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The effect of high power ultrasound on phenolic composition, chromatic characteristics, and aroma compounds of red wines

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Summary

High power ultrasound (HPU) is a novel, non-thermal technology the application of which has been primarily evaluated in managing food quality. The application of high power ultrasound in wine technology is therefore directed at modulating microbial activity during fermentation, extraction of phenolic and aroma compounds from grapes to must, as well as at accelerating aging reactions in wine. The main aim of this article was to evaluate the effect of different HPU process parameters on sustaining the phenolic and aroma composition of red wine and its colour characteristics. Three different red wines, including Cabernet Sauvignon, Merlot, and Plavac mali, were treated with high power ultrasound (20kHz), considering the variations in ultrasound probe diameter size (12.7 and 19 mm), amplitude level (20, 30, and 40 %), and processing time (2, 4, and 6 minutes). Total polyphenol content, total anthocyanin concentration, and chromatic characteristics were analyzed by spectrophotometry, free anthocyanins were analysed by high performance liquid chromatography, and wine aroma compounds were analyzed by gas chromatography combined with solid-phase microextraction. The obtained results show that ultrasonic irradiation induces chemical changes in phenolic composition, chromatic characteristics, and aroma compounds concentration, and accelerates chemical reactions responsible for wine aging. The intensity of the mentioned chemical changes depends on the selected processing parameters and on the treated variety. Among three different parameters, the selection of the probe diameter was showed to be most significant factor influencing chemical composition, followed by the amplitude level and processing time. The smaller diameter probe size (12.7 mm), lowest amplitude (20%), and a shorter processing time (2 minutes) showed a more favourable and lighter effect on the chemical composition of the treated red wines.

Keywords: high power ultrasound, red wine, polyphenols, colour, aroma

Introduction

High power ultrasound (HPU) is considered to be an efficient, non-thermal, environmentally friendly technology which typically utilizes sound intensities above 1 W/cm^2 and frequencies in the power ultrasound range (20-100 kHz) (Leighton, 1998; Villamiel and de Jong, 2000). Acoustic cavitation (formation and implosive collapse of unstable, high-energy bubbles), induced by ultrasonic irradiation in liquid, generates intense localized pressure and temperature gradients, inducing chemical, physical, or even mechanical effects (García Martín and Sun, 2013). Therefore, the application of HPU has been evaluated in numerous food and beverage processes (Knorr et al., 2004) and has yet to be applied to the winemaking industry, since it may yield potential benefits. High power ultrasound represents an attractive and promising green alternative, complementing SO_2 use, in order to reduce or to eliminate spoilage microorganisms present before fermentation or to control and modulate the microbial activity of spoilage or inoculated microorganisms during primary or secondary fermentation (Gracin et al., 2016; Jiranek et al., 2008; García Martín and Sun, 2013). Its application would be a worthy substitute for the further addition of additives or time-consuming wine filtration and it could be conceivably achieved via a flow-through system during juice, must, or wine transfer from tank to tank or alternatively by the direct treatment of a tank or a barrel. Additionally, HPU irradiation of wine post primary fermentation can not only reduce microbial population, but also liberate the essential growth factors and nutrients from disrupted cells (Jiranek et al., 2008). Furthermore, as steam treatments are not effective enough, HPU technology can be potentially used as a convenient method for barrel sanitation and decontamination in the incidence of *Dekkera*/*Brettanomyces* spoilage problems in oak (Yap et al., 2007; Jiranek et al., 2008). Moreover, some recent studies pointed to HPU as a simple and rapid technique for enhancing the extraction of polyphenols and wine flavour components from grape to must (Hernanz et al., 1999; Carrera et al., 2012), as well as a physical method for accelerating the aging process in wine by promoting the polymerization of phenolic

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compounds (Masuzawa et al., 2000; García Martín and Sun, 2013; Ferraretto and Celotti, 2016). High power ultrasound was not able to significantly influence the basic oenological parameters of wine, like pH, total and volatile acidity, the content of sulphur dioxide, ethanol, and sugars, which provide the overall wine quality (Cui et al., 2012; Zhang et al., 2016; García Martín and Sun, 2013).

There is little published data about the effect of HPU on the phenolic and aroma composition responsible for the colour, flavour, and taste of wine, and that is why more attention is needed in the selection of the treatment parameters for preserving the wine compounds which determine the mentioned sensorial properties (Masuzawa et al., 2000; Ferraretto and Celotti, 2016; Singleton and Draper, 1963; Zhang et al., 2016).

Therefore, the main aim of this research was to observe the impact of different HPU treatments (variating probe diameter, amplitude intensity, and processing time) on the chemical composition, namely polyphenol concentration, chromatic characteristics, and aroma compounds, of the three red wine varieties, Cabernet Sauvignon, Merlot, and Plavac mali.

Materials and methods

Wine samples

The research was conducted on three quality dry red wines, varieties Cabernet Sauvignon (Agrolaguna, Poreč, Croatia), Merlot (Agrolaguna, Poreč, Croatia), and Plavac mali (Premium, Skaramuča, Pelješac, Croatia); all vintage 2014.

Chemicals

Ethanol, hydrochloric acid (37%), and formic acid were obtained from Carlo Erba (Val del Reuil, France). Sodium hydrogen sulphite was purchased from Acros (Gell, Belgium). Ethanol (96%) was obtained from Gram-Mol, from Kemika (Zagreb, Croatia). The Folin Ciocalteu's reagents, gallic acid, sodium carbonate, as well as the aroma reference standards, were also purchased from Sigma Aldrich (Saint Louis, USA). Methanol and acetonitrile (HPLC grade) were purchased from J. T. Baker (Deventer, the Netherlands) and Panreac (Barcelona, Spain). Analytical standards (delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, malvidin-3-*O*-glucoside) were obtained from Polyphenols (Sanders, Norway).

Ultrasound equipment and treatments

An ultrasonic processor (S-4000, Misonix Sonicators, Newtown, CT, USA) with 12.7 and 19 mm diameter probes, set at the constant frequency of 20 kHz was used for sonication. The ultrasound probe was submerged to a depth of 20 mm and centred in a 250 mL glass baker containing 100 mL of the wine sample. To assess the possible effects of the HPU treatment, the experimental test basis included variations of amplitude levels (iii): 20% (A), 30% (B), and 40% (C), and processing time (iv): 2 minutes (a), 4 minutes (b), and 6 minutes (c) for each red wine cultivar (i): Cabernet Sauvignon (CS), Merlot (M), or Plavac mali (PL) and single diameter of the probe (ii): 12.7 mm (1) or 19 mm (2), while the sample temperature was maintained at 25 °C by ice-water cooling of the reactor during the whole period of treatment. All treated samples were line coded in a way that indicates the wine cultivar, the diameter of the probe, the amplitude level, and the processing time. Each treatment was conducted in duplicate. The control sample represents the wine sample not exposed to the treatment.

The chemical analysis was conducted on 3 control and 20 of the 54 total treated wine samples. The selection of the treated samples was based on the results of the sensory analysis, in which the overall impact of the HPU treatment on colour, aroma, and taste was evaluated. The sensorial analysis was carried out by a trained panel group (14 judges) from the Faculty of Food Technology and Biotechnology, University of Zagreb, using a verbal 9-point hedonic scale (1=dislike extremely, 2=dislike very much, 3=dislike moderately, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8= like very much, 9= like extremely). The selected samples were those graded with a 5 (neither like nor dislike), since they represented the lower limit of treatment acceptability, as well as those lowest-rated in the group, in order to gain the information on the possible final negative outcomes. The selected wine samples included (i) CS_1Ac, CS_1Bb, CS_1Cb, and CS_1Cc, (ii) CS_2Aa, CS_2Ab, and CS_2Cc, (iii) M_1Cb, M_1Bb, and M_1Cc, (iv) M_2Aa, M_2Ab, and CS_2Cb, (v) PL_1Aa, PL_1Ba, and PL_1Cc, (vi) PL_2Aa, PL_2Ba, PL_2Cb, and PL_2Cc.

Chemical analysis

Spectrophotometric analyses were performed on a double-beam Specord 50 Plus spectrophotometer (AnalytikJena, Jena, Germany) and were all

conducted in duplicate. The total phenolic content was determined using the Folin Ciocalteu method (Singleton and Rossi, 1965), the total anthocyanin content by the bisulphite bleaching method (Ribereau-Gayon and Stonestreet, 1965), and the analysis of the chromatic characteristics of wine [L (clarity), a (red/green colour component), b (blue/yellow colour component), C (chroma), and h (tone)] according to the CIELab (CIE, 1986).

The HPLC analysis of the nine free anthocyanin compounds (3-*O*-glucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin, 3-*O*-acetylglucosides and 3-(6-*O*-*p*-coumaroyl) glucosides of peonidin and malvidin) was performed using the Agilent 1200 Series HPLC (Agilent Technologies, Santa Clara, CA, SAD) coupled with a Diode Array Detector (DAD) (Lorrain et al., 2011).

The wine sample aroma was analyzed by gas chromatography coupled with mass spectrometry (GC-MS) using an Agilent Gas Chromatograph 6890 series connected with an Agilent 5973 Inert mass-selective detector (Agilent Technologies, Santa Clara, CA, USA) and operated according to the method conditions (Câmara et al., 2006). Prior to the GC/MS analysis, volatile compounds were extracted from the wine by headspace solid-phase microextraction (HS-SPME) using a 100 μm PDMS fibre (Supelco, Bellefonte, USA) by following the method (Tomašević et al., 2016). The identified volatile compounds included ethyl esters (ethyl acetate, ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, diethyl succinate), acetate esters (*i*-butyl acetate, *i*-amyl acetate, 2-phenylethyl acetate), higher alcohols (*i*-butanol, *i*-amyl alcohol, 2,3-butanediol, 1-hexanol and 2-phenylethanol), volatile phenols (vanillin, 4-ethyl guaiacol), and terpenes (linalool and α-terpineol).

Data analysis

The significant differences among the three red wine varieties for each of the constituents were determined by one-way analysis of variance (ANOVA) using the Statistica V.10 software (StatSoft Inc., Tulsa, USA). Tukey's honestly significant difference (HSD) test ($p<0.05$) was used for comparison when the samples differed significantly after ANOVA was performed. The principal component analysis (PCA) was used to examine any possible grouping of control and treated wine samples, due to the speciality of each variety and different HPU treatments, mainly different probe diameter.

Results and discussion

The spectrophotometrically obtained results of total polyphenols, total anthocyanins, and chromatic characteristics (L, a, b, c, H) of 3 control and 20 treated wine samples of Cabernet Sauvignon, Merlot and Plavac mali are showed in Table 1.

The results presented in Table 1, indicate that ultrasonic irradiation causes a statistically significant decrease in total polyphenols and total anthocyanins in the samples of all three red varieties. That is in accordance with the conclusions of previously conducted scientific studies. The conclusions they reached refer to the ability of the HPU treatment to promote the polymerization of the phenolic compounds in red wines, which is otherwise a natural consequence of the wine aging process (Zhang et al., 2016; Ferraretto and Celotti, 2016). The reduction share depends on the selected processing parameters and the properties of the treated variety. The particularly noticeable changes of the polyphenol concentrations are noticed in the Cabernet Sauvignon variety samples, where the concentration decreased for about 3%, compared to the control sample (2032.73 mg/L). The most significant change in phenolic concentration occurred in sample CS_2Cc (1960.00 mg/L) after treatment with the 19 mm diameter probe, 40% amplitude during 6 minutes, and sample CS_1Cc (1970.00 mg/L), in which the 12.7 mm diameter probe was used. The similar trend of total polyphenols concentration decrease can be seen in the two remaining varieties, Merlot and Plavac mali. The most significant concentration of total polyphenols in the control samples was detected in the Plavac mali variety samples (3272.27 mg/L). This amount decreased for 0.5-1.4% after the treatment with a 12.7 mm diameter probe, with regard to the usage of the 19 mm diameter probe when it caused a 0.9-2.2% concentration decrease, depending on the amplitude and the duration of the treatment. The detected concentration of total polyphenols in Merlot samples decreased from 1774.09 mg/L in the control sample to 1727.73 mg/L in sample M_2Cb treated with the larger diameter size probe with 40% amplitude level during 4 minutes.

Furthermore, the similar trend of slight concentration decrease is observed in the total anthocyanin concentration. The initial anthocyanin concentration of the Cabernet Sauvignon variety decreased from 133.56 mg/L in the control sample for 0.8% in CS_1Ac to 3.4% in CS_1Cc, of the Merlot variety from 127.04 mg/L in the control sample for 0.6% detected in M_1Bb to 2.5% in M_2Cb, and of the Plavac mali variety from 112.37 mg/L for 2.5% detected in PL_2Cc, depending on the treatment

applied. A slight decrease in the concentrations of treated samples, in comparison with the control ones, was not proved to be statistically significant, which agrees with the research from Zhang et al. (2016) and Ferraretto and Celotti (2016), where no significant decrease in anthocyanin concentration was noted, except in treatments which used high temperature.

The analyzed samples of Cabernet Sauvignon, Merlot, and Plavac mali showed statistically significant changes in the chromatic characteristics of wine (L, a, b, c, H), manifested as a slight decrease of each characteristic. The particular effect of HPU on the chromatic characteristics of red wine has occurred using a 19 mm diameter probe, higher amplitude intensity, and longer processing time. Even though little information can be found in literature about the effects of ultrasound on the colour of red wine, Masuzawa et al. (2000) reported that significant changes occurred in the analyzed chromatic composition of red wines submitted to maturation acceleration by applying ultrasound at different power levels during 10 days.

Furthermore, the concentrations of 9 free anthocyanins (delfinidine-3-*O*-glucoside, cyanidin-3-O-glucoside, petunidin-3-O-glucoside, peonidine-3-*O*-glucoside, malvidin-3-*O*-glucoside, peonidin-3-*O*-glucoside acetate, malvidin-3-*O*-glucoside acetate, peonidin-3-(6-*O*-*p*-coumaroyl)glucoside, and malvidin-3-(6-*O*-*p*-coumaroyl)glucoside) in control and treated samples of Cabernet Sauvignon, Merlot and Plavac mali wines were determined by using high performance liquid chromatography (HPLC) and the results obtained are listed in Table 2. The results confirmed that malvidin-3-*O*-glucoside is the most dominant free anthocyanin in red wine varieties. The composition of free anthocyanins, in which malvidin-3-*O*-glucoside occupies 38-49% of them, is characteristic for all *Vitis vinifera* L. varieties (Ky et al., 2014). The obtained anthocyanin concentration depends on the chosen processing parameters, the nature of the target compound, and the red variety treated. There are no statistically significant changes detected in the concentrations of anthocyanin glucosides, except for a decrease in malvidin-3-*O*-glucoside of the Plavac mali variety, where the concentration was reduced from the initial 16.76 mg/L detected in the control sample (PL) to 16.04 mg/L detected in PL_2Cb after the treatment with a 19 mm probe, 40 % amplitude during 4 minutes, and the Merlot variety, where the initial value was reduced from 18.07 mg/L detected in the control sample (M) for about 1% to the 17.75 mg/L in sample M_1Cc and 17.78 mg/L in M_2Cb. The statistically significant changes in the content of anthocyanin glucoside, in regard to the control samples, were also identified in the Cabernet Sauvignon variety, as a decrease of delphinidin-3-*O*-glucoside (samples CS_2Ab and CS_2Cc) and cyaniding-3-*O*-glucoside (sample CS_1Bb), and Plavac mali variety, where only the delphinidin-3-*O*-glucoside concentration significantly decreased. The intensity of the change was dependent on the default processing parameters.

The concentration of acylated anthocyanins, peonidin-3-*O*-glucoside acetate and malvidin-3-*O*-glucoside acetate, were not significantly diminished with the application of the HPU treatment, irrespective of the cultivated variety or processing parameters, indicating a higher resistance of the acylated forms of anthocyanins to the effect of the HPU treatment. The same can be deduced from the peonidin-3-(6-*O*-*p*-coumaroyl) glucoside and malvidin-3-(6-*O*-*p*-coumaroyl) glucoside results, where no significant change in concentrations occurred during treatment. The further comparison of the initial anthocyanins composition of the three varieties shows that the variety Cabernet Sauvignon contains a higher content of anthocyanin glucoside and anthocyanin glucoside acetate compared to the other two, while the most significant concentrations of anthocyanin glucoside coumarate are present in the Plavac mali variety. The analysis of the ultrasound treated samples suggests that the partial degradation and polymerization of free anthocyanins occurs during the treatment, as their concentration decreases.

Using the SPME-GC-MS technique in the aroma analysis of the control and the treated wine samples of three red varieties, 18 volatile compounds were identified. The list of compounds includes acetate esters (*i*-butyl acetate, *i*-amyl acetate, 2-phenylethylacetate), ethyl esters (ethyl acetate, ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, diethyl succinate), higher alcohols (*i*-butanol, i-amyl alcohol, 2,3-butanediol, 1-hexanol, 2-phenylethanol), volatile phenols (vanillin and 4-ethyl guaiacol), and terpenes (linalool and α-terpineol). The quantitative results of the identified compounds are presented in Table 3.

Earlier studies indicated that more attention should be given to the selection of the adequate process parameters, since the application of inadequate ones results in the reduction and formation of negative volatile compounds or the reduced overall aroma intensity due to the known degassing effect of ultrasound (Singleton and Draper, 1963). Such impact of ultrasound irradiation can be observed from the results presented in Table 3. HPU treatments have a negative effect on preserving the content of volatile acetate and ethyl esters, the concentration of which tends to decrease depending on the selected processing parameters and the red wine variety.

Table 1. Concentration of total polyphenols, total anthocyanin, and chromatic characteristics values in control and HPU treated wine samples of Cabernet Sauvignon, Merlot, and Plavac mali

Sample	TP	TA	L	A	b	C	H
CS	2032.73±10.29^{b}	133.56±2.54 ^{a}	28.19±0.43^{d}	52.43±0.34^{d}	39.62±0.43^{d}	65.72±0.54^{d}	0.65±0.00^{c}
CS_1Ac	1999.55±17.36^{a,b}	132.47±2.30^{a}	27.42±0.15^{a,b}	51.68±0.09^{a,b,c}	38.80±0.06^{a,b,c}	64.62±0.10^{a,b,c}	0.64±0.00^{a,b}
CS_1Bb	2009.55±5.79^{a,b}	132.14±3.72^{a}	27.70±0.04^{b,d}	51.98±0.01^{c,d}	39.14±0.01^{c,d}	65.07±0.02^{c,d}	0.65±0.00^{b,c}
CS_1Cb	1987.27±14.14^{a,b}	131.14±3.71^{a}	27.48±0.03^{a,b}	51.84±0.05^{b,c,d}	39.02±0.05^{b,c,d}	64.89±0.07^{b,c,d}	0.65±0.00^{b,c}
CS_1Cc	1970.00±21.86^{a}	131.14±3.01^{a}	27.21±0.08^{a,b,c}	51.59±0.06^{a,b,c}	38.74±0.07^{a,b}	64.51±0.09^{a,b,c}	0.64±0.00^{a,b}
CS_2Aa	1998.73±2.44^{a,b}	131.05±2.54^{a}	27.13±0.01^{a,b,c}	51.33±0.24^{a,b}	38.43±0.19^{a,b}	64.12±0.30^{a,b}	0.64±0.00^{a,b}
CS_2Ab	1969.55±22.50^{a}	131.47±1.89^{a}	26.950±0.04^{a,c}	51.36±0.00^{a,b}	38.41±0.01^{a,b}	64.13±0.01^{a,b}	0.64±0.00^{a}
CS_2Cc	1960.00±7.71^{a}	129.11±2.57^{a}	26.70±0.03^{a}	51.22±0.05^{a}	38.25±0.04^{a}	63.93±0.07^{a}	0.64±0.00^{a}
M	1774.09±9.64^{a}	127.04±2.68^{a}	28.52±0.20^{b}	55.14±0.36^{b}	42.07±0.10^{d}	69.36±0.34^{b}	0.65±0.00^{a}
M_1Cb	1759.55±37.93^{a}	126.02±1.77^{a}	28.34±0.01^{b}	55.05±0.00^{b}	41.78±0.01^{c}	69.11±0.01^{b}	0.65±0.00^{a}
M_1Bb	1749.55±4.50^{a}	126.35±2.00^{a}	28.66±0.25^{b}	55.17±0.04^{b}	41.86±0.01^{c}	69.25±0.02^{b}	0.65±0.00^{a}
M_1Cc	1732.27±13.50^{a}	125.83±2.39^{a}	28.28±0.05^{b}	55.04±0.00^{b}	41.78±0.01^{c}	69.10±0.01^{b}	0.65±0.00^{a}
M_2Aa	1739.55±13.50^{a}	124.06±1.06^{a}	27.68±0.10^{a}	54.25±0.03^{a}	41.27±0.04^{b}	68.16±0.05^{a}	0.65±0.00^{a}
M_2Ab	1740.00±9.00^{a}	124.03±0.69^{a}	27.69±0.04^{a}	54.29±0.03^{a}	41.34±0.04^{b}	68.23±0.05^{a}	0.65±0.00^{a}
M_2Cb	1727.73±7.17^{a}	123.91±2.12^{a}	27.40±0.02^{a}	54.09±0.03^{a}	41.08±0.03^{a}	67.92±0.04^{a}	0.65±0.00^{a}
PL	3272.27±27.64^{a}	112.37±3.13^{a}	27.32±0.03^{b,c}	50.67±0.14^{a,b}	40.71±0.13^{b}	65.00±0.19^{a,b}	0.68±0.00^{b,c}
PL_1Aa	3258.64±12.21^{a}	112.28±1.82^{a}	27.66±0.06^{d}	50.98±0.01^{b}	41.06±0.02^{d}	65.46±0.00^{c}	0.68±0.00^{c}
PL_1Ba	3253.18±16.07^{a}	111.49±1,12^{a}	27.52±0.02^{c,d}	50.98±0.02^{b}	41.00±0.03^{c,d}	65.42±0.03^{c}	0.68±0.00^{b,c}
PL_1Cc	3226.82±8.36^{a}	111.42±0.72^{a}	27.23±0.07^{a,b,c}	50.83±0.01^{a,b}	40.73±0.04^{b,c}	65.14±0.03^{b,c}	0.68±0.00^{a,b}
PL_2Aa	3245.91±14.78^{a}	110.78±1.06^{a}	27.19±0.00^{a,b}	50.78±0.03^{a,b}	40.72±0.03^{b}	65.09±0.04^{b,c}	0.68±0.00^{a,b}
PL_2Ba	3232.33±28.33^{a}	110.04±1.53^{a}	27.13±0.02^{a,b}	50.70±0.08^{a,b}	40.63±0.07^{a,b}	64.97±0.10^{a,b}	0.68±0.00^{a,b}
PL_2Cb	3229.55±12.21^{a}	109.91±1.01^{a}	27.02±0.08^{a,b}	50.70±0.05^{a,b}	40.55±0.10^{a,b}	64.92±0.11^{a,b}	0.68±0.00^{a}
PL_2Cc	3202.27±10.93^{a}	109.62±1.89^{a}	26.95±0.18^{a}	50.53±0.16^{a}	40.38±0.03^{a}	64.68±0.14^{a}	0.67±0.00^{a}

Values are expressed in mg/L as average value of two repetitions ± standard deviation (n=2). Abbreviations: TP: total polyphenols; TA: total anthocyanins; L:clarity; a: red/green colour component; b: blue/yellow colour component; c: chroma; H: tone; CS: Cabernet Sauvignon; M: Merlot; PL: Plavac mali; 1: 12.7 mm probe diameter; 2: 19 mm probe diameter; A: 20% amplitude; B: 30% amplitude; C: 40% amplitude; a: 2 minutes; b: 4 minutes; c: 6 minutes

Table 2. Concentration of free anthocyanins in control and HPU treated wine samples of Cabernet Sauvignon, Merlot, and Plavac mali

Sample	Df*	Cy*	Pt*	Pn*	Mv*	PnAc*	MvAc*	PnCm*	MvCm*
CS	2.91±0.06^{b}	0.63±0.01^{c}	2.59±0.06^{a}	2.08±0.01^{a}	20.34±0.20^{a}	0.86±0.01^{a}	5.35±0.08^{b}	0.32±0.00^{a}	1.35±0.03^{c}
CS_1Ac	2.82±0.02^{a,b}	0.52±0.01^{a,b}	2.51±0.01^{a}	2.06±0.01^{a}	19.89±0.14^{a}	0.86±0.00^{a}	5.16±0.03^{a,b}	0.30±0.01^{a}	1.08±0.04^{a,b,c}
CS_1Bb	2.82±0.03^{a,b}	0.48±0.01^{a}	2.50±0.04^{a}	2.06±0.02^{a}	19.92±0.22^{a}	0.86±0.03^{a}	5.21±0.10^{a,b}	0.29±0.03^{a}	1.03±0.10^{a,b}
CS_1Cb	2.84±0.02^{a,b}	0.49±0.01^{a,b}	2.53±0.04^{a}	2.07±0.02^{a}	19.98±0.03^{a}	0.85±0.02^{a}	5.21±0.01^{a,b}	0.31±0.01^{a}	1.30±0.02^{b,c}
CS_1Cc	2.85±0.04^{a,b}	0.49±0.01^{a,b}	2.54±0.02^{a}	2.05±0.06^{a}	19.88±0.37^{a}	0.85±0.00^{a}	5.23±0.04^{a,b}	0.31±0.00^{a}	1.19±0.14^{a,b,c}
CS_2Aa	2.84±0.01^{a,b}	0.55±0.06^{a,b,c}	2.51±0.03^{a}	2.04±0.02^{a}	19.77±0.14^{a}	0.84±0.00^{a}	5.10±0.07^{a}	0.29±0.02^{a}	1.01±0.07^{a,b}
CS_2Ab	2.70±0.02^{a}	0.57±0.01^{b,c}	2.51±0.00^{a}	2.06±0.02^{a}	19.75±0.21^{a}	0.83±0.03^{a}	5.12±0.07^{a,b}	0.29±0.02^{a}	1.00±0.08^{a,b}
CS_2Cc	2.74±0.08^{a}	0.49±0.01^{a,b}	2.49±0.01^{a}	2.05±0.01^{a}	19.69±0.02^{a}	0.84±0.02^{a}	5.04±0.02^{a}	0.30±0.02^{a}	0.99±0.07^{a}
M	2.87±0.02^{a}	0.18±0.00^{a}	2.53±0.02^{a}	2.04±0.00^{a}	18.07±0.02^{b}	0.62±0.03^{a}	3.29±0.07^{a}	0.36±0.03^{a}	1.50±0.14^{a}
M_1Cb	2.84±0.08^{a}	0.17±0.00^{a}	2.48±0.00^{a}	2.02±0.00^{a}	17.88±0.07^{a,b}	0.59±0.04^{a}	3.20±0.06^{a}	0.35±0.02^{a}	1.47±0.11^{a}
M_1Bb	2.84±0.08^{a}	0.18±0.00^{a}	2.46±0.02^{a}	1.92±0.02^{a}	17.82±0.08^{a}	0.61±0.00^{a}	3.21±0.01^{a}	0.35±0.02^{a}	1.47±0.11^{a}
M_1Cc	2.76±0.03^{a}	0.18±0.00^{a}	2.46±0.01^{a}	1.91±0.01^{a}	17.75±0.06^{a}	0.61±0.05^{a}	3.20±0.07^{a}	0.36±0.02^{a}	1.47±0.14^{a}
M_2Aa	2.77±0.06^{a}	0.17±0.00^{a}	2.52±0.04^{a}	1.93±0.01^{a}	17.85±0.07^{a,b}	0.61±0.02^{a}	3.23±0.12^{a}	0.35±0.04^{a}	1.46±0.18^{a}
M_2Ab	2.84±0.00^{a}	0.18±0.00^{a}	2.50±0.03^{a}	2.02±0.20^{a}	17.82±0.06^{a}	0.60±0.00^{a}	3.23±0.01^{a}	0.34±0.00^{a}	1.42±0.01^{a}
M_2Cb	2.85±0.06^{a}	0.18±0.00^{a}	2.49±0.06^{a}	2.00±0.13^{a}	17.78±0.04^{a}	0.61±0.00^{a}	3.22±0.08^{a}	0.36±0.03^{a}	1.46±0.14^{a}
PL	2.05±0.02^{b}	0.29±0.01^{a}	2.24±0.03^{a}	2.15±0.02^{a}	16.76±0.03^{b}	0.54±0.01^{b}	1.42±0.01^{a}	0.43 ±0.00^{a}	2.54±0.07^{a}
PL_1Aa	1.89±0.13^{a,b}	0.29±0.02^{a}	2.22±0.01^{a}	2.12±0.05^{a}	16.61±0.08^{a,b}	0.52±0.01^{a,b}	1.30±0.10^{a}	0.43 ±0.03^{a}	2.53±0.08^{a}
PL_1Ba	1.90±0.07^{a,b}	0.28±0.01^{a}	2.23±0.04^{a}	2.09±0.07^{a}	16.57±0.14^{a,b}	0.31±0.00^{a}	1.30±0.03^{a}	0.43±0.01^{a}	2.53±0.04^{a}
PL_1Cc	1.79±0.02^{a,b}	0.27±0.00^{a}	2.22±0.03^{a}	2.13±0.00^{a}	16.46±0.19^{a,b}	0.52±0.02^{b}	1.37±0.03^{a}	0.43±0.01^{a}	2.49±0.04^{a}
PL_2Aa	1.97±0.10^{a,b}	0.26±0.02^{a}	2.19±0.07^{a}	2.06±0.01^{a}	16.31±0.27^{a,b}	0.51±0.05^{a,b}	1.36±0.02^{a}	0.42±0.00^{a}	2.46±0.04^{a}
PL_2Ba	1.87±0.06^{a,b}	0.28±0.00^{a}	2.20±0.03^{a}	2.09±0.00^{a}	16.43±0.23^{a,b}	0.42±0.14^{a,b}	1.32±0.07^{a}	0.42±0.02^{a}	2.46±0.13^{a}
PL_2Cb	1.87±0.08^{a,b}	0.29±0.01^{a}	2.14±0.01^{a}	2.06±0.03^{a}	16.04±0.14^{a}	0.51±0.02^{a,b}	1.32±0.04^{a}	0.41±0.03^{a}	2.41±0.13^{a}
PL_2Cc	1.72±0.01^{a}	0.26±0.01^{a}	2.17±0.06^{a}	2.06±0.05^{a}	16.12±0.22^{a,b}	0.54±0.02^{b}	1.35±0.03^{a}	0.42±0.02^{a}	2.41±0.14^{a}

Values are expressed in mg/L as average value of two repetitions ± standard deviation (n=2). Abbreviations: Df: delphinidin-3-*O*-glucoside; Cy: cyanidin-3-*O*-glucoside; Pt: petunidin-3-*O*-glucoside; Pn: peonidin-3-*O*-glucoside; Mv- malvidin-3-*O*-glucoside; PnAc: peonidin-3-O-acetylglucoside; MvAc: malvidin-3-*O*-acetylglucoside; PnCm: peonidin-3-(6-O-p-coumaroyl) glucoside; MvCm: malvidin-3-(6-O-p-coumaroyl)glucoside; CS: Cabernet Sauvignon; M: Merlot; PL: Plavac mali; 1: 12.7 mm probe diameter; 2: 19 mm probe diameter; A: 20% amplitude; B: 30% amplitude; C: 40% amplitude; a: 2 minutes; b: 4 minutes; c: 6 minutes

Table 3. Aroma compounds in control and HPU treated wine samples of Cabernet Sauvignon, Merlot, and Plavac mali

Sample	ethyl acetate	*i*-butyl acetate	*i*-amyl acetate	2-fenylethyl acetate	ethyl hexanoate	ethyl octanoate	ethyl decanoate	2-phenylethanol	diethyl succinate
CS	101.01±4.87^{c}	0.06±0.00^{f}	0.43±0.00^{d}	0.04±0.00^{a}	0.23±0.01^{f}	0.12±0.00^{e}	0.02±0.00^{d}	36.53±2.01^{a}	12.71±0.47^{a}
CS_1Ac	87.69±0.56^{b}	0.06±0.00^{e}	0.40±0.00^{c}	0.04±0.00^{a}	0.15±0.01^{d,e}	0.07±0.00^{d}	0.01±0.00^{c}	38.18±0.71^{a}	15.35±0.18^{b}
CS_1Bb	86.33±1.55^{b}	0.06±0.00^{e}	0.36±0.00^{b}	0.04±0.00^{a}	0.13±0.01^{c,d}	0.06±0.02^{b,c,d}	0.01±0.00^{b,c}	42.07±0.68^{a,b}	15.27±0.29^{b}
CS_1Cb	80.31±2.17^{b}	0.05±0.00^{d}	0.37±0.01^{b}	0.04±0.01^{a}	0.12±0.00^{b,c}	0.04±0.00^{a,b,c}	0.01±0.00^{a,b}	46.20±1.53^{b,c}	17.27±0.24^{c,d}
CS_1Cc	69.17±1.02^{a}	0.05±0.00^{c}	0.29±0.01^{a}	0.04±0.00^{a}	0.09±0.00^{a}	0.02±0.00^{a}	0.00±0.00^{a}	50.68±1.95^{c,d}	18.94±0.53^{e}
CS_2Aa	85.20±2.14^{b}	0.05±0.00^{d}	0.40±0.01^{c}	0.04±0.00^{a}	0.15±0.00^{d,e}	0.07±0.00^{d}	0.01±0.00^{b,c}	38.78±0.41^{a}	16.08±0.48^{b,c}
CS_2Ab	81.91±1.92^{b}	0.04±0.00^{b}	0.36±0.01^{b}	0.04±0.00^{a}	0.16±0.01^{e}	0.06±0.00^{c,d}	0.01±0.00^{a,b}	50.76±0.91^{c,d}	17.89±0.61^{d,e}
CS_2Cc	66.65±0.49^{a}	0.04±0.00^{a}	0.29±0.00^{a}	0.03±0.00^{a}	0.10±0.00^{a,b}	0.03±0.00^{a,b}	0.00±0.00^{a}	56.23±2.36^{d}	18.89±0.26^{e}
M	97.23±2.06^{c}	0.07±0.00^{d}	0.46±0.01^{e}	0.03±0.00^{c,d}	0.26±0.05^{b}	0.15±0.00^{e}	0.03±0.00^{d}	32.91±0.88^{a}	17.27±0.26^{a}
M_1Cb	66.12±2.29^{a}	0.04±0.00^{b}	0.33±0.01^{b,c}	0.03±0.00^{a,b,c,d}	0.17±0.00^{a}	0.05±0.00 ^{c}	0.01±0.00^{b,c}	48.26±0.26^{c,d,e}	20.15±0.76^{b,c}
M_1Bb	71.96±2.87^{a}	0.05±0.00^{a}	0.31±0.00^{a}	0.03±0.00^{b,c,d}	0.15±0.01^{a}	0.08±0.00^{b}	0.01±0.00^{a,b}	47.36±1.92^{e}	19.40±0.40^{b,c}
M_1Cc	63.63±2.09^{b}	0.03±0.00^{c}	0.30±0.00^{d}	0.03±0.00^{d}	0.16±0.02^{a,b}	0.04±0.00^{c}	0.01±0.00^{b,c}	52.99±0.85^{b}	21.15±1.13^{b,c}
M_2Aa	82.39±3.18^{a}	0.06±0.00^{b,c}	0.40±0.00^{c}	0.04±0.00^{a,b,c}	0.20±0.00^{a}	0.06±0.00^{c}	0.01±0.00^{a,b}	40.44±1.11^{c}	21.08±0.44^{c}
M_2Ab	67.89±3.62^{a}	0.05±0.00^{b,c}	0.34±0.00^{a}	0.03±0.00^{a}	0.12±0.00^{a}	0.05±0.00^{a}	0.01±0.00^{a}	46.29±1.06^{d,e}	22.60±0.82^{d}
M_2Cb	63.65±2.13^{a,b}	0.05±0.00^{b,c}	0.30±0.01^{a,b}	0.03±0.00^{a,b}	0.11±0.00^{a}	0.02±0.00^{d}	0.00±0.00^{c}	52.62±2.40^{c,d}	25.80±0.62^{a,b}
PL	67.14±0.83^{d}	0.03±0.00^{e}	0.25±0.00^{b,c}	0.02±0.00^{a,b}	0.16±0.01^{e}	0.13±0.00^{f}	0.04±0.00^{d}	19.56±0.98^{a}	9.08±0.06^{a}
PL_1Aa	64.75±3.08^{d}	0.03±0.00^{d,e}	0.28±0.00^{c}	0.02±0.00^{b,c}	0.17±0.02^{e}	0.08±0.00^{d,e}	0.02±0.00^{b}	29.02±1.10^{b,c}	12.06±0.00^{b}
PL_1Ba	59.09±0.07^{b,c,d}	0.02±0.00^{b}	0.26±0.00^{b,c}	0.02±0.00^{b,c}	0.16±0.01^{e}	0.07±0.00^{d}	0.02±0.00^{b}	29.66±0.83^{b,c,d}	13.64±0.32^{c,d}
PL_1Cc	53.77±2.34^{a,b}	0.01±0.00^{a}	0.23±0.01^{a,b,c}	0.02±0.00^{b,c}	0.13±0.00^{d}	0.05±0.00^{c}	0.01±0.00^{a}	31.65±3.20^{a}	14.60±0.52^{d,e}
PL_2Aa	63.00±2.56^{c,d}	0.03±0.00^{e}	0.22±0.00^{a,b}	0.02±0.00^{a,b,c}	0.11±0.00^{c,d}	0.09±0.00^{e}	0.03±0.00^{c}	24.47±2.02^{a,b}	10.32±0.34^{a}
PL_2Ba	55.88±3.53^{a,b,c}	0.03±0.00^{d}	0.24±0.04^{b,c}	0.02±0.00^{c}	0.01±0.00^{a}	0.08±0.00^{d,e}	0.02±0.00^{b}	24.63±2.27^{a,b}	12.39±0.43^{b,c}
PL_2Cb	53.53±0.39^{a,b}	0.02±0.00^{c}	0.21±0.01$^{a.,b}$	0.02±0.00^{a}	0.09±0.00^{c}	0.03±0.00^{b}	0.01±0.00^{a}	36.22±0.91^{d}	13.66±0.44^{c,d}
PL_2Cc	50.41±0.37^{a}	0.02±0.00^{b,c}	0.18±0.00^{a}	0.02±0.00^{a,b,c}	0.06±0.00^{b}	0.01±0.00^{a}	0.01±0.00^{a}	36.43±1.15^{d}	15.09±0.26^{e}

Values are expressed in mg/L as average value of two repetitions ± standard deviation (n=2). Abbreviations: CS: Cabernet Sauvignon; M: Merlot; PL: Plavac mali; 1: 12.7 mm probe diameter; 2: 19 mm probe diameter; A: 20% amplitude; B: 30% amplitude; C: 40% amplitude; a: 2 minutes; b: 4 minutes; c: 6 minutes

Table 4. Aroma compounds in control and HPU treated wine samples of Cabernet Sauvignon, Merlot, and Plavac mali

Sample	*i*-butanol	*i*-amyl acohol	2,3-butanediol	1-hexanol	ethyl butyrate	vanillin*	4-ethyl guaiacol*	linalool*	α-terpineol*
CS	42.98±1.16^{e}	257.38±3.14^{d}	0.89±0.00^{a,b}	1.49±0.03^{b}	0.27±0.00^{c}	7.50±0.71^{b,c}	40.50±0.71^{c}	7.50±0.71^{a}	7.50±0.71^{a}
CS_1Ac	29.45±1.65^{b,c}	243.61±2.71^{b,c}	0.87±0.04^{a,b}	1.45±0.02^{a,b}	0.22±0.01^{a,b}	5.50±0.71^{a}	40.50±2.12^{c}	8.00±0.00^{a}	6.50±0.71^{a}
CS_1Bb	28.22±0.90 ^{b}	228.13±3.08^{a}	0.92±0.03^{a,b}	1.39±0.02^{a}	0.20±0.00^{a}	5.50±0.71^{a}	35.50±0.71^{b}	8.50±0.71^{a}	7.00±0.00^{a}
CS_1Cb	27.85±1.58^{b}	250.75±3.51^{c,d}	0.95±0.01^{b}	1.46±0.03^{a,b}	0.26±0.01^{b,c}	7.00±0.00^{a,b,c}	41.50±0.71^{c}	9.50±0.71^{a,b}	7.00±0.00^{a}
CS_1Cc	21.84±0.62^{a}	239.53±1.94^{b}	0.92±0.02^{a,b}	1.41±0.02^{a,b}	0.22±0.01^{a,b}	6.00±0.00^{a,b}	34.50±0.71^{b}	8.50±0.71^{a}	6.50±0.71^{a}
CS_2Aa	34.87±2.30^{d}	234.64±2.11^{a,b}	0.84±0.02^{a}	1.42±0.03^{a,b}	0.23±0.02^{a,b,c}	8.00±0.00^{c}	27.00±1.41^{a}	9.50±0.71^{a,b}	7.00±0.00^{a}
CS_2Ab	34.74±0.54^{c,d}	250.31±0.47^{c,d}	0.92±0.04^{a,b}	1.49±0.01^{b}	0.24±0.00^{b,c}	7.50±0.71^{b,c}	37.50±0.71^{b,c}	11.00±0.00^{b}	7.50±0.71^{a}
CS_2Cc	25.60±1.14^{a,b}	240.34±0.13^{b}	0.84±0.01^{a}	1.38±0.01^{a}	0.23±0.01^{a,b,c}	8.00±0.00^{c}	35.50±0.71^{b}	7.50±0.71^{a}	7.00±0.00^{a}
M	83.50±1.06^{d}	294.72±4.86^{a,b}	0.62±0.02^{a}	1.40±0.03^{c,d}	0.26±0.00^{d}	10.50±0.71^{a}	51.00±2.83^{a}	10.00±0.00^{a,b,c}	12.50±0.71^{b}
M_1Cb	56.07±0.24^{b}	274.20±2.45^{a}	0.59±0.01^{a}	1.31±0.02^{a,b}	0.21±0.01^{a,b}	18.50±0.71^{c}	56.00±2.83^{a}	9.50±0.71^{a,b}	12.50±0.71^{b}
M_1Bb	51.75±0.78^{b}	279.83±7.76^{a,b}	0.59±0.02^{a}	1.27±0.03^{c,d}	0.25±0.01^{a}	13.50±0.71^{b}	55.50±4.95^{a}	8.50±0.71^{a}	10.00±0.00^{a}
M_1Cc	47.71±2.22^{a}	286.92±11.80^{b}	0.63±0.06^{a}	1.42±0.02^{d}	0.19±0.00^{b,c}	16.50±0.71^{c}	46.50±2.12^{a}	10.50±0.71^{a,b,c}	13.50±0.71^{b}
M_2Aa	72.25±1.49^{c}	299.37±5.91^{a,b}	0.67±0.01^{a}	1.48±0.01^{d}	0.23±0.00^{a,b}	11.50±0.71^{a,b}	54.00±2.83^{a}	12.50±0.71^{c}	13.00±0.00^{b}
M_2Ab	70.84±1.50^{a,b}	295.32±2.91^{a,b}	0.65±0.01^{a}	1.49±0.03^{b,c}	0.22±0.00^{b,c}	12.50±0.71^{a,b}	49.00±1.41^{a}	11.50±0.71^{b,c}	12.50±0.71^{b}
M_2Cb	51.04±0.31^{a,b}	294.15±0.80^{a,b}	0.64±0.01^{a}	1.37±0.02^{a}	0.23±0.01^{c,d}	12.50±0.71^{a,b}	46.00±1.41^{a}	9.50±0.71^{a,b}	9.50±0.71^{a}
PL	19.84±0.47^{d}	239.80±1.14^{a}	1.54±0.06 ^{c}	1.52±0.01^{a}	0.22±0.00^{a,b}	n.d.	51.00±2.83^{b}	7.00±0.00^{a}	5.50±0.71^{a,b}
PL_1Aa	16.86±0.10^{c}	221.89±2.67 ^{a,b}	1.40±0.01^{b}	1.63±0.02^{a}	0.21±0.00^{a,b}	n.d.	58.00±0.00^{b}	10.00±1.41^{a,b,c}	6.50±0.71^{a,b,c}
PL_1Ba	14.37±0.64^{b}	218.08±3.30^{a,b}	1.37±0.01^{b}	1.65±0.02^{a}	0.24±0.02^{a,b}	n.d.	59.00±1.41^{b}	12.00±0.00^{c}	7.50±0.71^{b,c}
PL_1Cc	9.62±0.76^{a}	212.56±13.71^{a}	1.30±0.03^{a,b}	1.61±0.04^{a}	0.25±0.03^{b}	n.d.	53.00±4.24^{b}	11.00±0.00^{b,c}	8.00±0.00^{c}
PL_2Aa	16.46±0.04^{c}	215.45±6.64 ^{a,b}	1.56±0.02^{c}	1.59±0.09^{a}	0.23±0.00^{a,b}	n.d.	29.50±2.12^{a}	6.50±0.71^{a}	5.00±0.00^{a}
PL_2Ba	14.28±0.05^{b}	213.41±4.00^{a}	1.60±0.02^{c}	1.61±0.01^{a}	0.23±0.00^{a,b}	n.d.	50.00±1.41^{b}	8.50±0.71^{a,b,c}	6.50±0.71^{a,b,c}
PL_2Cb	14.13±0.41^{b}	210.23±6.77^{a}	1.36±0.03^{b}	1.59±0.00^{a}	0.22±0.00^{a,b}	n.d.	36.50±2.12^{a}	6.50±2.12^{a}	5.50±0.71^{a,b}
PL_2Cc	13.64±0.46^{a}	211.36±4.97^{a}	1.21±0.01^{a}	1.61±0.00^{a}	0.20±0.00^{a}	n.d.	30.50±2.12^{a}	7.50±0.71^{a,b}	6.50±0.71^{a,b,c}

Values are expressed in mg/L as average value of two repetitions ± standard deviation (n=2). Values for compounds marked with *(vanillin, 4-ethyl guaiacol, linalool, α-terpineol) are expressed in μg/L. Abbreviations: CS: Cabernet Sauvignon; M: Merlot; PL: Plavac mali; 1: 12.7 mm probe diameter; 2: 19 mm probe diameter; A: 20% amplitude; B: 30% amplitude; C: 40% amplitude; a: 2 minutes; b: 4 minutes; c: 6 minutes

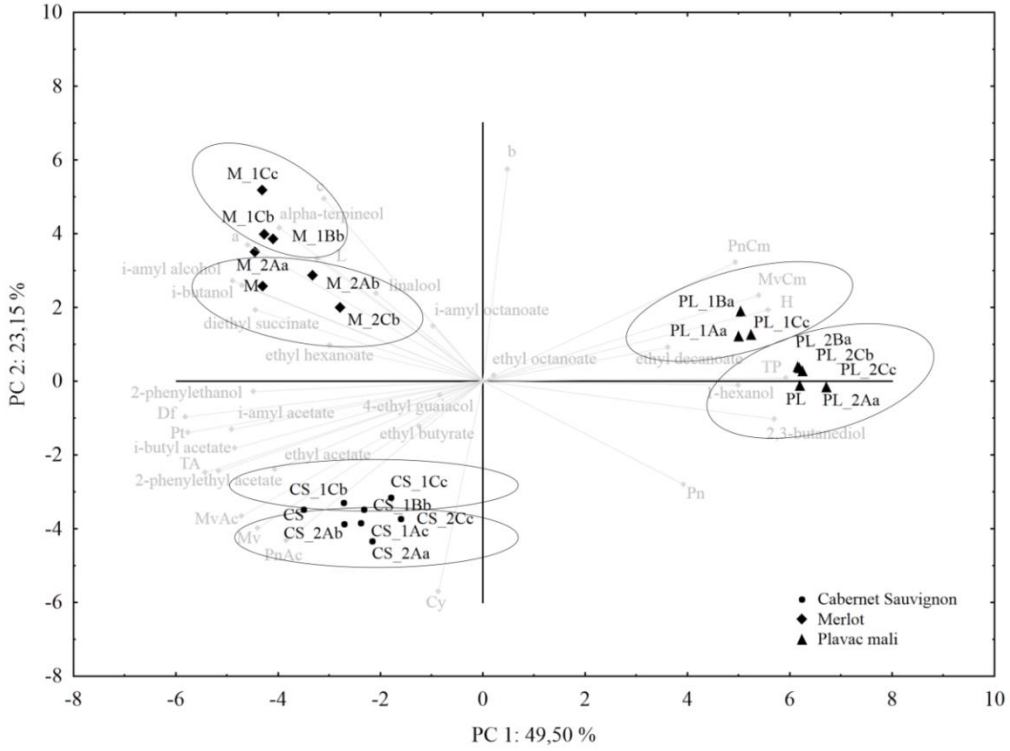

Fig. 1. Projection of the phenolic, colour, and aroma data, and the distribution of control and HPU treated wine samples in the two-dimensional system defined by PC1 and PC2

On the other hand, all treated wine samples showed higher content of diethyl succinate and 2-phenylethanol aroma compounds characteristic for the wine aging process. The concentration of 2-phenylethanol increased for 5-39 % in the case when the 12.7 mm diameter probe was used and for 6-47% when the 19 mm diameter probe was used, considering the applied amplitude, treatment duration, and treated sample variety. Furthermore, the concentration of diethyl succinate increased for 17-33% in Cabernet Sauvignon, for 11-34% in Merlot, and for about 25-40% in Plavac mali wine samples (depending on the selected treatment parameters). The most important influence of the HPU treatment on the formation of aroma compounds characteristic for the wine aging process is shown in the variety Plavac mali, followed by Merlot and Cabernet Sauvignon.

In accordance with earlier studies, all treated wines showed changes in phenolic composition, chromatic characteristics, as well as the aroma composition. However, these chemical changes showed to be treatment and cultivar dependent.

In order to compare the analyzed wine samples according to the phenolic, colour composition, and aroma compounds content, principal component analysis (PCA) was conducted. Applying PCA to the concentrations of variables (phenolic composition, colour parameters and aroma composition) of control and treated wine samples of all three used grape varieties, five factors were extracted with eigenvalues higher than 1, explaining 90.78% of the overall variance. The first two factors (PC1 and PC2) accounted for 72.65% of total variance.

The projection of samples and the analyzed chemical variables in the two-dimensional coordinate system defined by the first two variables is shown in Figure 1. The first variable, explaining 49.50% of the total variance, was strongly negatively correlated with total anthocyanins (-0.90) and certain free anthocyanins (delfinidin-3-*O*-glucoside (-0.97), petunidin-3-*O*-glucoside (-0.96), malvidin-3-*O*-glucoside (-0.73), and peonidin and malvidin acetyl glucosides (-0.64; -0.78)), as well as with volatile sample compounds: Ethyl acetate (-0.68), *i*-butyl acetate (-0.81), *i*-butanole (-0.78), *i*-amil acetate (-0.82), *i*-amil alcohol (-0.81), 2-phenylethyl acetate (-0.86), and diethyl succinate (-0.74), 2-phenylethanol (-0.75), and highly positively correlated with total polyphenols (0.99), free anthocyanin coumaroyl glucosides: Peonidin-3-(6-*O*-*p*-coumaroyl) glucoside (0.82) and malvidin-3-(6-*O*-*p*-coumaroyl) glucoside (0.90), and volatile 2,3-buthandiol (0.95) and 1-hexanol (0.83). The second principal component, explaining 23.15% of the total variance, showed a

strong negative correlation only with cyanidin-3-*O*-glucoside (-0.95) and peonidin-3-*O*-glucoside (-0.72), while showing a positive correlation with all chromatographic characteristics, especially b (0.96) and c (0.82).

The grouping of wine varieties according to the PCA analysis can be easily seen in Figure 1. The wine samples of Cabernet Sauvignon are placed on the left side of the first factorial plane and displaced from the other two wine varieties used in this experiment. They are characterized by higher concentrations of compounds which negatively correlate with both the first and the second factorial plane. The Merlot wine samples are placed on the left side of the first factorial plane and are characterized by high concentrations of compounds which positively correlate with the second factorial plane. The Plavac mali wine samples are located on the right side of the first factorial plane and are characterized by higher concentrations of total polyphenols, free coumaroyl anthocyanins, and wine esters (ethyl octanoate and ethyl decanoate). Also, there is a clear separation of treated wine samples according to the diameter size of the probe, which emphasizes the importance of appropriate probe selection among the other process parameters.

Conclusions

The application of HPU affects the chemical composition of treated red wines by inducing chemical reactions, which results in the decrease of phenolic content and chromatic characteristics, while on the other hand contributes to the aging aroma composition. The intensity of the mentioned chemical changes depends on the applied treatment and the treated red cultivar. Respectively, among the three different parameters, the selection of the probe diameter proved to be the most discriminatory parameter, since the PCA analysis showed a significant separation of the wine samples treated with the 12.7 and the 19 mm diameter probe, regardless of the amplitude level, processing time, or wine variety. A 12.7 mm diameter probe, lower amplitude (20%), and shorter processing time (2 minutes) showed a generally more favourable and lighter impact on the phenolic, colour, and aroma content of the treated wines, in comparison with the 19 mm diameter probe, higher amplitude (40%), and longer processing time (6 minutes).

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