent Delivery System 230 and a Varian Pro Star 330 photodiode array detector (Varian, Walnut Creek, CA, USA) using a Pinnacle II C-18 reversed-phase column (Restek, Bellefonte, PA, USA) (250×4.6 mm i.d., 5 μm particle size). The solvents consisted of water, 3 % formic acid (solvent A) and HPLC grade methanol (solvent B) at a flow rate of 1 mL/min. The elution was performed with a gradient starting at 2 % B to reach 32 % B at 20 min, 40 % B at 30 min and 95 % B at 40 min, and became isocratic for 5 min. Chromatograms were recorded at 278 nm.

Detection was performed with a photodiode array detector by scanning between 200 and 400 nm, with a resolution of 1.2 nm. Phenolic compounds were identified by comparing the retention times and spectral data with those of authentic standards. Quantitative determinations were performed using standard curves of determined hydroxycinnamic and hydroxybenzoic acids. The data acquisition and treatment were conducted using the Star Chromatography Workstation software v. 5 (Varian). All analyses were repeated three times.

HPLC analysis of organic acids

Organic acids (tartaric, malic, citric and succinic acids) were determined in grape juice and wine using an 1100 Agilent (Agilent Technologies, Santa Clara, CA, USA) high-performance liquid chromatograph (HPLC) coupled with diode array detector (DAD) and equipped with a reversed phase analytical column Synergi 4u Fusion-RP (Phenomenex, Torrance, CA, USA), 150 mm×4.6 mm i.d., 5 μm particle size. Chemstation software (Agilent Technologies) was used for data analysis.

Organic acids were extracted from the grape juice and wine samples using solid-phase extraction. Sample (10 mL) was alkalized with 1 M NaOH until pH=9.0±0.5. An aliquot of 2 mL was passed through a solid-phase extraction cartridge (55 μm, 70 Å; 500 mg/3 mL; Strata SAX, Phenomenex), which had previously been conditioned with 2 mL of HPLC grade methanol (Mallinckrodt Baker B.V., Deventer, The Netherlands) and 2 mL of deionized water. Neutral components (sugars and alcohols) were removed by elution with 1.5 mL of water, while the acid components were recovered with 2.5 mL of 1 M HCl. Eluate was filtered through a 0.2-μm Teflon membrane filter (Phenomenex).

HPLC analysis was performed under isocratic conditions (column temperature 30 °C, mobile phase flow rate 0.6 mL/min). Mobile phase was 0.02 M KH_2PO_4 (Fluka) water solution acidified with 85 % H_3PO_4 (Riedel-de Haën, Sigma-Aldrich, Seelze, Germany) until the pH=2.88±0.02. Extracted acids were detected at 210 nm with a DAD. For the quantification of acids, calibration curves were constructed using six standard solutions with acid concentration ranges of 0.5–10 g/L for tartaric, 0.2–8.0 g/L for malic, 0.05–1.5 g/L for citric and 0.2–2.0 g/L for succinic acid. Standards of organic acids were purchased from Fluka.

Statistical analysis

Data were analyzed by the analysis of variance (ANOVA) using the software package STATISTICA v. 9 (*34*). Least significant difference (LSD) test was used for *post hoc* comparison of significant treatment mean values.

Results and Discussion

Results of yield components and basic grape juice properties (soluble solids, titratable acidity and pH) had previously been reported (*35*). The average yield per vine ranged from 3.47 to 4.14 kg. Soluble solids varied from 22.9 to 24.1 °Brix and were significantly higher in the treatments at veraison and veraison+3 stages than at the stage before blooming and control. Higher soluble solid content in both veraison stage treatments could be a consequence of higher transpiration of water from berries in veraison stage treatments, which occurred after the grapes were suddenly exposed to sunlight following leaf removal. Titratable acidity varied from 5.9 to 6.3 g/L and pH varied from 3.43 to 3.48. Both titratable acidity and pH were not significantly different among treatments.

Significantly higher concentrations of tartaric acid were obtained in the control sample and both veraison stage treatments than at the stages before blooming and berry set (Table 1). Kozina *et al.* (*36*) found higher concentration of tartaric acid in the control sample in comparison with two intensities of leaf removal performed at veraison stage on Sauvignon blanc and Riesling cultivars. On the other hand, more severe defoliation (five to eight leaves per shoot), performed at the stage before blooming or at berry set, resulted in higher concentrations of tartaric acid in grapevine juice and corresponding wines (*30,32,33*). This reaction is explained by the hypothesis that sudden increase in light and temperature caused by early leaf removal might lead to greater synthesis of tartaric acid (*33*). In a study conducted by Tardaguila *et al.* (*33*) this reaction was found in Carignan cultivar, while in Graciano cultivar the leaf removal reduced tartaric acid in one year of investigation and did not change its concentration in another year. These differences between cultivars are explained by genotype and variations in grapevine canopy porosity, which impacts light exposure and temperature in the fruit zone, and in this case Graciano clusters were less exposed to sunlight than Carignan clusters. As Istrian Malvasia is prone to the formation of dense canopies with less exposed clusters due to the regrowth of lateral shoots after the leaf removal, this fact may explain lower concentration of tartaric acid when leaf removal was applied early in the season. Moreover, in our study in the treatments at stages before blooming and berry set, the leaf removal was less severe compared to the previously mentioned studies (*30,32, 33*), resulting in a less exposed fruit zone.

Table 1. Effects of fruit zone leaf removal on the concentration of organic acids in Istrian Malvasia grape juice

Organic acid	γ/(g/L)				
	Control	Before blooming	Berry set	Veraison	Veraison+3
tartaric	$(2.91\pm0.21)^{a}$	$(2.42\pm0.25)^{b}$	$(2.32\pm0.19)^{b}$	$(2.96\pm0.31)^{a}$	$(2.84\pm0.26)^{a}$
malic	$(2.45\pm0.27)^{ab}$	$(2.76\pm0.23)^{a}$	$(2.68\pm0.32)^{a}$	$(2.22\pm0.18)^{b}$	$(2.40\pm0.22)^{ab}$
citric	0.27±0.02	0.31±0.05	0.31±0.04	(0.27±0.03)	(0.32±0.06)

Results are means of nine values (3 independent repetitions×triplicate analyses)±standard deviation (S.D.). Means within a row designated by different letters are significantly different by the LSD test at $p<0.05$

Treatments at stages before blooming and berry set significantly increased the concentration of malic acid than veraison treatment. Malic acid is prone to respiratory degradation under high temperatures (*37*). Consequently, the degradation of malic acid was more intensive in grapes treated at veraison stage, which were more exposed to sunlight during the phase of ripening than grapes from other treatments. Significantly lower concentrations of malic acid following leaf removal at veraison stage resulted also in Sauvignon blanc, but not in Riesling cultivar (*36*). In most other studies the concentration of malic acid was lower when leaf removal treatments were performed at stages before blooming or berry set (*30*,*32*,*33*) due to higher exposure of clusters to sunlight and higher temperatures, which hastens the malic acid degradation (*37*). In our study, no significant differences were observed between the control treatment and early leaf removal treatments (at before blooming and berry set stages). The reason for such response is the lower severity of defoliation performed on Istrian Malvasia cultivar and the consequent formation of lateral shoots on early defoliated vines, resulting in similar microclimate inside the canopy as in control treatment vines, thus not affecting the rate of malic acid degradation.

No differences among treatments were observed in the concentration of citric acid (Table 1), which is usually present at low concentrations in grapevine berries (*3*).

Effects of fruit zone leaf removal on the concentration of hydroxycinnamic acids in Istrian Malvasia grape juice are presented in Table 2. Concentrations of all analyzed hydroxycinnamic acids were significantly lower in grape juice samples obtained from before blooming stage leaf removal treatment, while the samples from the treatment at berry set stage had the highest concentrations of most individual hydroxycinnamic acids. Concentrations of *trans*-caftaric and caffeic acids were the highest in the samples treated at berry set stage, although the difference between this treatment and the control was not significant. Veraison and veraison+3 samples had lower concentrations of *trans*-caftaric and caffeic acids than berry set and control samples, but higher concentrations than before blooming samples. Caffeic and *trans*-caftaric acids, as the most abundant hydroxycinnamic acids in grape juice and wine (*3*), have high antioxidant potency towards human LDL oxidation *in vitro* (*38*), implying that the enhancement of their concentrations with canopy management practices could lead to better antioxidant properties of the resultant wines.

The concentration of total hydroxycinnamic acids was the highest in samples treated at berry set stage, but it did not differ significantly among control, berry set, veraison and veraison+3 samples, while before blooming stage treatment resulted in significantly lower concentrations of total hydroxycinnamic acids.

Unlike with tannins and anthocyanins, up to now little work has been done to determine how hydroxycinnamic acids vary with different viticultural practices (*5*). In a fruit zone leaf removal trial conducted on Pinot noir cultivar, leaf removal treatment at berry set stage resulted in the highest concentrations of *trans*-caftaric, *cis*- and *trans*-coutaric acids throughout maturation, although for this class of phenolic compounds leaf removal was less effective than for anthocyanins and flavonols (*29*).

All analyzed hydroxybenzoic acids were present at significantly lower concentrations in grape juice samples from before blooming stage treatment in comparison with other treatments (Table 3). Control and berry set treatments caused significantly higher concentrations of gallic acid than veraison and veraison+3 treatments. Protocatechuic acid was the most abundant in grape juice from veraison and veraison+3 stage treatments. The concentration of *p*-hydroxybenzoic acid did not significantly differ

Table 2. Effects of fruit zone leaf removal on the concentration of hydroxycinnamic acids (HCA) in Istrian Malvasia grape juice

HCA	γ/(mg/L)				
	Control	Before blooming	Berry set	Veraison	Veraison+3
caffeic	$(2.84\pm0.29)^{a}$	$(2.54\pm0.56)^{c}$	$(2.92\pm0.50)^{a}$	$(2.70\pm0.84)^{b}$	$(2.66\pm0.80)^{bc}$
p-coumaric	$(2.48\pm0.70)^{b}$	$(2.26\pm0.56)^{c}$	$(2.65\pm0.65)^{a}$	$(2.41\pm0.64)^{b}$	$(2.35\pm0.62)^{bc}$
ferulic	$(0.85\pm0.28)^{b}$	$(0.51\pm0.23)^{d}$	$(0.99\pm0.24)^{a}$	$(0.82\pm0.30)^{bc}$	$(0.72\pm0.30)^{c}$
trans-caftaric	$(9.93\pm1.14)^{a}$	$(8.17\pm1.53)^{c}$	$(10.17\pm1.09)^{a}$	$(9.35\pm1.42)^{b}$	$(9.57\pm1.29)^{b}$
trans-coutaric	$(8.72\pm0.67)^{a}$	$(7.80\pm0.95)^{b}$	$(8.52\pm0.88)^{a}$	$(8.58\pm1.01)^{a}$	$(8.81\pm1.15)^{a}$
trans-fertaric	$(1.31\pm0.36)^{b}$	$(1.10\pm0.30)^{c}$	$(1.52\pm0.41)^{a}$	$(1.36\pm0.51)^{b}$	$(1.43\pm0.51)^{ab}$
total HCA	26.13^{a}	22.38^{b}	26.77^{a}	25.23^{a}	25.54^{a}

Results are means of nine values (3 independent repetitions×triplicate analyses)±S.D. Means within a row designated by different letters are significantly different by the LSD test at $p<0.05$

Table 3. Effects of fruit zone leaf removal on the concentration of hydroxybenzoic acids (HBA) in Istrian Malvasia grape juice

HBA	γ/(mg/L)				
	Control	Before blooming	Berry set	Veraison	Veraison+3
gallic	$(12.61\pm1.05)^{a}$	$(7.20\pm0.41)^{c}$	$(12.77\pm1.03)^{a}$	$(10.62\pm1.29)^{b}$	$(10.82\pm1.21)^{b}$
protocatechuic	$(3.22\pm0.61)^{ab}$	$(1.33\pm0.26)^{c}$	$(3.14\pm0.72)^{b}$	$(3.43\pm0.34)^{a}$	$(3.37\pm0.40)^{a}$
p-hydroxybenzoic	$(2.24\pm0.39)^{a}$	$(0.71\pm0.17)^{b}$	$(2.30\pm0.27)^{a}$	$(2.16\pm0.44)^{a}$	$(2.19\pm0.36)^{a}$
vanillic	$(0.86\pm0.23)^{b}$	$(0.44\pm0.12)^{c}$	$(0.91\pm0.13)^{b}$	$(0.96\pm0.19)^{ab}$	$(1.06\pm0.28)^{a}$
syringic	$(1.82\pm0.71)^{ab}$	$(1.02\pm0.14)^{c}$	$(1.92\pm0.60)^{a}$	$(1.87\pm0.28)^{a}$	$(1.74\pm0.31)^{b}$
total HBA	20.76^{a}	10.70^{c}	21.04^{a}	19.05^{b}	19.18^{b}

Results are means of nine values (3 independent repetitions×triplicate analyses)±S.D. Means within a row designated by different letters are significantly different by the LSD test at $p<0.05$

among control, berry set, veraison and veraison+3 samples. Veraison+3 sample had the highest concentration of vanillic acid. Syringic acid was the most abundant in grape juice from berry set and veraison stage treatments. Control and berry set treatments resulted in significantly higher concentrations of total hydroxybenzoic acids than other treatments. Veraison and veraison+3 samples had intermediate concentrations, while before blooming sample had the lowest concentration of total hydroxybenzoic acids.

As it is well known, sunlight exposure of leaves and grapes is important in the synthesis of phenolic compounds in grapes (*6,7*). Berry set treatment resulted in the highest values of most phenolic acids in our study, indicating that leaf removal at berry set stage and the exposure of clusters to sunlight in the early phases of berry development favoured the synthesis of phenolic acids in the grapes. Both veraison samples had similar values of hydroxycinnamic acids as control and berry set samples, and lower values of hydroxybenzoic acids, which implies that higher exposure of grapes to sunlight during ripening of grapes as a consequence of leaf removal at veraison stage does not affect hydroxycinnamic acids but leads to a lower concentration of hydroxybenzoic acids. It should be stressed here that clusters of grapes treated at before blooming and berry set stages were not as much exposed to sunlight during ripening as they were in veraison stage treatments, owing to the regrowth of lateral shoots in the fruit zone of grapes treated at before blooming and berry set stages.

Most studies that investigated the impact of leaf removal on phenolic compounds were performed on red grapevine cultivars. In the majority of cases higher concentrations of anthocyanins and total phenolics in grapes and resultant wines were obtained if five to eight leaves per shoot were removed early in the season, at before blooming or berry set stages (*30–33*). Although some discrepancies have been noted between the effects of leaf removal on phenolic acids in our study and the effects of this practice on anthocyanins and total phenolics (*30–33*), it should be noted here that phenolic acids make only a small part of total phenolic compounds in red grapes and wines, and anthocyanins are not present in grapes of white grapevine cultivars (*3*).

Fruit zone leaf removal affects also the concentration of volatile compounds in grapes of white grapevine cultivars (*39,40*). Concentrations of total glycosides, monoterpene alcohols geraniol, nerol and linalool, and bound aromatic alcohols (benzyl alcohol and 2-phenylethanol) in fruit were higher if leaf removal from Riesling vines was performed two to three weeks after the full bloom in comparison with control vines without defoliation (*39*). In a study conducted on Riesling and Chardonnay cultivars, leaf removal positively affected the concentrations of total and phenol-free glycosides in grapes (*40*).

The concentrations of organic acids in wines followed the same trend as organic acids in grape juice (Table 4), although the differences among treatments were not significant. Before blooming and berry set samples had lower concentrations of tartaric acid in comparison with control, veraison and veraison+3 samples. Wine sample of before blooming treatment had the highest concentration of malic acid, but the difference between this and veraison treatment, as a treatment with the lowest value, was only 0.14 g/L. Concentrations of citric and succinic acid did not differ considerably among treatments. Recent studies have shown that apart from the acidic taste, malic acid also tastes astringent and enhances the overall perception of astringency (*1,2*). By performing the fruit zone leaf removal at different grapevine phenological stages, it is possible to manipulate sensory characteristics of wine as a final product.

Table 4. Effects of fruit zone leaf removal on the concentration of organic acids in Istrian Malvasia wine

Organic acid	γ/(g/L)				
	Control	Before blooming	Berry set	Veraison	Veraison+3
tartaric	1.26±0.14	1.17±0.19	1.15±0.09	1.32±0.19	1.25±0.10
malic	2.32±0.17	2.43±0.41	2.36±0.25	2.29±0.17	2.30±0.16
citric	0.28±0.03	0.29±0.05	0.27±0.05	0.33±0.04	0.34±0.06
succinic	0.74±0.06	0.72±0.10	0.76±0.07	0.75±0.10	0.76±0.07

Results are mean values of triplicate analyses±S.D. No significant differences by the LSD test at $p<0.05$ were obtained among treatments

Before blooming treatment resulted in the lowest concentrations of most hydroxycinnamic acids in wine, except for *p*-coumaric acid, which was present at the lowest concentration in veraison+3 sample (Table 5). Control, berry set, veraison and veraison+3 wine samples had similar concentrations of most hydroxycinnamic acids, and the concentration of total hydroxycinnamic acids in these samples was significantly higher than in before blooming sample. As the concentration of hydroxycinnamic acids in wine is highly correlated with the antioxidant capacity of wines (*8,24*), leaf removal can be considered as a practice which impacts the antioxidant capacity of the resultant wines. The concentration of *p*-coumaric acid, a potential precursor of volatile phenols in wine (*3*), was similar in all treatments, meaning that before blooming treatment is not less prone to the potential risk of the formation of volatile phenols, although it resulted in the lowest concentration of total hydroxycinnamic acids. Furthermore, organoleptic properties of the resultant wines can be modified with leaf removal since hydroxycinnamic acids may contribute to astringency of grape juice and wine (*1,2*).

All analyzed hydroxybenzoic acids were present at the lowest concentrations in wines obtained from before blooming stage treatment (Table 6). Consequently, this treatment resulted in significantly lower concentration of total hydroxybenzoic acids (5.36 mg/L) in comparison with other treatments, which did not vary significantly in the concentration of total hydroxybenzoic acids, ranging from 7.51 to 7.69 mg/L. Concentrations of individual hydroxycinnamic and hydroxybenzoic acids were slightly higher than in a previous research concerning the phenolic composition of Istrian Malvasia wines (*24*), with the exception of ferulic and protocatechuic acids.

Principal component analysis was used to examine the effects of fruit zone leaf removal treatments using grape juice composition variables (Fig. 1) and wine composition variables (Fig. 2) which showed significant differences when applying the analysis of variance (ANOVA). For grape juice, PC1 explained 62.0 % of total variance, and was characterized by all phenolic acids on the positive side. PC1 separated the before blooming stage treatment from all other treatments, which is in accordance with the results obtained by the LSD test (Tables 2 and 3). PC2 explained 16.5 % of the variance and was mostly explained by malic acid having a positive loading and tartaric acid with a negative loading. PC2 separated the before blooming and berry set treatments from the other treatments, and especially from veraison and veraison+3 treatments, which were situated on the negative side of PC2.

Considering the differentiation of the wines, PC1 explained 42.2 % of total variance and was characterized by *p*-hydroxybenzoic, *trans*-caftaric, vanillic, ferulic and syringic acids having positive loadings. PC2 explained 25.3 % of the variance and had ferulic acid on the positive side and *trans*-fertaric acid on the negative side. It can be seen that wines obtained from before blooming treatment, which were situated on the negative side of PC1, were clearly separated from the wine samples corresponding to other treatments, which overlapped on the plot.

Table 5. Effects of fruit zone leaf removal on the concentration of hydroxycinnamic acids (HCA) in Istrian Malvasia wine

HCA	γ/(mg/L)				
	Control	Before blooming	Berry set	Veraison	Veraison+3
caffeic	3.33±0.34	3.01±0.38	3.47±0.45	3.34±0.46	3.30±0.52
p-coumaric	2.67±0.40	2.78±0.31	2.89±0.38	2.59±0.50	2.56±0.34
ferulic	$(0.95±0.26)^{ab}$	$(0.79±0.14)^{b}$	$(1.01±0.19)^{a}$	$(0.86±0.21)^{ab}$	$(0.89±0.21)^{ab}$
trans-caftaric	$(5.61±1.08)^{a}$	$(4.25±1.20)^{b}$	$(5.72±1.14)^{a}$	$(5.49±1.05)^{a}$	$(5.53±1.14)^{a}$
trans-coutaric	4.25±0.58	4.05±0.98	4.36±0.98	4.18±0.86	4.21±0.65
trans-fertaric	$(0.87±0.21)^{ab}$	$(0.67±0.11)^{b}$	$(0.99±0.25)^{a}$	$(0.78±0.30)^{ab}$	$(0.82±0.41)^{ab}$
total HCA	17.68^{a}	15.55^{b}	18.44^{a}	17.24^{a}	17.31^{a}

Results are mean values of triplicate analyses±S.D. Means within row designated by different letters are significantly different by the LSD test at $p<0.05$

Table 6. Effects of fruit zone leaf removal on the concentration of hydroxybenzoic acids (HBA) in Istrian Malvasia wine

HBA	γ/(mg/L)				
	Control	Before blooming	Berry set	Veraison	Veraison+3
gallic	3.59±0.55	2.94±0.77	3.65±0.61	3.45±0.58	3.50±0.79
protocatechuic	$(0.72±0.16)^{a}$	$(0.45±0.14)^{b}$	$(0.71±0.21)^{a}$	$(0.75±0.20)^{a}$	$(0.78±0.25)^{a}$
p-hydroxybenzoic	$(1.16±0.25)^{a}$	$(0.65±0.21)^{b}$	$(1.05±0.15)^{a}$	$(1.15±0.23)^{a}$	$(1.19±0.25)^{a}$
vanillic	$(0.95±0.18)^{a}$	$(0.60±0.11)^{b}$	$(1.01±0.19)^{a}$	$(1.05±0.10)^{a}$	$(1.07±0.31)^{a}$
syringic	$(1.20±0.35)^{a}$	$(0.72±0.16)^{b}$	$(1.25±0.37)^{a}$	$(1.11±0.25)^{a}$	$(1.15±0.21)^{a}$
total HBA	7.62^{a}	5.36^{b}	7.67^{a}	7.51^{a}	7.69^{a}

Results are mean values of triplicate analyses±S.D. Means within row designated by different letters are significantly different by the LSD test at $p<0.05$

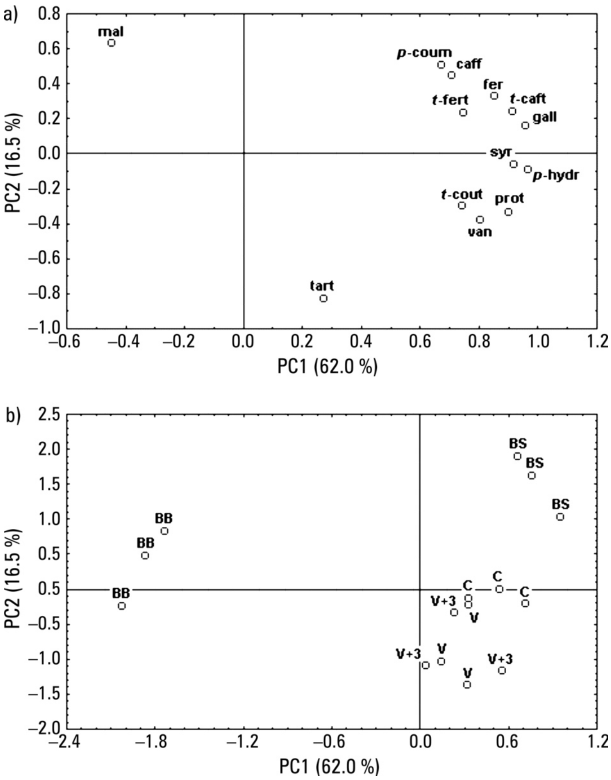

Fig. 1. Principal component analysis of: a) Istrian Malvasia grape juice composition variables which showed significant differences by ANOVA, and b) projection of grape juice samples on the two-dimensional PCA plot
Acids: caff=caffeic, *t*-caft=*trans*-caftaric, *p*-coum=*p*-coumaric, *t*-cout=*trans*-coutaric, fer=ferulic, *t*-fert=*trans*-fertaric, gall=gallic, *p*-hydr=*p*-hydroxybenzoic, mal=malic, prot=protocatechuic, syr=syringic, tart=tartaric, van=vanillic; treatments: BB=before blooming, BS=berry set, V=veraison, V+3=veraison+3, C=control

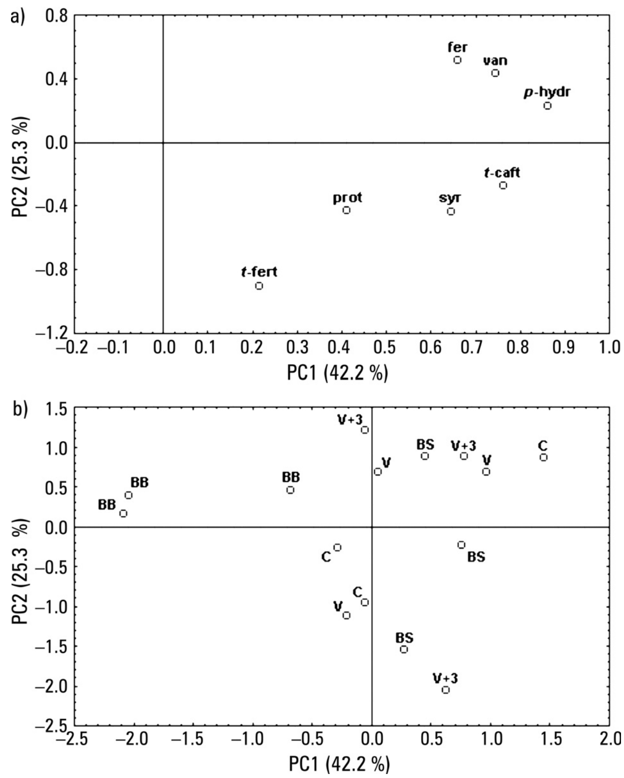

Fig. 2. Principal component analysis of: a) Istrian Malvasia wine composition variables which showed significant differences by ANOVA, and b) projection of wine samples on the two-dimensional PCA plot
For abbreviations see Fig. 1

Higher concentrations of phenolic acids in wine are generally considered desirable in terms of antioxidant capacity of wines and possible benefits on human health (*38*). On the other hand, enhanced concentration of phenolic compounds in white wines may have some undesirable effects such as higher sensitivity of wines to oxidation, a potential risk of the formation of volatile phenols and the contribution to undesirable astringency and bitterness (*3*).

Conclusions

Due to the limited knowledge about the impact of canopy management practices on the phenolic composition of grape juice and wine of white grapevine cultivars, our research represents one of the first studies in this field. Fruit zone leaf removal affected the concentrations of both hydroxycinnamic and hydroxybenzoic acids, as well as organic acids, in grape juice and wine. The highest impact on the concentration of phenolic acids was achieved with defoliation treatment at the stage before blooming, which resulted in the lowest concentration of hydroxycinnamic and hydroxybenzoic acids in grape juice and wine. Fruit zone leaf removal can therefore be used in order to manage grape juice and wine composition of white grapevine cultivars.

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