

**RELEASE OF CONTAMINANT ELEMENTS FROM BENTONITES
TO WINE: A CONTRIBUTION TO ACHIEVE A TEST SOLUTION**

**CEDÊNCIA DE ELEMENTOS CONTAMINANTES DE
BENTONITES AO VINHO: CONTRIBUTO PARA A
OPTIMIZAÇÃO DE UMA SOLUÇÃO TESTE**

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SUMMARY

The release of mineral elements from six bentonites to wine and to the test solution established by the OIV was evaluated in order to compare the extraction performance of both solutions. Significant differences between wine and tartaric solution results for thirty-four mineral elements analysed by AAS, ETAAS and ICP-MS were observed, suggesting that the extraction solution proposed by the OIV is not suitable for bentonite quality control purposes. Taking into account bentonite maximum extractable amounts defined by the OIV, some samples showed higher concentrations for Na (B3 and B8), Al (B8), Fe (B5) and As (B9). An additional extraction essay involving two bentonites, wine and two complex test solutions containing the major mineral elements of wine (K, Ca, Na and Mg) and protein (wine protein and BSA) was carried out. Significant differences were observed for all elements with exception of Sc, Mn, Co, W and Bi. For several elements, such as Na, Mg (test solution with BSA) V, Ni, Ga, Zr, Cd, In, Sb, Tl and U, the similarity of wine and test solutions content variations was satisfactory. The protein nature of test solutions seemed to have a decisive role in some element changes, probably related to wine protein and BSA distinct volume. In general, the test solution containing wine protein was a slightly better simulator of wine performance. However, the above mentioned test solutions did not simulated wine performance in a total satisfactory way, and further work should be performed on extraction solution composition.

Keywords: contaminant elements, bentonite, wine, test solution, ICP-MS

Palavras chave: elementos contaminantes, bentonite, vinho, solução teste, ICP-MS

INTRODUCTION

Bentonite is a technological additive with a high potential influence on mineral composition of the wine and one of its main sources of contamination. Over the last twenty years, several authors have reported important concentration increases of metals in wines due to bentonite addition (Postel *et al.*, 1986; Enkelmann, 1988; Wurzinger *et al.*, 1994; Gössinger *et al.*, 1997; Machado-Nunes *et al.*, 1998; Catarino *et al.*, 2004; Nicolini *et al.*, 2004; Gómez *et al.*, 2004). Wine enrichment in some mineral elements may affect its sensory quality, namely appearance, its legal quality as well as an impact on consumer health (Curvelo-Garcia and Catarino, 1998).

Usually, a general physical and chemical characterization as well application instructions are provided with the commercial bentonite, without information about its potential mineral release to wine. Considering that bentonite origin and type on the market is considerably diverse, each product may cause a particular effect on mineral composition of wine. This statement is supported by the results of a previous study, in which we have studied the release of contaminant elements from bentonites to wine, in relation with their physical and chemical characteristics (submitted for publication).

Assuming that quality control of bentonites is essential to assure its safe use as a technological additive, the International Organisation of Vine and Wine (OIV, 2003) defines limit values for a set of elements to be determined in a bentonite extraction solution. According to the OIV, the extraction trial should be carried out with a tartaric acid solution. Nevertheless, considering the wine natural complexity, it should be admitted that the results obtained using this test solution could be very different from wine results.

Due to the high cationic exchange capacity of bentonites, cations are released when positively charged proteins and other soluble cationic constituents in wine are adsorbed. Blade and Boulton (1988) have stated that adsorption of proteins suffers competition from other cations in the solution matrix and the solvent properties. These authors suggested that competition would be from K^+ , Ca^{2+} , Mg^{2+} , Na^+ and H^+ , most amino acids, some peptides and other cationic fractions. Concerning protein adsorption by bentonites, few studies have been carried out using wine model solutions (Blade and Boulton, 1988; Achaerandio *et al.*, 2001). However, as far as we know there are no published studies developed in view to attain a representative test solution of wine, in what concerns to its extraction effect on bentonites. Furthermore the natural diversity of wine composition represents an additional obstacle to a test solution establishment.

The main purpose of our work was to evaluate the release of mineral elements from bentonites employing: (1) the test solution indicated by the OIV, and (2) wine, in order to compare their performance. In addition, complex solutions

were also tested in order to improve a test solution fairly representative of wine.

MATERIAL AND METHODS

Six different bentonites follow designated by B1, B3, B4, B5, B8 and B9, obtained from four companies in Portugal, were used for extraction essays. Tartaric acid was added (3.20 g/L) to a white wine from 2001 vintage in order to obtain a pH 3.0. Wine pH value was controlled through the time until stability was reached then extraction trials with bentonites were carried out.

Test solutions

Test solutions were prepared using deionized water (conductivity < 0.1 mS/cm) obtained through a Seralpur Pro 90 CN (Seral, Ransbach-Baumbach, Germany).

Test solution A (OIV) - a L⁽⁺⁾ tartaric acid solution of 5 g/L (Merck) and pH level of 3 (adjusted with sodium hydroxide 10 N).

Test solution B and test solution C - 5 L of a solution with the follow composition was prepared: tartaric acid from Merck (5 g/L), potassium chloride from Merck (1.90 g/L); calcium carbonate from Riedel (250 mg/L) and hexahydrated magnesium chloride from Fluka (400 mg/L), in order to simulate K, Ca and Mg wine contents. The pH value was adjusted to 3 with sodium hydroxide 10 N. Afterwards, this solution was separated in two flasks and 120 mg/L of wine protein (test solution B) or standard protein BSA (bovinum serum albumin from Sigma) (test solution C) was added. Wine protein was obtained as described by Mesquita *et al.* (2001). Chosen concentration is considered typical in Portuguese white wines (Mesquita *et al.*, 2001).

Extraction essays

An amount of 5 g anhydrous bentonite was shaken together with 200 mL of wine (or test solution) in an Erlenmeyer vessel for 15 min. After 24 hours of decantation, the liquid obtained was centrifuged at 12000 rpm during 30 min and the supernatant was used for measurements. Samples were prepared in parallel, in triplicate, with control samples (without bentonite addition), similarly stirred and centrifuged. Only polypropylene material was used. All the material was soaked in 20% HNO₃ (v/v) for at least 24 hours and rinsed several times with deionized water before use.

Trial 1 – Extraction essay using wine and test solution A (OIV)

This study was based in a factorial experiment, with two factors: bentonite (B1, B3, B4, B5, B8 and B9) and extraction solution (wine, test solution A), with three replications (Figure 1).

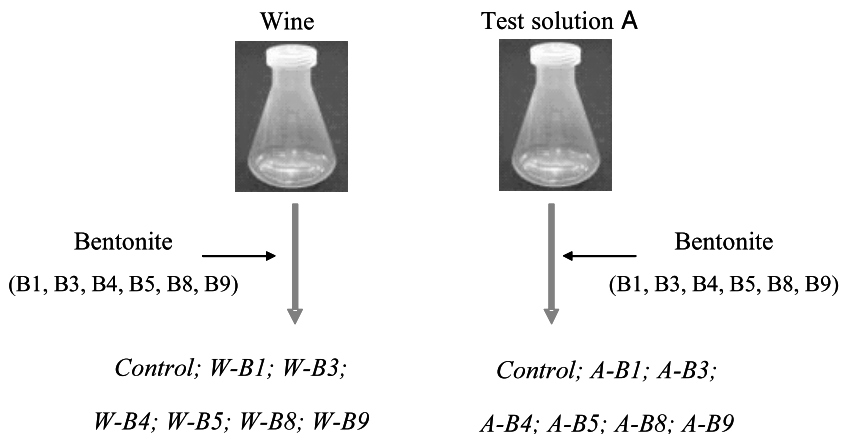


Fig. 1 - Trial 1 scheme
Esquema do ensaio 1

Trial 2 – Extraction essay using wine, test solution B and C

This study was also based in a factorial experiment, with two factors: bentonite (B1 and B9) and extraction solution (wine, test solution B, test solution C), with three replications (Figure 2).

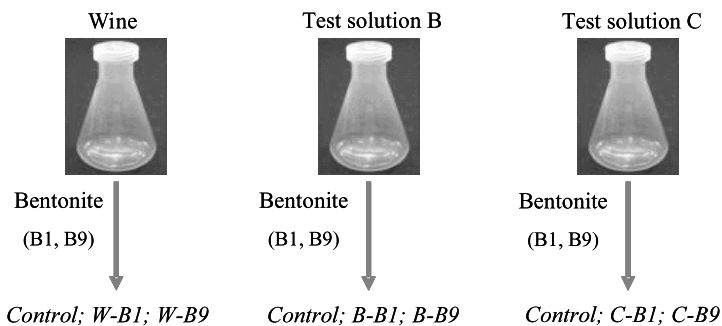


Fig. 2 – Trial 2 scheme
Esquema do ensaio 2

Mineral composition analysis

Na, Mg, K, Ca and Fe were analysed by flame atomic absorption spectrometry

(FAAS) (Catarino *et al.*, 2003), Al by electrothermal atomic absorption spectrometry (ETAAS) (Catarino *et al.*, 2002), while Li, Be, B, Sc, V, Mn, Fe, Co, Ni, Cu, Zn, Ga, Ge, As, Rb, Sr, Y, Zr, Nb, Mo, Cd, In, Sn, Sb, Ba, W, Tl, Pb, Bi and U were analysed by inductively coupled plasma mass spectrometry (ICP-MS) semi-quantitative approach (Catarino *et al.*, in press), using a Perkin Elmer SCIEX Elan 9000 apparatus, equipped with a crossflow nebulizer, a Rytan Scott-type spray chamber, nickel cones and a peristaltic sample delivery pump with four channels (Gilson) (application software Elan – 6100 Windows NT - version 2.4). An autosampler Perkin-Elmer AS-93 Plus was protected by a laminar-flow-chamber clean room class 100 (Reinraumtechnik Max Petek). Operating conditions used were as follows: RF power of 1200W; sample uptake rate of 0.85 mL/min; nebulizer argon flow between 0.85 and 0.95 L/min. A full mass spectrum ($m/z = 6-240$, omitting the mass ranges 16-18; 40, 41, 211-229) was obtained by full mass range scanning. A reference response table (Perkin-Elmer TotalQuant III) was updated with a multielement standard solution (Li, Be, Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Ag, Cd, In, Cs, Ba, Hg, Tl, Pb, Bi, U) at 10 mg/L, from Perkin-Elmer. Rh and Re were used as internal standards for elements in the mass range (m/z) 7-138 and 205-238, respectively. The software of the instrument performed automatic corrections of isobaric interferences. In order to get signal stabilization, a sample read delay of 75 s was chosen. Between samples or standards, the sampling system was rinsed with a 2% HNO_3 (v/v), 1% $\text{C}_2\text{H}_5\text{OH}$ (v/v), and 200 $\mu\text{g/L}$ of Au solution for 75 s. The experimental conditions used for the measurements were: dwell time = 50 ms; sweeps/reading = 6; reading/replicates = 1; replicates = 1; time per run = 67 s. Only high purity reagents and deionized water (conductivity < 0.1 mS/cm) were used. All the material (polypropylene and Teflon PFA) was soaked in 20% HNO_3 (v/v) for at least 24 h and rinsed several times with deionized water, before use.

Statistical analysis

The variance analysis was performed using *Statistica* version 98 edition (Statsoft Inc., E.U.A.). As control samples of wine and test solutions showed different mineral contents, concentration variations between control and bentonite treated samples were considered for statistical analysis.

RESULTS AND DISCUSSION

Table I shows the average variations in the concentration of several elements, both in wine and test solution A, caused by bentonite addition.

For each element, the variation of concentration between each modality (extraction solution and bentonite association) and the respective control was

TABLE I

Average changes in the concentrations of several elements in wine and test solution A caused by bentonites addition (Trial 1). The results are expressed in $\mu\text{g/L}$, except for Na, Mg, Al, K, Ca and Fe (mg/L).

Varição média da concentração de elementos minerais no vinho e na solução teste A em consequência da adição de bentonites (Ensaio 1). Os resultados são expressos em $\mu\text{g/L}$, excepto para o Na, Mg, Al, K, Ca and Fe (mg/L).

Element	Extraction solution effect	Wine mean (n=18)	Test sol. A mean (n=18)	Element	Extraction solution effect	Wine mean (n=18)	Test sol. A mean (n=18)
<i>Li</i>	**	9.80a	20.31b	<i>As</i>	**	24.34b	31.27a
<i>Be</i>	**	13.03b	4.03a	<i>Rb</i>	**	409.48b	28.89a
<i>B</i>	**	(-)331.31b	13.75a	<i>Sr</i>	**	665.95b	127.01a
<i>Na</i>	**	358.28b	247.67a	<i>Y</i>	**	4.597a	5.338b
<i>Mg</i>	**	50.00b	28.89a	<i>Zr</i>	**	269.30a	412.68b
<i>Al</i>	**	15.416a	25.804b	<i>Nb</i>	**	11.50b	8.68a
<i>K</i>	**	(-)193.6b	5.4a	<i>Mo</i>	**	7.55a	(-)46.55b
<i>Ca</i>	**	77.22b	2.27a	<i>Cd</i>	**	0.55b	0.20a
<i>Sc</i>	**	0.76a	2.11b	<i>In</i>	**	0.06b	(-)0.04a
<i>V</i>	**	27.10a	44.88b	<i>Sn</i>	**	2.60a	7.30b
<i>Mn</i>	**	1406.54b	964a	<i>Sb</i>	**	4.37b	2.88a
<i>Fe</i>	**	3.69a	8.02b	<i>Ba</i>	**	83.03a	218.46b
<i>Co</i>	**	19.77b	18.29a	<i>W</i>	**	0.736a	0.998b
<i>Ni</i>	**	45.85b	23.53a	<i>Tl</i>	**	2.41b	0.91a
<i>Cu</i>	**	(-)44.44b	16.10a	<i>Pb</i>	n.s.	13.13	13.33
<i>Zn</i>	**	(-)109.85a	125.83b	<i>Bi</i>	**	0.847a	66.749b
<i>Ga</i>	**	1.44a	5.88b	<i>U</i>	**	43.80a	64.34b
<i>Ge</i>	**	0.046a	0.250b				

calculated. Afterwards, for each extraction solution (wine and test solution A) the mean of the corresponding six modalities, considering the three independent replicates, was calculated.

The interpretation of the results should take into account that those were obtained under experimental conditions, using a bentonite dose higher than usually applied in winemaking. Thus, concerning wine enrichment or loss in different elements, the results could not be directly extrapolated to production scope. On the other hand, for some elements, it should be noted that significant differences between extraction solutions were not relevant, bearing in mind the low amounts involved.

Considering the whole mineral elements in study, with exception of Pb, the average concentration variation of wine and test solution A were significantly different (99% confidence level). At once, the results concerning to B, K, Cu

and Zn should be pointed out. In consequence of bentonite addition, their contents in wine suffered important decreases while in test solution A increases occurred. The different trends between the two extraction solutions were certainly related to their distinct composition, namely mineral and protein contents. The highest weight cation of wine, K, was present in test solution A as a trace element. Probably this element replaced bentonite Na and Ca positions being consequently partially removed from wine. In fact, proteins and K competition to bentonite adsorption was previously reported (Blade and Boulton, 1998). Similar explanation is credible for Rb decrease, since it is an element of low ionic potential, likewise K. The results concerning Mo element are probably explained by its distinct concentration in control samples. The control sample of test solution A presented a higher concentration of Mo, probably introduced as an impurity by tartaric acid. Likewise K and Rb, Mo probably replaced bentonite Na and Ca positions. The explanation for B, Cu and Zn depletions may be associated to their complexation with condensed tannins, which are in part bind to proteins. Moreover, the extraction solution A has no protein or tannins in its composition.

Regarding to bentonite major exchangeable cations, Na, Mg and Ca, important enrichments were verified in both extraction solutions, specially in wine. This observation suggests that cationic exchange phenomena were more intense in wine somehow as expected, since the test solution A contained no protein.

For the following elements, Al, Sc, V, Fe, Ga, Ge, Y, Zr, Sn, Ba, Bi and U, and in less extent Y, higher increases were observed in test solution A. Probably, the partial removal of these elements from wine by precipitation with other chemical species such as organic acids, proteins, tannins or even polysaccharides occurred. On the other hand, the release of the above mentioned elements from smectite structure or from other minerals and impurities could have been enhanced by the tartaric acid. Maujean (1993) stated that clays could be partial or totally destroyed by tartaric acid.

It should be remembered that the previous statements are based on average values. In fact, interaction between bentonite and extraction solution was observed for all the elements. Concerning Pb results, the similar average value observed in both extraction solutions were obtained by chance as for none of the individual studied bentonites the results were similar.

Additionally, for each element a linear least-squares adjustment of the results obtained using wine and test solution A was performed in order to evaluate their correlation (r). The most satisfactory results obtained, corresponding to Mg, Co, Fe, As, Zr, Sn, Sb, W, Tl and U elements, are shown in Figure 3 (the 95% confidence limits of the linear regression parameters are given).

A deviation of the slope from unity indicates proportional discrepancies

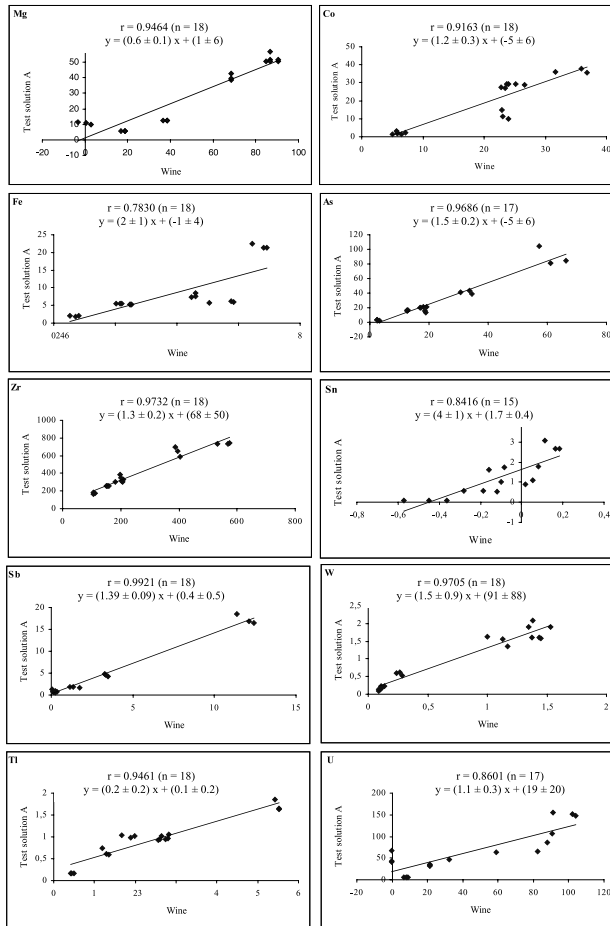


Fig. 3 - Relationship between the changes in the concentrations of Mg, Co, Fe, As, Zr, Sn, Sb, W, Tl and U in wine and test solution A, caused by bentonite addition. The linear least squares adjustment and correlation coefficient are shown (the 95% confidence limits of the linear regression parameters are given)

Relação entre as variações de concentração de Mg, Co, Fe, As, Zr, Sn, Sb, W, Tl and U no vinho e na solução teste A, em consequência da adição de bentonite. Regressão linear e coeficiente de correlação (apresenta-se os parâmetros da regressão linear e respectivos limites de confiança, P = 95%)

between wine and test solution A results. A non-zero intercept is diagnosed as a constant discrepancy.

The previous results confirmed that tartaric acid solution was not able to simulate wine extraction performance. Thus, concerning bentonite potential mineral contamination effect on wine, it could be stated that test solution A

is not a suitable extraction solution for bentonite quality control purposes.

Taking into account bentonite maximum extractable contents defined by the OIV (OIV, 2003) some observations should be emphasized: the extractable Al content of B8 in tartaric acid solution was of 2.6 mg/L, a slightly higher value than the established 2.5 mg/kg; the B9 soluble As content, of 2.5 mg/kg, exceeded the defined 2 mg/kg; B5 soluble Fe content was of 865 mg/kg (the established content is of 600 mg/kg). Finally, B3 and B8 samples both showing 13 g/kg exceeded the soluble Na content (less than 10 g/kg) defined for natural bentonites. These results are of major importance as to our knowledge the quality control of bentonites is not a regular practise.

The next step in our work involved essays carried out with two extraction solutions containing the major mineral elements of wine and also protein: (1) test solution B with wine protein and (2) test solution C with standard protein (BSA), both solutions tested in parallel with wine (Trial 2).

Table II shows the variations in the contents of several elements in wine, test solution B and test solution C, caused by bentonite addition.

The multielemental composition of control samples is shown at Table III. For each modality (extraction solution and bentonite association) and element, the difference between its content and the respective control was calculated.

Afterwards, for each type of extraction solution the mean of the corresponding two modalities, considering the three independent replicates, was calculated.

As previously stated cations release from bentonites depends on protein adsorption extent. Adsorption of proteins with distinct molecular weight by bentonite in a white wine model solution was studied by Achaerandio *et al.* (2001). These authors proposed that protein volume is the factor that most affects the adsorption process. The molecular weight of the large majority of wine proteins ranges from 20 to 30 kDa, while Bovine Serum Albumin (BSA), the standard protein also used in our study, has a considerable higher molecular weight, of approximately 66 kDa. Concerning to isoelectric points (pI), wine proteins and BSA show similar values, from 4.1 to 5.8 and of 4.7, respectively (Hsu and Heatherbell, 1987; Achaerandio *et al.*, 2001, Monteiro, 2001).

The protein nature of test solutions seemed to have a decisive role in some element changes, such as Mg, K, Zn and Pb. Mg and Zn and probably Pb are exchangeable cations of bentonite, suggesting that their release depend on the extent of cation adsorption. On the other hand, as shown on table III, the control solution of test solution B contained a high concentration of Zn, probably due to wine protein. K is a protein competitor, so its final content was strongly related to protein adsorption. In fact, higher K depletions were observed in test solution C modalities, probably because protein adsorption

TABLE II

Changes in the concentrations of several mineral elements in wine, test solution B and test solution C, caused by bentonite addition (Trial 2). The results are expressed in µg/L, except for Na, Mg, K, Ca and Fe (mg/L).

Variação da concentração de elementos minerais no vinho, na solução teste B e na solução teste C, em consequência da adição de bentonite (Ensaio 2). Os resultados são expressos em µg/L, excepto para o Na, Mg, K, Ca and Fe (mg/L).

		Wine	Test sol. B	Test sol. C	W-B1	B-B1	C-B1	W-B9	B-B9	C-B9
	Extraction solution effect	mean (n=6)	mean (n=6)	mean (n=6)	(n=3)	(n=3)	(n=3)	(n=3)	(n=3)	(n=3)
<i>Li</i>	**	76.17c	50.25b	47.79a	19.57b	20.68b	11.69a	132.77e	79.83c	83.88d
<i>Be</i>	**	33.04b	7.97a	8.69a	35.71e	5.48a	4.72a	30.38d	10.45b	12.67e
<i>B</i>	**	(-)193.06e	1.90a	12.59b	(-)388.93b	4.93a	8.26a	2.82a	(-)1.14a	16.92a
<i>Na</i>	**	276b	215a	313c	283d	220a	234b	269c	210a	391e
<i>Mg</i>	**	13.95b	7.38a	13.60b	23.77c	14.3b	23.87c	4.13a	0.48a	3.33a
<i>Al</i>	*	5169a	6809b	5283a	6060b	10465c	7709b	4280a	3152a	2856a
<i>K</i>	**	(-)75b	(-)4a	(-)258c	(-)121c	16a	(-)247d	(-)28b	(-)24b	(-)269d
<i>Ca</i>	**	98c	18a	28b	115e	(-)6a	12b	80d	42c	44c
<i>Sc</i>	n.s.	1.44	1.57	1.73	1.24a	1.92b	1.66a,b	1.63a,b	1.21a	1.81b
<i>V</i>	*	21.79b	19.69a,b	15.70a	39.71c	37.50c	28.34b	3.86a	1.89a	3.07a
<i>Mn</i>	n.s.	851.58	960.30	926.85	1424.60c	1521.24c	1371.54c	278.57a	399.26a,b	482.17b
<i>Fe</i>	**	3.94a	6.08b	6.20b	0.39a	1.47b	1.61b	7.49c	10.69d	10.79d
<i>Co</i>	n.s.	7.35	8.28	8.30	6.14a	7.01a	6.11a	8.57b	9.55b,c	10.48c
<i>Ni</i>	**	15.33a	17.01b	18.77c	(-)1.49b	4.59c	0.97a	32.15d	32.94d	33.06d
<i>Cu</i>	**	(-)96.38b	-52.72a	-54.13a	(-)85.02d	(-)68.50b	(-)72.68c	(-)107.74e	(-)36.94a	(-)35.58a
<i>Zn</i>	**	(-)176.75b	(-)8.98a	243.75c	(-)592.82e	(-)138.51b	17.99a	239.31c	120.56a	469.51d
<i>Ga</i>	**	1.97b	1.31a	1.34a	2.22e	1.22a,b	1.12a	1.74d	1.40b,c	1.57c,d
<i>As</i>	**	160.21b	51.38a	49.27a	10.02a	2.07a	1.94a	310.40c	100.69b	96.59b
<i>Rb</i>	**	(-)673.53b	18.04a	(-)16.37a	(-)784.62d	17.08a,b	11.79a	(-)562.44c	19.00b	20.94b
<i>Sr</i>	n.s.	1647.00	1633.73	1705.88	819.97a	778.03a	700.78a	2474.03b	2489.42b	2710.97c
<i>Y</i>	**	18.74a	33.77b	38.29c	4.41a	5.36a	6.90a	33.07b	62.18c	69.68d
<i>Zr</i>	**	660.89b	540.60a	523.60a	673.43d	495.69b	418.19a	648.34d	585.50c	629.01c,d
<i>Nb</i>	**	49.70c	19.04a	20.77b	28.89b	5.54a	4.82a	70.52e	32.55c	36.71d
<i>Mo</i>	**	27.45a	33.24b	36.12c	0.70a	1.32a	1.04a	54.20b	65.16c	71.20d
<i>Cd</i>	*	0.89a	1.03a,b	1.12b	0.78a	0.83a	0.84a	1.00a,b	1.22b,c	1.40e
<i>In</i>	**	0.330b	0.215a	0.22a	0.042b	0.001a	(-)0.002a	0.618d	0.429c	0.444c
<i>Sn</i>	**	10.80a	15.80b	17.12c	(-)0.18a	0.89b	0.75a,b	21.78c	30.70d	33.49e
<i>Sb</i>	*	10.20b	8.99a	9.90b	1.6a	1.55a	1.49a	18.78c	16.43b	18.32c
<i>Ba</i>	**	30.06a	599.90b	654.18b	41.52a	1062.50c	1136.90c	18.60a	137.31b	171.47b
<i>W</i>	n.s.	0.628	0.639	0.650	1.157b	1.185b	1.164b	0.099a	0.092a	0.136a
<i>Tl</i>	**	0.81b	0.50a	0.50a	0.44b	(-)0.05a	(-)0.05a	1.19c	1.05c	1.03c
<i>Pb</i>	**	29.18a	91.43b	170.50c	5.52a	10.97a	22.56b	52.85c	171.89d	286.75e
<i>Bi</i>	n.s.	4.22	4.02	4.05	(-)0.93b	(-)0.16a	(-)0.06a	9.37d	8.21c	8.16c
<i>U</i>	**	57.18b	54.28b	47.41a	(-)0.47a	(-)0.35a	2.30a	114.84d	108.91c	92.52b

*Averages followed by the same letter are not significantly different at $\alpha = 0.01^{**}$ or $\alpha = 0.05^*$; ns = without significant difference*

occurred at less extent due to BSA larger volume.

Significant different variations (95% confidence level) were observed between extraction solutions for all elements with exception of Sc, Mn, Co, W and Bi.

For several elements, such as Na, Mg (test solution C) V, Ni, Ga (B9), Zr (in

TABLE III

Mineral composition^a of wine and test solutions B and C control samples. The results are expressed in µg/L, except for Na, Mg, K, Ca and Fe (mg/L)

Composição mineral^a do vinho, solução teste B e solução teste C – amostras testemunha. Os resultados são expressos em µg/L, excepto para o Na, Mg, K, Ca e Fe (mg/L)

	Wine control (n = 3)	Test sol. B control (n = 3)	Test sol. C control (n = 3)		Wine control (n = 3)	Test sol. B control (n = 3)	Test sol. C control (n = 3)
<i>Li</i>	31.1 (0.1)	ND ^b	ND ^b	<i>As</i>	5.4 (0.3)	5.0 (0.3)	4.2 (0.9)
<i>Be</i>	0.21 (0.07)	ND ^b	ND ^b	<i>Rb</i>	1102 (21)	19 (1)	18.5 (0.6)
<i>B</i>	2921 (64)	37 (6)	9 (1)	<i>Sr</i>	207 (6)	114.1 (0.5)	112 (2)
<i>Na</i>	103.8 (0.7)	310 (3)	320 (3)	<i>Y</i>	0.287 (0.007)	0.41 (0.03)	0.45 (0.02)
<i>Mg</i>	78.6 (0.2)	64 (1)	62.6 (0.6)	<i>Zr</i>	3.8 (0.3)	4.1 (0.5)	5.2 (0.9)
<i>Al</i>	459 (30)	211 (35)	144 (8)	<i>Nb</i>	0.04 (0.01)	0.09 (0.02)	0.03 (0.01)
<i>K</i>	539 (15)	671 (28)	881 (3)	<i>Mo</i>	2.31 (0.09)	0.5 (0.2)	0.43 (0.08)
<i>Ca</i>	112 (2)	115.2 (0.4)	115.6 (0.7)	<i>Cd</i>	0.44 (0.03)	0.32 (0.09)	0.15 (0.04)
<i>Sc</i>	2.4 (0.2)	0.44 (0.04)	0.19 (0.04)	<i>In</i>	0.02 (0.01)	ND ^b	ND ^b
<i>V</i>	9.1 (0.3)	8.1 (0.4)	7.8 (0.4)	<i>Sn</i>	2.2 (0.2)	0.66 (0.01)	0.66 (0.02)
<i>Mn</i>	769 (16)	17.3 (0.6)	16.0 (0.8)	<i>Sb</i>	2.7 (0.1)	0.5 (0.3)	ND
<i>Fe</i>	1.93 (0.02)	0.05 (0.01)	0.04 (0.01)	<i>Ba</i>	35 (1)	16.1 (0.9)	12.9 (0.1)
<i>Co</i>	3.4 (0.2)	0.26 (0.02)	0.24 (0.03)	<i>W</i>	0.26 (0.01)	0.06 (0.01)	ND
<i>Ni</i>	40 (1)	5.2 (0.6)	4.2 (0.2)	<i>Tl</i>	0.13 (0.01)	1.5 (0.1)	1.56 (0.03)
<i>Cu</i>	121 (5)	79 (2)	78 (1)	<i>Pb</i>	23 (1)	0.9 (0.2)	0.67 (0.06)
<i>Zn</i>	1552 (46)	468 (20)	34 (1)	<i>Bi</i>	1.2 (0.9)	ND ^b	ND ^b
<i>Ga</i>	1.7 (0.2)	0.05 (0.04)	0.035 (0.005)	<i>U</i>	0.47 (0.05)	0.4 (0.2)	0.29 (0.03)

^aConcentration values correspond to the mean of three independent replicates and corresponding standard deviation.

^bND – not detected

special B9), Cd, In, Sb, Tl and U the similarity between wine and test solutions variations was satisfactory, in spite of those variations being statistically different.

In regard to Fe, Y and Sn contents, both test solutions showed similar variations with bentonite addition, higher than wine variations but nevertheless indicative. It should be pointed out that both extraction solutions showed similar variations in the contents of Fe, probably limited by its solubility.

Observing the elements which concentration decreased in wine with bentonite addition, B, K, Cu, Zn and Rb, the distinct concentrations of control samples

could explain, in part, the results. As a general trend, the solution presenting the initial higher concentration of the element showed the highest depletion with bentonite addition. An exception of this was observed for B in samples treated with B9. Wine control, with the highest concentration of Cu, Zn and Rb, suffered the major depletions. It could be concluded that in future studies the test solution should be prepared in order to present similar amounts of these elements to wine, in particular K amounts, since this element plays an important role in cationic exchange phenomena competing with proteins to bentonites complex exchange. Though the mineral profile of wines is widely variable it is difficult to establish a standard composition.

For Ca element the results were very unsatisfactory, with higher enrichment in wine than in test solutions. A probable explanation could be its reaction with tartaric acid originating calcium tartrate that could precipitate. This explanation is also valid for K element.

Concerning Li element and bentonite B1, its content variation in test solution B was similar to wine enrichment. Both test solutions, either with B1 or B9, verified low Be enrichments by comparison to wine.

The results concerning As and Nb elements were unsatisfactory, with wine presenting the highest enrichments, in spite of the similar values shown by both test solutions. On the contrary, Ba enrichments were much higher in test solutions than in wine.

Additionally, linear least-squares adjustment of the results (considering only the elements that have increased with bentonite treatment) obtained using wine and test solutions, were performed in order to evaluate their correlation (95% confidence level). For B1, the correlation value between wine and test solution B results was higher ($r=0.840$) than wine and test solution C correlation ($r = 0.791$). Similar results were obtained for B9, with correlation values of $r = 0.903$ and $r = 0.860$ between wine and test solutions B and C, respectively. Thus, in general it seems that test solution B, containing wine protein, was a better simulator of wine performance.

Finally, the previous results suggested that neither test solution B nor test solution C simulated wine performance in a total satisfactory way, and additional studies should be done in view to improve solution performance.

CONCLUSIONS

When using extraction solutions containing wine protein or BSA and some major mineral elements, similar average variations in the contents of Sc, Mn, Co, W and Bi in wine and test solutions were observed. In spite of significantly different, for Na, Mg, V, Ni, Ga, Zr, Cd, In, Sb, Tl and U similar results were

observed. The protein nature of test solutions seemed to have an important role in some element variations, perhaps due to wine protein and BSA different volumes that could affect the adsorption process. In fact, it was possible to observe that, globally, the test solution containing wine protein was a better simulator of wine performance. In spite of that, neither test solution B nor test solution C simulated wine performance in a total satisfactory way. Thus, further work should be carried out on test solution composition in view to improve its performance. Taking into account the active role played by the major mineral elements of wine in cationic exchange phenomena, it seems important that the test solution nearly reflects the wine composition. As the mineral profile of wines is widely variable it is difficult to settle a standard composition. Bearing in mind the different strength of acids could be advantageous to simulate the wine acidic composition.

It should be emphasized that the tartaric acid solution indicated by OIV was not able to simulate wine performance on bentonite extraction essays, leading to significant different average variations in the contents of thirty-four elements. Thus, as a principal conclusion, this work evidences the unsuitability of the test solution recommended by the OIV for bentonite quality control purposes, concerning its potential effect on wine mineral contamination.

At last, it was possible to verify that some bentonites were in disagreement with OIV resolutions, as maximum extractable contents of Na, Al, Fe and As were exceeded. These observations are of major importance as to our knowledge, the quality control of bentonites by control laboratories or by winemakers is not a regular practise in Portugal.

RESUMO

Cedência de elementos contaminantes de bentonites ao vinho: contributo para a optimização de uma solução teste

Tendo por objectivo principal avaliar e comparar a capacidade de extracção mineral da solução teste indicada pelo OIV relativamente à capacidade de extracção de um vinho, realizou-se um ensaio experimental envolvendo seis bentonites. A composição mineral das soluções de extracção foi determinada por FAAS, ETAAS e ICP-MS, observando-se diferenças significativas entre as variações dos teores de 34 elementos no vinho e na solução de ácido tartárico estabelecida pelo OIV, verificadas em consequência da adição de bentonite. A solução teste indicada pelo OIV parece assim inadequada para fins de controlo da qualidade das bentonites, uma vez que não reflecte o enriquecimento mineral observado no vinho. Algumas bentonites apresentaram teores extraíveis de Na, Al, Fe e As superiores aos estabelecidos pelo OIV. Um segundo ensaio de extracção foi realizado com duas bentonites, vinho e duas soluções teste contendo elementos minerais maioritários no vinho (K, Ca, Na e Mg), proteína de vinho (BSA, em alternativa). Observaram-se diferenças significativas entre as variações de teores no vinho e nas soluções teste para todos os elementos, com excepção do Sc, Mn, Co, W and Bi. Para os elementos Na, Mg (solução teste com BSA), V, Ni, Ga, Zr, Cd, In, Sb, Tl e U, os resultados obtidos foram considerados aceitáveis. A natureza da proteína utilizada nas soluções teste parece importante nas alterações de concentração verificadas por alguns elementos. De um modo geral a solução com proteína de vinho conduziu a resultados mais favoráveis. Contudo, os resultados não são

ainda completamente satisfatórios pelo que se considera ser necessário prosseguir os estudos com vista à obtenção de uma solução teste capaz de simular a capacidade de extração do vinho.

RÉSUMÉ

Transmission de quelques éléments minéraux des bentonites aux vins : une contribution pour l'optimisation d'une solution teste

On a effectué un essai expérimental concernant six bentonites, en envisageant l'objectif d'évaluer et comparer la capacité d'extraction minérale de la solution teste proposée par l'OIV, relativement à la capacité d'extraction d'un vin. La composition minérale des solutions d'extraction a été déterminée par FAAS, ETAAS et ICP-MS. On a observé des différences significatives entre les variations des teneurs de 34 éléments dans le vin et dans la solution de l'acide tartrique établie par l'OIV, vérifiées en conséquence de l'addition de bentonite. La solution teste proposée par l'OIV se montre ainsi impropre pour les fins de contrôle de la qualité des bentonites, une fois qu'elle n'est pas concernée à l'enrichissement minéral observé dans le vin. Quelques bentonites ont présenté des teneurs extractibles de Na, Al, Fe et As plus grandes aux établies par l'OIV. Dans un deuxième essai d'extraction, on a employé deux bentonites, un vin et deux solutions teste en contenant les éléments minéraux majoritaires dans les vins (K, Ca, Na et Mg) et une protéine de vin (BSA, en alternative). On a observé des différences significatives entre les variations des teneurs dans le vin et dans les solutions teste pour tous les éléments, sauf pour Sc, Mn, Co, W et Bi. Pour les éléments Na, Mg (solution teste avec BSA), V, Ni, Ga, Zr, Cd, In, Sb, Tl et U ; les résultats obtenus sont acceptables. La nature de la protéine employée dans les solutions teste paraît importante pour les modifications de concentration vérifiées pour quelques éléments. D'une façon générale, la solution avec la protéine de vin a donné des résultats plus favorables. Cependant, les résultats ne sont pas encore complètement satisfaisants. Ainsi, il y a besoin de continuer ces études en envisageant l'obtention d'une solution teste capable de simuler la capacité d'extraction du vin.

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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.
- Blade W.R., Boulton R., 1988. Adsorption of protein by bentonite in a model wine solution. *American Journal of Enology and Viticulture*, **39** (3), 193-199.
- Catarino S., Curvelo-Garcia A.S., Bruno de Sousa R., 2002. Determination of aluminum in wine by graphite furnace AAS: Validation of Analytical Method. *Atomic Spectroscopy*, **23** (6), 196-200.

- Catarino S., Pinto D., Curvelo-García A.S., 2003. Validação e comparação de métodos de análise em espectrofotometria de absorção atômica com chama para doseamento de cobre e ferro em vinhos e aguardentes. *Ciência e Técnica Vitivinícola*, **18** (2), 65-76.
- Catarino S., Soares J., Curvelo-García A.S., Bruno de Sousa R., 2004. Implicações da utilização de bentonites sobre a fracção mineral de vinhos: potássio, sódio, cálcio, alumínio e chumbo. Efeito do pH. *Ciência e Técnica Vitivinícola*, **19** (1), 29-45.
- Catarino S., A.S. Curvelo-García, R. Bruno de Sousa. Measurements of contaminant elements of wines by inductively coupled plasma-mass spectrometry: a comparison of two calibration approaches. *Talanta* (in press).
- Curvelo-García A.S., Catarino S., 1998. Os metais contaminantes dos vinhos. Origens da sua presença, teores, influência dos factores tecnológicos e definição de limites. *Ciência e Técnica Vitivinícola*, **13** (1-2), 49-70.
- Enkelmann R., 1988. Migration of heavy metals from bentonites to wine.1. Note: Bentonites. *Deutsche Lebensmittel-Rundschau*, **84**, 243-247.
- Gómez M.M.C., Brandt R., Jakubowski N., Anderson J.T., 2004. Changes of the metal composition in german white wines through the winemaking process. A study of 63 elements by inductively coupled plasma-mass spectrometry. *Journal of Food Agriculture and Food Chemistry*, **52**, 2953-2961.
- Gössinger M., Schödl H., Steidl R., Meier W., 1997. Comparison of commercial must and wine bentonites. *Mitteilungen Klosterneuburg, Rebe und Wein, Obstbau und Fruchteverwertung Austria*, **47** (1-2), 1-7.
- Hsu J.C., Heatherbell D.A., 1987. Isolation and characterization of soluble proteins in grapes, grape juice and wine. *American Journal of Enology and Viticulture*, **38**, 6-10.
- Machado-nunes M., Laureano O., Ricardo-da-Silva J.M., 1998. Influência do tipo de cola (caseína e bentonite) e da metodologia de aplicação nas características físico-químicas e sensoriais do vinho branco. *Ciência e Técnica Vitivinícola*, **13** (1-2), 7-28.
- Maujean A., 1993. Propriétés physico-chimiques des bentonites: applications oenologiques. *Revue Française d'Oenologie*, **143**, 43-53.
- Mesquita P., Piçarra-Pereira M.A., Monteiro S., Loureiro V., Teixeira A., Ferreira R.B., 2001. Effect of wine composition on protein stability. *American Journal of Enology and Viticulture*, **52** (4), 324-330.
- Monteiro S.A.V.S., 2001. *Análise molecular das proteínas responsáveis pela turvação dos vinhos*. PhD Thesis. Instituto Superior de Agronomia, Universidade Técnica de Lisboa, Lisboa.
- Nicolini G., Larcher R., Pangrazzi P., Bontempo L., 2004. Changes in the contents of micro- and trace-elements in wine due to winemaking treatments. *Vitis*, **43** (1), 41-45.
- OIV, 2003. Bentonites. Resolution OENO 11/2003. Office International de la Vigne et du Vin.
- Postel W., Meier B., Markert R., 1986. Influence on processing aids on the content of mineral compounds and trace elements in wine. I. Bentonite. *Mitteilungen Klosterneuburg, Rebe und Wein, Obstbau und Fruchteverwertung Austria*, **36** (1), 20-27.
- Wurzinger A., Netzer M., Heili K., Bandion F., 1994. Migration of components of bentonites during the fermentation of must. *Mitteilungen Klosterneuburg, Rebe und Wein, Obstbau und Fruchteverwertung Austria*, **44**, 218-221.