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Ochratoxin A removal from red wine by several oenological fining agents: bentonite, egg albumin, allergen-free adsorbents, chitin and chitosan

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The ability of several oenological fining agents to remove ochratoxin A (OTA) from red wine was studied. The adsorbents tested were activated sodium bentonite, egg albumin, allergen-free adsorbents (complex PVPP, plant protein and amorphous silica (complex) and high molecular weight gelatine), and the non-toxic biodegradable polymers (chitin and chitosan). Several dosages within the oenological use range were tested and the wine pH, colour parameters and polyphenol concentration impact associated with each fining agent were studied. Generally, OTA removal achieved in all treatments was higher when the adsorbent dosage increased, but the impact on wine quality also was higher. Chitin at 50 g hl⁻¹ removed 18% the OTA without affecting significantly the wine-quality parameters. At the highest dosage tested the gelatine and complex treatments achieved greater OTA removal (up to 39–40%) compared with bentonite, egg albumin and chitin. Moreover, the gelatine and the complex had a lower impact on colour parameters and polyphenol concentration compared with chitosan, whilst OTA was reduced to around 40%. Chitosan achieved the greatest OTA removal (67%), but it strongly affected the wine-quality parameters. Otherwise, bentonite showed a relative efficiency to remove OTA, but the CI value decreased considerably. The egg albumin treatment only removed OTA up to 16% and moreover affected strongly the CI value and CIELab parameters. The results of this survey showed that the non-toxic chitin adsorbent and the allergen-free adsorbents tested could be considered as alternative fining agents to reduce OTA in red wine.

Keywords: chromatography – HPLC; mycotoxins – ochratoxin A; wine

Introduction

Ochratoxin A (OTA) is a widely distributed mycotoxin produced as a secondary metabolite by certain *Penicillium* and *Aspergillus* fungal species. OTA possesses nephrotoxic, teratogenic, immunotoxic and possibly neurotoxic properties. It has been classified in category 2B as ‘possibly carcinogenic to humans’ by the International Agency for Research on Cancer (IARC) (1993). Exposure to OTA through the diet has been associated with Balkan endemic nephropathy and the development of urinary tract tumours in humans (European Food Safety Authority (EFSA) 2006).

Wine is estimated to be the second source of OTA in the diet after cereals in Europe; it represents up to 10% of total OTA intake (European Commission 2002). OTA was first detected in wine by Zimmerli and Dick (1996). Since then, surveys conducted in 40 different countries have shown the worldwide presence of OTA in wine (Filali et al. 2001; Pietri et al. 2001; European Commission 2002, 2012; Shephard et al. 2003; Rosa et al. 2004; Berente et al. 2005; Quintela et al. 2011, 2012). These surveys showed that red wines generally contain higher OTA amounts than white and

rosé wines. This fact evidences a clear relationship between the maceration of the must with grape skins and OTA solubilisation in the must. The average contamination in red wine ranged between 0.026 and 0.912 µg l⁻¹. The highest value reported was in Italy at 15.6 µg l⁻¹.

A maximum limit for OTA in wine of 2 µg l⁻¹ was regulated by the European Commission in Regulation (EC) No. 123/2005 in order to protect consumers (European Commission 2005), and a recommendation to lower OTA levels to the lowest technologically feasible level was also made.

There are several chemical, microbiological or physical methods with the purpose of removing OTA from contaminated wines (Amézqueta et al. 2009). Focusing on chemical methods, a variety of fining agents including potassium caseinate, activated carbon, bentonite, egg albumin, gelatine and polyvinylpyrrolidone (PVPP) have been previously tested in wine. These products have variable levels of efficiency for reducing the contents of OTA. Several authors found that activated carbon removed the highest amount of OTA from red wine, but a decrease

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of coloured polyphenols (Castellari et al. 2001) and key sensory odorants of wine was also observed (Gambuti et al. 2005). Egg albumin, gelatine, potassium caseinate and silica gel at a dosage of 100 g hl^{-1} showed a good affinity for OTA, removing this mycotoxin by between 30% and 42%, whereas bentonite and PVPP showed poor OTA reduction (6–8%) (Castellari et al. 2001).

After various clarification product treatments to remove OTA, sensorial repercussions on wine quality as well as allergenic reactions for some sensitive wine consumers cannot be totally excluded. In fact, the use of some adsorbents is limited by European Commission Directive 2007/68/CE (European Commission 2007) as it indicates that all wines produced and/or marketed in the European Union must indicate on the label if they have been treated with co-adjuncts derived from eggs, fish and milk. European Commission Regulation (EC) No. 415/2009 (European Commission 2009) fixed the end of the transition period foreseen for wine in Directive 2007/68/EC at 31 December 2010.

Recently, new adsorbents formulations have been created without allergens to be used as alternatives to casein and potassium caseinate, such as a complex based on PVPP, plant protein and amorphous silica. However, the high molecular weight gelatine in sheets could be an alternative to egg albumin.

Other alternative adsorbents could be the non-toxic biodegradable polymers such as chitin and chitosan. Chitin is a polysaccharide composed of several N-acetyl-D-glucosamine units interconnected by $\beta \rightarrow (1,4)$ -type linkages. It is the main structural component of molluscs, insects, crustaceans, fungi, algae, and marine invertebrates such as crabs and shrimps. It is obtained by hydrolysis of a chitin-rich extract. Chitosan is composed of glucosamine sugar units (deacetylated units) and N-acetyl-D-glucosamine units (acetylated units) interconnected by $\beta \rightarrow (1,4)$ -type linkages. Lately, resolution OIV/OENO 338A/2009 (International Organisation of Vine and Wine (OIV) 2009) introduced the treatment using chitosan in wine to the International Code of Oenological Practices and prescribed that the maximum chitosan dosage used to reduce OTA must not exceed 500 g hl^{-1} .

The aim of the present study was to evaluate the OTA removal with the treatment of red wine with bentonite, egg albumin, alternative allergen-free adsorbents to casein, potassium caseinate and egg albumin and non-toxic biodegradable polymers such as chitin and chitosan. Several dosages within the oenological use range have been tested and the wine pH, colour parameters and polyphenol concentration impact associated with each fining agent have been studied. Thereby, this study contributes to toxicity reduction and the improvement of safety in wines.

Materials and methods

Fining agents and OTA removal assays

Six fining agents were tested at dosages commonly used in current winemaking practice. Activated sodium bentonite (bentonite) was tested at 10, 20, 40 and 60 g hl^{-1} ; the complex based on PVPP, plant protein and amorphous silica (complex) was tested at 10, 23, 36 and 50 g hl^{-1} . Egg albumin and high molecular weight gelatine in sheets (gelatine) were tested at 10, 12, 14 and 16 g hl^{-1} . Finally, chitin and chitosan were tested at 10, 50, 200 and 500 g hl^{-1} . Removal assays were performed as follows. Red wine (25 ml) was fortified with OTA at a concentration of $2.5 \mu\text{g l}^{-1}$ and a fining agent was added according to its technical specifications. The solution was mixed for 30 s and after 2 h at room temperature the supernatant was filtered through a $0.45 \mu\text{m}$ filter. OTA concentration, pH, colour intensity, tone, CIELab parameters, total polyphenol index, and total anthocyanins content were analysed in the initial red wine and after the removal assay. Each fining agent and clarification experiment was carried out in triplicate.

Reagents

The OTA standard solution was purchased from Sigma-Aldrich (Seelze, Germany). The OTA concentration of the standard was determined accurately by UV spectroscopy at 333 nm and was stored at -20°C when not in use. Acetonitrile and glacial acetic acid were of HPLC grade and purchased from Panreac (Barcelona, Spain). The activated sodium bentonite and egg albumin were purchased from Laffort (Erretería, Spain), the high molecular weight gelatine in sheets, and the complex based on PVPP, plant protein and amorphous silica were purchased from Sepsa-Enartis (Trecate, Italy); chitin and chitosan were purchased from Sigma Aldrich (St. Louis, MO, USA).

OTA analysis: apparatus and chromatographic conditions

Chromatographic analysis was performed with HPLC equipment connected to a fluorescence spectrophotometer ($\lambda_{\text{ex}} = 333 \text{ nm}$ and $\lambda_{\text{em}} = 460 \text{ nm}$), both from Hewlett-Packard model 1100. The computer program used was an Agilent Chemstation for chromatographic analysis. The analytical column was a reversed-phase Synergi C_{12} ($150 \times 4.60 \text{ mm}$, $4 \mu\text{m}$ particles) (Phenomenex) preceded by guard cartridge AQ C_{18} ($4 \times 2 \text{ mm}$). Chromatographic separation was performed using an isocratic elution with water–acetonitrile–acetic acid (99:99:2, v/v/v) at a flow rate of 1 ml min^{-1} . A total of $100 \mu\text{l}$ of solution was injected into the chromatograph.

The calibration curve obtained by analysing OTA standard solutions at six concentrations levels (range = 0.327–7.029 $\mu\text{g l}^{-1}$) was linear ($r^2 = 0.9999$). Precision (within- and between-day) and accuracy were tested by duplicate analyses on a red wine sample containing OTA at a concentration of 0.391, 1.562 and 7.029 $\mu\text{g l}^{-1}$. The coefficient of variation was less than 5%, demonstrating good repeatability of the HPLC analysis. The analytical recovery for OTA, determined in duplicate on a red wine sample spiked at a level of 0.703 and 3.515 $\mu\text{g l}^{-1}$, was 100.61% and 100.63%, respectively. The limit of detection ($S/N = 3:1$) was 0.080 $\mu\text{g l}^{-1}$; the limit of quantitation ($S/N = 10:1$) was 0.327 $\mu\text{g l}^{-1}$.

The direct injection method was applied for the analysis of the red wine sample.

Other wine analyses

Phenolic compounds and colour were analysed with a UV-Vis spectrophotometer Lambda 35 (Perkin Elmer), and pH measurements were effected in a GLP21 (Crison). Colour intensity (CI), tone (T) and pH were analysed following the official methods prescribed by Commission Regulation No. 2676/90 (European Economic Community (EEC) 1990). CIELab parameters (L^* , a^* and b^*) were analysed according to the compendium of international methods of wine and must analysis by the OIV (2011). Total polyphenol index (TPI) is the absorption at 280 nm, by direct measurement of the diluted sample 1:100 (v/v). Total anthocyanin concentration was determined with the methodology described by Ribéreau-Gayon and Stonestreet (1965).

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) using the SPSS Statistics 17.0 software package program. A Tukey-honestly significant difference (Tukey-HSD) multiple range test was performed to determine significant differences between adsorbent type and adsorbent dosages on OTA removal and to determinate if significant differences occurred with different wine parameters.

Results and discussion

The chemical structure of OTA is (R)-N-[(5-chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1H-2-benzopyran-7-yl)-carbonyl]-L-phenylalanine. The carboxyl group of the phenylalanine moiety shows a pK_a value in the range of 4.2–4.4 (Ertan and İ. Mert 2010). Thus, OTA is partially dissociated at the pH of wine and may interact with a positively charged surface through the negative charge it carries. Moreover, the OTA phenol

moiety could be adsorbed onto a negatively charged surface by hydrogen bonding and/or charge transfer complexes.

The efficacy of OTA removal by adsorbent materials depends on the molecular size and physicochemical properties of OTA as well as the physical structure and properties of the adsorbent (Kurtbay et al. 2008).

The basic parameters describing wine quality have been also measured before and after each experiment to assess the relevant effect of each treatment.

Table 1 shows the effect of OTA removal treatments in wine OTA concentration, pH, colour parameters and polyphenol concentration. Bentonite, a claylike material of volcanic origin belonging to the sheet-structured montmorillonite group, removed OTA significantly between 22% and 33% with respect to the untreated wine. The adsorption of soluble cationic constituents by bentonites in the wine is primarily due to the cationic exchange properties of these clays. There were significant differences on the OTA removal between the 60 g hl^{-1} dosage with respect to the other dosages, and also between 10 and 40 g hl^{-1} . Bentonite showed a relative efficiency for OTA removal, but the wine resulted in a significantly smaller CI value (up to 12.5% reduction) and significantly higher CIELab parameters values at all the dosages tested. Likewise, the tone value increased significantly from the 20 g hl^{-1} dosage due to a further increase in the yellow component of the wine with respect to the red component. The results are in agreement with the results reported by Stanković et al. (2004), which showed a considerable decrease in the CI value and an increase in the colour tone of red wines treated with bentonite. Bentonite is known to affect the red wine colour directly by binding with positively charged anthocyanins (Stanković et al. 2004). The results from the current survey are in agreement with these authors, and showed that the total anthocyanins concentration decreased significantly from a 40 g hl^{-1} dosage.

The complex treatment in wine resulted in significant OTA removal in all dosages tested, achieving up to 40% removal. The mechanism of action of PVPP is hydrogen bond formation between carbonyl groups of the polyamide and the phenolic hydrogens. However, PVPP is like a protein-fining agent with an affinity for low molecular weight phenolics. This adsorbent significantly affected CI value, CIELab parameters, TPI and polyphenol concentration in wine at all the dosages tested with respect to the untreated wine. The CI value was reduced to 4.9%, while total anthocyanins and TPI were reduced to 8.5% and 15.7% respectively. Furthermore, the wine colour was lighter (higher L^*), with also higher a^* and b^* values.

Egg albumin and gelatine are proteins positively charged at the wine pH and they could interact with OTA through hydrogen bond formation.

Table 1. Effect of OTA removal treatments on wine OTA concentration, pH, colour parameters and polyphenol concentration (mean $\pm$ SD, n = 3).

Fining agent	Dosage (g hl ⁻¹)	OTA (μ g l ⁻¹)	pH	CI	T	CIELab parameters			TPI	Total anthocyanins (mg l ⁻¹)
						L*	a*	b*		
Untreated wine	-	2.478 $\pm$ 0.071	3.38 $\pm$ 0.07	8.660 $\pm$ 0.057	0.817 $\pm$ 0.003	16.175 $\pm$ 0.216	46.685 $\pm$ 0.270	27.442 $\pm$ 0.375	50 $\pm$ 1	211 $\pm$ 2
Activated sodium bentonite	10	1.936 $\pm$ 0.036 ^{a,d,e,l}	3.34 $\pm$ 0.01 ^l	8.216 $\pm$ 0.056 ^{f,h,j,k,l}	0.821 $\pm$ 0.001 ^{e,m}	17.385 $\pm$ 0.081 ^{f,m}	47.932 $\pm$ 0.010 ^{a,c,m}	29.346 $\pm$ 0.116 ^{f,m}	51 $\pm$ 1 ^{h,j}	211 $\pm$ 2 ^{d,e,h,j}
	20	1.867 $\pm$ 0.023 ^{a,e}	3.33 $\pm$ 0.01	7.987 $\pm$ 0.029 ^f	0.823 $\pm$ 0.001 ^{a,e}	18.041 $\pm$ 0.131 ^{a,b}	48.588 $\pm$ 0.166 ^{a,b}	30.346 $\pm$ 0.207 ^{a,b}	51 $\pm$ 0	210 $\pm$ 4 ^e
	40	1.782 $\pm$ 0.044 ^{a,b,e}	3.38 $\pm$ 0.01	7.806 $\pm$ 0.035 ^f	0.826 $\pm$ 0.002 ^{a,e}	18.198 $\pm$ 0.125 ^{a,b}	48.499 $\pm$ 0.140 ^a	30.483 $\pm$ 0.195 ^{a,b}	50 $\pm$ 1	205 $\pm$ 1 ^{a,b,e}
	60	1.656 $\pm$ 0.018 ^f	3.36 $\pm$ 0.06	7.577 $\pm$ 0.054 ^f	0.835 $\pm$ 0.002 ^f	18.459 $\pm$ 0.262 ^{a,b}	48.384 $\pm$ 0.336 ^a	30.718 $\pm$ 0.372 ^{a,b}	49 $\pm$ 0	195 $\pm$ 1 ^f
Complex PVPP, plant protein and amorphous silica	10	1.735 $\pm$ 0.025 ^{a,i,l}	3.32 $\pm$ 0.03 ^{k,l}	8.573 $\pm$ 0.034 ^{f,g,i,j}	0.816 $\pm$ 0.001 ^{d,e,g}	16.565 $\pm$ 0.122 ^{f,g,i,j}	47.123 $\pm$ 0.134 ^{f,m}	28.096 $\pm$ 0.200 ^{f,g,i,j}	44 $\pm$ 3 ^{a,m}	206 $\pm$ 13 ^{a,d,e,m}
	23	1.641 $\pm$ 0.041 ^a	3.32 $\pm$ 0.00	8.415 $\pm$ 0.021 ^{a,b,e}	0.813 $\pm$ 0.000	17.330 $\pm$ 0.110 ^{a,b,e}	47.936 $\pm$ 0.122 ^{a,b,e}	29.360 $\pm$ 0.183 ^{a,b,e}	44 $\pm$ 1 ^a	197 $\pm$ 6 ^{a,d,e}
	36	1.580 $\pm$ 0.031 ^a	3.35 $\pm$ 0.01	8.384 $\pm$ 0.046 ^{a,b,e}	0.811 $\pm$ 0.001 ^{a,b}	17.344 $\pm$ 0.112 ^{a,b,e}	47.955 $\pm$ 0.102 ^{a,b,e}	29.393 $\pm$ 0.179 ^{a,b,e}	44 $\pm$ 1 ^a	195 $\pm$ 1 ^{a,b,e}
	50	1.493 $\pm$ 0.050 ^a	3.37 $\pm$ 0.02	8.233 $\pm$ 0.014 ^f	0.809 $\pm$ 0.001 ^{a,b}	17.984 $\pm$ 0.075 ^f	48.588 $\pm$ 0.091 ^f	30.443 $\pm$ 0.123 ^f	42 $\pm$ 3 ^a	193 $\pm$ 9 ^{a,b,c}
	10	2.126 $\pm$ 0.091 ^{a,h,l}	3.32 $\pm$ 0.02 ^{k,l}	8.118 $\pm$ 0.062 ^{a,e,h,j,k,l}	0.814 $\pm$ 0.003 ^{e,g}	17.823 $\pm$ 0.182 ^{a,e,m}	48.314 $\pm$ 0.186 ^{a,e,m}	29.933 $\pm$ 0.260 ^{a,e,m}	50 $\pm$ 1 ^{h,j}	209 $\pm$ 1 ^{h,j}
Egg albumin	12	2.124 $\pm$ 0.105 ^{a,j}	3.36 $\pm$ 0.01	8.057 $\pm$ 0.062 ^{a,j}	0.811 $\pm$ 0.001 ^a	18.038 $\pm$ 0.187 ^{a,j}	48.558 $\pm$ 0.174 ^{a,j}	30.249 $\pm$ 0.269 ^{a,j}	49 $\pm$ 0 ^j	211 $\pm$ 4 ^j
	14	2.078 $\pm$ 0.077 ^{a,j}	3.33 $\pm$ 0.01	8.015 $\pm$ 0.025 ^{a,j}	0.810 $\pm$ 0.001 ^{a,j}	18.178 $\pm$ 0.063 ^{a,j}	48.690 $\pm$ 0.070 ^{a,j}	30.453 $\pm$ 0.088 ^{a,j}	49 $\pm$ 1 ^j	210 $\pm$ 1 ^j
	16	2.071 $\pm$ 0.036 ^{a,j}	3.30 $\pm$ 0.01	7.968 $\pm$ 0.034 ^{a,b,j}	0.808 $\pm$ 0.002 ^{a,b,j}	18.313 $\pm$ 0.146 ^{a,b,j}	48.816 $\pm$ 0.198 ^{a,b,j}	30.635 $\pm$ 0.230 ^{a,b,j}	50 $\pm$ 0 ^j	210 $\pm$ 2 ^j
	10	2.092 $\pm$ 0.126 ^{a,d,e,l}	3.34 $\pm$ 0.03 ^l	7.773 $\pm$ 0.021 ^{a,m}	0.816 $\pm$ 0.002 ^g	19.147 $\pm$ 0.052 ^{a,m}	49.645 $\pm$ 0.034 ^{a,d,m}	31.963 $\pm$ 0.065 ^{a,m}	48 $\pm$ 1 ^{a,d,m}	202 $\pm$ 1 ^{a,m}
Gelatin	12	1.755 $\pm$ 0.096 ^{a,j}	3.29 $\pm$ 0.00	7.634 $\pm$ 0.179 ^{a,i}	0.816 $\pm$ 0.004	19.542 $\pm$ 0.504 ^{a,i}	49.908 $\pm$ 0.368 ^{a,i}	32.500 $\pm$ 0.685 ^{a,i}	47 $\pm$ 0 ^{a,i}	202 $\pm$ 4 ^{a,i}
	14	1.543 $\pm$ 0.133 ^{a,b,i}	3.36 $\pm$ 0.03	7.573 $\pm$ 0.095 ^{a,i}	0.814 $\pm$ 0.001 ⁱ	19.785 $\pm$ 0.157 ^{a,i}	50.144 $\pm$ 0.017 ^{a,b,i}	31.834 $\pm$ 0.153 ^{a,i}	46 $\pm$ 0 ^{a,b,i}	205 $\pm$ 1 ^{a,i}
	16	1.503 $\pm$ 0.099 ^{a,b,i}	3.38 $\pm$ 0.04	7.726 $\pm$ 0.056 ^{a,i}	0.813 $\pm$ 0.001 ⁱ	19.409 $\pm$ 0.140 ^{a,i}	49.909 $\pm$ 0.089 ^{a,i}	32.345 $\pm$ 0.186 ^{a,i}	47 $\pm$ 0 ^{a,i}	203 $\pm$ 1 ^{a,i}
	10	2.099 $\pm$ 0.149 ^{a,l}	3.42 $\pm$ 0.00 ^{e,h,i}	8.543 $\pm$ 0.063 ^{d,e,g,i,j,l}	0.815 $\pm$ 0.002 ^g	16.597 $\pm$ 0.089 ^{d,e,g,i,j,l}	47.152 $\pm$ 0.049 ^{e,m}	28.133 $\pm$ 0.130 ^{d,e,g,i,j,l}	49 $\pm$ 0 ^{d,e,h,j,l}	210 $\pm$ 7 ^{d,e,h,j}
Chitin	50	2.040 $\pm$ 0.173 ^a	3.43 $\pm$ 0.01 ^l	8.570 $\pm$ 0.041 ^{d,e,l}	0.814 $\pm$ 0.000 ^l	16.423 $\pm$ 0.059 ^{d,e,l}	46.887 $\pm$ 0.037 ^{d,e,l}	27.842 $\pm$ 0.088 ^{d,e,l}	50 $\pm$ 1 ^{d,e,l}	207 $\pm$ 7 ^{d,e}
	200	1.861 $\pm$ 0.119 ^{a,l}	3.49 $\pm$ 0.00 ^h	8.255 $\pm$ 0.039 ^{f,i}	0.815 $\pm$ 0.000 ^l	17.254 $\pm$ 0.190 ^{f,i}	47.711 $\pm$ 0.225 ^{a,e,l}	29.165 $\pm$ 0.311 ^{f,i}	47 $\pm$ 0 ^{f,i}	191 $\pm$ 0 ^{a,b,e}
	500	1.749 $\pm$ 0.130 ^{a,l}	3.51 $\pm$ 0.01 ^{a,b,l}	7.848 $\pm$ 0.006 ^{f,l}	0.816 $\pm$ 0.002 ^l	18.046 $\pm$ 0.370 ^{f,l}	48.241 $\pm$ 0.559 ^{a,b,c,l}	30.331 $\pm$ 0.639 ^f	46 $\pm$ 0 ^{f,l}	185 $\pm$ 4 ^{a,b,c,l}
	10	2.395 $\pm$ 0.074 ^{f,m}	3.44 $\pm$ 0.06 ^{e,g,h,i,j}	8.499 $\pm$ 0.003 ^{c,d,e,g,i,j,k}	0.814 $\pm$ 0.000 ^{e,g}	16.437 $\pm$ 0.008 ^{c,d,e,g,i,j,k}	46.839 $\pm$ 0.028 ^{c,d,e,m}	27.823 $\pm$ 0.015 ^{c,d,e,g,i,j,k}	50 $\pm$ 1 ^{c,d,e,h,j,k}	207 $\pm$ 1 ^{c,d,e,h,j}
Chitosan	50	2.117 $\pm$ 0.025 ^f	3.56 $\pm$ 0.05 ^{e,k}	7.678 $\pm$ 0.033 ^{f,k}	0.811 $\pm$ 0.000 ^{e,k}	18.988 $\pm$ 0.155 ^{f,k}	49.260 $\pm$ 0.174 ^{f,k}	31.564 $\pm$ 0.234 ^{f,k}	46 $\pm$ 1 ^{f,k}	202 $\pm$ 2 ^f
	200	1.417 $\pm$ 0.124 ^{f,k}	3.54 $\pm$ 0.10 ^e	5.900 $\pm$ 0.129 ^{f,k}	0.810 $\pm$ 0.006 ^{e,k}	24.986 $\pm$ 0.694 ^{f,k}	52.749 $\pm$ 0.804 ^{f,k}	36.673 $\pm$ 0.965 ^{f,k}	38 $\pm$ 1 ^{f,k}	185 $\pm$ 1 ^f
	500	0.826 $\pm$ 0.050 ^{f,k}	3.78 $\pm$ 0.07 ^{f,k}	4.331 $\pm$ 0.058 ^{f,k}	0.801 $\pm$ 0.003 ^{f,k}	31.740 $\pm$ 0.213 ^{f,k}	50.642 $\pm$ 0.269 ^{f,k}	29.596 $\pm$ 0.370 ^f	31 $\pm$ 1 ^{f,k}	160 $\pm$ 1 ^{f,k}

Notes: Significant different means ($p < 0.05$) with: ^auntreated wine, ^bfirst fining agent dosage, ^csecond fining agent dosage, ^dthird fining agent dosage, ^efourth fining agent dosage, ^fall fining agent dosages (including untreated wine), ^gactivated sodium bentonite, ^hcomplex PVPP, plant protein and amorphous silica, ⁱegg albumin, ^jgelatine, ^kchitin, ^lchitosan, and ^mall fining agents.

OTA removal at all the dosage levels of egg albumin and gelatine was significant compared with the untreated wine. Percentage OTA removal was higher with the addition of gelatine (16–39%) compared with egg albumin (14–16%), this difference being significant at 12 g hl⁻¹ dosage. Treatment with these adsorbents resulted in wine with a significant reduction in CI value, as well as a significant increase in CIELab parameters at all dosages tested. The effect on CI, CIELab parameters, TPI and total anthocyanins was significantly higher with gelatine treatment than with egg albumin treatment. Moreover, gelatine induced a significant decrease in TPI value and total anthocyanins at all dosages tested, while egg albumin did not significantly affect these parameters. However, egg albumin significantly affected tone values from a 12 g hl⁻¹ dosage. No significant differences were observed in the wine parameters analysed when taking into account the different dosages used in the treatment with egg albumin, except for the 16 g hl⁻¹ dosage which showed a significantly greater effect on the CI, T and CIELab parameters with regard to the 10 g hl⁻¹ dosage. In the case of gelatine treatment, the 14 and 16 g hl⁻¹ dosages achieved a significant greater OTA reduction with regard to the 10 g hl⁻¹ dosage, while the a* parameter and TPI value had a significantly greater effect with the 14 g hl⁻¹ dosage with respect to the 10 g hl⁻¹ dosage.

On the other hand, the treatment with chitin and chitosan reduced OTA significantly from the untreated wine at all dosages tested, achieving reductions up to 29% and 67% respectively. Chitosan achieved a significantly higher OTA removal at 200 and 500 g hl⁻¹ with respect to chitin. The amino groups of chitosan and chitin at the wine pH are protonated ($-\text{NH}_3^+$), and to maintain neutrality negative counter-ions were adsorbed, producing an ionic exchange in which electrostatic interactions were involved. Chitosan showed significant differences in OTA removal depending on the dosage applied; with chitin treatment this did not happen. However, chitin only significantly affected most of the wine-quality parameters analysed from the 200 g hl⁻¹ dosage, while it occurred for chitosan at a 50 g hl⁻¹ dosage.

There are not many studies on the use of chitin and chitosan in wine to reduce OTA levels. Our results are consistent with those obtained by Bornet and Teissedre (2008), where they found that there is a dosage dependence with the highest removal of OTA. Kurtbay et al. (2008) achieved a reduction of 59% by adding 1000 g hl⁻¹ of chitosan bead in red wine. Moreover, these authors found that at a dosage of 1000 g hl⁻¹ the total polyphenols are adsorbed by natural bentonite, nonylammonium bentonite and dodecylammonium bentonite and chitosan beads at 1%, 12%, 18% and 63% respectively. However, these authors found that the adsorption of total anthocyan

was highest, by the different bentonites removing 24–32% and by the chitosan bead removing 69%.

At a dosage of 10 g hl⁻¹ the OTA adsorption ability of the adsorbents added to wine was different. The highest OTA removal was achieved with the complex, decreasing by 30% the OTA concentration. The OTA removal percentage by adding this complex was significantly higher than could be achieved with chitosan and egg albumin. By adding bentonite, OTA removal reached 22%, with a significantly higher OTA reduction with regards to chitosan. With egg albumin, chitin and gelatine a similar OTA removal was obtained, comprising between 14% and 16%. Finally, the lowest removal of OTA (3%) was achieved with chitosan. Concerning the effect of the removal of treatments on wine-quality parameters analysed, there were differences depending on the adsorbent added. All adsorbents showed a small effect on pH. The highest significant CI reduction was achieved with gelatine, whereas with the complex of chitin and chitosan the lowest significant reduction was obtained compared to the bentonite, egg albumin and gelatine. Tone value increased by treatment with bentonite, whereas with the other treatments it decreased. This fact was due to a further increase in the yellow component of the wine in the bentonite treatment with respect to the red component. Bentonite, egg albumin and gelatine were those more affected in the CIELab parameters. Gelatine and complex showed a significant higher reduction in TPI and total anthocyanins with respect to the other adsorbents.

The results differ from other authors, probably due to differences between the types of fining agents, dosages, as well as the initial OTA concentration in untreated wine. In the current study, a higher OTA reduction is achieved with lower concentrations of the adsorbents tested, except in the case of egg albumin. Castellari et al. (2001) have variable efficiency levels for reducing the OTA level from red wine with 3.78 µg l⁻¹ OTA concentration at 100 g hl⁻¹ of different adsorbents. For example, with potassium-ferrocyanide bentonite, PVPP, egg albumin and gelatine, these authors removed up to 8%, 6%, 40% and 30% OTA, respectively. Otherwise, Gambuti et al. (2005), among different fining agents, at 50 g hl⁻¹ of high sodic activation bentonite and PVPP achieve 20% and 14% OTA removal in red wine respectively. Kurtbay et al. (2008) obtained, at 1000 g hl⁻¹, 0%, 14% and 22% OTA removal in red wine with natural bentonite, dodecylammonium bentonite and nonylammonium bentonite, respectively.

If we regarded levels of OTA contamination in wine different from those tested in the current study, chitin at 50 g hl⁻¹ dosage could be an efficient treatment for the remove of OTA (up to 18%) in slightly contaminated wine, without affecting significantly the quality of the wine. Treatment with the complex and

gelatine could be the best options for moderately contaminated wine, but it affects significantly the colour parameters and polyphenol concentration. On the other hand, chitosan could be used for highly contaminated wine, but the wine-quality parameters are strongly affected. However, it should also be considered that the OTA adsorption abilities of fining agents varied depending on the concentration of the toxin. Castellari et al. (2001) tested 18 adsorbents in two red wines with 3.78 and 1.50 $\mu\text{g l}^{-1}$ OTA concentrations. These authors found that several adsorbents, such as activated carbon, showed a greater OTA removal (between 2% and 21%) in the most contaminated wine. While for other adsorbents, such as gelatine, the opposite effect occurred, with the less contaminated wine showing a 1–6% higher OTA adsorption. This presumably depends on the different composition of each wine.

Wineries often carry out fining trials to ensure the fining objective is achieved by using the smallest possible amount of fining agent. Small-scale results obtained in this study allow winemakers more knowledge about how the fining agent tested here will alter the wine before scaling up.

Conclusions

The results reported here indicate that the fining agents used at the recommended dosage range for wine-making practice caused the OTA removal and thereby improved wine safety. Nevertheless, the quantitative effect on the OTA removal as well as on the wine-quality parameters varied depending on the dosage and type of the agents applied.

Generally, an increase of the dosage in all treatments caused greater OTA removal, but also a higher impact on wine quality. The results of this study indicate that chitin was an efficient agent that could be used to remove OTA up to 18% without affecting wine quality significantly. The treatment with gelatine and the complex were the best to obtain a 39–40% OTA removal rate, causing the least possible impact on colour parameters and polyphenol concentration. The greatest OTA removal (67%) was achieved with chitosan, but the wine-quality parameters were strongly affected. On the other hand, bentonite treatment removed OTA with a relative efficiency, but it also caused a considerable decrease in the CI value. Finally, egg albumin treatment removed only up to 16% of OTA concentration and moreover it affected strongly the CI value and CIELab parameters.

The findings of this study suggest the potential use for the non-toxic chitin and the allergen-free adsorbents (complex and gelatine) as alternative adsorbents for OTA removal during the winemaking process.

References

- Amézqueta S, González-Peñas E, Murillo-Arbizu M, López de Cerain A. 2009. Ochratoxin A decontamination: a review. *Food Control*. 20:326–333.
- Berente B, Móricz Á, Otta KH, Záray G, Lékó L, Rácz L. 2005. Determination of ochratoxin A in Hungarian wines. *Microchem J*. 79:103–107.
- Bornet A, Teissedre PL. 2008. Chitosan, chitin-glucan and chitin effects on minerals (iron, lead, cadmium) and organic (ochratoxin A) contaminants in wines. *Eur Food Res Technol*. 226(4):681–689.
- Castellari M, Versari A, Fabiani A, Parpinello GP, Galassi S. 2001. Removal of ochratoxin A in red wines by means of adsorption treatments with commercial fining agents. *J Agric Food Chem*. 49:3917–3921.
- Ertan A, İ. Mert A. 2010. Ochratoxin A and brewing technology: a review. *J Inst Brew*. 116(1):23–32.
- European Commission. 2002. Reports on tasks for scientific cooperation. Report of experts participating in Task 3.2.7. Assessment of dietary intake of ochratoxin A by the population of EU Member States. Directorate-General Health and Consumer Protection. January 2002. Brussels (Belgium): European Commission.
- European Commission. 2005. Commission Regulation (EC) No. 123/2005 of 26 January 2005 amending Regulation (EC) No. 466/2001 as regards ochratoxin A. *Off J Eur Union*. L25:3–5.
- European Commission. 2007. Commission Directive 2007/68/EC of 27 November 2007 amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients. *Off J Eur Union*. L310:11–14.
- European Commission. 2009. Commission Regulation (EC) No. 415/2009 of 20 May 2009 amending Directive 2007/68/EC amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients. *Off J Eur Union*. L125:52–53.
- European Economic Community (EEC). 1990. Commission Regulation (EEC) No. 2676/90 determining Community methods for the analysis of wines. 17 September 1990. *Off J Eur Comm*. L272:1–192.
- European Food Safety Authority (EFSA). 2006. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the commission related to ochratoxin A in food. *EFSA J*. 365:1–56.
- Filali A, Ouammi L, Betbeder AM, Baudrimont I, Soulaymani R, Benayada A, Creppy EE. 2001. Ochratoxin A in beverages from Morocco: a preliminary survey. *Food Addit Contam*. 18(6):565–568.
- Gambuti A, Strollo D, Genovese A, Ugliano M, Ritieni A, Moio L. 2005. Influence of enological practices on ochratoxin A concentration in wine. *Am J Enol Vitic*. 56(2):155–162.
- International Agency for Research on Cancer (IARC). 1993. IARC Monographs on the evaluation of carcinogenic risks to humans. Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and

- mycotoxins. Summary of data reported and evaluation. IARC Sci Publ. 56:489–521.
- International Organisation of Vine and Wine (OIV). 2009. Resolution OIV/OENO 338A/2009. Wines – treatment using chitosan. Paris (France): International Organisation of Vine and Wine.
- International Organisation of Vine and Wine (OIV). 2011. Compendium of international methods of wine and must analysis. Edition 2011. Paris (France): International Organisation of Vine and Wine.
- Kurtbay HM, Bekçi Z, Merdivan M, Yurdakoç K. 2008. Reduction of ochratoxin A levels in red wine by bentonite, modified bentonites, and chitosan. *J Agric Food Chem.* 56:2541–2545.
- Pietri A, Bertuzzi T, Pallaroni L, Piva G. 2001. Occurrence of ochratoxin A in Italian wines. *Food Addit Contam.* 18(7):647–654.
- Quintela S, Villarán MC, López de Armentia I, Elejalde E. 2011. Occurrence of ochratoxin A in Rioja Alavesa wines. *Food Chem.* 126(1):302–305.
- Quintela S, Villarán MC, López de Armentia I, Elejalde E. 2012. Ochratoxin A in Spanish exportation wine. *Food Control.* 25:501–504.
- Ribéreau-Gayon P, Stonestreet E. 1965. Le dosage des anthocyanes dans le vin rouge. *Bull Soc Chim Fr.* 9:2649–2652.
- Rosa CAR, Magnoli CE, Fraga ME, Dalcero AM, Santana DMN. 2004. Occurrence of ochratoxin A in wine and grape juice marketed in Rio de Janeiro, Brazil. *Food Addit Contam.* 21(4):358–364.
- Shephard GS, Fabiani A, Stockenström S, Mshicileli N, Sewram V. 2003. Quantitation of ochratoxin A in South African wines. *J Agric Food Chem.* 51: 1102–1106.
- Stanković S, Jović S, Živković J. 2004. Bentonite and gelatine impact on the young red wine coloured matter. *Food Technol Biotechnol.* 42(3):183–188.
- Zimmerli B, Dick R. 1996. Ochratoxin A in table wine and grape-juice: occurrence and risk assessment. *Food Addit Contam.* 13(6):655–668.