Bentonite fining during fermentation reduces the dosage required and exhibits significant side-effects on phenols, free and bound aromas, and sensory quality of white wine

Horvat, Ivana; Radeka, Sanja; Plavša, Tomislav; Lukić, Igor

Source / Izvornik: **Food Chemistry, 2019, 285, 305 - 3015**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

https://doi.org/10.1016/j.foodchem.2019.01.172

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:159:801772

Food Chemistry 285 (2019) 305–315

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Bentonite fining during fermentation reduces the dosage required and exhibits significant side-effects on phenols, free and bound aromas, and sensory quality of white wine

Ivana Horvat[a], Sanja Radeka[a], Tomislav Plavša[a], Igor Lukić[a,b,*]

[a] Institute of Agriculture and Tourism, K. Huguesa 8, 52440 Poreč, Croatia
[b] Centre of Excellence for Biodiversity and Molecular Plant Breeding, Svetošimunska 25, 10000 Zagreb, Croatia

ARTICLE INFO

Keywords:
White wine
Protein stability
Bentonite
Phenols
Aroma
Malvazija istarska

ABSTRACT

To test the effects of bentonite addition at various stages of fermentation, five Malvazija istarska white grape must vinification treatments were performed with 100 g/hL of bentonite added in clear juice, at the beginning, in the middle, and at the end of fermentation, while control was fermented without bentonite. Phenols and free and bound volatile aromas were determined by HPLC-DAD and SPE-GC-MS. Wines were evaluated sensorially. Fining during fermentation reduced the total bentonite dose required, and was most effective near the end of fermentation with the reduction of 16% and 21%, depending on the protein stability test. All treated wines preserved more hydroxycinnamoyltartaric acids with respect to control. The side-effect of these treatments on varietal aromas was moderate, but enhanced the preservation of key fermentation volatiles in relation to control, and exhibited positive sensory effects. It was concluded that bentonite added during fermentation may positively affect wine quantity and quality.

1. Introduction

White wines with developed protein haze are perceived by consumers as faulty (Marangon et al., 2013). The formation of haze originates mostly from the presence of thaumatin-like proteins and chitinases, which therefore need to be removed by fining before wine marketing (Marangon et al., 2011). Bentonite is still the most efficient fining agent in achieving protein stability of white wines, although its use often reduces both wine quantity and quality (Waters et al., 2005). It was estimated that hidden costs of bentonite fining correspond to the loss of around one billion dollars of world's wine industry annual revenue, with a volume equivalent to the total white wine production of New Zealand (Majewski, Barbalet, & Waters, 2011). Because of its non-selectiveness, fining with bentonite may exhibit negative effects on the properties of final wine mainly by removing positive aromas (Armada & Falqué, 2007; Lambri, Dordoni, Silva, & De Faveri, 2010, 2012; Vincenzi, Panighel, Gazzola, Flamini, & Curioni, 2015). Bentonite exhibits a cation exchange effect, and particular commercial samples were shown to be able to enrich wine with metals in concentrations above the maximum recommended limits (Dordoni, Colangelo, et al., 2015). For these reasons, the efforts of scientists and experts have long been and still are focused on finding effective alternatives or protocols with reduced bentonite requirements, which are both of great interest for producers.

Several alternative fining aids, such as the combination of heat and proteolytic enzymes (Pocock, Høj, Adams, Kwiatkowski, & Waters, 2003), zirconium dioxide (Pashova et al., 2004), carrageenan (Marangon et al., 2013), etc., were found to be more or less effective, but are still on a research level and not implemented in wine industry.

Certain authors have found that treating grape juice with bentonite can reduce the total dose required in relation to standard wine fining (Ewart, Phipps, & Iland, 1980; Lambri et al., 2012), although others observed the contrary (Lira et al., 2015; Pocock, Salazar, & Waters, 2011; Vela, Hernández-Orte, Castro, Ferreira, & Lopez, 2017). However, in most such studies, a negative effect of juice treatment on the quantity of available nitrogen, varietal and fermentation aromas, and wine quality in general was observed (Armada & Falqué, 2007; Burin, Caliari, & Bordignon-Luiz, 2016; Lambri et al., 2010, 2012).

Another promising approach alternative to standard fining after fermentation, relatively easily applicable and in conformity with the current regulations, is bentonite fining during fermentation. Its potential to reduce the required bentonite dose and to improve wine quality was hinted by particular authors in the eighties (Ewart et al., 1980), but only a few studies on this topic were published from then on to date

* Corresponding author at: Institute of Agriculture and Tourism, K. Huguesa 8, 52440 Poreč, Croatia.
E-mail addresses: ihorvat@iptpo.hr (I. Horvat), sanja@iptpo.hr (S. Radeka), tomislav@iptpo.hr (T. Plavša), igor@iptpo.hr (I. Lukić).

https://doi.org/10.1016/j.foodchem.2019.01.172
Received 25 January 2018; Received in revised form 3 December 2018; Accepted 25 January 2019
Available online 02 February 2019
0308-8146/

(Lira et al., 2015, 2014; Pocock et al., 2011). These works respectively showed more or less positive effects, while Lira et al. (2015) achieved extraordinary results for Albariño wines, with an approximate 50% reduction of the bentonite dosage and significant improvements in wine chemical composition and sensory quality after bentonite addition in the middle or late in fermentation.

It is apparent that despite such promising indications, the effect of bentonite treatment during fermentation has generally been poorly investigated. The impact of such treatment on volatile compounds was not studied extensively up to date, and the published works mainly focused on the main free volatile compounds (Lira et al., 2015, 2014). To our knowledge, the response of important glycosidically bound volatile compounds has not been considered at all, although it is reasonable to assume various significant impacts of bentonite during fermentation depending on the time of addition, either by adsorption (Lambri, Dordoni, Silva, & De Faveri, 2013; Vincenzi et al., 2015) or indirectly by inhibiting β-glucosidase activity (Jaeckels et al., 2015). In light of the inhibitory activity of bentonite towards other enzymes, such as polyphenoloxidases (Main & Morris, 1991), it is to be assumed that its addition during fermentation would have a significant impact on the chemistry of hydroxycinnamates and other phenols. However, these compounds have not been studied from this viewpoint.

The main hypothesis of this work was that bentonite fining during alcoholic fermentation may reduce the required dose, and possibly contribute to the preservation or improvement of important chemical and sensory quality parameters of wine, which are both of large importance for wine industry. For this reason, the aim was to investigate the possibility to minimise the total dose of bentonite required for achieving protein stability by adding it at different fermentation stages, and at the same time gain a deeper insight into the response of key wine constituents, such as free and bound volatile aromas and phenols, to such treatments. The repercussions on the sensory characteristics of the produced wines were also considered. To achieve more general conclusions, the study was conducted with a cultivar problematic with respect to protein stability, Malvazija istarska (*Vitis vinifera* L.), whose wines often require high doses of standard sodium-based bentonite, in certain years even up to 300 g/hL.

2. Materials and methods

2.1. Chemicals and reagents

Methanol, dichloromethane, and pentane were purchased from Sigma–Aldrich (Sigma-Aldrich, St. Louis, MO, USA). Sodium sulphate anhydrous was purchased from Kemika d.d. (Zagreb, Croatia). Isolute ENV+ (1 g, 6 mL) SPE cartridges were obtained from Biotage (Uppsala, Sweden), and C-18 Bond Elut (500 mg, 6 mL) SPE cartridges were obtained from Agilent Technologies (Palo Alto, CA, USA). A mixture of pectinases and glycosidases, Rapidase AR2000 enzyme was purchased from DSM Food Specialties B.V. (Delft, The Netherlands). Pure standards of volatile compounds were purchased from Merck (Darmstadt, Germany), Sigma-Aldrich, and Fluka (Buchs, Switzerland), and pure standards of phenols were purchased from Sigma-Aldrich, Acros Organics (Geel, Belgium), and Extrasynthese (Genay, France) (Table S1). Qualitative standards of *trans*-coutaric acid, *trans*-fertaric acid, and *cis*-piceid were kindly donated by Dr Urska Vrhovsek from Fondazione Edmund Mach (FEM), San Michele all'Adige, Italy. *Cis*-isomers of hydroxycinnamates were obtained by UV illumination of a methanol solution of the *trans*-isomers for 4 h. Standard solutions were prepared in synthetic wine containing 12 vol% of ethanol and 5 g/L of tartaric acid, adjusted to pH 3.2.

2.2. Winemaking and bentonite treatments

The experiment was performed in the Istria region of Croatia, with Malvazija istarska (*Vitis vinifera* L.), the most widespread and important native white grape variety in Croatia, cultivated also in Slovenia and Italy. The grapes were harvested manually on September 15, 2015, from the experimental vineyard of the Institute of Agriculture and Tourism in Poreč (Istria, Croatia) and were immediately destemmed, crushed, mashed, and pressed using a closed-type pneumatic press of 500 L capacity (Letina Inox d.o.o., Čakovec, Croatia) with pressure not exceeding 1 bar. The obtained juice was cold settled with the aid of Endozym Rapid pectolytic enzymes at 2 g/hL (AEB s.p.a. Brescia, Italy) for 36 h at 12 °C. Total acidity was adjusted with the addition of 1 g/L of tartaric acid. The clear juice was homogenised and divided in 15 equal portions prepared for fermentation in stainless steel tanks of 80 L capacity.

Juices were inoculated with selected yeasts *Saccharomyces cerevisiae* Lalvin QA 23 (Lallemand SA, Montreal, Canada) at 20 g/hL, rehydrated with Go-Ferm Protect Evolution (Lallemand) at 30 g/hL. Yeast supplements (25 g/hL of Fermaid E, Lallemand) were added at the 2nd and 5th day of fermentation. Initial sugar concentration was 230 g/L.

Granular activated sodium bentonite, montmorillonite based, was used for protein stabilisation. Five treatments were set based on the time of bentonite addition, in triplicates: the same bentonite dose of 100 g/hL was added in the clear juice (JU), at the beginning (BE, reducing sugars at 170–180 g/L), in the middle (MD, sugars at 90–100 g/L), and near the end of fermentation (EN, sugars at 40–50 g/L), while control (CO) received no bentonite. During over more than 20 years of practical experience with Malvazija istarska wine protein stabilisation (data not shown) it was observed that the doses required for complete stabilization have been lower than 150 g/L in extremely rare occasions. Considering this, the arbitrarily chosen dose of 100 g/hL in this work was considered suitable for avoiding overfining and for achieving partial protein stabilization during fermentation, which allowed the comparison of treatments afterwards.

Fermentation was performed at 17 °C and lasted for 13 days (reducing sugars < 2 g/L). After fermentation, partially stabilized wines were racked, and left to spontaneously settle for 2 months. A portion from each treatment was subjected to chemical and sensory analyses (code: AFerm), while the rest of wine was fined with additional doses of bentonite required to achieve protein stability, as determined by the standard heat stability test (heating at 80 °C; Pocock et al., 2011; Lira et al., 2015). After 15 days of contact with bentonite, protein stable wines were subjected to chemical and sensory analyses (code: ProStab). The level of free SO_2 was monitored throughout the whole process and was corrected to 25–30 mg/L after fermentation, before and after racking, and before sampling, if needed.

2.3. Protein stability tests

Bentonite dosage rates for heat stability of wine were determined to the nearest 10 g/hL by preliminary tests, using a variety of different dosages (10–300 g/hL), followed by fine tuning to the nearest 5 g/hL. For each dosage two stability tests were applied. In the standard heat stability test, wine sample (20 mL) was filtered through a PTFE 0.45 µm syringe filter and heated at 80 °C for 2 h. Sample was then shortly cooled in tap water, placed at 4 °C for another 2 h, and then left to reach room temperature. The amount of haze produced was measured by a nephelometric turbidity meter Hanna Instruments HI 83749 (Padova, Italy). A sample was considered to be protein stable when the difference between a heated sample and an unheated control was lower than 2 nephelometry turbidity units (NTU) (Lira et al., 2015; Pocock et al., 2011). In heating with tannins stability test, a portion of tannic acid solution was added to filtered wine, which was then heated at 80 °C for 2 h, and after it reached the room temperature nephelometric turbidity measurements were performed. A sample was considered to be protein stable when NTU < 5 (Radeka, Peršurić, Lukić, Bocca, & Plavša, 2009).

2.4. Standard physico-chemical analyses

Standard physico-chemical parameters were determined according to the OIV methods (OIV, 2015). No significant differences between wines analysed after fermentation and after additional bentonite fining were observed. Average values with standard deviations were: relative density 0.9912 ± 0.0001 after fermentation and 0.9913 ± 0.0001 after additional fining, alcoholic strength by volume (%) 13.34 ± 0.09 and 13.16 ± 0.07, total dry extract without reducing sugars (g/L) 19.3 ± 0.03 and 19.3 ± 0.02, total acidity (g/L) 5.6 ± 0.01 and 5.5 ± 0.01, volatile acidity (g/L) 0.41 ± 0.05 and 0.36 ± 0.04, and pH 3.27 ± 0.01 and 3.25 ± 0.01, respectively.

2.5. Analysis of free and bound volatile aroma compounds

The sample preparation method reported previously (Lukić, Lotti, & Vrhovsek, 2017) was adopted with modifications. A 40-mL aliquot of wine was diluted with deionised water to 100 mL, and 1-heptanol (100 µL, 230 mg/L in ethanol) was added as internal standard. Isolute ENV+ solid-phase extraction (SPE) cartridges were conditioned (15 mL each of methanol followed by deionised water) and the diluted wine was loaded. The cartridges were washed with 20 mL of water to remove water-soluble impurities. Free volatiles were released by eluting 25 mL of dichloromethane; this eluate was collected in a 100-mL flask and 50 mL of pentane were added to it followed by the addition of anhydrous Na_2SO_4 to remove water. Subsequently, the whole fraction was carefully concentrated prior to analysis up to 500 µL using a Vigreux column. To release the glycosylated precursors, cartridges were eluted with 25 mL of methanol; this eluate was evaporated to dryness by using a rotary vacuum evaporator. The flask was rinsed with 10 mL of the mixture pentane:dichloromethane 2:1, v/v, to remove any remaining traces of free volatile compounds. The bound fraction was then re-dissolved in 4 mL of citrate buffer (pH 5); 200 µL of AR2000 (70 mg/mL) were added and tubes were kept at 40 °C for 24 h. Later, 25 µL of internal standard were added and released bound volatiles were extracted by loading a sample onto a C-18 cartridge activated by methanol and deionised water. Further elution, dehydration, and pre-concentration to 250 µL were performed in the same manner as for the free volatiles, with 5 times lower solvent volumes.

Identification and quantification of volatile compounds was performed using a Varian 3900 GC coupled with a Varian Saturn 2100 T ion trap mass spectrometer (Varian Inc., Harbour City, CA, USA). The column used was a 60 m × 0.25 mm i.d. × 0.25 µm d.f. Rtx-WAX (Restek, Bellefonte, PA, USA). Initial oven temperature was 40 °C, then increased at 2 °C/min to 240 °C, and then kept at 240 °C for 10 min. Injector, transfer line and ion trap temperatures were 245, 80 and 120 °C, respectively. Mass spectra were acquired in EI mode (70 eV) at 1 s/scan, full scan with a range of 30–450 m/z. The carrier gas was helium (1 mL/min). Identification was performed by comparing retention times and mass spectra with those of pure standards when available, and with mass spectra from NIST05 library. Linear retention indices (relative to n-alkanes from C10 to C28) were calculated and compared to those from literature. When standards were available, standard calibration curves were constructed (Table S1). For other compounds semi-quantitative analysis was carried out, and their concentrations were expressed as equivalents of compounds with similar chemical structure for which standards were available, assuming a relative response factor equal to one.

2.6. Analysis of phenols

Analyses of phenols were carried out by high performance liquid chromatography (HPLC), according to the modified method proposed by Pati et al. (2014), using an Agilent Infinity 1260 system (Agilent) equipped with a G1311B quaternary pump, a G1329B autosampler, a G1316A column oven, and a G4212B DAD detector. Wine samples were filtered through 0.45 µm PTFE filters, and 10 µL were injected onto a Poroshell 120 EC-C18 column (150 × 4.6 mm i.d., particle size 2.7 µm, Agilent) with a guard (Poroshell 120 EC-C18, 5 × 4.6 mm i.d., particle size 2.7 µm, Agilent). The following gradient system was used with water/formic acid (99:1, v/v) (solvent A) and acetonitrile (solvent B): 0 min, 2% B (flow 0.3 mL/min); 10 min, 13% B (0.3 mL/min); 25 min, 15% B (0.3 mL/min); 30 min, 22% B (0.3 mL/min); 46 min 22% B (0.3 mL/min); 47 min, 99% B (0.7 mL/min); 56 min 99% B (0.7 mL/min); 49 min, 2% B (0.7 mL/min); 64 min 2% B (0.7 mL/min); 65 min, 2% B (0.3 mL/min); 74 min 2% B (0.3 mL/min). The column temperature was 26 °C. UV–Vis detection wavelengths were 280 nm (for hydroxybenzoic acids, flavan-3-ols, stilbenes, taxifolin, and tyrosol), and 330 nm (for hydroxycinnamic acids), and spectra were registered from 200 to 600 nm. Identification was performed by comparing retention times and spectra with those of pure standards. Standard calibration curves were constructed (Table S1). For phenols for which only qualitative standards were available, semi-quantitative analysis was carried out.

2.7. Sensory analysis

Quantitative descriptive sensory analysis was performed by a panel of five trained tasters (three male, two female, age between 25 and 45), all of them highly experienced in Malvazija istarska wine sensory analysis. Four out of five tasters were members of Croatian Enological Society, certified and authorised by the Croatian Ministry of Agriculture for official commercial wine sensory analysis. Qualitative (selection of main descriptors by consensus and standardisation of vocabulary) and quantitative (intensity of perception) criteria of the tasters were attuned by tasting representative samples of Malvazija istarska wine through several preliminary training sessions and at the beginning of each sensory analysis session. The same representative Malvazija istarska wine sample was tasted for attuning before each session.

Tasters were seated in separate booths, and the environment was free of interference in terms of noise, visual stimulation, and ambient odour. Wine samples stored at 11 °C were served (40 mL) in standard ISO 3591:1977 wine tasting glasses at room temperature (20 °C). Samples were served in random order and were coded by three-digit numbers for identification, divided in three sessions. The tasters used an 11-point structured scale to rate the aroma or taste intensity of each descriptor (0 = descriptor not perceptible, 10 = descriptor strongly perceptible). The tasters were also asked to rate the varietal typicity of the investigated wines regarding their Malvazija istarska wine concept on an 11-point structured scale (0 = not typical, 10 = very typical). Wines were also assessed by the hedonic 100-point OIV method.

2.8. Data elaboration

One-way analysis of variance (ANOVA) and Fischer's least significant difference (LSD) test were used to compare the means (n = 3) at the level of significance of $p < 0.05$. Statistical data elaboration was performed with Statistica v. 13.2 software (Stat-Soft Inc., Tulsa, OK, USA).

3. Results and discussion

3.1. Protein stability

Initial (added during fermentation), additional (added after fermentation), and total (initial + additional) doses of bentonite applied in order to achieve protein stable Malvazija istarska wines are shown in Table 1. Heating with tannins test resulted with slightly higher bentonite dosages than the standard heating test, which was as expected since the former is known to give higher turbidity values (Ribereau-Gayon, Dubourdieu, Doneche, & Lonvaud, 2006). The results of both tests correlated and confirmed that bentonite added during

Table 1
Initial (added at different points of fermentation), additional, and total doses of bentonite applied in order to achieve protein stable Malvazija istarska wines.

Treatment (dosing time)	Protein stability test[a]	Dose (g/hL)		
		Initial	Additional	Total
CO	A	0	220	220
	B	0	260	260
JU	A	100	115	215
	B	100	135	235
BE	A	100	105	205
	B	100	125	225
MD	A	100	90	190
	B	100	110	210
EN	A	100	85	185
	B	100	105	205

[a] A = heating at 80 °C; B = heating at 80 °C with tannins. CO – control wine without bentonite in fermentation, JU – initial bentonite dose (100 g/hL) added into clear grape juice, BE – initial bentonite dose (100 g/hL) added at the beginning of fermentation, MD – initial bentonite dose (100 g/hL) added at the middle of fermentation, EN – initial bentonite dose (100 g/hL) added at the end of fermentation. The wines were treated by additional bentonite doses after fermentation.

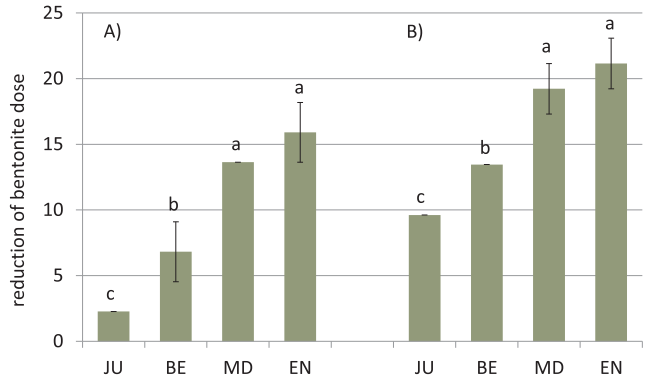

Fig. 1. Reduction of the total dose (means and standard deviations; %) required to achieve protein stability of Malvazija istarska wine with respect to the moment of initial bentonite dosing (100 g/hL), according to (A) standard heating at 80 °C test and (B) heating with tannins test. Abbreviations: CO – control wine without bentonite in fermentation, JU – initial bentonite dose added into clear grape juice, BE – initial bentonite dose added at the beginning of fermentation, MD – initial bentonite dose added at the middle of fermentation, and EN – initial bentonite dose added at the end of fermentation. The wines were treated by additional bentonite doses after fermentation to achieve total protein stability. Different lowercase superscript letters represent statistically significant differences between treatments with respect to bentonite dosing time, at $p < 0.05$ obtained by one-way ANOVA and least significant difference (LSD) test.

fermentation significantly reduced the total amount required in relation to the fining of the fermented CO wine. The most effective were the treatments MD and EN, with the reduction with respect to CO of approximately 14% and 16%, and 19% and 21%, as determined by the two heating tests, standard heat stability test and heating with tannins stability test, respectively (Fig. 1). It is probable that the overall must or wine matrix composition, which certainly varied depending on the time of addition, has had a significant influence on the efficacy of fining. It was shown earlier that various wine components, such as ions (Dordoni, Colangelo, et al., 2015), tannins (Dordoni, Galasi, Colangelo, De Faveri, & Lambri, 2015; Marangon, Vincenzi, Lucchetta, & Curioni, 2010), and polysaccharides (Gazzola, Van Sluyter, Curioni, Waters, & Marangon, 2012), significantly affect protein stability and aggregation. Variable quantities of other cations in wine matrix competitive to pathogenesis-related protein adsorption, such as particular alkali and alkaline earth metal ions, amino acids, some peptides, and other proteins, could have also had an effect, as presumed earlier by Blade and Boulton (1988). The value of wine pH was shown to be an important factor determining bentonite activity, especially in the case of medium and high molecular mass proteins, either directly or by influencing the exchange of sodium and potassium between bentonite and wine (Dordoni, Colangelo, et al., 2015). Slight modulation of the pH and the composition of the mentioned and other metals, as well as their interactions during fermentation could have possibly affected the differences between the efficiency of bentonite in the treatments in this work. Total must protein amount decreases during the course of fermentation (Pocock et al., 2011), so it is possible that when added earlier, although longer in contact, a part of bentonite is spent by adsorbing particular non- or less pathogen proteins present in higher concentration at that stage of fermentation. Among other components, ethanol and reducing sugars varied more dramatically among the treatments with respect to the moment of bentonite addition. Xifang et al. (2007) have demonstrated that a particular bentonite has had a maximum chicken egg albumin (ovalbumin) adsorption capacity in wine model solution at around 11 vol% of ethanol, which corresponds to the alcoholic strength of wine at the moment of the bentonite addition in EN treatment in this work. Blade and Boulton (1988) suggested that ethanol increases the swelling and the adsorption capacity of bentonite by displacing smaller water molecules in bentonite layers. It was shown that its effectiveness depends on protein volume: the adsorption of smaller proteins by bentonite was enhanced by increasing ethanol levels up to 10 vol% for bovine serum albumin (BSA), and up to 12 vol% for lysozyme, while ethanol had no significant effect on ovalbumin (Achaerandio, Pachova, Güell, & López, 2001). Contradictory results for ovalbumin (Achaerandio et al., 2001; Xifang et al., 2007) suggest that other factors, such as bentonite type and wine matrix composition, may have a large influence, as reported by Dordoni, Colangelo, et al. (2015), and Lambri, Dordoni, Silva, and De Faveri (2010). Pathogenesis-related fractions (20–30 kDa, Pashova et al., 2004) are generally among the smaller proteins in wine, so it is reasonable to assume that ethanol has the ability to separate bentonite layers enough to enhance their adsorption to a certain degree. It is possible that the varying alcoholic strength was indeed the main reason why the degree of the reduction of bentonite dose depended on the time of addition. Such a result corresponds to that obtained by other groups (Lira et al., 2015; Pocock et al., 2011), who also found later additions to be more effective than those applied to grape juice or at the beginning of fermentation, and more effective than fining after fermentation. Lira et al. (2015) observed a reduction of around 50% of the total bentonite dose after fining late in fermentation in relation to control for Albariño variety, which was more effective than 16% and 21%, as determined by the standard heat stability test and heating with tannins stability test, respectively, for Malvazija istarska wine in this work (Fig. 1).

3.2. Phenols

Bentonite addition exhibited a strong effect on the concentrations of individual phenols (Table 2). All the treatments fermented with bentonite apparently lowered the concentrations of the majority of hydroxybenzoic acids (HBAs). After fermentation, the concentration of gallic acid was the highest in CO, but the additional fining lowered this concentration the most in comparison to other treatments. In MD and EN wines the concentration of gallic acid was significantly reduced after additional protein stabilization. Additional application of bentonite resulted with increased protocatechuic acid content in most treatments, possibly as a consequence of the conversion of other phenols.

Interesting pattern was observed for hydroxycinnamoyltartaric acids and the corresponding free derivatives (HCAs). All wines fermented with bentonite preserved higher levels of the major HCA tartaric acid esters (*trans*-caftaric, *trans*-coutaric, and *trans*-fertaric acid) in relation to CO. CO wine had higher levels of the corresponding free HCA derivatives, caffeic, *p*-coumaric, and ferulic acid. It is probable that

Table 2
Concentrations (means ± standard deviations; mg/L) of phenols in Malvazija istarska wines obtained after partial fining with bentonite (100 g/hL) at different points of fermentation, and in final protein stable wines.

Phenols	Stage	Treatment				
		CO	JU	BE	MD	EN
Hydroxybenzoic acids						
Gallic acid	AFerm	$1.44^{aA} \pm 0.01$	$1.25^{c} \pm 0.04$	$1.14^{d} \pm 0.04$	$1.31^{bA} \pm 0.04$	$1.36^{bA} \pm 0.02$
	ProStab	$1.01^{cB} \pm 0.03$	$1.26^{a} \pm 0.04$	$1.18^{b} \pm 0.02$	$1.22^{abB} \pm 0.02$	$1.20^{bB} \pm 0.02$
Protocatechuic acid	AFerm	$0.89^{B} \pm 0.04$	$0.81^{B} \pm 0.03$	0.85 ± 0.07	$0.84^{B} \pm 0.02$	0.76 ± 0.11
	ProStab	$1.07^{aA} \pm 0.03$	$0.96^{bA} \pm 0.07$	$0.93^{b} \pm 0.08$	$0.92^{bA} \pm 0.01$	$0.91^{b} \pm 0.01$
p-Hydroxybenzoic acid	AFerm	$0.51^{a} \pm 0.01$	$0.50^{a} \pm 0.01$	$0.48^{ab} \pm 0.02$	$0.48^{bA} \pm 0.00$	$0.47^{b} \pm 0.01$
	ProStab	$0.53^{a} \pm 0.01$	$0.49^{b} \pm 0.02$	$0.47^{b} \pm 0.02$	$0.43^{cB} \pm 0.03$	$0.46^{bc} \pm 0.01$
2,5-Dihydroxybenzoic acid	AFerm	$0.31^{a} \pm 0.04$	$0.18^{b} \pm 0.03$	$0.22^{b} \pm 0.03$	$0.19^{b} \pm 0.01$	$0.18^{b} \pm 0.00$
	ProStab	$0.31^{a} \pm 0.03$	$0.18^{b} \pm 0.01$	$0.21^{b} \pm 0.03$	$0.19^{b} \pm 0.01$	$0.18^{b} \pm 0.00$
Syringic acid	AFerm	$0.11^{bA} \pm 0.00$	$0.15^{aA} \pm 0.01$	$0.12^{bA} \pm 0.01$	$0.12^{bA} \pm 0.00$	$0.11^{bA} \pm 0.01$
	ProStab	$0.074^{cB} \pm 0.004$	$0.11^{aB} \pm 0.00$	$0.092^{bB} \pm 0.003$	$0.091^{bB} \pm 0.008$	$0.088^{bB} \pm 0.008$
Hydroxycinnamic acids						
trans-Caftaric acid	AFerm	$2.95^{b} \pm 0.43$	$5.30^{aA} \pm 0.16$	$5.10^{a} \pm 0.88$	$5.36^{aA} \pm 0.24$	$5.30^{aA} \pm 0.06$
	ProStab	$1.92^{b} \pm 0.30$	$4.28^{aB} \pm 0.08$	$4.12^{a} \pm 0.91$	$4.20^{aB} \pm 0.22$	$4.09^{aB} \pm 0.02$
cis-Coutaric acid[a]	AFerm	$0.63^{dB} \pm 0.00$	$0.73^{a} \pm 0.01$	$0.68^{bB} \pm 0.01$	$0.66^{bcB} \pm 0.02$	$0.66^{cB} \pm 0.02$
	ProStab	$0.64^{dA} \pm 0.00$	$0.73^{a} \pm 0.01$	$0.72^{bA} \pm 0.01$	$0.71^{bcA} \pm 0.01$	$0.70^{cA} \pm 0.01$
trans-Coutaric acid[a]	AFerm	$0.11^{b} \pm 0.06$	$0.65^{aA} \pm 0.08$	$0.69^{a} \pm 0.25$	$0.80^{aA} \pm 0.10$	$0.83^{aA} \pm 0.02$
	ProStab	$0.031^{b} \pm 0.003$	$0.32^{aB} \pm 0.02$	$0.35^{a} \pm 0.18$	$0.37^{aB} \pm 0.04$	$0.41^{aB} \pm 0.01$
Caffeic acid	AFerm	$2.72^{aA} \pm 0.00$	$1.85^{b} \pm 0.05$	$1.88^{b} \pm 0.01$	$1.85^{b} \pm 0.03$	$1.88^{b} \pm 0.05$
	ProStab	$2.63^{aB} \pm 0.03$	$1.78^{d} \pm 0.02$	$1.85^{b} \pm 0.02$	$1.82^{c} \pm 0.01$	$1.81^{cd} \pm 0.02$
cis-Fertaric acid[a]	AFerm	$0.59^{B} \pm 0.01$	$0.56^{B} \pm 0.01$	0.57 ± 0.06	$0.56^{B} \pm 0.01$	$0.58^{B} \pm 0.01$
	ProStab	$0.68^{A} \pm 0.01$	$0.62^{A} \pm 0.01$	0.65 ± 0.07	$0.65^{A} \pm 0.01$	$0.66^{A} \pm 0.01$
trans-Fertaric acid[a]	AFerm	$1.86^{b} \pm 0.03$	$2.20^{a} \pm 0.03$	$2.14^{a} \pm 0.13$	$2.17^{a} \pm 0.03$	$2.17^{aA} \pm 0.00$
	ProStab	$1.78^{b} \pm 0.02$	$2.15^{a} \pm 0.02$	$2.09^{a} \pm 0.14$	$2.12^{a} \pm 0.04$	$2.10^{aB} \pm 0.01$
p-Coumaric acid	AFerm	$1.22^{a} \pm 0.09$	$0.75^{b} \pm 0.07$	$0.71^{bc} \pm 0.10$	$0.67^{bcA} \pm 0.01$	$0.63^{cA} \pm 0.03$
	ProStab	$1.06^{a} \pm 0.12$	$0.67^{b} \pm 0.03$	$0.63^{b} \pm 0.10$	$0.57^{bB} \pm 0.02$	$0.57^{bB} \pm 0.01$
Ferulic acid	AFerm	$1.17^{a} \pm 0.07$	$0.88^{b} \pm 0.04$	$0.86^{b} \pm 0.03$	$0.84^{b} \pm 0.01$	$0.84^{bA} \pm 0.01$
	ProStab	$1.14^{a} \pm 0.06$	$0.87^{b} \pm 0.04$	$0.84^{b} \pm 0.05$	$0.81^{b} \pm 0.02$	$0.81^{bB} \pm 0.01$
Flavan-3-ols						
Catechin	AFerm	2.21 ± 0.01	$2.22^{A} \pm 0.05$	2.19 ± 0.08	2.25 ± 0.03	2.22 ± 0.01
	ProStab	$2.24^{a} \pm 0.05$	$2.10^{bB} \pm 0.05$	$2.20^{a} \pm 0.08$	$2.26^{a} \pm 0.02$	$2.22^{a} \pm 0.02$
Procyanidin B1	AFerm	2.93 ± 0.04	$2.88^{B} \pm 0.10$	2.79 ± 0.25	$2.92^{B} \pm 0.04$	2.90 ± 0.07
	ProStab	2.90 ± 0.04	$3.14^{A} \pm 0.05$	2.93 ± 0.31	$3.03^{A} \pm 0.05$	2.96 ± 0.02
Procyanidin B2	AFerm	$1.26^{ab} \pm 0.02$	$1.11^{bB} \pm 0.02$	$1.22^{ab} \pm 0.17$	$1.19^{ab} \pm 0.05$	$1.28^{aA} \pm 0.02$
	ProStab	1.21 ± 0.01	$1.20^{A} \pm 0.01$	1.18 ± 0.12	1.21 ± 0.01	$1.22^{B} \pm 0.01$
Other phenols						
Tyrosol	AFerm	11.8 ± 2.9	12.3 ± 1.1	11.7 ± 1.0	11.8 ± 0.8	12.4 ± 0.5
	ProStab	12.0 ± 2.8	12.5 ± 1.0	12.0 ± 1.0	12.1 ± 0.8	12.7 ± 0.4
Taxifolin	AFerm	$0.17^{a} \pm 0.01$	$0.090^{c} \pm 0.005$	$0.10^{bc} \pm 0.01$	$0.10^{b} \pm 0.01$	$0.10^{b} \pm 0.00$
	ProStab	$0.18^{a} \pm 0.01$	$0.10^{b} \pm 0.00$	$0.10^{b} \pm 0.01$	$0.10^{b} \pm 0.01$	$0.10^{b} \pm 0.00$

CO – control wine without bentonite in fermentation, JU – initial bentonite dose (100 g/hL) added into clear grape juice, BE – initial bentonite dose (100 g/hL) added at the beginning of fermentation, MD – initial bentonite dose (100 g/hL) added at the middle of fermentation, EN – initial bentonite dose (100 g/hL) added at the end of fermentation. AFerm – wines analysed after fermentation, ProStab – wines analysed after total protein stabilisation by additional post-fermentation fining with bentonite.

Different lowercase superscript letters in a row represent statistically significant differences between treatments with respect to bentonite dosing time, and different uppercase superscripts in a column represent statistically significant differences between the concentrations after fermentation (AFerm) and after total protein stabilisation (ProStab), both at $p < 0.05$ obtained by one-way ANOVA and least significant difference (LSD) test.

[a] Semi-quantitative determination, concentrations expressed as equivalents of *trans*-caftaric acid assuming a relative response factor = 1.

bentonite, known for its inhibiting activity towards enzymes (Jaeckels et al., 2015; Main & Morris, 1991), reduced the activity of esterases responsible for the hydrolysis of HCA tartaric acid esters and liberation of fee HCAs. The same relation between the concentrations of HCAs and their tartaric esters in wines fermented with bentonite and in CO wine was retained after achieving protein stability (ProStab in Table 2). However, the additional doses of bentonite caused a decrease in the levels of particular HCA esters in the majority of treatments, especially that of *trans*-coutaric and *trans*-caftaric acid. Since the concentrations of the corresponding free forms (caffeic and *p*-coumaric acid) did not increase notably, it is probable that a part of the HCA ester amounts was adsorbed by bentonite and removed as shown for polyphenols in general by Dordoni, Colangelo, et al. (2015), or was lost due to oxidation into *o*-quinones and other products.

Only slight differences were found between the concentrations of monomeric and dimeric flavanols in different treatments, both after fermentation and after additional fining (Table 2). The concentration of tyrosol was not affected either by bentonite addition or by dosing time. Taxifolin concentration was significantly lower in wines fermented with bentonite, but remained the same after final protein stabilization. It was presumed that in fermentation bentonite interacted with a particular taxifolin precursor either by direct adsorption, or indirectly by limiting its degradation. Dosing time did not have any effect.

3.3. Free volatile aroma compounds

With respect to free volatile varietal aroma compounds, a significant negative effect of bentonite fining during fermentation was observed for citronellol (Table 3). After the second fining, the amounts of particular monoterpenes increased. The observed was probably not a consequence of the activity of bentonite, but simply a result of the gradual liberation of volatile glycons by chemically induced hydrolysis of glycosides

Table 3
Concentrations (means ± standard deviations) and odour perception thresholds (OPT) in μg/L, except * in mg/L, of free volatile aroma compounds in Malvazija istarska wines obtained after partial fining with bentonite (100 g/hL) at different points of fermentation, and in final protein stable wines.

Free aroma compounds	ID	LRI	OPT[a]	Odour description	Stage	Treatment				
						CO	JU	BE	MD	EN
Monoterpenes										
cis-Linalool furan oxide	MS,LRI	1464	3000	floral	AFerm	0.29^{B} ± 0.06	0.25 ± 0.04	0.30^{B} ± 0.03	0.32^{B} ± 0.14	0.26^{B} ± 0.07
					ProStab	0.77^{A} ± 0.05	0.72 ± 0.25	0.92^{A} ± 0.11	0.92^{A} ± 0.09	0.92^{A} ± 0.00
Linalool	S,MS,LRI	1542	15	floral	AFerm	30.1^{B} ± 1.5	30.9^{B} ± 1.0	29.4^{B} ± 1.1	30.9^{B} ± 0.8	29.7^{B} ± 1.0
					ProStab	48.4^{cA} ± 0.4	50.7^{bcA} ± 0.3	55.1^{aA} ± 1.6	52.1^{bA} ± 2.2	57.5^{aA} ± 1.9
Ho-trienol	MS,LRI	1601	n/a	floral	AFerm	11.2^{ab} ± 0.9	9.75^{bB} ± 1.56	10.5^{abB} ± 0.1	9.77^{bB} ± 0.36	11.7^{aB} ± 0.6
					ProStab	12.6^{d} ± 0.4	13.1^{cdA} ± 0.5	14.7^{abA} ± 0.4	13.8^{bcA} ± 0.7	15.6^{aA} ± 0.7
α-Terpineol	S,MS,LRI	1684	250	lilac, camphor	AFerm	9.27^{abB} ± 0.16	9.69^{aB} ± 0.04	9.16^{bcB} ± 0.15	9.59^{aB} ± 0.24	8.78^{cB} ± 0.20
					ProStab	32.0^{cA} ± 0.2	34.2^{bA} ± 1.2	35.9^{aA} ± 0.8	33.6^{bcA} ± 0.7	36.5^{aA} ± 0.9
trans-Linalool pyran oxide	MS,LRI	1726	n/a	floral	AFerm	0.18 ± 0.02	0.16 ± 0.00	0.21 ± 0.01	0.18^{B} ± 0.03	0.20 ± 0.14
					ProStab	0.22 ± 0.03	0.32 ± 0.09	0.28 ± 0.10	0.36^{A} ± 0.10	0.27 ± 0.01
Citronellol	S,MS,LRI	1758	18	citrus	AFerm	10.8^{a} ± 0.2	6.81^{bB} ± 0.56	7.10^{b} ± 1.70	7.01^{b} ± 0.25	7.19^{b} ± 0.32
					ProStab	10.1^{a} ± 0.5	8.97^{abA} ± 0.20	8.85^{ab} ± 0.54	8.26^{bc} ± 0.92	7.55^{c} ± 0.59
Geraniol	S,MS,LRI	1838	30	rose, geranium	AFerm	11.8^{A} ± 0.8	12.2 ± 1.8	11.5 ± 0.1	12.0^{A} ± 0.7	11.1 ± 1.2
					ProStab	9.02^{B} ± 0.26	9.65 ± 1.60	10.8 ± 0.7	9.24^{B} ± 0.14	9.10 ± 1.69
C_{13}-norisoprenoids										
Vitispirane I	MS,LRI	1521	n/a	camphor, woody	AFerm	0.088^{abB} ± 0.001	0.10^{abB} ± 0.00	0.090^{abB} ± 0.014	0.076^{bB} ± 0.019	0.12^{aB} ± 0.02
					ProStab	0.32^{abA} ± 0.07	0.36^{aA} ± 0.03	0.30^{abA} ± 0.01	0.28^{bA} ± 0.02	0.32^{abA} ± 0.04
Vitispirane II	MS,LRI	1523	n/a	camphor, woody	AFerm	0.10 ± 0.01	0.088^{B} ± 0.011	0.12^{B} ± 0.03	0.089^{B} ± 0.012	0.10^{B} ± 0.02
					ProStab	0.32 ± 0.09	0.34^{A} ± 0.02	0.33^{A} ± 0.01	0.30^{A} ± 0.05	0.29^{A} ± 0.08
Actinidol ethyl ether	MS,LRI	1690	n/a	camphor, woody	AFerm	0.045^{B} ± 0.033	0.018^{B} ± 0.014	0.043^{B} ± 0.012	0.040^{B} ± 0.023	0.063^{B} ± 0.049
					ProStab	0.71^{A} ± 0.13	0.54^{A} ± 0.08	0.65^{A} ± 0.24	0.48^{A} ± 0.08	0.70^{A} ± 0.20
β-Damascenone	S,MS,LRI	1809	0.05	sweet, fruity, stewed apple	AFerm	3.65^{cA} ± 0.10	3.65^{cA} ± 0.07	4.13^{bA} ± 0.03	4.38^{aA} ± 0.10	4.22^{abA} ± 0.16
					ProStab	2.65^{B} ± 0.11	2.31^{B} ± 0.11	2.27^{B} ± 0.47	2.23^{B} ± 0.02	2.40^{B} ± 0.16
Actinidol I	MS,LRI	1914	n/a	camphor, woody	AFerm	0.87^{B} ± 0.10	0.90^{B} ± 0.00	0.84^{B} ± 0.13	0.89^{B} ± 0.01	0.90^{B} ± 0.05
					ProStab	6.57^{bA} ± 0.20	7.18^{abA} ± 0.29	7.27^{aA} ± 0.43	6.93^{abA} ± 0.23	7.26^{aA} ± 0.30
Actinidol II	MS,LRI	1927	n/a	camphor, woody	AFerm	1.67^{B} ± 0.12	1.72^{B} ± 0.01	1.59^{B} ± 0.14	1.70^{B} ± 0.04	1.65^{B} ± 0.09
					ProStab	11.3^{bA} ± 0.3	12.3^{aA} ± 0.4	12.3^{aA} ± 0.8	11.7^{abA} ± 0.2	12.3^{aA} ± 0.3
Alcohols										
1-Hexanol*	S,MS,LRI	1356	1.62	fresh cut grass	AFerm	1.88^{a} ± 0.05	1.48^{bB} ± 0.00	1.92^{a} ± 0.07	1.94^{a} ± 0.17	1.85^{aB} ± 0.04
					ProStab	1.96^{ab} ± 0.04	1.72^{cA} ± 0.08	1.98^{a} ± 0.08	1.85^{b} ± 0.01	1.99^{aA} ± 0.05
trans-3-Hexen-1-ol	S,MS,LRI	1361	400	fresh cut grass	AFerm	127^{bc} ± 4	113^{c} ± 0	140^{ab} ± 4	140^{ab} ± 9	141^{a} ± 5
					ProStab	130^{bc} ± 2	123^{c} ± 6	148^{a} ± 5	134^{b} ± 5	148^{a} ± 4
cis-3-Hexen-1-ol	S,MS,LRI	1379	400	green grass	AFerm	61.4^{bB} ± 1.2	60.3^{bB} ± 1.3	74.8^{a} ± 4.9	75.3^{a} ± 6.2	74.6^{a} ± 3.7
					ProStab	66.5^{cA} ± 1.0	66.5^{cA} ± 2.4	77.2^{a} ± 2.1	72.2^{b} ± 1.8	76.6^{a} ± 2.2
1-Octen-4-ol	MS,LRI	1535	n/a	n/a	AFerm	8.79^{B} ± 1.70	9.22^{B} ± 0.08	9.60^{B} ± 1.79	11.3^{B} ± 1.0	10.7^{B} ± 1.2
					ProStab	21.4^{bA} ± 3.7	25.3^{aA} ± 1.7	24.0^{abA} ± 1.5	23.4^{abA} ± 0.5	25.3^{aA} ± 1.1
Benzyl alcohol	MS,LRI	1857	100,000	fruity, walnut	AFerm	5.36^{aB} ± 0.07	4.45^{bB} ± 0.13	4.33^{bB} ± 0.11	4.54^{bB} ± 0.18	4.55^{bB} ± 0.29
					ProStab	9.90^{aA} ± 0.44	7.35^{bA} ± 0.23	7.76^{bA} ± 0.56	7.14^{bA} ± 0.39	7.35^{bA} ± 0.22
2-Phenylethanol*	S,MS,LRI	1891	10	rose, honey	AFerm	18.3^{b} ± 4.2	19.0^{ab} ± 0.6	22.3^{ab} ± 2.6	23.9^{a} ± 2.0	23.3^{ab} ± 2.4
					ProStab	18.8 ± 4.5	21.5 ± 1.3	22.0 ± 2.0	21.0 ± 1.1	23.0 ± 2.4
Fatty acids										
Butanoic acid	S,MS,LRI	1612	173	cheese, rancid	AFerm	32.7^{b} ± 0.6	30.9^{bB} ± 1.0	40.3^{a} ± 2.0	40.8^{a} ± 3.4	40.7^{a} ± 3.4
					ProStab	34.1^{c} ± 1.1	37.1^{bcA} ± 1.0	38.1^{b} ± 3.1	37.4^{bc} ± 1.5	42.1^{a} ± 1.6
Hexanoic acid*	S,MS,LRI	1830	0.42	cheese, rancid	AFerm	3.79^{c} ± 0.44	5.08^{b} ± 0.08	5.65^{ab} ± 0.45	5.33^{ab} ± 0.25	5.72^{aB} ± 0.20
					ProStab	3.59^{d} ± 0.29	4.96^{c} ± 0.20	5.69^{ab} ± 0.43	5.45^{bc} ± 0.36	6.23^{aA} ± 0.17
Octanoic Acid*	S,MS,LRI	2043	0.50	cheese, rancid, fat	AFerm	1.62^{c} ± 0.18	2.45^{b} ± 0.10	3.03^{a} ± 0.14	2.72^{ab} ± 0.33	3.04^{aB} ± 0.13
					ProStab	1.88^{d} ± 0.16	2.49^{c} ± 0.11	3.11^{ab} ± 0.31	2.89^{bc} ± 0.30	3.39^{aA} ± 0.06
Decanoic acid*	S,MS,LRI	2257	1	rancid, wax, plasticine	AFerm	0.65^{c} ± 0.06	0.97^{bA} ± 0.04	1.07^{bA} ± 0.03	1.01^{bA} ± 0.04	1.22^{aA} ± 0.09
					ProStab	0.69^{b} ± 0.01	0.66^{bB} ± 0.06	0.81^{abB} ± 0.08	0.79^{abB} ± 0.11	0.94^{aB} ± 0.12
Ethyl esters										
Ethyl hexanoate*	S,MS,LRI	1236	0.014	fruity, green apple	AFerm	0.45^{cA} ± 0.03	0.57^{bA} ± 0.02	0.58^{bA} ± 0.03	0.59^{bA} ± 0.01	0.65^{aA} ± 0.01
					ProStab	0.30^{cB} ± 0.01	0.31^{cB} ± 0.01	0.38^{bB} ± 0.04	0.36^{bB} ± 0.01	0.43^{aB} ± 0.03
Ethyl octanoate*	S,MS,LRI	1435	0.005	sweet, apple, pineapple	AFerm	0.78^{bA} ± 0.01	0.95^{abA} ± 0.01	0.89^{abA} ± 0.17	0.96^{abA} ± 0.09	1.08^{aA} ± 0.06
					ProStab	0.34^{Bb} ± 0.02	0.41^{aB} ± 0.02	0.45^{aB} ± 0.04	0.44^{aB} ± 0.01	0.45^{aB} ± 0.03
Ethyl decanoate*	S,MS,LRI	1637	0.2	fruity, grape	AFerm	0.25^{cA} ± 0.01	0.30^{bcA} ± 0.05	0.30^{abA} ± 0.00	0.29^{bcA} ± 0.02	0.35^{aA} ± 0.01

(*continued on next page*)

Table 3 (*continued*)

Free aroma compounds	ID	LRI	OPT[a]	Odour description	Stage	Treatment				
						CO	JU	BE	MD	EN
					ProStab	$0.084^{cB} \pm 0.001$	$0.11^{bB} \pm 0.01$	$0.12^{abB} \pm 0.01$	$0.12^{bB} \pm 0.01$	$0.13^{aB} \pm 0.01$
Acetates										
Isoamyl acetate*	S,MS,LRI	1120	0.03	fruity, sweet, banana	AFerm	$0.73^{cA} \pm 0.00$	$1.00^{bA} \pm 0.00$	$1.35^{aA} \pm 0.18$	$1.35^{aA} \pm 0.14$	$1.32^{aA} \pm 0.03$
					ProStab	$0.46^{cB} \pm 0.04$	$0.67^{bB} \pm 0.02$	$0.79^{abB} \pm 0.14$	$0.72^{abB} \pm 0.05$	$0.86^{aB} \pm 0.04$
Hexyl acetate*	S,MS,LRI	1272	0.67	apple, cherry, pear, floral	AFerm	$0.10^{bA} \pm 0.00$	$0.14^{aA} \pm 0.00$	$0.13^{aA} \pm 0.02$	$0.13^{aA} \pm 0.01$	$0.13^{aA} \pm 0.00$
					ProStab	$0.019^{bB} \pm 0.001$	$0.035^{aB} \pm 0.005$	$0.044^{aB} \pm 0.013$	$0.039^{aB} \pm 0.004$	$0.047^{aB} \pm 0.006$
2-Phenethyl acetate*	S,MS,LRI	1803	0.25	fruity, honey, floral	AFerm	$0.12^{b} \pm 0.02$	$0.18^{aA} \pm 0.00$	$0.16^{aA} \pm 0.01$	$0.18^{aA} \pm 0.01$	$0.18^{aA} \pm 0.01$
					ProStab	$0.085^{c} \pm 0.012$	$0.12^{abB} \pm 0.01$	$0.11^{bB} \pm 0.01$	$0.12^{abB} \pm 0.00$	$0.12^{aB} \pm 0.00$
Other esters										
Ethyl lactate*	S,MS,LRI	1341	100	sweet, buttery	AFerm	$6.29^{B} \pm 0.88$	$5.96^{B} \pm 0.23$	$5.40^{B} \pm 0.44$	$6.30^{B} \pm 0.51$	$5.76^{B} \pm 0.33$
					ProStab	$16.5^{A} \pm 1.5$	$16.0^{A} \pm 1.3$	$17.4^{A} \pm 0.6$	$16.9^{A} \pm 0.6$	$17.1^{A} \pm 0.9$
Isoamyl hexanoate	MS,LRI	1457	n/a	fruity	AFerm	$1.10^{bB} \pm 0.20$	$1.38^{abB} \pm 0.10$	$1.69^{abA} \pm 0.49$	$1.82^{aB} \pm 0.24$	$1.90^{aB} \pm 0.34$
					ProStab	$2.68^{bA} \pm 0.13$	$3.41^{abA} \pm 0.13$	$3.16^{abB} \pm 0.45$	$3.69^{aA} \pm 0.53$	$3.16^{abA} \pm 0.30$
Isoamyl octanoate	MS,LRI	1655	125	fruity	AFerm	$2.07^{b} \pm 0.19$	$2.75^{ab} \pm 0.82$	$2.78^{ab} \pm 0.49$	$2.60^{ab} \pm 0.17$	$3.18^{aB} \pm 0.42$
					ProStab	$2.09^{b} \pm 0.02$	$2.72^{b} \pm 0.27$	$6.82^{a} \pm 3.26$	$3.09^{b} \pm 0.84$	$9.64^{aA} \pm 0.35$
Diethyl succinate*	S,MS,LRI	1667	6	overripe melon, vinous	AFerm	$0.22^{B} \pm 0.06$	$0.28^{B} \pm 0.02$	$0.18^{B} \pm 0.00$	$0.26^{B} \pm 0.07$	$0.22^{B} \pm 0.02$
					ProStab	$1.66^{bA} \pm 0.30$	$2.24^{aA} \pm 0.08$	$1.94^{abA} \pm 0.16$	$1.97^{abA} \pm 0.18$	$2.03^{aA} \pm 0.11$
Other										
Furfural	MS,LRI	1451	14,100	sweet, woody, almond	AFerm	$1.51^{bB} \pm 0.25$	$1.55^{bB} \pm 0.28$	$1.60^{abB} \pm 0.23$	$1.75^{abB} \pm 0.35$	$2.12^{aB} \pm 0.11$
					ProStab	$11.0^{aA} \pm 0.0$	$8.66^{bA} \pm 0.80$	$11.3^{aA} \pm 0.9$	$10.1^{aA} \pm 0.9$	$11.1^{aA} \pm 0.4$
Benzaldehyde	S,MS,LRI	1508	2000	bitter almond, nutty, cherry	AFerm	$4.87^{b} \pm 0.49$	$5.30^{b} \pm 0.03$	$7.10^{ab} \pm 1.58$	$6.79^{ab} \pm 1.15$	$7.77^{a} \pm 0.72$
					ProStab	$5.69^{c} \pm 0.54$	$5.55^{c} \pm 0.20$	$7.25^{ab} \pm 1.13$	$6.29^{bc} \pm 0.14$	$8.03^{a} \pm 0.43$
Dihydro-3(2H)-thiophenone	MS	1510	n/a	n/a	AFerm	6.65 ± 4.22	$8.64^{A} \pm 0.30$	6.93 ± 2.32	$9.23^{A} \pm 1.85$	8.98 ± 1.30
					ProStab	4.61 ± 2.40	$6.45^{B} \pm 0.62$	4.88 ± 1.48	$5.42^{B} \pm 1.17$	6.00 ± 1.59
Ethyl benzeneacetate	MS,LRI	1773	n/a	n/a	AFerm	$1.11^{B} \pm 0.06$	$0.96^{B} \pm 0.03$	$0.82^{B} \pm 0.18$	$0.93^{B} \pm 0.12$	$1.03^{B} \pm 0.25$
					ProStab	$2.42^{A} \pm 0.04$	$2.24^{A} \pm 0.10$	$2.12^{A} \pm 0.28$	$2.12^{A} \pm 0.14$	$2.31^{A} \pm 0.28$
4-Ethylphenol	MS,LRI	2156	600	phenolic, leather	AFerm	0.65 ± 0.01	$0.69^{B} \pm 0.02$	0.67 ± 0.01	1.25 ± 0.60	0.95 ± 0.25
					ProStab	$0.77^{b} \pm 0.11$	$0.89^{abA} \pm 0.06$	$0.87^{ab} \pm 0.09$	$0.94^{ab} \pm 0.09$	$1.01^{a} \pm 0.11$
4-Vinylguaiacol	MS,LRI	2175	40	spices, smoke	AFerm	101 ± 13	$114^{A} \pm 5$	117 ± 7	114 ± 9	107 ± 2
					ProStab	$111^{ab} \pm 4$	$105^{abB} \pm 2$	$121^{a} \pm 17$	$100^{b} \pm 3$	$106^{ab} \pm 7$

CO – control wine without bentonite in fermentation, JU – initial bentonite dose (100 g/hL) added into clear grape juice, BE – initial bentonite dose (100 g/hL) added at the beginning of fermentation, MD – initial bentonite dose (100 g/hL) added at the middle of fermentation, EN – initial bentonite dose (100 g/hL) added at the end of fermentation. AFerm – wines analysed after fermentation, ProStab – wines analysed after total protein stabilisation by additional post-fermentation fining with bentonite.

Identification of compounds (ID): S – retention time and mass spectrum consistent with that of the pure standard and with NIST05 mass spectra electronic library; LRI – linear retention index consistent with that found in literature; MS – mass spectra consistent with that from NIST05 mass spectra electronic library or literature. The concentration of compounds for which pure standards were not available (without symbol S in ID column) were expressed semi-quantitatively as equivalents of compounds with similar chemical structure assuming a relative response factor = 1.

Different lowercase superscript letters in a row represent statistically significant differences between treatments with respect to bentonite dosing time, and different uppercase superscripts in a column represent statistically significant differences between the concentrations after fermentation (AFerm) and after total protein stabilisation (ProStab), both at $p < 0.05$ obtained by one-way ANOVA and least significant difference (LSD) test.

[a] Odour perception thresholds (μg/L) and odour descriptors reported in the literature (Ferreira, López, & Cacho, 2000; Guth, 1997; Moreno, Zea, Moyano, & Medina, 2005; Noguerol-Pato, González-Álvarez, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2013).

(linalool, α-terpineol, and citronellol) or oxidation and other conversions (ho-trienol, α-terpineol, and linalool oxides). The additional bentonite treatment exhibited a negative effect on free geraniol. Armada and Falqué (2007) observed a decrease in linalool, nerol, and geraniol, and an increase in α-terpineol and citronellol concentrations after fining of Albariño grape juice with 60 g/hL of bentonite, while Burin et al. (2016) noted a loss of all the major monoterpenols after a similar treatment. In a two year study, Lambri et al. (2012) observed significant losses of total and particular monoterpenols after treating grape must before fermentation with 100 g/hL of bentonite, with the effect being significant mostly only in a single vintage, except for linalool which decreased in both years. The application of an additional bentonite treatment after fermentation in both wines (must treated or not treated with bentonite) caused mixed effects depending on the monoterpene compound and the harvest year. Vincenzi et al. (2015) did not observed significant effect on relevant free monoterpenes after bentonite treatment applied in a model solution.

The most important C_{13}-norisoprenoid from a sensorial point of view, β-damascenone, was found in higher concentration in BE, MD, and EN in relation to JU and CO treatments (Table 3). It is possible that bentonite added in the earliest phase (JU) removed a portion of β-damascenone precursors. The formation of β-damascenone was previously shown to be negatively correlated with the amount of particle matter in fermentation medium, with an assumed inhibitory activity of solids by providing either competitive substrates or inhibitors, or by adsorbing β-damascenone precursors (Lukić et al., 2017). It is possible that this was the reason for the higher concentration found in BE, MD, and EN treatments, in which a portion of solid particles was removed by

bentonite. Additional fining reduced β-damascenone concentration in all the treatments, but also changed the relationship between them, leaving CO with the highest content. Besides removal by the additional bentonite dose, β-damascenone possibly partly decreased after reacting with sulphur dioxide during short period of wine aging between the two sampling times, as suggested earlier (Oliveira, Oliveira, Baumes, & Maia, 2008). The amounts of other norisoprenoids, such as vitispirane and actinidol isomers, significantly increased after additional fining, probably as a result of the transformation of norisoprenoidic precursors during aging, as reported earlier by Oliveira et al. (2008).

Significantly the lowest C_6-alcohol concentration was found in JU (Table 3), which is in agreement with the findings from Armada and Falqué (2007) who noted a decrease after bentonite treatment of grape juice before fermentation. Bentonite added in such an early stage of winemaking possibly limited the action of lipoxygenase and alcohol dehydrogenase enzymes responsible for the cleavage of long-chain fatty acids and the formation of C_6 aldehydes and alcohols. In contrast to the findings from Lambri et al. (2010) who observed a rather significant decrease of 1-hexanol after bentonite fining of wine, in this work fining during fermentation and additional wine fining apparently did not remove a significant portion of this compound. Bentonite treated wines produced less benzyl alcohol than CO. After additional fining, the concentrations of 1-octen-4-ol and benzaldehyde significantly increased.

In general, bentonite treatments during fermentation significantly affected the synthesis and preservation of fermentation volatile compounds. 2-Phenylethanol was found in the lowest concentration in JU and CO treatments, but after the additional fining, the concentrations levelled off (Table 3). The wines treated with bentonite preserved higher concentrations of major fermentation acids and esters with respect to CO. This effect was especially conspicuous in the case of acetates. An exception was the JU treatment, with in some cases lower concentrations than in other bentonite treated wines. Burin et al. (2016) observed a similar effect after treating Chardonnay juice with 70 g/hL of bentonite, and partly attributed it to a decrease of total yeast assimilable nitrogen content. The results of this study are mostly in accordance to those from Lira et al. (2015), who found wines treated with bentonite in later phases of fermentation to be richer in volatile odoriferous esters than juice-treated and untreated wines. One of the possible reasons for this was the protective effect of bentonite against the action of cellular esterases responsible for the cleavage of volatile esters, which are more active near the end of fermentation (Mauricio et al., 1993). Bentonite inhibition of PPO enzymes, as discussed in a previous section, could have limited the rate of the oxidation of phenols, which is known to initiate a series of chemical transformations in which semiquinone radicals and quinones are formed, while in the presence of transition metals oxygen is reduced to hydrogen peroxide, which may all oxidise and decrease the level of esters (Patrianakou & Roussis, 2013). Lukić et al. (2017) observed that solids in fermentation inhibit the formation of esters, which could be related to the lower ester concentrations in more turbid CO must and wine after fermentation (Table 3).

After the final bentonite treatment and the achievement of protein stability, the levels of all the investigated ethyl and acetate esters dropped significantly (Table 3). This could have partly been the result of oxidation, despite wines were protected by sulphur dioxide, since the racking process in such experimental conditions was partly aerobic. On the other hand, this result is in accordance with earlier studies, where bentonite treatment of wine model solutions was shown to dramatically decrease the concentration of esters (Vincenzi et al., 2015). Lambri et al. (2010) observed rather high losses of the majority of important volatiles after bentonite treatment of finished wine, depending on the compound, and the type and dose of bentonite. The authors (Lambri et al., 2010; Vincenzi et al., 2015) hypothesised the possibility of losing volatiles by deproteinisation, that is by hydrogen bonding of hydrophilic ones on protein surface and bonding of more hydrophobic molecules to interior protein sites, followed by subsequent removal by bentonite. Lambri et al. (2013) showed that various volatile compounds can be adsorbed directly on the surface of bentonite without the involvement of macromolecules, by both physical and chemical mechanisms. For example, the adsorption of ethyl esters was assumed to be driven mostly by chemical interactions. However, Lambri et al. (2013) showed that the degree of adsorption depends more on the characteristics of bentonite, such as the proportion between specific surface area and charge density, than on compound properties, which can explain the mixed results depending on the harvest year obtained by Vela et al. (2017), where in some cases bentonite treatment even increased the amounts of particular esters.

The concentration of other esters increased after the second fining (Table 3), probably mainly due to esterification reactions to reach equilibrium concentrations with respect to alcohol and acid precursors.

3.4. Bound volatile aroma compounds

Bound volatile aroma compounds represent the so-called varietal aroma reserve, which can gradually hydrolyse and release volatile odoriferous aglycones during wine storage. Generally, the effect of bentonite treatments during fermentation on bound volatile aroma compounds was weak to moderate (Table 4). Among many specific outcomes, it is worth emphasizing the behaviour of citronellol and nerol that had the lowest amounts in CO wine. This could be tentatively related to the highest free citronellol concentration found in CO (Table 3), possibly as a result of enzymatic hydrolysis of its bound precursor, unhindered because of the absence of bentonite as an inhibitor. Similar was also observed for other, less important bound compounds.

The difference between bound volatiles composition after fermentation and after the additional fining was again the result of the combination of chemical hydrolysis and other transformations during the (short) aging period. The amount of bound linalool decreased most dramatically, followed by that of citronellol, geraniol, and 3-hydroxydamascone. The decreased amounts of linalool and citronellol (Table 4) corresponded roughly to the increase observed for the corresponding free forms after the additional fining and aging (Table 3).

3.5. Sensory analysis

After fermentation, the intensity of floral, fruity, and tropical odours was generally higher in bentonite treated wines than in CO (Fig. 2a, Table S2), without statistically significant differences between them. Such a result corresponded to the differences between the amounts of the odoriferous fermentation esters in these wines, which are known to be responsible for the mentioned odours, especially those with concentrations surpassing their corresponding odour perception thresholds (Table 3). Similar results were obtained earlier by Lira et al. (2015). Since, except for JU, no significant difference between the treatments was observed for the concentration of 1-hexanol, a carrier of herbaceous odour but in this work barely surpassing its odour perception threshold (Table 3), it was supposed that a greater expression of herbaceous notes in CO wine was mainly an indirect consequence of the lower intensities of the competing floral, fruity, tropical, and honey nuances observed for this treatment (Fig. 2). Apparently, bentonite treatment generally enhanced wine body, while the contrary was observed for other taste attributes, such as acidity, bitterness and astringency, which were the most intense in CO wine (Fig. 2a, Table S2). Although a direct cause-effect relationship between the amount of hydroxycinnamates and wine body was not established up to date, a positive correlation found in this work could be compared to that found between the amount of these phenols and the viscosity of white wine observed in a recent study (Gawel et al., 2014). Other previous studies on white wines have also showed that increase in phenolic compounds may be associated to increased mouthfeel attributes (Olejar, Fedrizzi, &

Table 4
Concentrations (means ± standard deviations; μg/L) of bound volatile aroma compounds in Malvazija istarska wines obtained after partial fining with bentonite (100 g/hL) at different points of fermentation, and in final protein stable wines.

Bound aroma compounds	ID	LRI	Stage	Treatment				
				CO	JU	BE	MD	EN
Monoterpenes								
β-Pinene	MS, LRI	1122	AFerm	0.28 ± 0.02	0.27 ± 0.03	0.18^{B} ± 0.03	0.19 ± 0.06	0.21 ± 0.06
			ProStab	0.25^{a} ± 0.02	0.27^{a} ± 0.02	0.26^{aA} ± 0.02	0.25^{a} ± 0.01	0.19^{b} ± 0.02
trans-Ocimene	MS, LRI	1253	AFerm	0.14^{B} ± 0.00	0.20 ± 0.10	0.28 ± 0.15	0.29 ± 0.07	0.26 ± 0.11
			ProStab	0.22^{A} ± 0.02	0.23 ± 0.09	0.25 ± 0.08	0.29 ± 0.06	0.26 ± 0.02
trans-Linalool furan oxide	MS, LRI	1436	AFerm	0.60^{B} ± 0.03	0.66 ± 0.03	0.69 ± 0.13	0.62^{B} ± 0.02	0.65^{B} ± 0.07
			ProStab	0.83^{abA} ± 0.02	0.81^{ab} ± 0.09	0.73^{b} ± 0.05	0.85^{aA} ± 0.03	0.83^{aA} ± 0.03
cis-Linalool furan oxide	MS, LRI	1464	AFerm	0.18^{cdB} ± 0.02	0.29^{ab} ± 0.04	0.32^{a} ± 0.04	0.23^{bc} ± 0.03	0.14^{d} ± 0.02
			ProStab	0.29^{bA} ± 0.02	0.22^{b} ± 0.02	0.51^{a} ± 0.11	0.23^{b} ± 0.03	0.25^{b} ± 0.15
Linalool	S, MS, LRI	1542	AFerm	28.3^{A} ± 3.9	28.5^{A} ± 1.8	30.7^{A} ± 5.9	30.2^{A} ± 3.2	27.4^{A} ± 0.8
			ProStab	4.46^{B} ± 0.15	4.02^{B} ± 0.53	4.06^{B} ± 0.87	4.40^{B} ± 0.57	3.76^{B} ± 0.41
Ho-trienol	MS, LRI	1601	AFerm	0.31^{ab} ± 0.06	0.28^{b} ± 0.06	0.34^{ab} ± 0.06	0.41^{aA} ± 0.03	0.31^{ab} ± 0.05
			ProStab	0.36 ± 0.06	0.28 ± 0.09	0.28 ± 0.08	0.23^{B} ± 0.06	0.34 ± 0.03
α-Terpineol	S, MS, LRI	1684	AFerm	2.41^{ab} ± 0.21	2.15^{b} ± 0.04	2.46^{ab} ± 0.28	2.07^{b} ± 0.73	3.37^{aA} ± 0.35
			ProStab	2.24 ± 0.67	3.84 ± 1.40	3.94 ± 2.30	3.66 ± 2.01	2.25^{B} ± 0.20
trans-Linalool pyran oxide	MS, LRI	1726	AFerm	1.58^{B} ± 0.12	1.71 ± 0.30	1.81 ± 0.20	1.39 ± 0.29	1.56 ± 0.36
			ProStab	2.37^{aA} ± 0.22	1.76^{b} ± 0.24	1.61^{b} ± 0.15	1.72^{b} ± 0.11	1.66^{b} ± 0.25
Citronellol	S, MS, LRI	1758	AFerm	6.54^{b} ± 0.78	11.6^{abA} ± 0.2	15.5^{a} ± 4.6	9.63^{ab} ± 1.77	14.9^{a} ± 3.6
			ProStab	7.02 ± 0.41	3.62^{B} ± 0.27	7.31 ± 1.40	5.85 ± 3.68	5.94 ± 4.35
Nerol	S, MS, LRI	1791	AFerm	9.59^{b} ± 1.81	11.7^{abA} ± 0.6	11.0^{abA} ± 0.7	11.9^{aA} ± 0.7	12.6^{aA} ± 1.1
			ProStab	8.97 ± 0.84	8.71^{B} ± 0.86	8.08^{B} ± 1.06	8.92^{B} ± 0.55	7.45^{B} ± 0.96
Geraniol	S, MS, LRI	1838	AFerm	72.4 ± 4.1	79.3^{A} ± 6.0	82.5 ± 14.4	83.5^{A} ± 4.4	81.5^{A} ± 9.3
			ProStab	69.8^{a} ± 5.0	59.7^{bB} ± 3.3	62.5^{ab} ± 7.0	65.8^{abB} ± 3.2	63.2^{abB} ± 3.4
C_{13}-norisoprenoids								
3-Hydroxy-β-damascone	MS, LRI	2634	AFerm	49.8^{abA} ± 4.2	51.4^{aA} ± 3.9	41.2^{b} ± 5.6	43.4^{abA} ± 4.3	50.5^{aA} ± 3.0
			ProStab	35.2^{B} ± 1.0	29.7^{B} ± 0.9	29.3 ± 5.5	31.6^{B} ± 1.2	30.8^{B} ± 3.4
Alcohols								
1-Hexanol	S, MS, LRI	1356	AFerm	133 ± 46	134 ± 9	134^{B} ± 1	140 ± 11	151 ± 20
			ProStab	139^{c} ± 7	172^{b} ± 21	226^{aA} ± 15	115^{c} ± 16	124^{c} ± 3
trans-3-Hexen-1-ol	S, MS, LRI	1361	AFerm	0.64^{bB} ± 0.26	1.34^{ab} ± 0.36	1.39^{ab} ± 0.43	1.30^{ab} ± 0.40	1.44^{aB} ± 0.07
			ProStab	1.86^{A} ± 0.05	1.75 ± 0.25	1.63 ± 0.18	1.71 ± 0.24	1.89^{A} ± 0.04
cis-3-Hexen-1-ol	S, MS, LRI	1379	AFerm	28.8^{B} ± 0.6	29.0 ± 0.5	29.3 ± 2.0	28.9 ± 2.2	29.9 ± 1.9
			ProStab	33.8^{A} ± 1.3	31.2 ± 2.0	30.1 ± 2.9	31.0 ± 1.8	32.9 ± 1.8
1-Octen-3-ol	MS, LRI	1535	AFerm	1.48^{B} ± 0.01	1.62 ± 0.26	1.69 ± 0.17	1.59^{B} ± 0.16	1.70^{B} ± 0.04
			ProStab	1.96^{cdA} ± 0.11	3.10^{c} ± 0.74	1.97^{d} ± 0.62	6.49^{aA} ± 0.54	4.22^{bA} ± 0.58
Benzyl Alcohol	MS, LRI	1857	AFerm	68.7^{B} ± 3.1	67.1 ± 5.5	70.1 ± 13.7	71.0^{B} ± 5.3	80.2 ± 5.1
			ProStab	92.5^{aA} ± 1.3	79.7^{ab} ± 7.1	78.6^{b} ± 8.4	87.2^{abA} ± 7.3	81.1^{ab} ± 5.1
2-Phenylethanol	S, MS, LRI	1891	AFerm	334^{bc} ± 28	371^{abA} ± 13	318^{c} ± 30	355^{abc} ± 16	384^{aA} ± 20
			ProStab	368^{a} ± 20	287^{bB} ± 15	348^{a} ± 41	335^{ab} ± 31	277^{bB} ± 37
Other								
Benzaldehyde	S, MS, LRI	1508	AFerm	14.3^{a} ± 0.2	13.4^{abA} ± 0.1	12.2^{b} ± 0.2	13.8^{a} ± 0.1	14.7^{a} ± 1.1
			ProStab	15.8^{a} ± 0.5	10.6^{cB} ± 1.1	12.3^{bc} ± 1.8	13.8^{ab} ± 0.5	15.4^{a} ± 1.0

CO – control wine without bentonite in fermentation, JU – initial bentonite dose (100 g/hL) added into clear grape juice, BE – initial bentonite dose (100 g/hL) added at the beginning of fermentation, MD – initial bentonite dose (100 g/hL) added at the middle of fermentation, EN – initial bentonite dose (100 g/hL) added at the end of fermentation. AFerm – wines analysed after fermentation, ProStab – wines analysed after total protein stabilisation by additional post-fermentation fining with bentonite.

Identification of compounds (ID): S – retention time and mass spectrum consistent with that of the pure standard and with NIST05 mass spectra electronic library; LRI – linear retention index consistent with that found in literature; MS – mass spectra consistent with that from NIST05 mass spectra electronic library or literature. The concentration of compounds for which pure standards were not available (without symbol S in ID column) were expressed semi-quantitatively as equivalents of compounds with similar chemical structure assuming a relative response factor = 1.

Different lowercase superscript letters in a row represent statistically significant differences between treatments with respect to bentonite dosing time, and different uppercase superscripts in a column represent statistically significant differences between the concentrations after fermentation (AFerm) and after total protein stabilisation (ProStab), both at $p < 0.05$ obtained by one-way ANOVA and least significant difference (LSD) test.

Kilmartin, 2016).

Similar relations were observed after sensory analysis of protein stable wines obtained after additional fining (Fig. 2b, Table S2). After this phase BE wine was described by the highest intensities of the majority of positive odour and taste attributes, followed by MD and EN, while the difference between these treatments and JU became more evident. CO was distinguished from the other treatments by the same attributes as after fermentation. After additional bentonite fining, the intensities of the majority of positive descriptors decreased in all the treatments, in some cases with statistical significance (Fig. 2b, Table S2), which in a way corroborated the widely accepted opinion that bentonite applied to finished wine reduces its sensory quality. Previously mentioned possible oxidation cascade (Patrianakou & Roussis, 2013) and evaporation during and after the additional racking step could have also had a significant effect on the loss of impacting volatiles, such as esters. Probably the sole comparative advantage of the wines obtained after additional fining with respect to those analysed after fermentation was the increase in the concentration of free linalool (Table 3). Linalool possibly contributed positively, but this effect did not reflect on the results of the sensory analysis, probably due to a larger sensory impact of the previously mentioned decrease in the concentration of esters in protein stable wines. Average hedonic sensory scores for overall quality obtained by the OIV method generally correlated positively very strongly ($r > 0.9$) with the intensities of all the

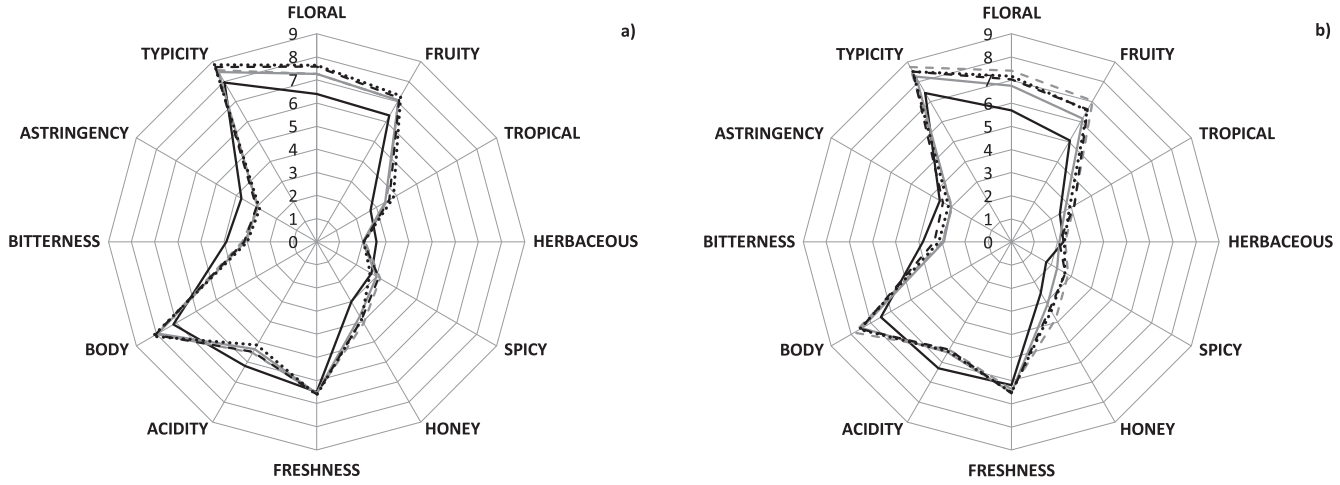

Fig. 2. Sensory profiles of Malvazija istarska wines (a) after fermentation and (b) after additional fining with bentonite, with respect to the moment of initial bentonite dosing (100 g/hL): control wine without bentonite in fermentation (CO, solid black line), initial bentonite dose added into clear grape juice (JU, solid grey line), initial bentonite dose added at the beginning of fermentation (BE, dashed grey line), initial bentonite dose added at the middle of fermentation (MD, dotted black line), and initial bentonite dose added at the end of fermentation (EN, dashed black line). The wines were treated by additional bentonite doses after fermentation to achieve total protein stability.

positive odour and taste attributes, and negatively ($r < 0.9$) with herbaceous odour, acidity, bitterness and astringency, in both wines assessed after fermentation and after the final fining. BE, MD, and EN were rated with the highest scores, and CO with the lowest. Wines assessed after final fining were rated with lower average scores for overall quality with respect to the corresponding wines of the same treatment assessed after fermentation, with statistical significance for JU and EN (Table S2).

4. Conclusions

Fining during fermentation reduced the total bentonite dose required, and its efficacy depended on the moment of dosage, being most effective near the end of fermentation. Various, mostly positive side-effects were observed. All wines treated during fermentation preserved higher amounts of hydroxycinnamoyltartaric acids with respect to the control, in all probability due to the inhibition of the activity of enzymes responsible for their hydrolysis and oxidation. Hydroxycinnamoyltartaric acids are known to be important contributors to wine antioxidant activity, meaning that such treatments may ensure additional protection of volatiles from oxidation and improved wine oxidative stability in general. Although a strong impact of bentonite on β-glucosidase activity and varietal aroma compound chemistry was expected, the response of monoterpenes and C_{13}-norisporenoids was negligible to moderate. Probably most important from the compositional and sensorial points of view, bentonite fining in fermentation resulted with preserved amounts of key odoriferous fermentation volatiles in relation to control, which exhibited significant positive sensory effects. Treating clear juice before fermentation produced lower concentrations, but still higher than in the control wine.

The reduction of the bentonite dose of up to 16% or 21% (depending on the test) after its addition during fermentation might turn out to be significant from an economical point of view. Also, it seems that bentonite added during fermentation induces important positive effects on white wine chemical composition and sensory quality regardless of the time of addition and the dose reduction rate. However, additional fining after fermentation in achieving complete protein stability exhibited negative effects. This suggests that fining during fermentation with the minimum bentonite dose required to achieve absolute protein stability could have multiple positive outcomes, and could enable avoiding or shortening particular production steps, such as additional fining, racking, and other manipulation that may reduce wine quality and delay wine marketing. Finding the method for the determination of such dose imposes itself as one of the important goals of the research on this topic in the future.

Conflict of interest

There is not any conflict of interests.

Acknowledgements

This work has been supported in part by Croatian Science Foundation – Croatia under the project “Unravelling the varietal typicity of wines and olive oils from Croatian domestic varieties” (UIP-2014-09-1194).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.01.172.

References

Achaerandio, I., Pachova, V., Güell, C., & López, F. (2001). Protein adsorption by bentonite in a white wine model solution: Effect of protein molecular weight and ethanol concentration. *American Journal of Enology and Viticulture, 52*(2), 122–126.

Armada, L., & Falqué, E. (2007). Repercussion of the clarification treatment agents before the alcoholic fermentation on volatile composition of white wines. *European Food Research and Technology, 225*(3–4), 553–558.

Blade, W. H., & Boulton, R. (1988). Adsorption of protein by bentonite in a model wine solution. *American Journal of Enology and Viticulture, 39*(3), 193–199.

Burin, V. M., Caliari, V., & Bordignon-Luiz, M. T. (2016). Nitrogen compounds in must and volatile profile of white wine: Influence of clarification process before alcoholic fermentation. *Food Chemistry, 202*, 417–425.

Dordoni, R., Colangelo, D., Giribaldi, M., Giuffrida, M. G., De Faveri, D. M., & Lambri, M. (2015). Effect of bentonite characteristics on wine proteins, polyphenols, and metals under conditions of different pH. *American Journal of Enology and Viticulture, 66*, 518–530.

Dordoni, R., Galasi, R., Colangelo, D., De Faveri, D. M., & Lambri, M. (2015). Effects of fining with different bentonite labels and doses on colloidal stability and colour of a Valpolicella red wine. *International Journal of Food Science and Technology, 50*, 2246–2254.

Ewart, A. J. W., Phipps, G. J., & Iland, P. G. (1980). Bentonite additions to wine: Before, during or after fermentation? *Australian & New Zealand Grapegrower & Winemaker, 196*, 46–47.

Ferreira, V., López, R., & Cacho, J. F. (2000). Quantitative determination of the odorants of young red wines from different grape varieties. *Journal of the Science of Food and Agriculture, 80*, 1659–1667.

Gawel, R., Day, M., Van Sluyter, S. C., Holt, H., Waters, E. J., & Smith, P. A. (2014). White

wine taste and mouthfeel as affected by juice extraction and processing. *Journal of Agricultural and Food Chemistry, 62*, 10008–10014.
Gazzola, D., Van Sluyter, S. C., Curioni, A., Waters, E. J., & Marangon, M. (2012). Roles of proteins, polysaccharides, and phenolics in haze formation in white wine via reconstitution experiments. *Journal of Agricultural and Food Chemistry, 60*, 10666–10673.
Guth, H. (1997). Quantitation and sensory studies of character impact odorants of different white wine varieties. *Journal of Agricultural and Food Chemistry, 45*, 3027–3032.
Jaeckels, N., Tenzer, S., Rosch, A., Scholten, G., Decker, H., & Fronk, P. (2015). β-Glucosidase removal due to bentonite fining during winemaking. *European Food Research and Technology, 241*, 253–262.
Lambri, M., Dordoni, R., Silva, A., & De Faveri, D. M. (2010). Effect of bentonite fining on odor-active compounds in two different white wine styles. *American Journal of Enology and Viticulture, 61*(2), 225–233.
Lambri, M., Dordoni, R., Silva, A., & De Faveri, D. M. (2012). Comparing the impact of bentonite addition for both must clarification and wine fining on the chemical profile of wine from Chambave Muscat grapes. *International Journal of Food Science and Technology, 47*, 1–12.
Lambri, M., Dordoni, R., Silva, A., & De Faveri, D. M. (2013). Odor-active compound adsorption onto bentonite in a model white wine solution. *Chemical Engineering Transactions, 32*, 1741–1746.
Lira, E., Rodríguez-Bencomo, J. J., Salazar, F. N., Orriols, I., Fornos, D., & López, F. (2015). Impact of bentonite additions during vinification on protein stability and volatile compounds of Albariño wines. *Journal of Agricultural and Food Chemistry, 63*, 3004–3011.
Lira, E., Salazar, F. N., Rodríguez-Bencomo, J. J., Vincenzi, S., Curioni, A., & López, F. (2014). Effect of using bentonite during fermentation on protein stabilisation and sensory properties of white wine. *International Journal of Food Science and Technology, 49*, 1070–1078.
Lukić, I., Lotti, C., & Vrhovsek, U. (2017). Evolution of free and bound volatile aroma compounds and phenols during fermentation of Muscat blanc grape juice with and without skins. *Food Chemistry, 232*, 25–35.
Main, G. L., & Morris, J. R. (1991). Color of Riesling and Vidal wines as affected by bentonite, Cufex®, and sulfur dioxide juice treatments. *American Journal of Enology and Viticulture, 42*, 354–357.
Majewski, P., Barbalet, A., & Waters, E. (2011). $1 billion hidden cost of bentonite fining. *Australian & New Zealand Grapegrower & Winemaker, 569*, 58–62.
Marangon, M., Stockdale, V. J., Munro, P., Trethewey, T., Schulkin, A., Holt, H. E., & Smith, P. A. (2013). Addition of carrageenan at different stages of winemaking for white wine protein stabilization. *Journal of Agricultural and Food Chemistry, 61*, 6516–6524.
Marangon, M., Van Sluyter, S. C., Neilson, K. A., Chan, C., Haynes, P. A., Waters, E. J., & Falconer, R. J. (2011). Roles of grape thaumatin like protein and chitinase in white wine haze formation. *Journal of Agricultural and Food Chemistry, 59*, 733–740.
Marangon, M., Vincenzi, S., Lucchetta, M., & Curioni, A. (2010). Heating and reduction affect the reaction with tannins of wine protein fractions differing in hydrophobicity. *Analytica Chimica Acta, 660*, 110–118.
Mauricio, J. C., Moreno, J., Valero, E. M., Zea, L., Medina, M., & Ortega, J. M. (1993). Ester formation and specific activities of in vitro alcohol acetyltransferase and esterase by Saccharomyces cerevisiae during grape must fermentation. *Journal of Agricultural and Food Chemistry, 41*, 2086–2091.
Moreno, J. A., Zea, L., Moyano, L., & Medina, M. (2005). Aroma compounds as markers of the changes in sherry wines subjected to biological ageing. *Food Control, 16*, 333–338.
Noguerol-Pato, R., González-Álvarez, M., González-Barreiro, C., Cancho-Grande, B., & Simal-Gándara, J. (2013). Evolution of the aromatic profile in Garnacha Tintorera grapes during raisining and comparison with that of the naturally sweet wine obtained. *Food Chemistry, 139*, 1052–1061.
OIV (2015). *Compendium of international methods of wine and must analysis*, *Vol. 2*. Paris: International Organisation of Vine and Wine (OIV).
Olejar, K. J., Fedrizzi, B., & Kilmartin, P. A. (2016). Enhancement of Chardonnay antioxidant activity and sensory perception through maceration technique. *LWT – Food Science and Technology, 65*, 152–157.
Oliveira, J. M., Oliveira, P., Baumes, R. L., & Maia, O. (2008). Changes in aromatic characteristics of Loureiro and Alvarinho wines during maturation. *Journal of Food Composition and Analysis, 21*, 695–707.
Pashova, V., Güell, C., Pueyo, E., López-Barajas, M., Polo, M. C., & López, F. (2004). White wine protein stabilization by a continuous process using a packed column. *American Journal of Enology and Viticulture, 55*, 195–198.
Pati, S., Crupi, P., Benucci, I., Antonacci, D., Di Luccia, A., & Esti, M. (2014). HPLC-DAD–MS/MS characterization of phenolic compounds in white wine stored without added sulfite. *Food Research International, 66*, 207–215.
Patrianakou, M., & Roussis, I. G. (2013). Decrease of wine volatile aroma esters by oxidation. *South African Journal of Enology and Viticulture, 34*(2), 241–245.
Pocock, K. F., Høj, P. B., Adams, K. S., Kwiatkowski, M. J., & Waters, E. J. (2003). Combined heat and proteolytic enzyme treatment of white wines reduces haze forming protein content without detrimental effect. *Australian Journal of Grape and Wine Research, 9*, 56–63.
Pocock, K. F., Salazar, F. N., & Waters, E. J. (2011). The effect of bentonite fining at different stages of white winemaking on protein stability. *Australian Journal of Grape and Wine Research, 17*, 280–284.
Radeka, S., Peršurić, Đ., Lukić, I., Bocca, E., & Plavša, T. (2009). Influence of the addition of tannins of different origin on protein stability and aromatic profile of Malvazija istarska wine. In: *Presentation summaries and final papers: 32nd World Congress of Vine and Wine, 7th General Assembly of the OIV (proceedings)*, Zagreb, Croatia.
Ribereau-Gayon, P., Dubourdieu, D., Doneche, B., & Lonvaud, A. (2006). 2nd ed.*The Chemistry of Wine: Stabilization and Treatments, Handbook of Enology*, *Vol. 2*. Chichester, UK: John Wiley & Sons Ltd.
Vela, E., Hernández-Orte, P., Castro, E., Ferreira, V., & Lopez, R. (2017). Effect of bentonite fining on polyfunctional mercaptans and other volatile compounds in Sauvignon blanc wines. *American Journal of Enology and Viticulture, 68*, 30–38.
Vincenzi, S., Panighel, A., Gazzola, D., Flamini, R., & Curioni, A. (2015). Study of combined effect of proteins and bentonite fining on the wine aroma loss. *Journal of Agricultural and Food Chemistry, 63*, 2314–2320.
Waters, E. J., Alexander, G., Muhlack, R., Pocock, K. F., Colby, C., O'Neill, B. K., ... Jones, P. (2005). Preventing protein haze in bottled white wine. *Australian Journal of Grape and Wine Research, 11*, 215–225.
Xifang, S., Chun, L., Zhansheng, W., Xiaolin, X., Ling, R., & Hongsheng, Z. (2007). Adsorption of protein from model wine solution by different bentonites. *Chinese Journal of Chemical Engineering, 15*(5), 632–638.