

Vitis 46 (3), 138–147 (2007)

Metal reduction in wine using PVI-PVP copolymer and its effects on chemical and sensory characters

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Summary

We studied the influence of an adsorbent PVI-PVP resin (a copolymer of vinylimidazole and vinylpyrrolidone), on the removal of heavy metals in wines, mainly copper (Cu), iron (Fe), lead (Pb), cadmium (Cd) and aluminium (Al). The study also investigated the influence of PVI-PVP on the physical-chemical and sensory characteristics of white and red wines, comparing its effect when applied in the must and in the wine. The removal of metals was more effective when PVI-PVP was applied to the wine than to the must. The removal of Fe and Pb was more effective in white wines than in red wines, while the removal of Cu and Al was higher in red wines. In general, the higher the PVI-PVP dose, the greater the quantity of metallic elements (copper, iron, lead and aluminium) that are removed. PVI-PVP had a minor effect on phenolic composition. The wines showed some decrease in total acidity and an increase in pH with PVI-PVP. The application of PVI-PVP at the dose rates employed here did not affect the wine's sensory characteristics significantly.

Key words: PVI-PVP, vinylimidazole, vinylpyrrolidone, metals, wine.

Introduction

The mineral constituents of must and of wine are present in ionic form and also as colloidal complexes with other compounds such as some organic acids and polysaccharides. Their primary presence is due to the natural transfer from soil-to-root-to-grapevine, in which the elements are absorbed by the roots according to their bio-availability in the soil, these being afterwards metabolised by the plant and further modified in the winemaking process and during the aging of the wine. These natural levels can also be increased by contamination (SEPPI and SPERANDIO 1978; MEDINA and SUDRAUD 1979; MCKINNON *et al.* 1992; OUGH 1993; ESCHNAUER *et al.* 1996; BAUER *et al.* 2001). A number of different factors can be responsible for the presence of exogenous metal contaminants in wines such as plant protection products, the winemaking equipment, and accidental contaminations of the must.

With technological progress, and especially with increased use of stainless steel in oenological equipment a

significant decrease in the level of residual Fe in wine has resulted. According to EDER *et al.* (2003) clouding problems are often the result of Cu levels above about 0.5 mg·l⁻¹. The use of copper-containing plant protection products can result in grape residues which are at least partially transferred to the wine. It is also possible that levels of this cation can be further raised by treatment of the wine with copper sulphate to eliminate unpleasant smells caused by sulphydric acid and by thiols (MATTIVI *et al.* 2000).

The presence of Pb in wine can originate from atmospheric pollution, from industrial emissions and from road traffic (presumably from the old, leaded petrol) (TEISSÈDRE *et al.* 1993 a; AUGAGNEUR *et al.* 1997; FOURNIER *et al.* 1998), or from plant protection and oenological products containing Pb as an impurity (TEISSÈDRE *et al.* 1993 b; MINGUEZ *et al.* 1997).

Contamination with Cd is the result mainly of contact with zinc materials rich in Cd and from some stainless steels (CURVELO-GARCIA 1988). GILBERT (1979) and MEDINA and SUDRAUD (1979) refer to a possible cadmium enrichment in musts from grapes growing close to major roads. ESCHNAUER *et al.* (1996) cite also plant protection products as possible sources of Cd.

High levels of metal contamination in wine such as Fe, Cu, Pb or Cd can influence the physico-chemical stability of the wine (BOULTON *et al.* 1995), causing browning, clouding and the formation of precipitates. This is quite separate from issues of food safety and of breaching legal upper limits. The present OIV (International Organisation of Vine and Wine) upper limits for Pb in wine are 150 µg·l⁻¹; for Cd 10 µg·l⁻¹, and for Cu 1 mg·l⁻¹ (OIV 2005, 2006) making it sometimes necessary to remove excess metals so as to lower the concentrations to below these legal limits. This also brings their contents to levels that are both safe for the consumer and favourable for the stability of the wine. These OIV limits are accepted within the European Community but not necessarily outside.

The classic removal process for excess metals in wine (mainly iron, but also sometimes copper, zinc, manganese, nickel, silver, lead, cadmium and aluminium) is by treatment with potassium ferrocyanide (K₄[Fe(CN)₆]·3H₂O). This is sometimes called 'blue fining'. The use of potassium ferrocyanide in wines is a technique which has limitations such as the need to carry out preliminary assays to determine the right dose rate. Obviously, 'over fining' by the addition of too much potassium ferrocyanide can result in the formation of free cyanide ions so that potassium

ferrocyanide use must be very carefully controlled indeed. Potassium ferrocyanide residues also raise environmental problems in disposal of waste. The addition of potassium ferrocyanide to the must is not allowed. From a technological viewpoint, this method has not anyway been very effective in eliminating Cu in wines that also contain low levels of Fe.

Because of these limitations, alternative methods have been explored such as those using adsorbent or exchanger resins (FENG *et al.* 1997; PALACIOS *et al.* 2001; EDER *et al.* 2001, 2003; BENITEZ *et al.* 2002). Many authors have developed methods based on the use of just one adsorbent resin to lower the levels of all of the contaminant metals present in wines (MATTIVI *et al.* 1994, 2000; EDER *et al.* 2001, 2003; NICOLINI *et al.* 2004), to values which are both safe for the consumer and which enhance the stability of the wine. The adsorbent resin referred to by FUSSENER *et al.* (1992) is known as PVI-PVP or just PVI, and is a vinylimidazole and vinylpyrrolidone copolymer (ratio 9:1). PVI-PVP is insoluble in water and in most other liquids, and it has a number of functional imidazole groups which connect selectively to the various metals. Research has been carried out into the use of different doses of PVI-PVP in the must and in the wine with different times of contact, and its influence on wine colour, and on the various phenolic and aroma compounds of wine.

Besides its effect on the metals, PVI-PVP seems to have a relevant action in wine clarification. Here, as well as decreasing the level of hydroxycinnamic acid derivatives, mainly caftaric acid (MATTIVI *et al.* 1994, 2000; NICOLINI *et al.* 2004; EDER *et al.* 2001, 2003) it also minimises browning.

The treatment of musts and/or wines with PVI-PVP for lowering levels of metals such as Fe, Cu, Zn and Al, has been under discussion at OIV by experts under both the "Wine Technology" and, also the "Food Security" groups. The authorisation dossier is currently under evaluation. In May 2006, the French Agency of Food Security in an advice (AFSSA 2006) commented that utilisation of PVI-PVP at a dosage of 80g/hL did not appear to present any risk to consumer health.

The aim of this work was to study the influence of PVI-PVP on metal reduction (Fe, Cu, Pb, Cd, Al) and, especially to confirm that there were no important changes in the chemical and sensory characteristics of either white or red wines. We also wanted to compare its effects on wine characteristics when it was used either in the must or, later in the wine.

Material and Methods

Finning treatment applied to must and wine: **Wines:** White wines were prepared using grapes of the variety 'Fernão Pires' (*Vitis vinifera* L.), and red wines using 'Castelão' (*Vitis vinifera* L.). Grapes were harvested in 2002 from the Ribatejo region, Portugal.

PVI - PVP: The PVI-PVP (Divergan®HM, BASF, Germany) was applied at 10 and 20 g·hl⁻¹ (must) and 30 and 50 g·hl⁻¹ (wine). Experiments are summarised in Fig. 1. These application doses are those recommended by the supplier.

For the musts, the PVI-PVP was introduced before fermentation. They were immediately shaken for 20 min,

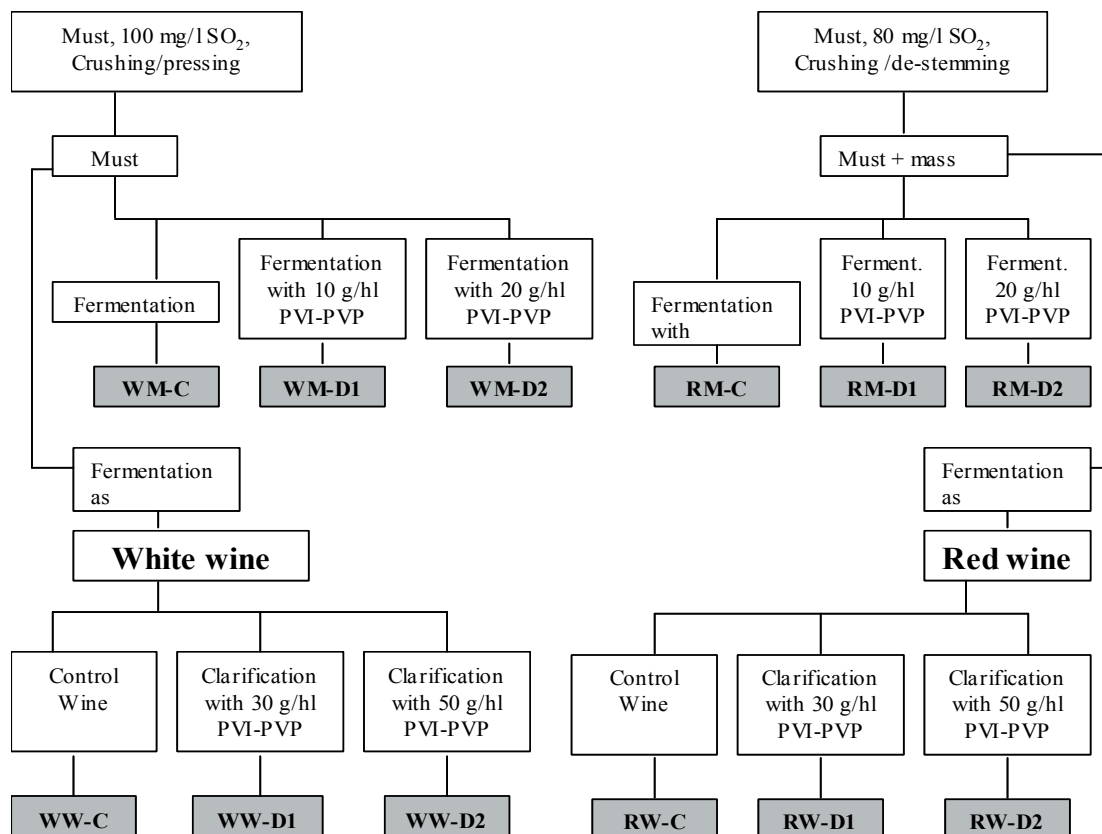


Fig. 1: Flowchart of the fining treatment (PVI-PVP) applied and wines.

after which they remained at rest during fermentation. Separation occurred at first racking. The wines were shaken for 15 min immediately after introduction of the PVI-PVP, after which they too remained at rest for 48 h.

Finishing treatment applied in wine with metal additions: With the aim of confirming the influence of PVI-PVP on Fe and Cu we added these elements to matured white and red wines: 'Addition A' (5 mg·l⁻¹ of Fe and 0.5 mg·l⁻¹ of Cu). 'Addition B' (15 mg·l⁻¹ of Fe and 1 mg·l⁻¹ of Cu). We also added two levels of PVI-PVP 25 and 50 g·hl⁻¹ (the amounts '1' and '2' respectively).

Mineral composition: The concentration of potassium (K), calcium (Ca), sodium (Na), magnesium (Mg), iron (Fe) and copper (Cu) was assayed by Flame Atomic Absorption Spectrometry (FAAS) using a Varian Spectra 10/20 (Victoria, Australia), according to the official method of OIV (OIV 1990).

The Pb, Cd and Al content were determined by Electrothermal Atomic Absorption Spectrometry (ETAAS), according to the method described by CATARINO (2000) and CATARINO *et al.* (2002).

Colour and phenolic composition: The total content of phenolic compounds was measured by the absorbance at 280 nm (RIBÉREAU-GAYON 1970); colour intensity by the sum ($A_{420} + A_{520} + A_{620}$), where tonality is defined by the ratio (A_{420}/A_{520}). For the white wine, colour intensity was measured using absorbance at 420 nm. Colour measurements of the wines were also performed according to the CIELAB 76 method (McLAREN 1980). Spectral readings, transmittance every 10 nm over the visible spectrum 380-770 nm, were performed with a UV4 Unicam Visible Spectrometer (Cambridge, UK), using quartz cells of path length 1 mm (red wine), and 10 mm (white wine). The software *Chroma 2.0 colour measurement* was used to calculate the CIELAB coordinates directly. The L*, a* and b* values describe a three-dimensional colour space.

For the red wine, the total pigments were estimated using the method of SOMERS and EVANS (1977); the polymeric pigments index was determined by a method proposed by GLORIES (1978) and total anthocyanins were evaluated according to the method of RIBÉREAU-GAYON and STONESTREET (1965).

Organic acid composition: Organic acids (tartaric, malic, citric, lactic and shikimic acid) were analysed by High Performance Liquid Chromatography (HPLC) using a method described by TUSSEAU and BENOIT (1986 a, b). Two columns with reverse phase Lichrospher 100 RP 8 (Merck, Darmstadt, Germany) (particle size 5 µm, 250 x 4 mm) were used. Detection was made with a UVIS 206 PHD (KONIK Instruments, Barcelona, Spain) set at 210 nm, and the peak areas were determined with Konikchrom 5.2 software.

Current analysis: pH, titratable and volatile acidity were determined by Fourier Transform Infrared Spectrometry – FTIR (WineScan FT120, Foss, Slangerupgade, Denmark) (MOREIRA *et al.* 2002 a, b).

Sensory analysis: Sensory analyses were performed by six expert panellists who were members of "Comissão Vitivinícola Regional of Ribatejo (CVRR)".

These persons were all properly trained and had good previous experience. We asked panellists to look for differences between the control wine and each sample of treated wine. The wine attributes included visual, nose and taste senses, as well as overall (global) appreciation.

Statistic analysis: The data were analyzed by Analysis of Variance using SPSS 12.0 for Windows. The treatment means were separated by the Scheffé test at the 5 % significance level.

Results and Discussion

Finishing treatment applied in must and wine: Must and white wine: The results of applying PVI-PVP in must and wines were analysed separately. The summary of our variance analysis results with white wine is shown in Tab. 1.

For phenolic composition we confirm that PVI-PVP, either in the must or in wine caused a slight, but significant decrease in the level of phenolic compounds in the wine. This was expressed in a reduction in the total phenols index (Ipt). However, no significant differences appeared between the two PVI-PVP dose rates used. This is consistent with the observations of MATTIVI *et al.* (2000). According to these authors, PVI-PVP removes also some phenolic compounds from the wine and, in particular the low molecular weight ones. It is these that are mainly involved in the oxidative browning of white wines. Also NICOLINI *et al.* (2001, 2004) and EDER *et al.* (2001, 2003) refer to the influence of PVI-PVP on the reduction of hydroxycinnamates, thus improving colour stability.

In fact, with regard to the wines' chromatic characteristics, we confirm a slight, but not significant decrease in A420 and in the co-ordinates L*, a* and b*.

As for mineral composition, we found that PVI-PVP had no effect on Ca, Mg and Na contents, regardless of whether the product was used in the must or, later in the wine. This result is in line with that of other authors (EDER *et al.* 2001, 2003; NICOLINI *et al.* 2004). However, the application of PVI-PVP in the must did have a slight, but significant effect on the potassium content when compared with the control. This seems to indicate that the application of PVI-PVP in the must causes a premature elimination of this cation, and this probably contributes to tartaric stabilisation in the subsequent wine.

In the particular case of Cu, the application of PVI-PVP in the must or in the wine did not have a significant effect on the content of this metal. This result may be due to the very low copper content of our wine, or to our experimental conditions. Also, the discontinuous shaking may have resulted in insufficient contact between PVI-PVP and the wine. Our result could be explained by that of NICOLINI *et al.* (2004), who found that removal was faster and more complete when mixing was more thorough. Also, EDER *et al.* (2003), suggest that metal removal can occur quite rapidly allowing PVI-PVP to be separated from the wine after only 16 h of contact.

Under our conditions, no effect on Fe content was detected with PVI-PVP either in white must or in wine;

Table 1
Effects of PVI-PVP application in must and wine on some white wine characteristics

	Effect	WM-C	WM-D1	WM-D2	WW-C	WW-D1	WW-D2	Effect
Colour and phenolic composition:								
Total phenol index (ua)	**	$\bar{\chi}$ 10.8b	10.4a	10.2a	10.8c	9.6b	9.2a	**
		s 0.1	0.0	0.1	0.0	0.1	0.1	
A420 (ua)	ns	$\bar{\chi}$ 0.113a	0.104a	0.116a	0.113a	0.103a	0.103a	ns
		s 0.035	0.007	0.009	0.002	0.001	0.005	
L*	ns	$\bar{\chi}$ 98.1a	97.8a	98.2a	98.9a	98.1a	98.1a	ns
		s 0.2	0.3	0.0	1.7	0.1	0.0	
a*	ns	$\bar{\chi}$ -0.9a	-0.7a	-1.0a	-0.3a	-0.7a	-0.6a	ns
		s 0.14	0.06	0.24	0.45	0.16	0.05	
b*	ns	$\bar{\chi}$ 7.6a	7.5a	8.0a	4.0a	6.9a	6.6a	ns
		s 0.08	0.10	0.98	0.75	0.09	0.04	
Mineral composition:								
Potassium (mg·l ⁻¹)	*	$\bar{\chi}$ 605b	578a	565a	565a	565a	568a	ns
		s 10	3	4	10	13	0	
Calcium (mg·l ⁻¹)	ns	$\bar{\chi}$ 72a	69a	70a	71a	70a	69a	ns
		s 1	1	1	0	1	0	
Sodium (mg·l ⁻¹)	ns	$\bar{\chi}$ 47a	46a	47a	48a	48a	48a	ns
		s 1	0	0	1	0	1	
Magnesium (mg·l ⁻¹)	ns	$\bar{\chi}$ 93a	91a	92a	91a	90a	91a	ns
		s 1	1	1	1	1	1	
Copper (mg·l ⁻¹)	ns	$\bar{\chi}$ 0.1a	0.1a	0.1a	0.1a	0.1a	0.1a	ns
		s 0.1	0.1	0.1	0.1	0.1	0.1	
Iron (mg·l ⁻¹)	ns	$\bar{\chi}$ 0.4a	0.4a	0.4a	0.4a	0.3a	0.3a	ns
		s 0.1	0.0	0.0	0.0	0.0	0.1	
Lead (µg·l ⁻¹)	*	$\bar{\chi}$ 79.0b	48.5a	50.0a	34.4b	25.5a	19.6a	*
		s 10.7	4.2	1.1	1.2	0.8	2.1	
Cadmium (µg·l ⁻¹)	ns	$\bar{\chi}$ < 0.3a	< 0.3a	< 0.3a	< 0.3a	< 0.3a	< 0.3a	ns
		s 0.45	0.00	0.92	0.30	0.04	0.00	
Acid composition:								
Tartaric ac. (g·l ⁻¹)	ns	$\bar{\chi}$ 2.4a	2.4a	2.3a	2.4a	2.4a	2.4a	ns
		s 0.0	0.0	0.0	0.0	0.0	0.0	
Malic ac. (g·l ⁻¹)	ns	$\bar{\chi}$ 1.3a	1.4a	1.3a	1.2a	1.2a	1.2a	ns
		s 0.0	0.1	0.0	0.0	0.0	0.0	
Shikimic ac. (mg·l ⁻¹)	ns	$\bar{\chi}$ 33a	34a	33a	34a	33a	34a	ns
		s 0	0	0	0	0	0	
Lactic ac. (g·l ⁻¹)	ns	$\bar{\chi}$ 0.6a	0.7a	0.6a	0.8a	0.6a	0.6a	ns
		s 0.1	0.0	0.0	0.0	0.1	0.0	
Citric ac. (mg·l ⁻¹)	ns	$\bar{\chi}$ 744a	707a	709a	724a	673a	683a	ns
		s 45	29	2	0	32	17	
Current analysis:								
Density (g·ml ⁻¹)	ns	$\bar{\chi}$ 0.9912a	0.9904a	0.9897a	0.9927a	0.9905a	0.9903a	ns
		s 0.0005	0.0004	0.000	0.0001	0.0001	0.0001	
Alcohol content (% v/v)	ns	$\bar{\chi}$ 12.7a	12.8a	12.8a	12.8a	12.7a	12.8a	ns
		s 0.1	0.1	0.1	0.0	0.0	0.1	
Titrable acidity (g·l ⁻¹ tartaric ac.)	**	$\bar{\chi}$ 5.7b	5.4a	5.3a	5.5b	5.3a	5.2a	**
		s 0.0	0.0	0.1	0.0	0.1	0.0	
Volatile acidity (g·l ⁻¹ acetic ac.)	ns	$\bar{\chi}$ 0.52a	0.49a	0.50a	0.52b	0.45a	0.47ab	*
		s 0.10	0.01	0.01	0.02	0.01	0.01	
pH	ns	$\bar{\chi}$ 3.08a	3.11a	3.14a	3.14a	3.17ab	3.20b	*
		s 0.01	0.00	0.03	0.12	0.01	0.01	

The two experiments (must and wine) were analysed in separate. $\bar{\chi}$ – mean; s – standard error. Treatment means were separated by the Scheffé test at the 5% significance level. Different letters in the same line indicate statistically significant differences: * (p≤0.05), ** (p≤0.01), ns-not significant; nd - not detected. WM-C: white wine control for PVI-PVP must applications; WM-D1 or D2: white wine, resulting from the PVI-PVP application in must; WW-C: white wine control for PVI-PVP wine applications; WW-D1 or D2: white wine, resulting from the PVI-PVP application in wine. C – control wine; D1 - amount 1 (low); D2 - amount 2 (high). L*.a*.b* - CIELAB coordinates, au – absorbance units.

however the Fe content in the wine was also very low. This may explain the partial disagreement with previous work (MATTIVI *et al.* 2000, EDER *et al.* 2003, NICOLINI *et al.* 2004). This difference too, may be related to an inadequate contact between PVI-PVP and the wine.

For Pb, we see a decrease in content with PVI-PVP, both in the must and in the wine. However, increasing the PVI-PVP dosage did not bring about a corresponding increase in Pb removal.

For Cd, all must and wine samples showed concentrations below our detection limit for this metal, so no conclusion about their removal is possible. Portuguese wines generally have very low cadmium concentrations (CATARINO 2000).

We did not find significant changes in the content of organic acids when the PVI-PVP was applied to the must or to the wine at either dosage. For the white wine, we observed a slight but significant decrease in total acidity, but an increase in pH, with PVI-PVP when this was added either to the must or the wine. This confirmed the results of EDER *et al.* (2001, 2003). PVI-PVP did not seem to have any influence on wine density or alcohol content.

The sensory analysis results from the test panel did not find significant differences between the wines regardless of PVI-PVP dosage and of whether it was added to the must or to the wine (Tab. 2). This represents an important technological result.

Table 2

Sensory analysis results for the wine. Values of the scores from 1 (minimum) to 5 (maximum) for each attribute, except global appreciation that is scaled from 1 to 20

		Visual	Nose	Taste	Global appreciation
White wine	WM-C	3.0	3.0	2.9	13.5
	WM-D1	3.4	2.9	2.8	13.2
	WM-D2	3.3	2.9	2.8	12.9
	WW-C	3.3	3.0	3.0	13.5
	WW-D1	3.2	2.7	2.7	12.6
	WW-D2	3.5	2.8	2.9	13.2
Red wine	RM-C	3.7	3.0	2.3	12.6
	RM-D1	3.6	2.8	2.5	13.0
	RM-D2	3.5	2.9	2.4	12.7
	RW-C	3.8	3.0	2.6	13.5
	RW-D1	3.9	3.3	2.4	13.1
	RW-D2	3.7	3.2	2.5	13.1

Legend: WM-C: white wine control for PVI-PVP must application; WM-D1 or D2: white wine, resulting from the PVI-PVP application in must; WW-C: white wine control for PVI-PVP wine applications; WW-D1 or D2: white wine, resulting from the PVI-PVP application in wine. RM-C: red wine control for PVI-PVP must application; RM-D1 or D2: red wine, resulting from the PVI-PVP application in must; RW-C: red wine control for PVI-PVP wine applications; RW-D1 or D2: red wine, resulting from the PVI-PVP application in wine. C – control wine; D1 - amount 1 (low); D2 - amount 2 (high).

Must and red wine: When PVI-PVP was added to the must, the phenolic composition of the wine was not significantly affected (data not shown). On the other hand, when it was added to the wine (Tab. 4), we saw a small but significant reduction in the total phenol index, in the colour tonality, total pigments and in the total anthocyanin content. The latter effect usually reveals a decrease in the value of all of the parameters analysed. PVI-PVP dosage had no significant effect. MATTIVI *et al.* (2000) also found a slight decrease in total phenols and anthocyanins in red wine.

PVI-PVP did not remove significant amounts of K, Ca, Mg or Na when added to either the must or the wine (Tabs 3 and 4). This confirms the results of EDER *et al.* (2001, 2003) for must and for white wine.

Table 3

Effects of PVI-PVP application in red must on mineral composition

	Effect		RM-C	RM-D1	RM-D2
Potassium (mg·l ⁻¹)	ns	$\bar{\chi}$	785a	771a	767a
		s	13	1	1
Calcium (mg·l ⁻¹)	ns	$\bar{\chi}$	83a	82a	87a
		s	0	3	1
Sodium (mg·l ⁻¹)	ns	$\bar{\chi}$	14a	14a	14a
		s	0	1	1
Magnesium (mg·l ⁻¹)	ns	$\bar{\chi}$	70a	70a	70a
		s	0	2	0
Copper (mg·l ⁻¹)	*	$\bar{\chi}$	0.04b	0.02ab	0.01a
		s	0.01	0.00	0.00
Iron (mg·l ⁻¹)	ns	$\bar{\chi}$	7.3a	6.6a	6.0a
		s	0.4	0.2	0.5
Lead (mg·l ⁻¹)	*	$\bar{\chi}$	91.9b	64.4a	64.4a
		s	8.3	2.2	3.0
Cadmium (mg·l ⁻¹)	ns	$\bar{\chi}$	< 0.3a	< 0.3a	< 0.3a
		s			

$\bar{\chi}$ – mean; s – standard error. Treatment means were separated by the Scheffé test at the 5% significance level. Different letters in the same line indicate statistically significant differences: * ($p \leq 0.05$), ** ($p \leq 0.01$), ns-not significant. RM-C: red wine control for PVI-PVP must applications; RM-D1 or D2: red wine, resulting from the PVI-PVP application in must; C - control wine; D1 - amount 1 (low); D2 - amount 2 (high).

We found a significant decrease in Cu content when PVI-PVP was added to the must.

When added to the wine, we observed a tendency, but not statistically significant, for decrease in Cu content. Copper levels were lowered to about 57 % of the initial value, with greater removal in red wine than in white. The probable explanation for this is copper's association with the anthocyanins as metal complexes (ESPARZA *et al.* 2004). The decrease in total anthocyanin content with PVI-PVP supports this hypothesis - see Tab. 4.

Table 4

Effects of PVI-PVP application in wine on some red wine characteristics

	Effect	RW-C	RW-D1	RW-D2
Colour and phenolic composition:				
Total phenol index (ua)	**	$\bar{\chi}$ 63.6b s 0.3	61.1a 0.8	60.9a 0.2
Color intensity (ua)	ns	$\bar{\chi}$ 19.73a s 0.51	19.14a 0.31	19.01a 0.46
Tonality (ua)	**	$\bar{\chi}$ 0.569b s 0.052	0.549a 0.063	0.555a 0.017
L*	ns	$\bar{\chi}$ 58.4a s 0.7	57.6a 0.4	58.0a 0.3
a*	ns	$\bar{\chi}$ 46.4a s 1.00	47.2a 0.31	46.5a 0.36
b*	ns	$\bar{\chi}$ 0.31a s 0.30	0.35a 0.19	0.43a 0.22
Total anthocyanins (mg dm ⁻³ Mv-3-glc)	**	$\bar{\chi}$ 367.5b s 6.8	358.0ab 5.3	346.6a 2.2
Total pigments colour (au)	*	$\bar{\chi}$ 26.54b s 1.33	23.86ab 0.10	22.72a 1.21
Polymerised pigments index (%)	ns	$\bar{\chi}$ 56.31a s 1.59	57.47a 0.77	56.81a 0.36
Mineral composition:				
Potassium (mg·l ⁻¹)	ns	$\bar{\chi}$ 955a s 8	951a 8	957a 8
Calcium (mg·l ⁻¹)	ns	$\bar{\chi}$ 78a s 1	78a 1	77a 1
Sodium (mg·l ⁻¹)	ns	$\bar{\chi}$ 15a s 1	15a 0	16a 1
Magnesium (mg·l ⁻¹)	ns	$\bar{\chi}$ 84a s 1	85a 1	85a 0
Copper (mg·l ⁻¹)	ns	$\bar{\chi}$ 0.23a s 0.13	0.10a 0.11	0.10a 0.08
Iron (mg·l ⁻¹)	ns	$\bar{\chi}$ 6.6b s 0.2	5.6a 0.2	5.1a 0.6
Lead (µg·l ⁻¹)	**	$\bar{\chi}$ 133.6a s 30.2	121.3a 15.2	124.2a 37.9
Cadmium (µg·l ⁻¹)	ns	$\bar{\chi}$ < 0.3a s	< 0.3a	< 0.3a
Acid composition:				
Tartaric ac. (g·l ⁻¹)	*	$\bar{\chi}$ 2.5b s 0.0	2.4a 0.0	2.4a 0.0
Malic ac. (g·l ⁻¹)	ns	$\bar{\chi}$ 0.2a s 1.4	0.2a 0.0	0.2a 0.0
Shikimic ac. (mg·l ⁻¹)	ns	$\bar{\chi}$ 55a s 1	56a 0	55a 3
Lactic ac. (g·l ⁻¹)	ns	$\bar{\chi}$ 4.4a s 1.4	4.5a 0.2	4.4a 0.1
Citric ac. (mg·l ⁻¹)	ns	$\bar{\chi}$ 973a s 10	967a 12	972a 3
Current analysis:				
Density (g·ml ⁻¹)	ns	$\bar{\chi}$ 0.9947a s 0.0002	0.9946a 0.0001	0.9945a 0.0001
Alcohol content (% v/v)	ns	$\bar{\chi}$ 11.4a s 0.1	11.4a 0.0	11.4a 0.0
Titration acidity (g·l ⁻¹ tartaric ac.)	**	$\bar{\chi}$ 7.6c s 0.0	7.4b 0.0	7.3a 0.2
Volatile acidity (g·l ⁻¹ acetic ac.)	ns	$\bar{\chi}$ 0.28a s 0.05	0.31a 0.01	0.32a 0.01
pH	**	$\bar{\chi}$ 3.12a s 0.02	3.15a 0.00	3.18a 0.01

When PVI-PVP was added to the must, although the decrease in Fe content was not statistically significant it represented about a 15 % reduction for the lower dosage and 23% for the higher one. When added to the wine, PVI-PVP caused a very significant decrease in Fe content and this was enhanced by increasing the PVI-PVP dosage.

Lead levels were significantly lowered (30 %) by PVI-PVP when this was added to the must, but when added to the wine a non-significant reduction of 9 % was found (NB Pb determinations were very variable and standard deviations were high).

For Cd, the contents were below our detection limits for red wines, so no conclusions can be drawn. The same was true for must and for white wine.

No effect on organic acid content (tartaric, malic, lactic, citric or shikimic) was detected with PVI-PVP when this was added either to the must or to the wine. However, a significant effect was detected in wine on total acidity and on pH. The significant decrease in total acidity and the increase in pH had already been observed in the assay with white wine (Tab. 1), and in agreement with the results of EDER *et al.* (2003). The density and alcohol content were not affected by PVI-PVP.

The taste panel could not find significant differences between the wines for the attributes evaluated by them. This can be considered a good result from a technological point of view with PVI-PVP not affecting sensory characteristics at either of the dosages used in this study (Tab. 2).

Finishing treatment applied in wine with metals addition: To confirm the influence of PVI-PVP on Fe and Cu content of the wine, these metal elements were added to a white and a red wine as described above. In addition, we have analysed also the content of the other metals (Cd, Pb, Al).

White wine: The physical-chemical test results are shown in Tab. 5. As noted above, PVI-PVP had some influence on the total phenol index, on the optical density at 420 nm, and in the lightness of the wine (L*). Titrable acidity was significantly reduced by increasing PVI-PVP dosage. This was accompanied by an increase in pH, again confirming our earlier results (Tab. 1). EDER *et al.* (2001, 2003) also found a slight reduction in titrable acidity with PVI-PVP. The other physical-chemical parameters, density, alcohol content, and volatile acidity were not significantly affected.

Relatively to the remove of the metals in study (Cu, Fe, Cd, Pb and Al), we obtained a more effective reduction in Cu with the higher PVI-PVP dosage and with the highest Cu addition (59 %), as seen in Fig. 2. This result was similar to that of MATTIVI *et al.* (2000). The removal of Fe was very significant. The removal percent was nearly 70 % (except with the addition B having the lower level of PVI-

$\bar{\chi}$ – mean; s – standard error. Treatment means were separated by the Scheffée test at the 5 % significance level. Different letters in the same line indicate statistically significant differences: * (p ≤ 0.05), ** (p ≤ 0.01), ns-not significant. RW-C: red wine control for PVI-PVP wine applications; RW-D1 or D2: red wine, resulting from the PVI-PVP application in wine. C – control wine; D1 – amount 1 (low); D2 – amount 2 (high). L* a* b* – CIELAB coordinates; au – absorbance units.

Table 5

Effects of PVI-PVP application on some white wine characteristics

	Effect	Addition A Fe: 5 mg·l ⁻¹ Cu: 0.5 mg·l ⁻¹			Addition B Fe: 15 mg·l ⁻¹ Cu: 1 mg·l ⁻¹			Effect
		Control wine	PVI-PVP 25 g·hl ⁻¹	PVI-PVP 50 mg·l ⁻¹	Control wine	PVI-PVP 25 g·hl ⁻¹	PVI-PVP 50 mg·l ⁻¹	
Colour and phenolic composition:								
Total phenol index (ua)	**	$\bar{\chi}$ 7.9c s 0.0	7.4b 0.0	7.1a 0.1	7.9c 0.1	7.4b 0.0	7.1a 0.1	**
A420 (ua)	**	$\bar{\chi}$ 0.160c s 0.002	0.126b 0.001	0.117a 0.001	0.165c 0.001	0.131b 0.001	0.121a 0.002	**
L*	*	$\bar{\chi}$ 97.5a s 0.3	98.5b 0.0	98.7b 0.2	97.4a 0.1	98.2ab 0.3	98.4b 0.2	*
a*	ns	$\bar{\chi}$ -1.1a s 0.07	-1.2a 0.00	-1.2a 0.06	-1.4a 0.09	-1.2a 0.00	-1.3a 0.04	ns
b*	**	$\bar{\chi}$ 9.6c s 0.27	7.9b 0.03	7.2a 0.06	10.2b 0.37	8.0a 0.03	7.6a 0.01	**
Mineral composition:								
Copper (mg·l ⁻¹)	*	$\bar{\chi}$ 0.5b s 0.0	0.3a 0.0	0.3a 0.0	0.7b 0.0	0.3a 0.1	0.3a 0.0	**
Iron (mg·l ⁻¹)	**	$\bar{\chi}$ 4.9b s 0.0	1.5a 0.1	1.4a 0.4	9.4b 0.4	5.3ab 0.9	2.6a 1.6	*
Lead (µg·l ⁻¹)	**	$\bar{\chi}$ 35.6b s 1.3	29.4a 0.4	26.6a 4.7	35.4b 0.1	29.8a 0.6	27.0a 1.1	**
Cadmium (µg·l ⁻¹)	ns	$\bar{\chi}$ 0.7a s 0.0	0.7a 0.1	0.6a 0.0	0.7a 0.0	0.8a 0.0	0.7a 0.1	ns
Aluminium (µg·l ⁻¹)	**	$\bar{\chi}$ 414b s 8	334a 25	267a 16	429b 6	345ab 26	259a 13	*
Current analysis:								
Density (g·ml ⁻¹)	ns	$\bar{\chi}$ 0.9902a s 0.0000	0.9901a 0.0000	0.9901a 0.0000	0.9903a 0.0001	0.9902a 0.0001	0.9901a 0.0000	ns
Alcohol content (% v/v)	ns	$\bar{\chi}$ 11.7a s 0.0	11.8a 0.0	11.8a 0.0	11.6a 0.1	11.7a 0.0	11.7a 0.0	ns
Titrate acidity (g·l ⁻¹ tartaric ac.)	**	$\bar{\chi}$ 6.3b s 0.0	6.2b 0.0	6.0a 0.0	6.3b 0.0	6.2b 0.0	6.0a 0.0	**
Volatile acidity (g·l ⁻¹ acetic ac.)	ns	$\bar{\chi}$ 0.4a s 0.01	0.4a 0.00	0.4a 0.00	0.4a 0.00	0.4a 0.01	0.4a 0.01	ns
pH	**	$\bar{\chi}$ 3.16a s 0.00	3.20b 0.00	3.24c 0.01	3.16a 0.00	3.19b 0.01	3.24c 0.01	**

The two experiments (addition A and addition B) were analysed separately. $\bar{\chi}$ – mean; s – standard error. Treatment means were separated by the Scheffée test at the 5 % significance level. Different letters in the same line indicate statistically significant differences: * (p ≤ 0.05), ** (p ≤ 0.01), ns-not significant. Control wine – wine without PVI-PVP application. L* a* b* – CIELAB coordinates; au – absorbance units.

PVP). In wine with addition A, increasing levels of PVI-PVP did not result in more effective Fe removal.

Lastly, PVI-PVP seems to have some capacity to deplete Pb and Al. The lowest dosage had some Pb reduction effect (18 %) and this was improved by the highest dosage. This fitted with the previous results for Pb. Nevertheless, PVI-PVP was not effective in removing Cd. Aluminium depletion (from 19 % to 40 %) has also been reported by NICOLINI *et al.* (2001, 2004).

Red wine: The results of PVI-PVP additions on the removal of metals and on other physical-chemical characteristics of red wine are presented in Tab. 6.

PVI-PVP affected the total phenols index of the wines slightly but still significantly with removal percentages of about 6 %. However, it did not significantly affect anthocy-

anin content, or the polymeric pigment, or the total pigments (expressed as the colour due to the total pigments). These results have confirmed those of our previous assays (Tab. 4). Confirming our previous results (Tab. 4), PVI-PVP reduces the total acidity of the wine, and significantly increases pH. As for metal removal, there was an efficient reduction of Cu, Fe, Pb and Al by PVI-PVP, but the greatest reduction was found for Cu (74 %). This agrees with MATTIVI *et al.* (2000) and NICOLINI *et al.* (2004). The removal percentage increased with dosage (Fig. 3) and was greater in the wines and with addition B. These results are consistent with those of Mattivi *et al.* (2000), in which adsorption efficiency is greater when the metal content is higher.

PVI-PVP also showed some ability to remove Pb, which confirms the results obtained in the previous assays.

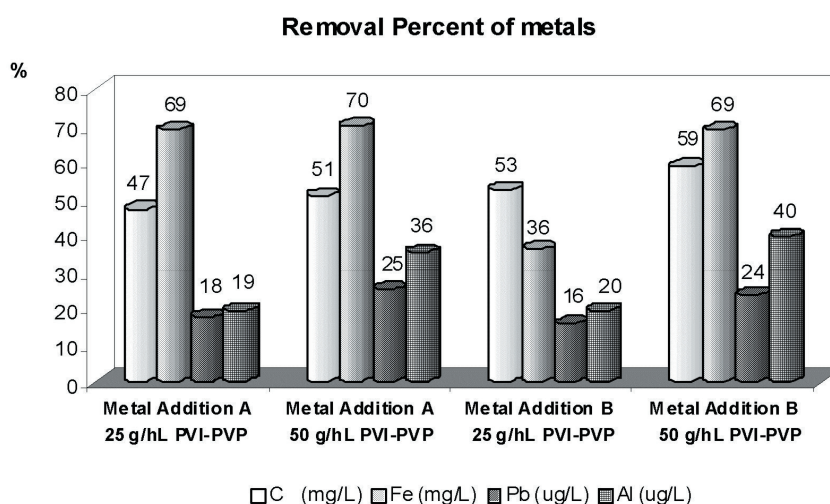


Fig. 2: Removal percent of copper, iron, lead and aluminium in white wine (metal addition A: 5 mg·ml⁻¹ of iron and 0.5 mg·ml⁻¹ of copper; metal addition B: 15 mg·ml⁻¹ of Fe and 1 mg·ml⁻¹ of copper).

However, in wines with a higher content of metals (addition B) the percentage of removal was slightly lower compared to the wines having the lower metal content addition A (Fig. 3).

Conclusion

Generally, PVI-PVP removed Cu, Fe, Pb and Al more effectively when it was added at the highest dosage and when these metal contents were initially high. However, PVI-PVP had no significant effect on the contents of Ca, Mg, Na and Cd. However, the wines used in this study were very low in Cd, lower than our detection limit as is usual in Portuguese wines.

The removal of metals was more efficient when PVI-PVP was used in the wine than in the must. This probably reflects the complex macromolecular composition of the must. In the metal addition assays, the removal of Fe and Pb was more efficient in white wine than in red, while the

removal of Cu and Al was more efficient for the red wine than for the white. For Pb, the complexes formed with pectic polysaccharide Rhamnogalacturonan II (PELLERIN and O'NEILL 1998), which are very important in red wines, could explain the smaller removal of Pb in red wines compared to white wines. Again, the percentage removal of all of these metallic elements increased with increasing dosages of PVI-PVP. In future work other (greater and lesser) application doses will be assessed.

PVI-PVP application had only a slight influence on the colour and phenolic composition of the wines. Organic acid content (tartaric, malic, lactic, citric and shikimic acids) of the wine was not influenced by PVI-PVP, either in the must or in the wine. The wines showed significant decreases in total acidity and increases in pH, with PVI-PVP additions. However, sensory analysis did not reveal significant differences between any of the wines from any of the many attributes evaluated by the test panel. The application of PVI-PVP in must or wine seems not to affect its sensory characteristics, for the dosages used in this study which is a

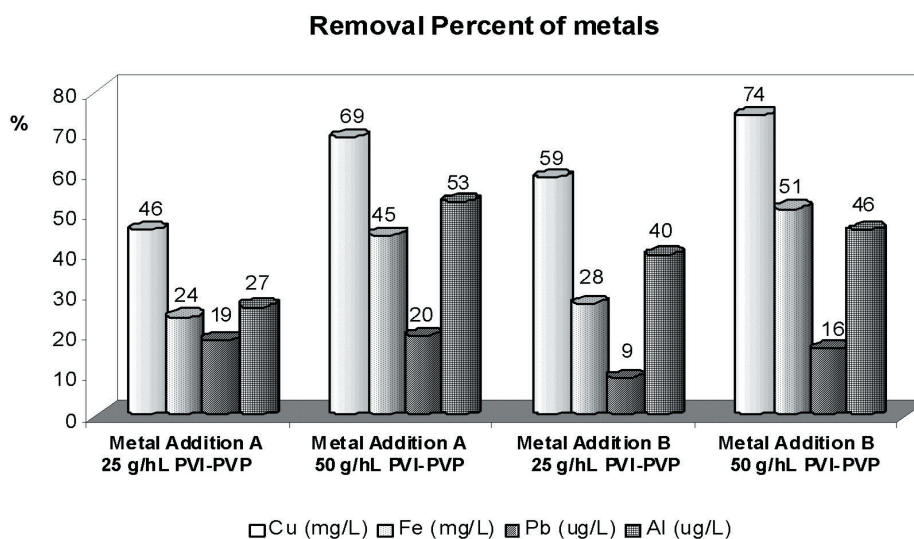


Fig. 3: Removal percent of copper, iron, lead and aluminium in red wine. (metal addition A: 5 mg·ml⁻¹ of iron and 0.5 mg·ml⁻¹ of copper; metal addition B: 15 mg·ml⁻¹ of Fe and 1 mg·ml⁻¹ of copper).

Table 6

Effects of PVI-PVP application on some red wine characteristics

	Effect	Addition A Fe: 5 mg·l ⁻¹ Cu: 0.5 mg·l ⁻¹			Addition B Fe: 15 mg·l ⁻¹ Cu: 1 mg·l ⁻¹			Effect
		Control wine	PVI-PVP 25 g·hl ⁻¹	PVI-PVP 50 mg·l ⁻¹	Control wine	PVI-PVP 25 g·hl ⁻¹	PVI-PVP 50 mg·l ⁻¹	
Colour and phenolic composition:								
Total phenol index (ua)	*	$\bar{\chi}$ 57.4b s 1.0	55.1ab 0.1	54.1a 0.6	57.3b 0.4	54.1ab 1.4	53.5a 0.1	*
Colour intensity (ua)	ns	$\bar{\chi}$ 10.97c s 0.05	10.72b 0.09	10.52a 0.09	11.02a 0.04	10.96a 0.24	10.68a 0.09	ns
Tonality (ua)	ns	$\bar{\chi}$ 0.790b s 0.006	0.785a 0.007	0.784a 0.004	0.792a 0.002	0.795a 0.010	0.797a 0.006	ns
L*	*	$\bar{\chi}$ 51.5a s 0.1	52.2ab 0.2	52.7b 0.2	51.4a 0.0	52.1a 0.1	52.7a 0.1	**
a*	ns	$\bar{\chi}$ 43.03a s 0.02	42.58a 0.31	42.07 0.56	42.44a 0.09	41.45a 0.39	41.03a 0.26	ns
b*	ns	$\bar{\chi}$ 15.73a s 0.01	15.61a 0.10	15.44a 0.13	15.77a 0.10	15.68a 0.03	15.59a 0.07	ns
Total anthocyanins (mg dm ⁻³ Mv-3-glc)	ns	$\bar{\chi}$ 47.8a s 1.5	50.2a 2.8	51.3a 2.6	49.0a 0.65	50.9a 2.1	47.3a 1.3	ns
Polymerised pigments index (%)	ns	$\bar{\chi}$ 73.43a s 0.50	73.74a 0.36	74.66a 0.23	75.40a 0.25	75.58a 0.78	75.42a 1.97	ns
Total pigments colour (ua)	ns	$\bar{\chi}$ 12.28a s 0.18	11.98a 0.11	11.95a 0.14	12.08a 0.18	12.08a 0.04	11.70a 0.14	ns
Mineral composition:								
Copper (mg·l ⁻¹)	**	$\bar{\chi}$ 0.48c s 0.03	0.26b 0.03	0.15a 0.01	0.97b 0.10	0.40a 0.02	0.25a 0.01	*
Iron (mg·l ⁻¹)	**	$\bar{\chi}$ 4.9c s 0.03	3.8b 0.01	2.8a 0.20	10.0b 0.71	7.9ab 1.26	5.3a 0.09	*
Lead (µg·l ⁻¹)	*	$\bar{\chi}$ 41.4b s 2.3	33.9a 1.9	33.7a 1.0	40.6b 2.6	36.9a 0.9	35.0a 0.1	*
Cadmium (µg·l ⁻¹)	ns	$\bar{\chi}$ 0.3a s 0.0	0.4a 0.0	0.4a 0.0	0.3a 0.0	0.4a 0.1	0.4a 0.2	ns
Aluminium (µg·l ⁻¹)	*	$\bar{\chi}$ 928b s 11	679ab 129	437a 18	752b 130	454ab 33	409a 31	*
Current analysis:								
Density (g·ml ⁻¹)	ns	$\bar{\chi}$ 0.9926a s 0.0000	0.9926a 0.0000	0.9926a 0.0001	0.9928a 0.0001	0.9927a 0.0001	0.9925a 0.0000	ns
Alcohol content (% v/v)	ns	$\bar{\chi}$ 13.1a s 0.0	13.1a 0.0	13.0a 0.0	13.0a 0.1	13.0a 0.0	13.0a 0.0	ns
Titrate acidity (g·l ⁻¹ tartaric ac.)	**	$\bar{\chi}$ 6.1c s 0.0	5.9b 0.0	5.8a 0.0	6.1c 0.0	5.9b 0.0	5.8a 0.0	**
Volatile acidity (g·l ⁻¹ acetic ac.)	ns	$\bar{\chi}$ 1.1a s 0.01	1.1a 0.01	1.1a 0.01	1.1a 0.01	1.1a 0.01	1.1a 0.00	ns
pH	**	$\bar{\chi}$ 3.42a s 0.01	3.46b 0.00	3.49b 0.00	3.43a 0.00	3.47b 0.00	3.50c 0.01	**

The two experiments (addition A and addition B) were analysed separately. $\bar{\chi}$ – mean; s – standard error. Treatment means were separated by the Scheffé test at the 5% significance level. Different letters in the same line indicate statistically significant differences: * (p≤0.05), ** (p≤0.01), ns-not significant. Control wine – wine without PVI-PVP application; L*.a*.b* - CIELAB coordinates; au – absorbance units.

useful result. In our opinion, these findings make an important, practical contribution, showing that oenologists could use this product without major impact on the chemical and sensory properties of their wines. Finally, PVI-PVP efficiently removes Cu and Fe from wines and does not seem

to have some of the limitations and dangers associated with traditional potassium ferrocyanide treatment. In addition, PVI-PVP does not impact negatively upon the principal characteristics of the wine making this copolymer a useful likely replacement.

Acknowledgements

The authors thank the AGRO Program (project n°23, measure 8.1 of the Ministry of Agriculture, Portugal) for financial support for this work; also, PRODEP – Action 5.3 – Advanced training of university teachers (Ministry of Education, Portugal); also, Comissão Vitivinícola Regional do Ribatejo (CVRR) for providing the wines and, lastly BASF, Germany for supplying the PVI-PVP copolymer.

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Received November 30, 2006