



Performance of purified grape pomace as a fining agent to reduce the levels of some contaminants from wine

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ABSTRACT

The quality of red wine depends on the absence of compounds which may affect its safety and/or stability such as ochratoxin A, biogenic amines and some metals and trace compounds. The presence of ochratoxin A in musts and wines is due to fungal contamination of the grapes and has been classified as a possible human carcinogen. Biogenic amines are formed by the micro-biological decarboxylation of the corresponding amino acid precursors during the fermentation or ageing and storage, and, at high concentrations, they may induce adverse reactions in sensitive people. Trace elements may have both a nutritional and a toxic effect on health, but also can cause turbidity and stability problems. Their presence is affected mainly by natural factors such as soil mineral content and direct contact with tank surfaces and metallic tubing during winemaking. One of the best options to remove these compounds when present in excess in wine is fining. However, some fining agents commonly used may themselves present problems related with their allergenic properties or with their propensity to increase the protein content, which can cause turbidity problems. In an attempt to avoid such these problems, purified grape pomace was tested as a fining alternative since it has been seen to have a high capacity to reduce the astringency, turbidity and also the ochratoxin A content. The main aim of this work, therefore, was to study if this material can limit the presence of ochratoxin A, biogenic amines and metals and some trace elements in a Monastrell red wine, thus increasing the value and safety of this product.

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Introduction

Some compounds present in wine may affect its safety and quality. Among these compounds are some metabolites whose origin is microorganism-related [biogenic amines (BA) and ochratoxin A (OTA)] and metal and some trace compounds, which not only may affect wine safety but also its quality by affecting the chemistry of the wine and participating, for example, in oxidation reactions leading to wine colour and flavour degradation (Cacho et al. 1995).

OTA is a mycotoxin which has been associated with renal diseases and is classified as a possible human carcinogen. The presence of OTA in musts and wines is due to the fungal contamination of grapes and is mainly related to grape contamination with *A. carbonarius* (Garcia-Moruno et al. 2005). It may develop both in the pre-harvest and

post-harvest periods before the actual winemaking process. Several factors, such as climate, cultivation practices, infection, and the interaction between different fungi and winemaking techniques, affect the presence of OTA in wines (Ertan-Anli et al. 2011). The concentration of the toxin is usually higher in red wines than in rosé and white (Visconti et al. 1999; Grazioli et al. 2006; Solfrizzo et al. 2010). A correlation also seems to exist with the geographic area of production; the further south the wine origin, the more frequent the occurrence and the greater the OTA concentration (Ottender and Majerus 2000; Pietri et al. 2001). Previous studies showed that OTA is not produced during winemaking, but that individual operations during winemaking can modify the OTA content. For example, some of the OTA present in grapes may be released to the juice during crushing. Maceration increases the OTA content,

while alcoholic and malolactic fermentation may cause a reduction in OTA in the wine (Grazioli et al. 2006). The Codex Alimentarius Commission estimates that 15% of the total intake of OTA is due to wine consumption (Castellari et al. 2001). In 2005, the European Union established a limit of 2 µg/L of OTA in wines, and the same maximum OTA concentration was established by the Office International de la Vigne et du Vin (Leong et al. 2006). Several oenological fining agents have been tested as a way of reducing the OTA content of wines, although with different degrees of success, depending on the fining agent used, the dosage applied and the OTA concentration in the wine (Ertan-Anli et al. 2011).

BA are low-molecular-weight organic compounds that are mainly formed in wine by the microbiological decarboxylation of the corresponding amino acid precursors during fermentation or ageing and storage (Kosmerl et al. 2013). It is important to control the BA content of wine because, while low concentrations are needed for human metabolic and physiological functions – for example, histamine and serotonin can work as neurotransmitters or as vasoactive substances on blood pressure (Grossmann et al. 2007), they can induce adverse reactions in sensitive people at high concentrations. Moreover, in the case of wine, the toxic effects of BA may be enhanced by the alcohol, which prevents the detoxifying mechanisms of the organism from working properly (Izquierdo-Cañas et al. 2008). Once they are present in the wine, fining is the best oenological treatment to decrease their content. This can be carried out by physical methods (sedimentation, flotation, centrifugation and filtration) or by adding a fining agent. Some studies have suggested that, among these oenological fining agents, bentonite seems to be the most effective at decreasing the BA content in wines.

Metals and some trace elements have both a nutritional and a toxic effect on health. Wine consumption can contribute to the daily nutritional requirements of many essential metals, although their bioavailability depends on the physicochemical forms in which they are present. Other metals may present some toxic effects and their content in wine is regulated (Pohl 2007). Several factors, such as the fertilisers used for cultivation, the contact of wine with materials made of copper, iron, glass, aluminium, wood, stainless steel, etc., and the use of clarifying substances, can contribute to the content of these elements in wine.

As stated before, it is clear that one of the best options to remove these three types of prejudicial compounds once they are found in the wine is fining. Fining, usually by the addition of an adsorptive compound, can help reduce levels of these compounds from wine. In recent years, some studies have shown the binding capacity of the cell wall material (CWM) to some phenolic compounds, such as proanthocyanidins (PA) and anthocyanins (Bindon et al. 2010a, 2010b; Bautista-Ortín et al. 2014, 2016). Due to this capacity, its potential use as fining agent for reducing tannins and astringency in wine was studied by some research groups. In this way, the role and behaviour of grape pomace in terms of fining and clarification was first reported by Guerrero et al. (2013), who used fibres extracted from Cabernet Sauvignon grape pomace, and they observed a decrease in phenolic concentration and in the turbidity values of the treated wines. Bautista-Ortín et al. (2015) and Jiménez-Martínez et al. (2017) used purified CWM for wine fining, demonstrating its capacity for modulating wine phenolic content. However, the purification and extraction of cell walls is a time-consuming process so our objective is to test the direct use of purified grape pomaces and their role, not only in the modulation of PA and anthocyanidin concentrations in wines but also in the control of some undesirable compounds. In this way, Solfrizzo et al. (2010) found that the repassage of wine over grape pomaces could reduce OTA levels in must and wine during vinification. However, no studies could be found on their effect on removing BA, metals and some trace elements.

Therefore, the aim of this research was to study the possibilities of using purified grape pomace as a fining agent to limit the presence in wine of BA, OTA, and some metals and trace elements. The effect of this material on the red wines is discussed and compared with the application of other conventional fining agents.

Materials and methods

Isolation of grape pomace for fining purposes

For this study, Monastrell grape pomace was obtained after the devatting and pressing steps of a standard red wine vinification. Grape pomace was separated from seeds with a scalpel, and then treated with two different solvents to eliminate PA and

other phenolic compounds. Two different purification methods were tested: the use of ethanol (70% v/v) or a solution of acetone:water (2:1). Both were carried out for 24 h in an orbital shaker at 150 rpm in the dark and repeated twice. After this step, the purified grape pomace was washed with Milli-Q water and lyophilised and ground to a fine particle size with a grinder before using.

Samples preparation

To ensure the presence of OTA and BA in the wines, 10 L of Monastrell red wine was spiked with a 0.5 µg/L OTA solution (Fluka, Buchs, Switzerland) and with frequently found BA (histamine, tyramine, putrescine, cadaverine and phenylethylamine, purchased from Sigma Aldrich, St. Louis, USA) at 100 mg/L. As regard the inorganic elements content of the wine, no spiking was carried out.

Fining

For the fining trial, wine (100 mL aliquots) was transferred into 125 mL glass bottles. The fining agents were added, and the wine was shaken to ensure a homogeneous distribution. Treatments were conducted in triplicate. A control wine with no added fining agent was also prepared. For the wine fining, grape pomace purified with ethanol (PGP EtOH) or acetone (PGP Acetone) was used at a dose of 13 mg/mL and its effect was compared with seven different commercial fining agents, tested at the maximum dose recommended by the respective manufacturers. Fining agents included bentonite (0.75 g/L, Enoproma, Murcia, Spain), potassium caseinate (0.75 g/L, Laffort SA, Bordeaux Cedex, France), a gelatine with high degree of hydrolysis (Vinigel Forte, 0.15 g/L), a gelatine with low degree of hydrolysis (Vinigel Platinum, 0.10 g/L), an egg albumin powder (Ovovin, 0.15 g/L), a vegetable protein from pea (Proveget100, 0.20 g/L) and a manno-protein-rich yeast autolysate (SuperBouquet, 0.5 g/L). The last five products were obtained from Agrovin S.A. (Alcazar de San Juan, Spain). After mixing the wine with the fining agents, it was held at room temperature for 21 days and then analysed. All the samples were filtered through a 0.45 µm nylon filter before analysis.

Analysis of OTA by HPLC

The method developed to determine the concentration of OTA in each sample is based on that described by Castellari et al. (2000), using Ochraprep immunoaffinity columns (R-Biopharm Rhone) to clean up the samples. Wine samples (10 mL), adjusted to pH 7.8 using 2 M NaOH, were diluted with 10 mL of phosphate buffer saline (PBS), centrifuged (5 min at 1500 rpm) and directly loaded on the immunoaffinity column at a flow rate of about 2–3 mL/min using a vacuum manifold. The column was washed with 20 mL PBS at 5 mL/min and dried under a gentle stream of air. The OTA was eluted by passing 1.5 mL of desorption solution (acetic acid: methanol, 2:98) through the column and collected in an amber vial. To complete the elution, 1.5 mL of ultrapure water were passed giving a final volume of 3 mL. OTA was quantified by reversed-phase high resolution liquid chromatography (HPLC) with fluorescence detection using a Waters liquid chromatograph 2695 (Waters, Milford, MA, USA) equipped with a Waters 2475 fluorescence detector. Chromatographic separations were performed on a Cortecs C18 column, 2.7 µm size particle, 4.6 mm × 75 mm (Waters Milford, MA, USA). The isocratic method used a mobile phase consisting of a mixture of acetonitrile:water:acetic acid (51:47:2 v/v/v) eluted at a flow rate of 1.0 mL/min with a column temperature of 35°C. Detection was carried out at an excitation wavelength of 333 nm and an emission wavelength of 443 nm. For the quantification, a calibration curve was made for a known amount of the pure standard.

Analysis of BA by HPLC

The method used in this study was developed by Gómez-Alonso et al. (2007). The BA were analysed by a derivatisation reaction with diethyl ethoxymethylenemalonate (DEEMM) to form aminoenones. First, 1 mL of the sample was mixed with 1.75 mL of borate buffer 1M (pH 9), 750 µL of methanol and 30 µL of DEEMM in a screw-cap vial and left for 30 min in an ultrasound bath. Then, the samples were heated at 70°C for 2 h to allow complete degradation of excess DEEMM and reagent by-products.

The chromatographic analysis was carried out with a Waters 2695 HPLC system (Waters, Milford, MA, USA)

equipped with a system autosampler, and a Waters 2996 photodiode array detector (Waters). The column used to separate the compounds was an ACE HPLC column (5 C18-HL, Aberdeen, Scotland), 5 µm particle size, 250 mm × 4.6 mm, thermostatted at 25°C. A binary gradient was achieved by mixing two solvents (phase A, 25 mM acetate buffer pH 5.8 with 0.02% sodium azide; phase B, 80:20 mixture of acetonitrile and methanol) with a flow rate of 0.9 mL/min. For detection, the photodiode array detector was monitored at 280 nm. BA were quantified using their respective external standards and their concentration was expressed in mg/L.

Analysis of metals and trace elements by ICP-MS

These compounds were determined before and after fining the wine samples by inductively coupled plasma mass spectrometry (ICP-MS). Wine samples were previously diluted (1:10, v/v) using 2% v/v HNO₃. An Agilent 7900 (Agilent, Tokyo, Japan) instrument was used with a Micro Mist nebuliser and a Scott-type double-pass spray chamber and continuous nebulisation. The operating conditions are nebuliser gas flow rates: 1.05 L/min; auxiliary gas flow: 0.9 L/min; plasma gas flow: 15 L/min; and ICP RF power: 1500 W. A calibration curve up to 100 mg/L was used for Na, K, Mg, S, Ca and P, and a calibration curve up to 1 mg/L for the minor elements, such as Fe, Mn, Cu, Zn, Al and B.

Statistical analysis

To evaluate the differences in the values obtained after the treating the wine with the fining agents tested, an analysis of variance was carried out using Statgraphics Centurion XVI (Statpoint Technologies Inc., Warrenton, VA, USA). The level of significance was established as $p \leq 0.05$.

Results and discussion

Removal of OTA by fining agents

Purified grape pomace and seven different commercial fining agents (all of them at the maximum doses recommended by the company) were tested. Some were added at doses sevenfold greater than others due to their different nature. The highest dose was used with the PGP, and it was similar to the dose

and working conditions used in another study carried out by our research group, where the PGP's role on the wine PA and anthocyanidin concentrations was studied (Jiménez-Martínez et al. 2017).

The wine was spiked with 0.5 µg/L of OTA since the data on OTA occurrence shows that in most wines average concentrations of 0.5 µg/L were reported (Gentile et al. 2016). Table 1 shows the wine OTA content after adding the different fining agents used in this study. Two gelatines, Vinigel Forte and Vinigel Platinum, showed the lowest percentage of OTA retention, probably because wine polyphenols compete with the OTA when these fining agents are used (Castellari et al. 2000), occupying the reactive sites in which the OTA could be adsorbed. The interaction of proteins and polyphenols is a well-known phenomenon that is associated with a wine's organoleptic characteristics such as astringency (Haslam 1974). These protein fining agents therefore showed very little retention. Castellari et al. (2001) also proposed that polyphenols in red wine would compete with OTA for binding sites when gelatine is used, predicting that gelatine would be less effective in red wine than in white wine.

The use of casein and Ovovin slightly decreased the OTA content in wines, although differences with control wine were not significant. Ertan-Anli et al. (2011) found that egg albumin was a more effective fining agent for OTA removal than gelatine, producing a decrease of OTA levels of around 20%, whereas casein and polyvinyl polypyrrolidone (PVPP) did not appear to play a significant role in the removal of OTA from white wine. Castellari et al. (2001) also found in their experiences that a high percentage of OTA was removed with egg albumin and potassium caseinate (40% and 35%,

Table 1. Effect of the addition of commercial fining agents and purified grape pomace (PGP) on OTA.

Samples	OTA (µg/L)	OTA recovery (%)	OTA reduction (%)
Control wine	0.5 d	90	
Wine + bentonite	0.4 c	73	17
Wine + caseinate	0.4 c	79	11
Wine + Vinigel Forte	0.5 d	91	0
Wine + Vinigel Platinum	0.4 c	87	3
Wine + Ovovin	0.4 c	81	9
Wine + Proveget 100	0.4 c	79	11
Wine + SuperBouquet	0.3 b	69	21
Wine + PGP EtOH	0.2 a	32	58
Wine + PGP acetone	0.2 a	36	54

Different letters in the same column show significant differences according to Least significant difference (LSD) test ($p > 95\%$).

respectively), although the added dose of these agents was much higher than that used in this study, and the same authors demonstrated that the OTA content of wine depended on fining agent dosage. However, contrary to these results, Leong et al. (2006) reported that proteinaceous fining agents, including gelatine and egg albumin, barely decreased the OTA concentration in Syrah wine and any decrease was only significant for gelatine at the highest doses. They explained that the protein content of wine is an important factor, and the Syrah wine they used was very poor in proteins. In the absence of proteins in the wine, OTA is likely to be present in a freely soluble form, thus readily bound to the gelatine, whereas in wines rich in proteins, grape-derived proteins may compete with gelatine for the binding of OTA.

Also, a vegetable protein from pea was used, Proveget 100. This agent showed a slightly higher retention of OTA than gelatines, but again differences were not significant. According to previous researches (Iturmendi et al. 2010), some vegetable protein fining agents are negatively charged, and their interaction with polyphenols may not be strong because these too are negatively charged, consequently there would be more sites where OTA could be adsorbed.

Bentonite, having a negatively charged surface, was expected to have a greater OTA retention capacity than casein and egg albumin due to the presence on the surface of this layered aluminium silicate of polar terminal regions that are locally positive. OTA is a weak acid with a pKa value for the carboxyl group of the phenylalanine moiety of 4.4, so OTA is partially dissociated at the pH of wine (3.5) and carries a negative charge (Iturmendi et al. 2010). Therefore, molecules like OTA, which is charged negatively at the pH of wine, could be adsorbed by a hydrogen bonding mechanism to bentonite, as Castellari et al. (2001) suggested. These authors also formulated the hypothesis that OTA could be adsorbed within the interlaminar spaces of bentonite by a cation exchange mechanism. Moreover, Valenta (1998) also claimed that OTA could be bound by ionic interactions to the grape-derived proteins present in the wine, which, in turn, could be bound to bentonite and precipitated from the wine. This model is supported by data from Lataste et al. (2004), who demonstrated that a combination of gelatine (to bind OTA) and bentonite (to bind and precipitate the gelatine) was more effective for removing OTA than combinations of gelatine and silica gel or gelatine

and gallotannins. Our result points to a significant decrease of OTA when bentonite was used.

SuperBouquet is a mannoprotein-rich yeast autolysate, which reduced OTA by 21%, similar to the reduction observed by Garcia-Moruno et al. (2005) using an active dry yeast after a 7-day treatment. Together with Silva et al. (2003), these authors demonstrated the efficacy of yeast hulls added to wine as OTA fining agents, which is supported by findings on the binding properties of yeast material during and after fermentation. Bejaoui et al. (2004) conducted experiments in which the yeast derivatives were added to synthetic grape juice and the percentage of OTA adsorption reached 60%. The absorption mechanism involved could be similar to that expected for the cell walls in grape pomace. Cell walls contain polysaccharides, lipids, and proteins, exhibiting numerous and easily accessible adsorption sites that would allow different adsorption mechanisms, e.g. hydrogen bonding, ionic or hydrophobic interactions.

The use of grape pomace, purified with ethanol or acetone (the absence of OTA in the pomace was previously tested), removed 57% or 54% of ochratoxin, respectively. Solfrizzo et al. (2010) studied the reduction of OTA by repassing wine over grape pomaces and found out that they could reduce OTA concentration by 50–100%, and it was possible by the reuse of these pomaces to remove more OTA. They also observed that repassing wine over pomaces from the same grape variety as that used for the winemaking process did not affect the quality parameters of the treated wine (Solfrizzo et al. 2010). This pronounced ability to grape pomaces to reduce OTA could be due, as stated above, to their complex composition since the cell walls comprising the grape pomace are a network of 30% neutral polysaccharides, 20% acidic pectic substances, 15% insoluble PA, lignin and structural proteins and phenols (Apolinar-Valiente et al. 2015). This composition would allow numerous different and easily accessible adsorption sites to which OTA could be bound and also different adsorption mechanisms.

Removal of BA by fining agents

Some studies propose that fining is one of the best oenological treatments to decrease the BA content of wine (Kosmerl et al. 2013). In our work, the wine was spiked with the five most frequently found BA –

histamine, tyramine, putrescine, cadaverine and phenylethylamine – at a 100 mg/L concentration. The wines were spiked with an important amount of BA since it has been reported that the individual concentrations of these compounds can vary between 0.8 and 47.3 mg/L (Landete et al. 2005) in a large number of wines, the total amount varying between 25.2 and 96.8 mg/L (Arrieta and Prats-Moya 2012).

Only one BA, histamine, was significantly reduced by two of the fining agents used in the study (Table 2), bentonite and purified grape pomace. At wine pH, all amines are positively charged and can be associated with the surface of a fining agent by an ion-exchange mechanism, but they can also form hydrogen bonds involving, among others, water molecules and superficial groups of the adsorbent (Amghouz et al. 2014). As mentioned above, only histamine was affected to any great extent by the fining agents, suggesting that the difference between histamine and the rest of the amines could lie in its structure. Histamine, on the opposite side to the amino group, has an imidazole group, constituting a heterocyclic amine with two nitrogen atoms whose free electrons are conjugated with π -electrons of the aromatic ring. As a consequence, these nitrogen atoms have low basicity. In the case of the rest of amines, the alkyl groups may impart electron density to the nitrogen atom by an inductive effect: being more available, the free electrons around the nitrogen atom may be shared with a Lewis acid and the basicity of the nitrogen maybe favoured by inductive effects (Amghouz et al. 2014). Moreover, the pKa values reported by Gugliucci (2004) and Kvasnicka et al. (2006) for these BA indicate that, in the case of histamine, the nitrogen atom located in the aromatic ring is much less basic than that linked to the alkyl carbon, and the basicity of histamine is higher than that of tyramine or phenylethylamine, which would

explain why histamine has the highest affinity for the superficial acid groups of bentonite. This was already reported by Mayer and Pause (1978), who showed that only the bentonite treatment reduced certain BA levels, mainly histamine. Kallay and Body-Szalkai (1996) also observed that in red wines 0.8 g/L of bentonite reduced histamine content by 60%. In the same way, Mannino et al. (2006) found that the most effective fining agent for BA reduction was bentonite, the decrease being directly correlated with the amount of bentonite used. This effect was even more pronounced when bentonite was used in the must (Grossmann et al. 2007).

Purified grape pomace was also partially effective in reducing BA in the wine, showing around 10% of retention. Grape pomace would retain the histamine because of its complex composition and because some of the compounds it contains may have a strong ionic interaction with BA, possibly in a similar manner to that which takes place between this cell material and the anthocyanins (Padayachee et al. 2012). The other BA were not removed by the fining agents.

Removal of inorganic compounds by fining agents

The concentration of some metals and trace elements in wine can be a significant parameter affecting quality, consumption and conservation of wine. In this way the reduction of K and Ca is important and may have a significant effect on preventing the precipitation of tartaric salts, thus improving wine stability and maintaining its pH and acidity. High concentrations of magnesium (Mg) and sodium (Na) are often responsible for imparting a salty character in wines that is often described as a negative sensory attribute (Cabello-Pasini et al. 2013). Elemental sulphur (S_0) residues on grapes not only can produce H_2S during fermentation but also can

Table 2. Effect of the addition of commercial fining agents and purified grape pomace (PGP) on BA (mg/L).

Samples	Histamine	Tyramine	Putrescine	Cadaverine	Phenylethylamine	Histamine absorption (%)
Control wine	93 bc	103 abc	112 a	60 a	103 ab	
Wine + bentonite	84 a	104 bcd	115 ab	61 a	105 ab	10
Wine + caseinate	95 c	105 cd	116 ab	62 ab	105 ab	0
Wine + Vinigel Forte	95 c	105 bcd	116 ab	62 ab	105 ab	0
Wine + Vinigel Platinum	94 bc	104 abcd	112 a	62 ab	100 a	0
Wine + Ovovin	90 b	105 cd	122 b	65 b	107 ab	3
Wine + Proveget 100	93 bc	106 cd	116 ab	63 ab	106 b	1
Wine + SuperBouquet	93 bc	106 d	118 ab	64 ab	106 ab	0
Wine + PGP EtOH	84 a	102 ab	114 a	62 ab	101 a	10
Wine + PGP acetone	82 a	102 a	116 ab	63 ab	101 a	12

Different letters in the same column show significant differences according to LSD test ($p > 95\%$).

form precursors capable of generating additional H₂S after bottle storage for 3 months (Jastrzembski et al. 2017).

Metallic ions may have an important role in redox reactions resulting in wine browning, turbidity, cloudiness and astringency. Moreover, metals in wine may affect human health. In this way, Tariba (2011) reported that several elements, including Al, Cu, Fe and Zn, contribute to haze formation and colour changes of them tend to form complexes with anthocyanins and tannins, Mn may favour acetaldehyde formation during oxidation, influencing the disappearance of phenolic compounds, and tannins and aluminium clouding is considered to form at Al concentrations above 10 mg/L.

Tables 3 and 4 show the changes in mineral composition after the fining treatments. The most significant observation was the reduction of K and Ca when grape pomace was used as fining agents. The quantities found in the studied control wine were similar to those reported by other authors, who found K and Ca concentrations of around 826 and 47 mg/L, respectively (González et al. 2009). In our study, their content was 673 of K and 100 mg/L of Ca, as can be seen in Table 3. The

other fining agents tested did not affect K and Ca concentrations, only Proveget reduced a significant amount of Ca. The use of grape pomace led to reductions of K and Ca of around 15–25% and 18–21%, respectively, the greatest reduction being obtained when grape pomace purified with ethanol was used (see Table 3). Such reductions are very important and, for this reason, purified grape pomace may be a good option for eliminating an excess amount of K and Ca. In fact, when the calcium content is over 60 mg/L there is a real risk of tartrate deposits (Ribéreau-Gayon et al. 2006), so the use of grape pomace in wines with high calcium content could help avoid the precipitation of these salts. The traditional technology for tartrate stabilisation is refrigeration, but calcium tartrate is not very temperature-sensitive, so that elimination of the cation will be the best solution.

Another positive aspect of grape pomace as regard tartaric stabilisation is that they can introduce to the medium macromolecular compounds like proteins and glucide polymers (such as pectins and gums, neutral polysaccharides), all of them known as 'protective colloids' that inhibit the growth of nuclei and

Table 3. Effect of the addition of commercial fining agents and purified grape pomace (PGP) on Na, Mg, P, S, K and Ca (mg/L).

Samples	Na	% r	Mg	% r	P	% r	S	% r	K	% r	Ca	% r
Control wine	45 bc		129 a		187.5 a		153 a		673 cd		100 cd	
Wine + bentonite	47 c	−4	128 a	1	197 abc	−5	155 ab	−2	659 cd	2	104 d	−3
Wine + caseinate	44 ab	2	127 a	1	203 bc	−8	163 bcd	−7	668 cd	1	98 bcd	2
Wine + Vinigel Forte	44 ab	3	128 a	1	205 c	−9.5	166 cd	−9	668 cd	1	100 bcd	1
Wine + Vinigel Platinum	44 ab	4	126 a	3	200 abc	−6.7	171 de	−12	662 cd	2	97 bc	3
Wine + Ovovin	44 ab	3	131 a	−1	206 c	−9.8	178 e	−16	678 d	−1	99 bcd	1
Wine + Proveget 100	42 a	7	123 a	5	191 ab	−1.7	163 bcd	−7	640 cd	5	94 b	7
Wine + SuperBouquet	44 ab	3	127 a	1	193 abc	−3	163 bcd	−7	659 cd	2	97 bc	4
Wine + PGP EtOH	43 ab	6	126 a	2	196 abc	−4.6	155 ab	−2	502 a	26	79 a	21
Wine + PGP acetone	44 ab	4	126 a	2	198 abc	−5.5	159 abc	−4	569 b	16	83 a	18

r: recovery.

Different letters in the same column show significant differences according to LSD test ($p > 95\%$).

Table 4. Effect of the addition of commercial fining agents and purified grape pomace (PGP) on B, Al, Mn, Fe, Cu and Zn (mg/L).

Samples	B	% r	Al	% r	Mn	% r	Fe	% r	Cu	% r	Zn	% r
Control wine	13,016 ab		172 b		539 abc		726 ab		49 d		414 bc	
Wine + bentonite	13,115 ab	−1	480 c	−179	542 bcd	−1	1563 d	−115	27 a	45	406 abc	2
Wine + caseinate	13,177 ab	−1	147 ab	15	560 cde	−4	715 ab	1	43 b	12	454 c	−10
Wine + Vinigel Forte	13,609 b	−5	175 b	−2	577 e	−7	736 ab	−1	51 d	−6	425 bc	−3
Wine + Vinigel Platinum	12,966 ab	0	160 ab	7	557 bcde	−3	702 ab	3	49 cd	−1	392 abc	5
Wine + Ovovin	13,162 ab	−1	165 b	4	569 de	−6	611 a	16	48 bcd	2	335 a	19
Wine + Proveget 100	12,601 a	3	129 a	25	530 ab	2	715 ab	1	46 bc	5	365 ab	12
Wine + SuperBouquet	13,030 ab	−0	161 ab	7	540 bcd	−0	704 ab	3	45 bc	8	356 ab	14
Wine + PGP EtOH	12,401 a	5	180 b	−5	508 a	6	809 bc	−12	48 bcd	2	557 d	−35
Wine + PGP acetone	12,412 a	5	174 b	−1	528 abc	2	942 c	−30	60 e	−24	567 d	−37

r: recovery.

Different letters in the same column show significant differences according to LSD test ($p > 95\%$).

crystallisation of tartaric salts (Ribéreau-Gayon et al. 2006).

The other minerals present at high concentrations, like Na and Mg, which in high concentration may impart salty notes to wines, were barely affected by the fining agents assayed, the small increase in Na observed when bentonite was used perhaps being due to its sodium content.

The presence of sulphur can produce H₂S during fermentation and during storage (Jastrzembski et al. 2017). S did not decrease with the fining agents and neither did P, but a small increase was observed with some of the proteinaceous fining agents which would contain these minerals in their compositions.

The concentration of B did not change with the fining treatments. As stated before, Al, Cu, Fe and Zn may contribute to haze formation and change of colour as they tend to participate in oxidation reactions (Tariba 2011). Al and Fe increased with bentonite, which is an aluminosilicate. The use of GP did not reduce any of these minor minerals and only slightly increased Fe, Cu (with PGP purified with acetone) and Zn, although the final concentrations of these elements were still low (Phol 2007). The range of Zn reported in wines is 0.14–4 mg/L (Ribéreau-Gayon et al. 2006), and in our study none of the samples showed Zn values higher than 1 mg/L. The values of Cu were also low even following the use of grape pomace. The concentration of Fe should not be over 5–6 mg/L (Dippong and Mihali 2016), and lower values will lead to a stable wine (Bornet and Teissedre 2008) and be beneficial since the formation of reactive oxygen species, responsible for staleness of aroma and taste of wine, will be reduced.

Conclusions

The use of fining agents can be useful to decrease the presence of some undesirable compounds in wine. Among the tested fining agents, purified grape pomace led to the best results and resulted in a significant reduction of OTA, histamine and of K and Ca, which could lead to a reduction in tartrate instability. The results indicate that this material can be considered a good alternative for using as a protein-based fining agent in red wine. Its use would also avoid allergen-related effects

and could increase the added value of this abundant winery by-product.

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