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Elimination of ochratoxin A from white and red wines: Critical characteristics of activated carbons and impact on wine quality

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ABSTRACT

Ochratoxin A (OTA) is a mycotoxin frequently found in wines, with an allowed maximum limit of 2.0 μg/L. To optimise OTA removal from white and red wines at high levels (10.0 μg/L) using activated carbons (ACs) different commercial deodorising ACs were tested. OTA elimination from white wines was less dependent on ACs characteristics, with 100% removal with all but one AC with the lowest total pore volume. For red wines, only one of the ACs with higher abundance of mesopores was successful in completely removing OTA. This decrease in performance was due to the competition of anthocyanins for ACs mesopores. ACs treatment impacted positively on white wine chromatic characteristics, decreasing the yellowness-brownish colour but for the red wine it was observed a higher but limited impact (maximum 24% decrease) on wine colour. ACs pore volume distribution, with pore sizes in the range 42.6–55.9 Å and 125.6–137.4 Å were important for efficient OTA removal. These results will allow the rational design of ACs with pore volume characteristics for OTA elimination with less impact on wine colour. Therefore, it was shown for the first time that AC with appropriate pore size distribution can remove completely OTA from white and also red wines.

1. Introduction

The occurrence of mycotoxins in foodstuffs, including wine, represents a serious risk for consumer's health. Mycotoxins are toxic secondary metabolites produced by certain species of moulds with ochratoxin A (OTA) being one of the most important (Quintela et al., 2012). OTA is a potent nephrotoxin with teratogenic, mutagenic and immunosuppressive effects (Heussner & Bingle, 2015). In 1993, the International Agency for Research on Cancer (IARC) classified OTA as possibly carcinogenic for humans (group 2B) (IARC, 1993).

OTA occurrence in wine has been described in several works (Bellver Soto, Fernandez-Franzon, Ruiz, & Juan-Garcia, 2014; Gil-Serna, Vazquez, Gonzalez-Jaen, & Patino, 2018; Oteiza et al., 2017; Silva, Rodrigues, Pereira, Lino, & Pena, 2019; Zimmerli & Dick, 1996), being typically higher in red (<0.01–7.63 μg/L), followed by rosé (<0.01–2.40 μg/L) and white wines (<0.01–1.72 μg/L) (Blesa, Soriano, Molto, & Manes, 2006), with high levels of incidence (OTA was present in 53% of 521 red wines, 69% of 98 rosé wines and 61% of 301 white wines analysed, Blesa et al., 2006). In Europe wine is estimated to be the second source of OTA intake, after cereals. Since 2006, the maximum limit for OTA in wine is 2 μg/L, according to Regulation (EC) No. 1881/2006 of the European Commission.

Although the prevention of its appearance in wines is of the utmost importance when present, efficient removal treatments are necessary to achieve safe levels of OTA for human consumption (Ubeda, Hornedo-Ortega, Cerezo, & Troncoso, 2020). Several microbiological, physical and chemical methods have been described for its elimination from foods (Abrunhosa et al., 2014; Amézqueta, González-Peñas, Murillo-Arbizu, & de Cerain, 2009; Quintela, Villarán, de Armentia, & Elejalde, 2013; Sun et al., 2017). Numerous studies of detoxification of mycotoxins using microorganisms such as bacteria, yeast, and fungi have been published (Chen et al., 2018; Hathout & Aly, 2014). Biological transformation can occur by degradation or enzymatic transformation of mycotoxins to less toxic compounds (the most important mechanism of OTA biodegradation is to OTα, Loi, Fanelli, Liuzzi, Logrieco, & Mulè, 2017, and L-β-phenylalanine) these degradation products

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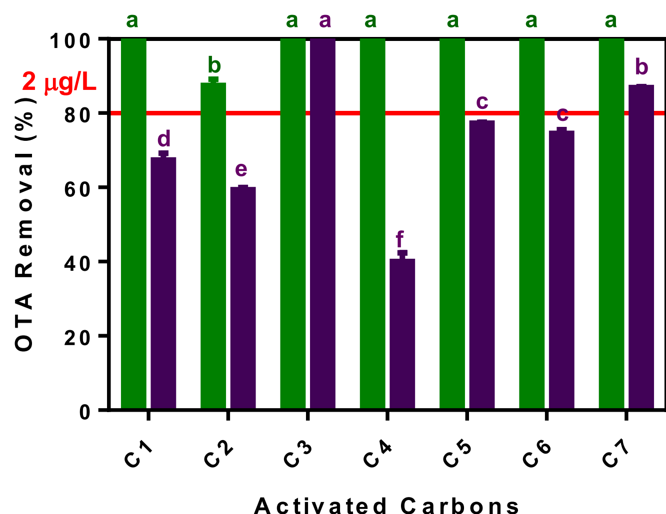

Fig. 1. Removal of OTA in white (■) and red (■) wine. Activated carbons C1–C7. Means within a column for each wine, followed by the same letter are not significantly different (Tukey $p < 0.05$). The red line in the figure correspond to a removal percentage of 80% and therefore to a level of 2 μg/L in the treated wine. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

are formed by the hydrolysis of the amide bond via hydrolytic enzymes, such as carboxypeptidase A, protease A, lipase A, or ochratoxinase (Abrunhosa, Santos, & Venâncio, 2006; Dobritzsch, Wang, Schneider, & Yu, 2014; Liuzzi et al., 2017) or by binding the toxin on the cell wall surface of these microorganisms (Piotrowska, 2014). Bacteria, such as, *Oenococcus oeni* and *Lactobacillus plantarum*, have been found to adsorb OTA, being the peptidoglycan and polysaccharides probably involved in the toxin binding (Del Prete et al., 2007). According to Piotrowska (2014), OTA binding was higher in the case of thermally inactivated bacterial biomass (46.2%–59.8%). OTA biodegradation by *Pediococcus parvulus* UTAD 473 was also studied, and it can degrade 100% of OTA (1000 μg/L) within 7 days at 30 °C in MRS broth medium, 80% of OTA (7 μg/L) after 6 days incubation in grape must, however, no obvious degradation of OTA (7 μg/L) was observed in synthetic wine, probably due to the presence of ethanol, which may inhibit the enzyme responsible for the OTA hydrolysis (Abrunhosa et al., 2014). Csutorás et al. (2013) also observed that OTA (4000 μg/mL) adsorbed to *S. cerevisiae* during the fermentation process (90 days) of 73, 85, and 90% in white, rosé, and red wine musts, respectively. Petruzzi et al. (2014) made a review concerning OTA removal mediated by yeasts live cells, cell walls, cell wall extracts and yeast lees, as yeast biomass may be regarded as a good adsorbing tool, due to the presence in the cell wall of some specific macromolecules, such as mannoproteins and β-glucans. Also, physical approaches have been studied such as wine filtration through a 0.45 μm membrane that showed an 80% decrease of OTA (Gambuti et al., 2005). Solfrizzo, Avantaggiato, Panzarini, and Visconti (2010), showed that the levels of OTA in wine reduced by repassage of contaminated musts or wines over grape pomaces. The authors observed that, if grape pomaces from red wines of the same grape variety are used it did not affect wine quality parameters, including colour intensity and health-promoting phenolic content. Nevertheless, in the case of wines, the effectiveness of the available treatment for OTA removal is limited, and at present, the use of adsorbents is the most common practice. Galvano et al. (1998) showed that activated carbon (AC) has a good capacity to absorb OTA in model solution (125 μg of OTA by milligram of AC). Nevertheless, the complex wine matrix, especially in red wines, decreases significantly the OTA adsorption capacity of ACs. Var, Kabak, and Erginkaya (2008) showed OTA reductions up to 98.3% for white wines (5 μg/L OTA contamination level), using 1 g/L of AC (no physicochemical characteristics specified). On the other hand, Castellari, Versari, Fagiani, Parpinello, and Galassi (2001) showed that AC was able to remove 32–61% of the original OTA concentration (3.78 μg/L OTA contamination level), depending on the physicochemical characteristics of the ACs used (with an active area 500–1500 m^2/g and at 0.05 g/L carbon dose presenting the best results) showed an OTA decrease in red wines, up to 72% (5 μg/L OTA contamination level), using oenological decolourising carbon (no characteristics specified) at 0.030 g/L. Olivares-Marín et al. (2009) using different ACs made from cherry stones at 0.012 g/L obtained an OTA removal percentage from practically negligible to a maximum of 54% in red wine (7.38 μg/L OTA contamination level), with the AC showing the best performance being characterised by a macroporosity made up of larger size pores (Olivares-Marín et al., 2009). Similar reduction results were obtained in sweet wines by Espejo and Armada (2009), using ACs (Brunauer-Emmett-Teller (BET) surface area, S_{BET} = 900–1100 m^2/g) at 0.40 g/L dose were able to remove ~90% of the OTA present, but the OTA levels present were low (0.979 μg/L OTA contamination level).

AC is a fining agent used in oenology (Commission Regulation (EC) No 606/2009) with a maximum dose of 1 g/L. AC adsorption efficiency is related to their physicochemical characteristics namely pore structure, magnitude, distribution of pore volume and surface area are determinant for their adsorption characteristics and efficiency (Karanfil, 2006).

Table 1
Effect of activated carbons application on phenolic compounds, colour ($Abs_{420\ nm}$) and chromatic characteristics in white wine.

	Total phenolics (mg/L GAE)	Total flavonoids (mg/L GAE)	Total non-flavonoid phenolics (mg/L GAE)	Colour (Abs_{420nm})	*L**	*a**	*b**	*C**	*h*°	*ΔE**
T	385 ± 2[a]	228 ± 4[a]	157 ± 2[a]	0.107 ± 0.001[a]	96.6 ± 0.1[a]	- 0.11 ± 0.01[a]	8.15 ± 0.05[a]	8.15 ± 0.05[a]	178.44 ± 0.00[a]	
C1	353 ± 4[cd]	212 ± 5[d]	141 ± 1[c]	0.073 ± 0.000[c]	96.9 ± 0.2[a]	- 0.21 ± 0.04[a]	5.89 ± 0.15[c]	5.89 ± 0.14[c]	178.47 ± 0.01[a]	2.29 ± 0.15[e]
C2	365 ± 3[b]	231 ± 1[a]	134 ± 3[d]	0.069 ± 0.001[d]	97.7 ± 0.1[b]	- 0.38 ± 0.07[a]	5.62 ± 0.01[c]	5.63 ± 0.00[c]	178.50 ± 0.01[a]	2.75 ± 0.05[d]
C3	341 ± 3[e]	208 ± 2[d]	133 ± 4[d]	0.039 ± 0.001[g]	98.1 ± 0.1[b]	- 0.28 ± 0.13[a]	3.54 ± 0.06[f]	3.55 ± 0.07[f]	178.51 ± 0.03[a]	4.84 ± 0.08[a]
C4	369 ± 3[b]	222 ± 5[b]	147 ± 3[b]	0.088 ± 0.001[b]	97.1 ± 0.0[a]	- 0.27 ± 0.02[a]	6.98 ± 0.07[b]	6.98 ± 0.07[b]	178.47 ± 0.00[a]	1.28 ± 0.05[f]
C5	356 ± 2[c]	216 ± 2[c]	140 ± 1[c]	0.050 ± 0.001[f]	98.0 ± 0.3[b]	- 0.34 ± 0.14[a]	4.37 ± 0.12[e]	4.38 ± 0.11[e]	178.51 ± 0.03[a]	4.05 ± 0.21[b]
C6	365± 2[b]	222 ± 1[b]	143 ± 1[bc]	0.056 ± 0.000[e]	97.3 ± 0.2[a]	- 0.28 ± 0.16[a]	4.80 ± 0.13[d]	4.81 ± 0.14[d]	178.49 ± 0.03[a]	3.42 ± 0.05[c]
C7	349 ± 3[d]	207 ± 5[e]	142 ± 2[c]	0.039 ± 0.001[g]	97.8 ± 0.3[b]	- 0.25 ± 0.11[a]	3.78 ± 0.00[f]	3.79 ± 0.01[f]	178.49 ± 0.03[a]	4.55 ± 0.06[a]

Values are presented as mean ± standard deviation (n = 2); Unfined wine (T). Activated carbons C1–C7. Means within a column followed by the same letter are not significantly different (Tukey $p < 0.05$). L* – lightness, a* - redness, b* - yellowness, C*– chroma, h° – hue angle, ΔE* –colour difference. The values corresponding to ΔE* were obtained taking as a reference the untreated wine (T), and wine treated with activated carbons (C1–C7). GAE – Gallic acid equivalents.

Table 2
Effect of activated carbons application on phenolic compounds, total and coloured anthocyanins, total and polymeric pigments, and chromatic characteristics in red wine.

	Total phenolics (mg/L GAE)	Total flavonoids (mg/L GAE)	Total non-flavonoid phenolics (mg/L GAE)	Total anthocyanins (mg/L)	Coloured anthocyanins (a.u)	Polymeric pigments (a.u.)	Total pigments (a.u.)	Colour intensity (a.u.)	Hue	L*	a*	b*	C*	h°	ΔE*
T	2056 ± 16^{a}	1764 ± 14^{a}	292 ± 3^{a}	381 ± 6^{a}	3.63 ± 0.01^{a}	5.81 ± 0.01^{a}	18.33 ± 0.06^{b}	11.20 ± 0.06^{a}	0.689 ± 0.001^{a}	10.3 ± 0.7^{a}	40.54 ± 1.17^{a}	38.64 ± 1.16^{a}	56.00 ± 1.65^{a}	0.76 ± 0.00^{a}	
C1	1838 ± 31^{b}	1574 ± 30^{bc}	264 ± 3^{cd}	347 ± 1^{b}	3.38 ± 0.14^{b}	5.42 ± 0.12^{b}	18.71 ± 0.21^{b}	10.44 ± 0.21^{c}	0.690 ± 0.001^{a}	12.0 ± 0.2^{b}	42.79 ± 0.26^{ab}	40.11 ± 0.15^{a}	58.65 ± 0.29^{ab}	0.75 ± 0.00^{a}	1.98 ± 0.36^{a}
C2	1878 ± 10^{b}	1608 ± 8^{b}	270 ± 3^{bc}	306 ± 4^{d}	3.39 ± 0.09^{b}	5.09 ± 0.09^{c}	16.21 ± 0.35^{d}	9.98 ± 0.08^{d}	0.717 ± 0.019^{b}	12.2 ± 0.1^{b}	42.79 ± 0.24^{ab}	39.61 ± 0.28^{a}	58.31 ± 0.37^{a}	0.75 ± 0.00^{b}	2.31 ± 0.14^{a}
C3	1783 ± 12^{c}	1522 ± 12^{c}	261 ± 3^{d}	284 ± 8^{f}	1.95 ± 0.04^{e}	4.43 ± 0.02^{f}	19.47 ± 0.56^{a}	8.50 ± 0.01^{g}	0.727 ± 0.008^{bc}	15.4 ± 0.3^{c}	46.38 ± 0.32^{c}	40.33 ± 0.12^{a}	61.47 ± 0.33^{b}	0.72 ± 0.00^{d}	7.30 ± 0.47^{c}
C4	1866 ± 39^{b}	1591 ± 43^{b}	275 ± 4^{b}	323 ± 2^{c}	3.43 ± 0.05^{b}	5.51 ± 0.05^{b}	17.47 ± 0.35^{c}	10.68 ± 0.09^{b}	0.696 ± 0.005^{a}	11.7 ± 0.5^{a}	42.28 ± 0.60^{a}	39.53 ± 0.10^{a}	57.88 ± 0.50^{a}	0.75 ± 0.00^{a}	1.30 ± 0.78^{a}
C5	1841 ± 17^{b}	1567 ± 18^{bc}	275 ± 2^{b}	292 ± 8^{ef}	2.91 ± 0.03^{c}	4.95 ± 0.04^{d}	16.24 ± 0.32^{d}	9.56 ± 0.12^{e}	0.692 ± 0.013^{a}	13.5 ± 0.0^{b}	44.32 ± 0.02^{bc}	40.16 ± 0.09^{a}	59.804 ± 0.07^{b}	0.74 ± 0.00^{bc}	4.06 ± 0.02^{b}
C6	1884 ± 16^{b}	1567 ± 15^{b}	287 ± 2^{a}	297 ± 1de	2.96 ± 0.03^{c}	4.72 ± 0.01^{e}	16.26 ± 0.23^{d}	9.26 ± 0.01^{f}	0.720 ± 0.000^{bc}	13.8 ± 0.5^{c}	44.66 ± 0.70^{bc}	40.12 ± 0.23^{a}	60.03 ± 0.67^{b}	0.73 ± 0.01^{c}	5.39 ± 0.33^{bc}
C7	1725 ± 16^{d}	1462 ± 18^{d}	263 ± 4^{d}	260 ± 8^{g}	2.24 ± 0.01^{d}	4.34 ± 0.00^{f}	17.98 ± 0.00^{c}	8.59 ± 0.02^{g}	0.735 ± 0.003^{c}	15.4 ± 0.5^{c}	46.34 ± 0.63^{c}	40.35 ± 0.21^{a}	61.45 ± 0.62^{b}	0.72 ± 0.01^{d}	6.87 ± 0.82^{c}

Values are presented as mean ± standard deviation (n = 2); Unfined wine (T). Activated carbons C1–C7. Means within a column followed by the same letter are not significantly different (Tukey $p < 0.05$). GAE – Gallic acid equivalents; a.u. – absorbance units; L* – lightness, a* - redness, b* - yellowness, C* – chroma, h° – hue angle, ΔE* – colour difference. The values corresponding to ΔE* were obtained taking as a reference the untreated wine (T), and wine treated with activated carbons (C1–C7).

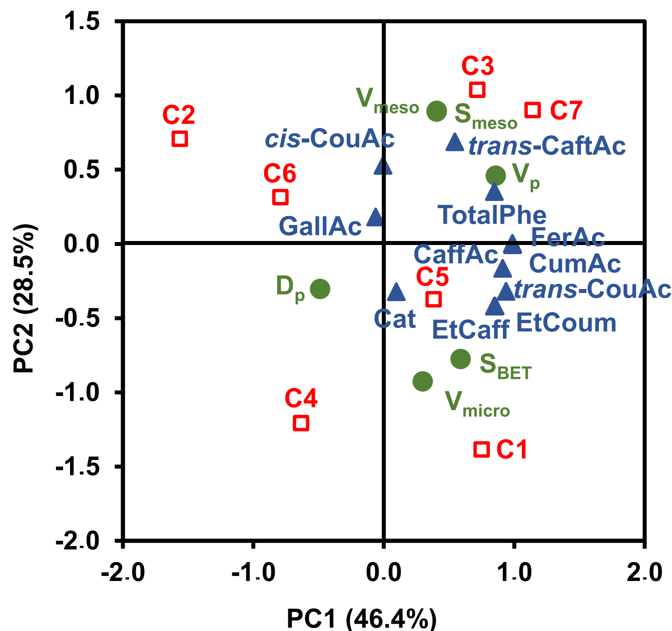

Fig. 2. Projection of PCA data from activated carbons treated white wine samples (□), Phenolic compounds removal (mg/L) (▲) and activated carbons physicochemical characteristics (●). S_{BET} – Brunauer-Emmett-Teller (BET) surface area; S_{meso} – surface area of mesopores; V_p – total volume of pores; V_{micro} – micropore volume; D_p – average pore diameter; *trans*-CaftAc – *trans*-Caftaric acid; Total PhenAc – sum of all phenolic acids determined by HPLC, *cis*-CouAc – *cis*-Coutaric acid; *trans*-CouAc – *trans*-Coutaric acid; GallAc – Gallic acid; CaffAc – Caffeic acid; FerAc – Ferulic acid; CoumAc – *p*-Coumaric acid; EtCoum – Ethyl ester of *p*-Coumaric acid; EtCaff – Ethyl ester of Caffeic acid; Cat – Catechin.

Although the use of ACs for removal of OTA from white and red wines is a potential oenological treatment that needs to be optimised, its non-specific adsorption behaviour can have a negative impact on wine quality, especially for red wines, but it can be limited by careful selection of ACs based on their physicochemical properties, being a good trade-off solution, especially when compared to the quality and safety improvement attained by removal of undesirable compounds (Filipe-Ribeiro, Milheiro, Matos, Cosme, & Nunes, 2017a). Therefore the knowledge of the relation between ACs physicochemical characteristics and their efficiency in removing OTA and phenolic compounds from the wine will help to minimise its impact on wine quality.

This work aims to study for the first time, as far as we known, the efficiency of different well-characterised deodorising oenological ACs in the removal of OTA from white and red wines as well as their impact on wines physicochemical characteristics, aiming understanding the ACs structural features that are critical for the complete elimination of OTA minimising their impact on wine quality.

2. Material and methods

2.1. Wine sample

A commercial white wine from the Vinho Verde region (vintage 2013) and a commercial red wine from the Douro Valley (vintage 2014) were used with the following characteristics respectively: alcohol content (10.4%. v/v; 13.4%. v/v), density at 20 °C (0.9917 g/mL; 0.9914 g/mL), titratable acidity (6.8 g/L; 5.0 g/L expressed as tartaric acid), pH (3.14; 3.49), volatile acidity (0.16 g/L; 0.35 g/L expressed as acetic acid). Oenological parameters were analysed using a Fourier transform infrared spectroscopy (FTIR) Bacchus Micro (Microderm, France). Analyses were performed in duplicate.

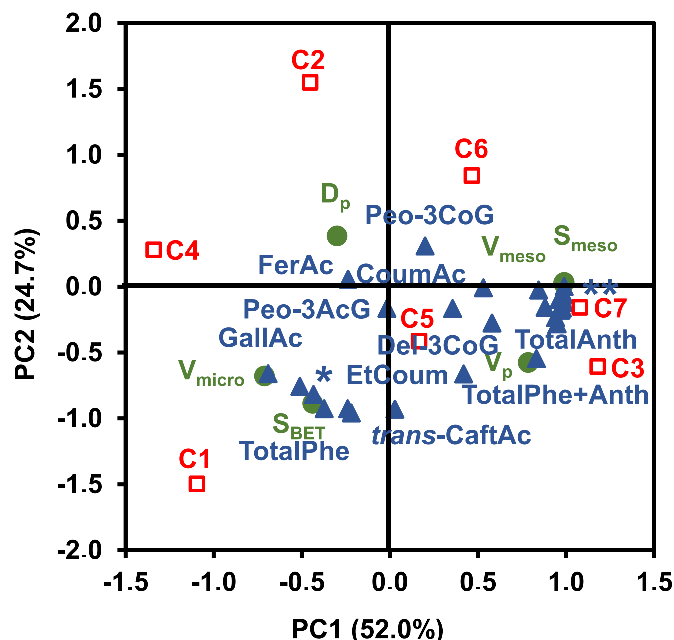

Fig. 3. Projection of PCA data from activated carbons treated red wine samples (□), Phenolic compounds and anthocyanins removal (mg/L) (▲, ×) and activated carbons physicochemical characteristics (●) (see legend Fig. 2). * – Catechin, Coutaric acid isomer, Coutaric acid, Caffeic acid-CaffAc; ** – Caffeic acid ethyl ester, Delphinidin-3-glucoside, Cyanidin-3-glucoside, Petunidin-3-glucoside, Peonidin-3-glucoside, Malvidin-3-glucoside, Delphinidin-3-acetylglucoside, Cyanidin-3-acetylglucoside, Peonidin-3-acetylglucoside, Malvidin-3-acetylglucoside, Petunidin-3-coumaroylglucoside, Malvidin-3-coumaroylglucoside. Total Anth – sum of all anthocyanins determined by HPLC; Total Phe – sum of all phenolic acids and anthocyanins determined by HPLC; Peo-3CoG – Peonidin-3-coumaroylglucoside; Peo-3AcG – Pedonidin-3-acetylglucoside; Del-3CoG – Delphinidin-3-coumaroylglucoside. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

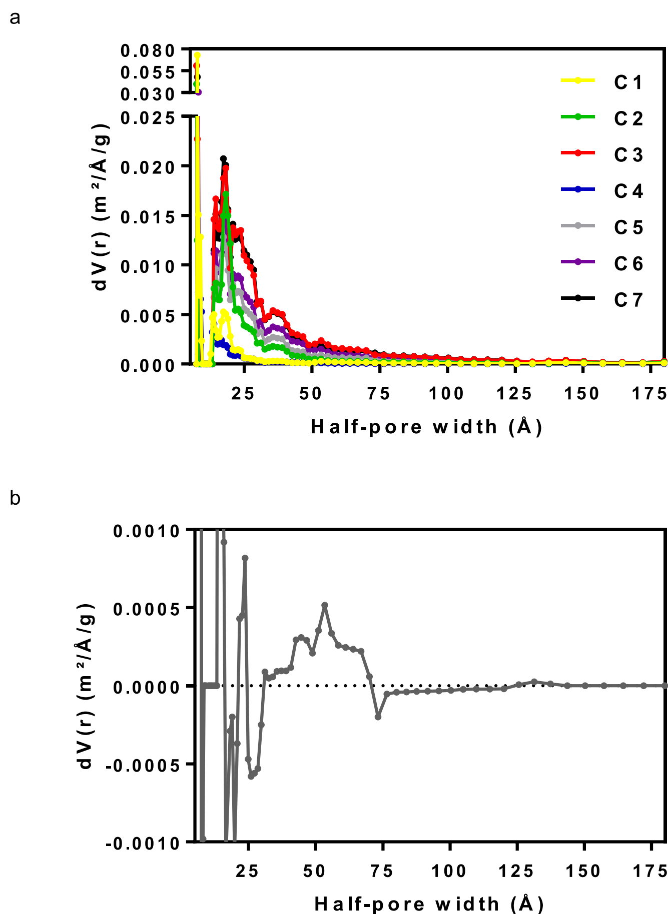

Fig. 4. Pore size distribution of activated carbons (C1-C7) obtained by the DFT method.

2.2. Activated carbons

The ACs of vegetable origin used in this study were supplied by the oenological supplier company SAI Enology Lda (Paredes, Portugal). In wine fining only ACs of vegetable origin are allowed. ACs were coded from C1 to C7, and their main characteristics (Filipe-Ribeiro et al., 2017a; Filipe-Ribeiro, Milheiro, Matos, Cosme, & Nunes, 2017b) are presented in Table S.1. Detailed procedures used in this work are presented in Supplementary Material.

2.3. Fining experiments

White and red wine samples were supplemented with OTA at a final concentration of 10 μg/L before the fining experiments. This OTA concentration was chosen as the worst-case scenario of wine contamination according to the values present in the literature (Visconti, Pascale, & Centonze, 1999). The ACs were applied at 1 g/L (the maximum allowed dosage for white wines (Commission Regulation (EC) No 606/2009), to white and red wines placed in 250 mL graduated cylinders, mixed and allowed to remain in contact with the wines for 7 days at 20 °C, simulating the standard oenological practices. Red and white wines without any AC added were used as controls. All the experiments were performed in duplicate.

2.4. High-performance liquid chromatography (HPLC) OTA analysis in white and red wine

Analyses were carried out with by HPLC with fluorescence detection (Varian Prostar 210 pump, Varian Prostar 410 autosampler, a Jasco FP-920 fluorescence detector and a Jones Chromatography 7971 column heater set at 30 °C). The chromatographic separation was performed on a C18 reversed-phase YMC-Pack ODS-AQ analytical column (250 × 4.6 mm I.D. 5 mm), fitted with a pre-column, with a flow rate of 1 mL/min, at 30 °C. The injection volume was 50 μL and parameters for detection: λex = 333 nm, λem = 460 nm and gain = 1000. The OTA retention time was approximately 12 min (Fig. S.1.). The determinations were performed in duplicate. Detailed procedures used in this work are presented in Supplementary Material.

2.5. Quantification of non-flavonoids, flavonoids and total phenols

The non-flavonoid phenolics of the white and red wine were quantified according to Kramling and Singleton (1969), and the total phenolic compounds, according to Ribéreau-Gayon, Peynaud, and Sudraud (1982). The flavonoid phenolics were obtained by difference between total phenolic compounds and non-flavonoid phenolics (Kramling & Singleton, 1969). All analyses were performed in duplicate.

2.6. Colour, chromatic characteristics and pigments

White wine colour, red wine colour intensity and hue and chromatic characteristics using the CIELab method were quantified as described in the O.I.V. methods (OIV, 2015). The concentration of total and coloured anthocyanins, total, and polymeric pigments from red wine were determined according to Somers and Evans (1977). All analyses were performed in duplicate.

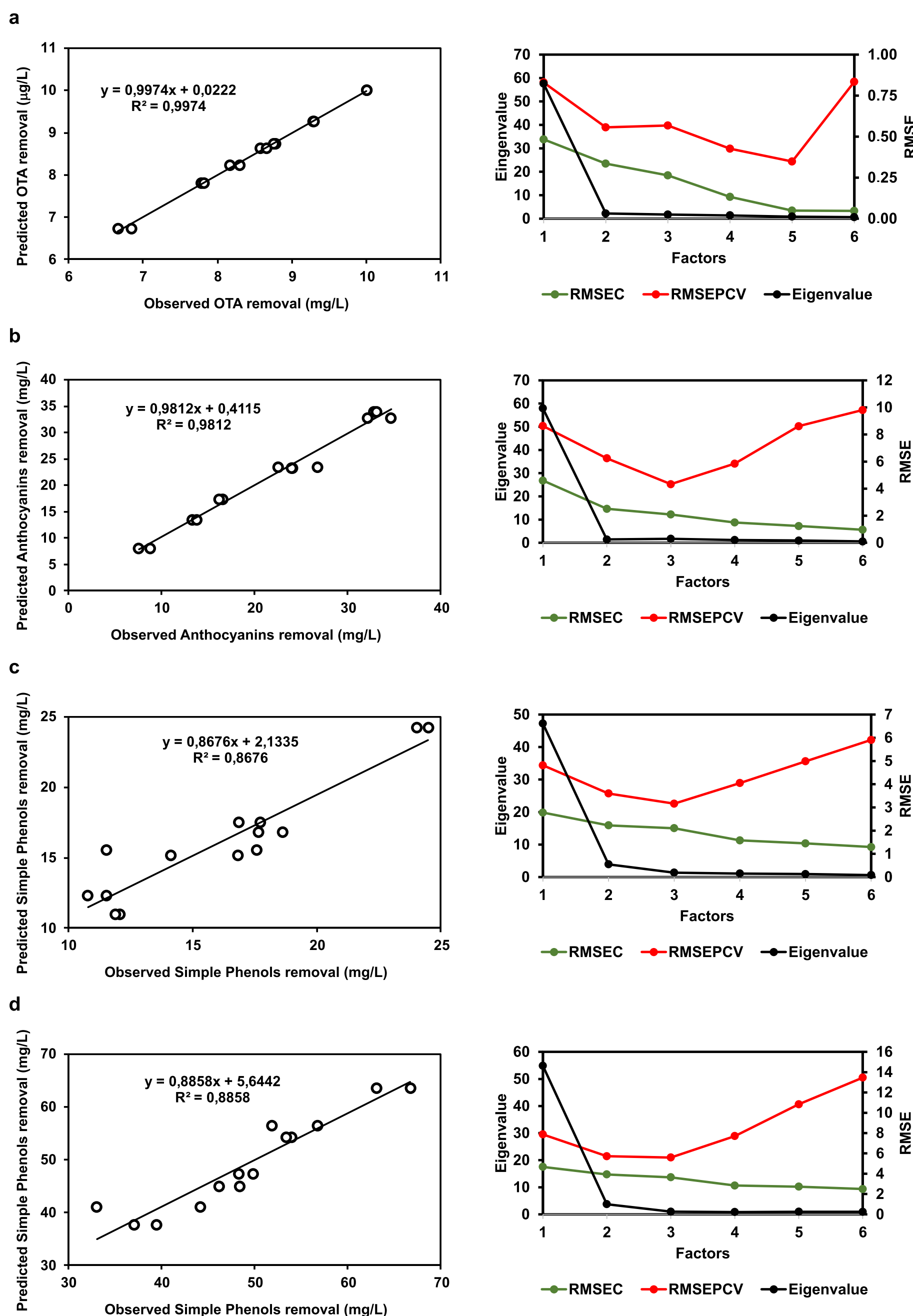

Fig. 5. Calibration curves obtained after PLS regression and Plot of Eigenvalue, root mean square error of calibration (RMSEC), root mean square error of prediction by leave-one out cross validation (RMSEPCV) versus the number of principal components obtained by PLS regression of the (a) OTA removal (b) Total anthocyanins, (c) Red wine phenolic acids and catechin, (d) White wine phenolic acids and catechin on the activated carbons pore volume size distribution. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.7. High-performance liquid chromatography (HPLC) analysis of anthocyanins, catechin and phenolic acids

Analyses were carried out with an Ultimate 3000 Dionex HPLC equipped with a PDA-100 photodiode array detector and an Ultimate 3000 Dionex pump. The separation was performed on a C18 column (250 mm × 4.6 mm, 5 µm particle size, ACE, Scotland) with a flow rate of 1 mL/min, at 35 °C. The injection volume was 50 µL, and the detection was performed from 200 to 650 nm. The analysis conditions were carried out using 5% aqueous formic acid (A) and methanol (B) and the gradient was as follows: 5% B from zero to 5 min followed by a linear gradient up to 65% B until 65 min and from 65 to 67 min down to 5% B (Filipe-Ribeiro et al., 2017a, 2017b; Guise et al., 2014).

2.8. Statistical analysis

The data are presented as means ± standard deviation. Analysis of variance (ANOVA) and a post-hoc test, Tukey honestly significant difference (HSD, 5% level) test was applied to physicochemical data to determine significant differences between the fining treatments using the Statistica 7 software (Statsoft. OK, USA). Partial Least Square (PLS) analysis was employed to access the ACs critical characteristics for OTA removal from white and red wines by developing a structure-efficiency relationship between the pore volume size distribution of ACs with the removal efficiencies of OTA (Mehmood, Liland, Snipen, & Sæbø, 2012). Also, the removal of anthocyanins and simple phenolic compounds by the same ACs were accessed by this method. Cross-validation (Vinzi, Chin, Henseler, & Wang, 2010) was used to determine the number of components to include in the analysis, based on the number of PLS components with the smallest prediction error sum of squares (PRESS).

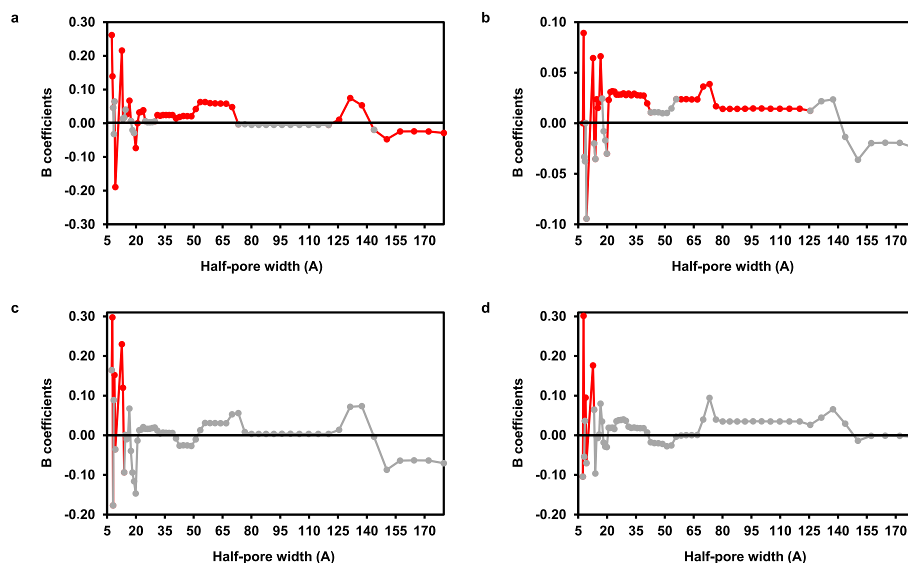

Fig. 6. B coefficients plots for determination of (a) OTA, (b) anthocyanins, (c) red wine simple phenols and (d) white wine phenolic compounds removal, by activated carbons in red wine in function of the pore volume distribution. B coefficients significantly different from zero are shown in red; otherwise coefficients are shown in grey. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Effect of activated carbons application on phenolic acids (mg/L) and flavonoids (mg/L) in white wine.

	Gallic acid	Catechin	*trans*-Caftaric acid	*cis*-Coutaric acid	*trans*-Coutaric acid	Caffeic acid	*p*-Coumaric acid	Ferulic acid	Ethyl caffeic acid	Ethyl coumaric acid
T	12.03 ± 0.10^{a}	8.67 ± 2.45^{a}	53.21 ± 5.05^{a}	1.62 ± 0.31^{a}	27.64 ± 0.60^{a}	10.94 ± 1.33^{a}	5.81 ± 0.71^{a}	2.81 ± 0.23^{a}	3.05 ± 0.33^{a}	1.29 ± 0.09^{a}
C1	9.70 ± 0.61^{b}	5.76 ± 1.30^{b}	37.52 ± 2.51^{ab}	1.17 ± 0.49^{ab}	14.38 ± 0.68^{d}	5.41 ± 0.08^{c}	0.63 ± 0.00^{c}	0.27 ± 0.00^{c}	0.23 ± 0.00^{c}	0.09 ± 0.03^{c}
C2	9.12 ± 0.53^{b}	7.62 ± 2.14^{ab}	40.20 ± 6.83^{ab}	0.86 ± 0.01^{b}	20.79 ± 1.86^{b}	8.39 ± 0.82^{b}	1.30 ± 0.05^{b}	0.80 ± 0.10^{b}	0.72 ± 0.02^{b}	0.24 ± 0.02^{b}
C3	9.25 ± 0.51^{b}	9.17 ± 0.75^{a}	32.25 ± 1.46^{ab}	0.83 ± 0.00^{b}	16.14 ± 0.60^{cd}	5.49 ± 0.13^{c}	0.68 ± 0.01^{c}	0.22 ± 0.01^{c}	0.33 ± 0.01^{c}	0.14 ± 0.01^{bc}
C4	8.48 ± 1.65^{b}	6.37 ± 1.01^{ab}	48.60 ± 5.21^{ab}	0.88 ± 0.16^{b}	17.51 ± 0.43^{c}	7.60 ± 0.12^{b}	0.90 ± 0.05^{bc}	0.58 ± 0.05^{b}	0.28 ± 0.00^{c}	0.12 ± 0.02^{c}
C5	11.03 ± 1.35^{a}	5.22 ± 0.58^{b}	41.91 ± 5.71^{ab}	0.93 ± 0.20^{b}	15.22 ± 0.60^{d}	5.78 ± 0.24^{c}	0.75 ± 0.12^{c}	0.29 ± 0.04^{c}	0.29 ± 0.04^{c}	0.12 ± 0.02^{c}
C6	9.83 ± 1.48^{ab}	4.49 ± 0.14^{b}	34.73 ± 0.00^{ab}	1.13 ± 0.07^{ab}	19.53 ± 0.50^{b}	7.38 ± 0.25^{b}	1.40 ± 0.12^{b}	0.61 ± 0.07^{b}	0.50 ± 0.00^{bc}	0.17 ± 0.01^{bc}
C7	8.45 ± 0.21^{b}	4.96 ± 1.09^{b}	28.04 ± 1.32^{b}	0.85 ± 0.04^{b}	15.18 ± 0.38^{d}	5.32 ± 0.07^{c}	0.57 ± 0.08^{c}	0.22 ± 0.05^{c}	0.22 ± 0.12^{c}	0.08 ± 0.08^{c}

Values are presented as mean ± standard deviation (n = 2); Unfined wine (T). Activated carbons C1–C7. Means within a column followed by the same letter are not significantly different (Tukey $p < 0.05$).

PLS-R calculations were performed using the XLSTAT-v2006.06 package (Addinsoft, Inc).

3. Results and discussion

3.1. OTA removal from white and red wine using different ACs

As it can be observed in Fig. 1, the efficiency of OTA removal for all ACs was dependent on the wine matrix. For the white wines, all ACs (except for C2) were able to remove completely the OTA added to the wine; however, for the red wines, a lower removal efficiency for most of the ACs tested was observed. For red wine, only AC C3 was able to remove OTA completely and AC C4 was the least efficient with an OTA removal efficiency of only 40%. The results obtained for the OTA removal efficiency from red wine shows that the structural features of ACs are essential for their performance in this more complex matrix. It was observed a significant correlation between the ACs removal efficiency and ACs mesopore volume (V_{meso}; r = 0.889, $p < 0.0031$). The fact that ACs presented higher efficiency of OTA removal for the white wine when compared to red wine (both with 10 μg/L of OTA) can be due to the higher amount of phenolic compounds present in red wine, especially anthocyanins (further discussed below).

The comparison of the results obtained in this work with those described in the literature is not straightforward due to the variation in AC application dose, OTA contamination level of the wines treated, and sometimes the absence of information regarding the structural characteristics of the ACs used. For the white wine, the results obtained for almost all the ACs used in this work, with exception of AC2, were better than those described by Var et al. (2008) that using the same AC application dose for the treatment of wine with a lower OTA contamination level (5 μg/L) resulted in the removal of 98.3% of the OTA present. Nevertheless, as the authors didn't present the physicochemical characteristics of the AC used, the reason for the different results cannot be disclosed. For the red wine treatment, the results obtained with AC3 were significantly better than those described by Castellari et al. (2001) who obtained only a 32–61% removal of OTA (wine OTA contamination level of 3.78 μg/L), nevertheless, in this work, the application dose of AC was only 0.05 g/L. This difference in performance can be due to the

Table 4
Effect of activated carbons application on phenolic acids (mg/L) and flavonoids (mg/L) in red wine.

	Gallic acid	Catechin	*trans*-Caftaric acid	*cis*-Coutaric acid	*trans*-Coutaric acid	Caffeic acid	*p*-Coumaric acid	Ferulic acid	Ethyl caffeic acid	Ethyl coumaric acid
T	15.92 ± 0.02^{a}	17.50 ± 0.03^{a}	30.74 ± 0.13^{a}	0.34 ± 0.00^{a}	11.23 ± 0.03^{a}	3.35 ± 0.35^{a}	2.74 ± 0.01^{a}	2.31 ± 0.03^{a}	0.33 ± 0.00^{a}	0.72 ± 0.01^{a}
C1	9.38 ± 0.08^{b}	11.42 ± 0.02^{d}	25.88 ± 0.13^{b}	0.28 ± 0.00^{b}	8.82 ± 0.03^{c}	2.31 ± 0.02^{b}	1.66 ± 0.0^{c}	0.99 ± 0.02^{cd}	0.15 ± 0.08^{c}	0.02 ± 0.01^{c}
C2	13.12 ± 0.10^{a}	15.19 ± 0.05^{b}	28.78 ± 0.08^{ab}	0.31 ± 0.00^{ab}	10.35 ± 0.03^{b}	2.80 ± 0.01^{ab}	2.11 ± 0.16^{b}	1.02 ± 0.08^{cd}	0.16 ± 0.00^{c}	0.18 ± 0.00^{b}
C3	12.00 ± 2.48^{b}	13.39 ± 0.49^{c}	26.38 ± 3.81^{b}	0.30 ± 0.04^{ab}	10.15 ± 0.17^{bc}	2.62 ± 0.39^{b}	2.00 ± 0.01^{b}	1.50 ± 0.00^{b}	0.04 ± 0.02^{d}	n.d
C4	9.94 ± 0.06^{b}	13.62 ± 0.07^{c}	28.19 ± 0.14^{ab}	0.31 ± 0.02^{ab}	9.95 ± 0.10^{b}	2.54 ± 0.52^{b}	2.12 ± 0.05^{b}	1.05 ± 0.00^{c}	0.22 ± 0.01^{b}	0.05 ± 0.00^{c}
C5	11.29 ± 0.05^{b}	12.61 ± 0.70^{cd}	27.52 ± 0.04^{b}	0.30 ± 0.00^{ab}	9.81 ± 0.01^{b}	2.77 ± 0.01^{ab}	1.75 ± 0.00^{c}	0.89 ± 0.01^{cd}	0.10 ± 0.00^{c}	n.d
C6	13.97 ± 0.12^{a}	14.72 ± 0.10^{b}	28.48 ± 0.15^{ab}	0.31 ± 0.00^{ab}	10.33 ± 0.00^{b}	2.90 ± 0.28^{ab}	1.31 ± 0.04^{d}	1.09 ± 0.16^{c}	0.11 ± 0.00^{c}	n.d.
C7	13.15 ± 0.21^{a}	13.50 ± 0.25^{c}	27.88 ± 0.62^{ab}	0.30 ± 0.01^{ab}	9.74 ± 0.56^{b}	2.70 ± 0.27^{ab}	1.59 ± 0.01^{c}	0.79 ± 0.00^{d}	0.05 ± 0.01^{d}	n.d.

Values are presented as mean ± standard deviation (n = 2); Unfined wine (T). n.d. – not detected; activated carbons C1–C7. Means within a column followed by the same letter are not significantly different (Tukey $p < 0.05$).

Table 5
Effect of activated carbons application on monomeric anthocyanins (mg/L) in red wine.

	D-3-G	C-3-G	Pet-3-G	Peo-3-G	M-3-G	D-3-A	C-3-A	Pet-3-A	Peo-3-A	M-3-A	D-3-C	Pet-3-C	Peo-3-C	M-3-C
T	1.39 ± 0.14^{a}	5.42 ± 0.29^{a}	8.74 ± 0.04^{a}	10.33 ± 0.18^{a}	47.88 ± 0.09^{a}	3.14 ± 0.09^{a}	0.52 ± 0.01^{a}	0.38 ± 0.15^{a}	1.29 ± 0.03^{a}	8.03 ± 0.20^{a}	0.10 ± 0.14^{a}	0.48 ± 0.03^{a}	1.12 ± 0.16^{a}	7.15 ± 0.08^{a}
C1	1.40 ± 0.00^{a}	4.74 ± 0.08^{ab}	7.57 ± 0.19^{b}	8.70 ± 0.63^{b}	41.84 ± 0.58^{c}	2.94 ± 0.01^{a}	0.36 ± 0.07^{ab}	0.25 ± 0.04^{ab}	0.90 ± 0.18^{ab}	6.90 ± 0.06^{a}	0.29 ± 0.02^{b}	0.47 ± 0.13^{a}	0.54 ± 0.46^{ab}	5.77 ± 0.02^{c}
C2	1.37 ± 0.08^{a}	4.73 ± 0.05^{ab}	7.56 ± 0.11^{b}	9.04 ± 0.79^{b}	41.40 ± 0.36^{c}	2.43 ± 0.02^{b}	0.36 ± 0.07^{ab}	0.20 ± 0.07^{ab}	0.92 ± 0.12^{ab}	6.19 ± 0.31^{b}	0.10 ± 0.13^{a}	0.34 ± 0.05^{ab}	0.05 ± 0.07^{b}	4.97 ± 0.04^{d}
C3	1.18 ± 0.01^{b}	4.18 ± 0.04^{c}	6.38 ± 0.17^{c}	6.83 ± 0.03^{d}	34.69 ± 0.45^{e}	1.38 ± 0.14^{c}	0.16 ± 0.02^{b}	0.19 ± 0.03^{b}	0.57 ± 0.01^{b}	3.82 ± 0.06^{c}	nd	nd	0.64 ± 0.05^{ab}	3.00 ± 0.08^{f}
C4	1.44 ± 0.07^{a}	5.05 ± 0.16^{a}	8.27 ± 0.18^{a}	8.92 ± 0.19^{b}	44.56 ± 0.8^{b}	3.03 ± 0.10^{a}	0.44 ± 0.05^{a}	0.17 ± 0.02^{b}	1.15 ± 0.21^{a}	7.31 ± 0.09^{a}	nd	0.26 ± 0.01^{ab}	0.94 ± 0.07^{a}	6.30 ± 0.06^{b}
C5	1.33 ± 0.11^{ab}	4.46 ± 0.03^{c}	7.02 ± 0.12^{bc}	7.85 ± 0.02^{c}	37.60 ± 0.0^{d}	2.13 ± 0.10^{b}	0.27 ± 0.12^{ab}	0.15 ± 0.12^{b}	0.61 ± 0.02^{b}	5.85 ± 0.02^{b}	nd	0.19 ± 0.07^{bc}	0.62 ± 0.06^{ab}	3.91 ± 0.03^{e}
C6	1.39 ± 0.04^{a}	4.25 ± 0.32^{c}	6.89 ± 0.28^{bc}	7.45 ± 0.47^{c}	37.61 ± 1.4^{d}	2.09 ± 0.12^{b}	0.18 ± 0.06^{b}	0.34 ± 0.08^{a}	0.60 ± 0.01^{b}	5.45 ± 0.45^{b}	nd	0.24 ± 0.03^{b}	0.73 ± 0.07^{ab}	4.12 ± 0.07^{e}
C7	1.37 ± 0.09^{a}	3.96 ± 0.31^{c}	6.34 ± 0.26^{c}	7.06 ± 0.47^{cd}	33.93 ± 1.48^{e}	1.62 ± 0.02^{c}	0.16 ± 0.05^{b}	0.18 ± 0.04^{b}	0.69 ± 0.21^{b}	4.30 ± 0.80^{c}	nd	nd	0.29 ± 0.17^{b}	2.67 ± 0.05^{h}

Values are presented as mean ± standard deviation (n = 2); Delphinidin-3-glucoside (D-3-G). Cyanidin-3-glucoside (C-3-G). Petunidin-3-glucoside (Pet-3-G). Peonidin-3-glucoside (Peo-3-G). Malvidin-3-glucoside (M-3-G). Delphinidin-3-acetylglucoside (D-3-A). Cyanidin-3-acetylglucoside (C-3-A). Petunidin-3-acetylglucoside (Pet-3-A). Peonidin-3-acetylglucoside (Peo-3-A). Malvidin-3-acetylglucoside (M-3-A). Cyanidin-3-coumaroylglucoside (C-3-C). Malvidin-3-coumaroylglucoside (M-3-C). Means within a column followed by the same letter are not significantly different (Tukey $p < 0.05$).

structural features of the ACs used as the only characteristic described by Castellari et al. (2001) was the total active area of ACs used (500–1500 m^2/g) that as shown above, doesn't correlate well with the removal efficiency of ACs. Also, Gambuti et al. (2005) showed an OTA removal up to 72% in red wines (5 μg/L OTA contamination level), using oenological decolourising carbon (no characteristics specified) at 0.030 g/L. Olivares-Marín et al. (2009) obtained their best results in OTA removal from red wine (7.38 μg/L OTA contamination level) when using ACs with larger size pores (54% of OTA removal), nevertheless, the AC application level using 0.012 g/L of AC. Therefore, the different performances found in the literature for the OTA removal efficiency by ACs can be the result of the use of ACs with different physicochemical characteristics (Castellari et al., 2001; Espejo & Armada, 2009; Gambuti et al., 2005; Olivares-Marín et al., 2009). It should be highlighted that in red wine, AC C3 was able to remove OTA completely, even for an OTA concentration higher than those used in other studies and well above the levels of OTA reported to be present in red wines (Visconti et al., 1999). The higher efficiency of AC C3 can be explained by its specific physicochemical characteristic: a higher V_{meso} when compared to the other ACs used, with the exception of AC C7. Although AC C7 presented similar values of V_{meso} than C3 (Table S.2), the AC C7 OTA removal efficiency was lower than that obtained by the use of C3, therefore V_{meso} alone cannot fully explain the AC C3 efficiency (further discussed below). Nevertheless, AC C7, also presenting high values of V_{meso}, was the second most efficient AC for OTA reduction. This AC was still able to reduce OTA levels to values below the maximum allowable limit of 2 μg/L imposed by Regulation No. 1881/2006 of the European Commission (European Commission, 2006).

The adsorption mechanism of organic molecules on activated carbons is a multifactorial process that depends on both activated carbons physicochemical properties like pore texture, surface chemistry, and mineral content and on the adsorbate properties like molecular size, solubility, pK_a, and the presence of aromatic structures (Moreno-Castilla, 2004). The correlation observed between the OTA removal and V_{meso} of activated carbons shows that as expected, the adsorption capacity will depend on the accessibility of OTA to the inner surface of the adsorbent, which depends on its size and competition with other components present for the pores available. At the wine pH (pH of 3.14 and 3.49 for white and red wines respectively) OTA is mainly present in the neutral form (4.2–4.4 and 7.0–7.3, the carboxyl group and the phenolic hydroxyl group, respectively, (Kőszegi & Poór, 2016), presents a high log K_{ow} (4.74) (http://www.hmdb.ca/metabolites/HMDB0029399), therefore, very low solubility in water and its structure contains two aromatic groups being expected that non-electrostatic

interactions essentially due to dispersion and hydrophobic interactions are responsible for the adsorption of OTA on activated carbons (Moreno-Castilla, 2004).

When compared to other alternative OTA removal treatments applied to wine described in the literature, the use of ACs with optimal structural features, like those presented by AC3 used in this work, show better results when compared to wine filtration through a 0.45 μm membrane where an 80% OTA reduction was observed (Gambuti et al., 2005) or repassage of contaminated wines over grape pomaces where a reduction up to 65% of OTA could be obtained (2–10 μg/kg OTA contamination level). Also, the use of *Pediococcus parvulus* UTAD 473 for OTA biodegradation in wines was not possible (Abrunhosa et al., 2014).

3.2. Effect of ACs on wine chromatic characteristics, total phenolic compounds, flavonoid, non-flavonoid and individual phenolic compounds

To study the impact of the application of the different ACs on wine quality after OTA removal, the wine colour and chromatic properties were measured for white (Table 1) and red (Table 2) wines. White wine colour (Abs 420 nm) decreased significantly with the application of ACs, with C3 and C7 being the ACs that resulted in white wine with lower colour (Table 1). This decrease in wine colour correlated with the decrease in total phenols and non-flavonoid phenols (Table S.2). The treatment of white wine with ACs resulted in a colour difference (ΔE^*) value of 1.2 for C4, a value that is not perceptible to the human eye (Spagna, Barbagallo, & Pifferi, 2000), but for the other ACs all treated wines presented a colour difference higher than 2, and ACs C3 and C7 presented a colour difference higher than 4. Nevertheless, for white wines, the removal of the brown-yellow colours is considered a positive effect from a sensory point of view and therefore the use of ACs for OTA removal in white wines will not have a negative impact on white wine colour. On the other hand for red wines, although all ACs were intended for wine deodorisation, there was observed a significant decrease in the colour intensity with the application of ACs, with a higher decrease observed for ACs C3 and C7 (Table 2). This decrease was correlated with the decrease in total phenols, flavonoid phenols, total anthocyanins, coloured anthocyanins and polymeric pigments (Table S.1). The application of ACs in red wine resulted in colour differences (ΔE^*) higher than 1 (Gonnet, 1998) for all ACs and for the ACs C3 and C7 the colour difference was the highest ($\sim$7). Although these colour differences can be probably detected by the human eye and considered negative by the consumers, the colour intensity of wines only decreased by 24%.

The application of ACs on white wines resulted in an overall decrease in phenolic compounds (obtained by the sum of all determined phenolic compounds) from 36 mg/L (C4) to 63 mg/L (C7). The most affected phenolic compound in white wines was *trans*-caftaric acid, followed by coutaric acid that together corresponds to 41% (C4) to 62% (C3) of all removed phenolic compounds. The removal extent of the different phenolic acids and catechin observed by the application of ACs were related to their concentration in white wine (Fig. S.2.). As can be observed in Fig. 2, for white wines, the removal of *trans*-caftaric acid correlated with the V_{meso} and total volume of pores (V_p). For all the other phenolic compounds, including coutaric acid, their removal correlated with V_p.

For red wines the phenolic compounds removed by ACs was smaller than that observed for the white wines and ranged from 25 mg/L (C4) to 50 mg/L (C3), nevertheless, this decrease included the phenolic acids and catechin that were removed in smaller amount 11 mg/L (C2) to 24 mg/L (C1), and the amount of anthocyanins removed ranged from 8 mg/L (C4) to 33 mg/L (C7). Again, for red wines, the removal extent of phenolic acids, catechin, and anthocyanins observed by the application of ACs was related to their concentrations in red wine (Fig. S.3 and Fig. S.4.). Although the white wine used in this study presented a higher concentration of phenolic acids and catechin when compared to red wines (+44%) the decrease in phenolic acids and catechin observed for the red wine was 114% lower compared to that observed for white wines. Therefore, these results suggest that the presence of anthocyanins in the red wine matrix change significantly the phenolic acids and catechin removal capacity of the ACs. C1 and C4 were the ACs where the amount of phenolic acids removed were higher than the amount of anthocyanins removed, these being also the ACs with higher V_{micro}. For the other ACs, especially for C3, C6 and C7, the amount of anthocyanins removed was higher than the amount of phenolic acids removed. This change in removal profile is related to the different physicochemical characteristics of these ACs. As can be seen in Fig. 3, the removal of anthocyanins was related to V_{meso} and V_p and in red wine, also the removal of the phenolic acids was related to micropore volume (V_{micro}). There was a change in the removal behaviour of *trans*-caftaric acid that in red wine, contrarily to white wine, was also correlated to V_{micro} and not to V_{meso}. This can be explained by the competition of anthocyanins present in higher concentration in red wines to the ACs mesopores. This competition lowers the removal of *trans*-caftaric acid by the mesopores and therefore its removal in red wine is dependent on the lower sized pores present in the ACs. This competition mechanism of anthocyanins in red wines for the mesopores of ACs can also explain the lower efficiency of OTA removal in red wine when compared to the white wine.

For ethical reasons, no sensory analysis was performed in the final wines after treatment with different ACs. Nevertheless, for red wines, it was previously shown that for those activated carbons resulting in a significant decrease in anthocyanins (C5, C6, C7), expert tasters were able to detect the colour differences in relation to the untreated wine (Filipe-Ribeiro et al., 2017a, 2017b). This was the only parameter shown to be negatively affected by the expert panel.

3.3. Effect of pore volume distribution of ACs on OTA, anthocyanins, and phenolic acids removal efficiency

Although V_{meso}, and V_p can explain a significant amount of the variation of OTA, anthocyanins and phenolic acids removal by ACs, a closer look at the results, especially those obtained for ACs C3 and C7, where they present similar V_{meso} but different removal performances, indicates that a different pore volume distribution present in these ACs can have an impact on their removal performance. To explore this hypothesis, the volume distribution of different sized pores was determined by the density functional theory (DFT) method, and the pore volume distribution is shown in Fig. 4a. As can be observed, ACs presented a different distribution of pore volumes showing two maxima, one below a half-pore width of 9 Å and a second local maximum at half-pore width of 17–19 Å. ACs C3 and C7 are those presenting a more similar pore volume distribution when compared to the other ACs used, nevertheless, presenting some differences, especially in the half-pore size range from 17 to 75 Å (Fig. 4b).

To have a deeper understanding of the effect of the pore volume distribution on the efficiency of the AC in the removal of OTA and also on the removal of anthocyanins, phenolic acids, and catechin, a Partial Least Squares (PLS1) regression of the standardised removal efficiency of OTA, anthocyanins and phenolic acids and catechin (Y) on the standardised pore volume distribution of ACs (X) was performed. The number of factors for each dependent variable analysed, estimated by internal cross-validation (leave-one-out procedure) are shown in Table S.3 and Fig. 5. Fig. 6a to Fig. 6d show the B coefficients vector plot in which it is possible to characterise the most important pore sizes for prediction of OTA, anthocyanins, phenolic acids, and catechin removal, either in white or red wines. These coefficients are used to predict the removal efficiency of ACs from the explanatory variables (pore volume distribution) and can be thought of like the directions in the explanatory variable space that result in the largest increase in the dependent variable (ACs removal efficiency). The regression curves obtained for each variable are presented in Fig. 5 a-d. For the OTA removal, 96.5% of the variance in the five PLS components related to the pore volume distribution of ACs explained 99.7% of the removal efficiency of ACs (Table S.3). Fig. 6a shows that different sized pores are essential for

describing the OTA removal efficiency, either lower sized pores of below a half-pore width of 10 Å, but also pores in the range of 12–73 Å and 120 to 137 Å. ACs C3 and C7 contain a high volume of these pore sizes (Fig. 4a).

For the anthocyanins removal, 88.4% of the variance in the three first PLS components related to the pore volume distribution of ACs explained 98.1% of the removal efficiency of ACs (Table S.3). For anthocyanins removal, lower sized pores of half-pore width of 7.7 Å, but also pores in the range of 12–17 Å, 21 to 41 Å, and 61 to 120 Å are important for their removal. Anthocyanins removal and OTA removal share much of the same pores in the range 12.6–23.8 Å, 31.1–40.8 Å, 58.4–69.9 Å, explaining the lower efficiency of ACs for OTA removal in red wines as anthocyanins are present in a much higher amount than OTA. This is in accordance with the predicted molecular volume of OTA and anthocyanins (Table S.4). Nevertheless, anthocyanin removal also presents some unique regions at 24.9–29.7 Å and 73.2 to 120.1 Å. On the other hand, the B coefficients obtained for OTA removal also show some non-overlapping regions between 42.6 and 55.9 Å and 125.6 to 137.4 Å. In fact, when we compare the pore volume distribution between C3 and C7 (Fig, 4b), we can observe that these are the regions where AC C3 presents a higher abundance of pore volume when compared to C7, and therefore it can be inferred that this fine structure of C3, when compared to C7, is the main reason for the higher efficiency of OTA removal in red wine matrix. Tables 3–5.

For the phenolic acids and catechin present in red and white wines, the pore sizes showing a stronger relationship with their removal are the lowest sized pores (Fig. 6c and d), this result also being in accordance with the lower predicted molecular volume of phenolic acids and catechin when compared to OTA and anthocyanins (Table S.4).

4. Conclusion

The results obtained in this work showed for the first time that ACs, within the authorised application levels of the European legislation (606/2009) for wine use, and with adequate structural characteristics can eliminate OTA in white and red wines. The structural characteristics of ACs needed for this complete OTA removal in wines are more critical when applied to red wines when compared to white wines, due to the presence of anthocyanins in the red wine matrix that competes with OTA for the mesopores present in the AC structure. Nevertheless, the presence of a high abundance of pores with a half-pore width between 42.6 and 55.9 Å and 125.6 to 137.4 Å, are important for efficient OTA removal in the presence of anthocyanins. For white wines, there was observed an improvement of the chromatic characteristics due to a decrease in the yellowness-brownish colour. For the red wine, the impact on chromatic characteristics was more intense; nevertheless, the highest amount of colour reduced was only 24% of the initial colour intensity. Nevertheless, the production of ACs with pore sizes in the range between 42.6 and 55.9 Å and 125.6 to 137.4 Å could allow to remove OTA without significant anthocyanin competition and at the same time to lower the amount of ACs needed for complete OTA removal and therefore with a lower impact on red wine colour. Therefore, the selection of ACs with proper pore size distribution is a very promising solution for mitigation of the safety risk due to the common presence of this mycotoxin in wines, even for high levels of OTA.

Conflict of interest statement

The authors have no conflit of interest to declare.

CRediT authorship contribution statement

Fernanda Cosme: Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition. **António Inês:** Writing - review & editing, Supervision, Project administration, Funding acquisition. **Davide Silva:** Formal analysis, Investigation. **Luís Filipe-Ribeiro:** Formal analysis, Writing - original draft, Writing - review & editing. **Luís Abrunhosa:** Formal analysis, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Fernando M. Nunes:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing, Supervision.

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Appendix A. Supplementary data

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References

Abrunhosa, L., Inês, A., Rodrigues, A. I., Guimarães, A., Pereira, V. L., Parpot, P., et al. (2014). Biodegradation of ochratoxin A by *Pediococcus parvulus* isolated from Douro wines. *International Journal of Food Microbiology, 188*, 45–52. https://doi.org/10.1016/j.ijfoodmicro.2014.07.019

Abrunhosa, L., Santos, L., & Venâncio, A. (2006). Degradation of Ochratoxin A by proteases and by a crude enzyme of *Aspergillus niger*. *Food Biotechnology, 20*, 231–242. https://doi.org/10.1080/08905430600904369

Amézqueta, S., González-Peñas, E., Murillo-Arbizu, M., & de Cerain, L. A. (2009). Ochratoxin A decontamination: A review. *Food Control, 20*, 326–333.

Bellver Soto, J., Fernandez-Franzon, M., Ruiz, M. J., & Juan-Garcia, A. (2014). Presence of ochratoxin A (OTA) mycotoxin in alcoholic drinks from southern European countries: Wine and beer. *Journal of Agricultural and Food Chemistry, 62*, 7643–7651.

Blesa, J., Soriano, J. M., Molto, J. C., & Manes, J. (2006). Factors affecting the presence of Ochratoxin A in wines. *Critical Reviews in Food Science and Nutrition, 46*, 473–478.

Castellari, M., Versari, A., Fagiani, A., Parpinello, G. P., & Galassi, S. (2001). Removal of ochratoxin A in red wines by means of adsorption treatments with commercial fining agents. *Journal of Agricultural and Food Chemistry, 49*, 3917–3921.

Chen, W., Li, C., Zhang, B., Zhou, Z., Shen, Y., Liao, X., et al. (2018). Advances in biodetoxification of ochratoxin A-A review of the past five decades. *Frontiers in Microbiology, 9*, 1386.

Commission Regulation. (2006). Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (consolidated version from 12/12/2014). *Off. J. Eur. Union, 2014*, L364/5–L364/24.

Commission Regulation. (2009). Commission regulation (EC) No 606/2009 of 10 July 2009 laying down certain detailed rules for implementing council regulation (EC) No 479/2008 as regards the categories of grapevine products, oenological practices and the applicable restrictions. *Off. J. Eur. Union*, 1–59, 606/20(No 479/2008). Brussels: Commission Regulation.

Csutorás, C., Rácz, L., Rácz, K., Futo, P., Forgó, P., & Kiss, A. (2013). Monitoring of Ochratoxin A during the fermentation of different wines by applying high toxin concentrations. *Microchemical Journal, 107*, 182–184. https://doi.org/10.1016/j.microc.2012.07.001

Del Prete, V., Rodriguez, H., Carrascosa, A. V., De Las Rivas, B., Garcia-Moruno, E., & Munoz, R. (2007). In vitro removal of ochratoxin A by wine lactic acid bacteria. *Journal of Food Protection, 70*, 2155–2160.

Dobritzsch, D., Wang, H., Schneider, G., & Yu, S. (2014). Structural and functional characterization of ochratoxinase, a novel mycotoxin-degrading enzyme. *Biochemical Journal, 462*, 441–452. https://doi.org/10.1042/bj20140382

Espejo, F. J., & Armada, S. (2009). Effect of activated carbon on ochratoxin A reduction in "Pedro Ximenez" sweet wine made from off-vine dried grapes. *European Food Research and Technology, 229*, 255–262.

Filipe-Ribeiro, L., Milheiro, J., Matos, C. C., Cosme, F., & Nunes, F. M. (2017a). Reduction of 4-ethylphenol and 4-ethylguaiacol in red wine by activated carbons with different physicochemical characteristics: Impact on wine quality. *Food Chemistry, 229*, 242–251.

Filipe-Ribeiro, L., Milheiro, J., Matos, C. C., Cosme, F., & Nunes, F. M. (2017b). Data on changes in red wine phenolic compounds, headspace aroma compounds and sensory

profile after treatment of red wines with activated carbons with different physicochemical characteristics. *Data in Brief, 12*, 188–202.

Galvano, F., Pietri, A., Bertuzzi, T., Piva, A., Chies, L., & Galvano, M. (1998). Activated carbons: In vitro affinity for ochratoxin A and deoxynivalenol and relation of adsorption ability to physicochemical parameters. *Journal of Food Protection, 61*, 469–475.

Gambuti, A., Strollo, D., Genovese, A., Ugliano, M., Ritieni, A., & Moio, L. (2005). Influence of enological practices on ochratoxin A concentration in wine. *American Journal of Enology and Viticulture, 56*, 155–162.

Gil-Serna, J., Vazquez, C., Gonzalez-Jaen, M. T., & Patino, B. (2018). Wine contamination with ochratoxins: A review. *Beverages, 4*, 1–21.

Gonnet, J. F. (1998). Colour effects of co-pigmention of anthocyanins revisited-1. A colorimetric definition using the CIElab scale. *Food Chemistry, 63*, 409–415.

Guise, R., Filipe-Ribeiro, L., Nascimento, D., Bessa, O., Nunes, F. M., & Cosme, F. (2014). Comparison between different types of carboxylmethylcellulose and other oenological additives used for white wine tartaric stabilization. *Food Chemistry, 156*, 250–257.

Hathout, A. S., & Aly, S. E. (2014). Biological detoxification of mycotoxins: A review. *Annals of Microbiology, 64*, 905–919.

Heussner, A., & Bingle, L. (2015). Comparative ochratoxin toxicity: A review of the available data. *Toxins, 7*, 4253–4282.

IARC. (1993). *Monographs on the evaluation of carcinogenic risks to humans* (Vol. 56). Geneva. Switzerland: IARC.

Karanfil, T. (2006). Activated carbon adsorption in drinking water treatment. In *Activated carbon surfaces in environmental remediation* (pp. 345–373) (Elsevier Ltd).

Kramling, T. E., & Singleton, V. L. (1969). An estimate of the nonflavonoid phenols in wines. *American Journal of Enology and Viticulture, 20*, 86–92.

Kőszegi, T., & Poór, M. (2016). Ochratoxin A: Molecular interactions, mechanisms of toxicity and prevention at the molecular level. *Toxins, 8*(4), 111. https://doi.org/10.3390/toxins8040111

Liuzzi, V. C., Fanelli, F., Tristezza, M., Haidukowski, M., Picardi, E., Manzari, C., et al. (2017). Transcriptional analysis of *Acinetobacter* sp. *neg1* capable of degrading Ochratoxin A. *Frontiers in Microbiology, 7*, 2162. https://doi.org/10.3389/fmicb.2016.02162

Loi, M., Fanelli, F., Liuzzi, V. C., Logrieco, A. F., & Mulè, G. (2017). Mycotoxin biotransformation by native and commercial enzymes: Present and future perspectives. *Toxins, 9*, 111. https://doi.org/10.3390/toxins9040111

Mehmood, T., Liland, K. H., Snipen, L., & Sæbø, S. (2012). A review of variable selection methods in Partial Least Squares Regression. *Chemometrics and Intelligent Laboratory Systems, 118*, 62–69.

Moreno-Castilla, C. (2004). Adsorption of organic molecules from aqueous solutions on carbon materials. *Carbon, 42*, 83–94.

OIV. Organisation International de la Vigne et du Vin. (2015). *Récueil de Méthodes Internationales d'Analyse des Vins et des Moûts*. Paris: Edition Offlcielle.

Olivares-Marín, M., Del Prete, V., Garcia-Moruno, E., Fernández-González, C., Macías-García, A., & Gómez-Serrano, V. (2009). The development of an activated carbon from cherry stones and its use in the removal of ochratoxin A from red wine. *Food Control, 20*, 298–303.

Oteiza, J. M., Khaneghah, A. M., Campagnollo, F. B., Granato, D., Mahmoudi, M. R., Sant'Ana, A. S., et al. (2017). Influence of production on the presence of patulin and ochratoxin A in fruit juices and wines of Argentina. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 80*, 200–207.

Petruzzi, L., Sinigaglia, M., Corbo, M. R., Campaniello, D., Speranza, B., & Bevilacqua, A. (2014). Decontamination of ochratoxin A by yeasts: Possible approaches and factors leading to toxin removal in wine. *Applied Microbiology and Biotechnology, 98*(15), 6555–6567.

Piotrowska, M. (2014). The adsorption of ochratoxin A by *Lactobacillus* species. *Toxins, 6*(9), 2826–2839. https://doi.org/10.3390/toxins6092826

Quintela, S., Villarán, M. C., de Armentia, I. L., & Elejalde, E. (2013). Ochratoxin A removal in wine: A review. *Food Control, 30*, 439–445.

Quintela, S., Villarán, M. C., de Armentia, I. L., Pisters, R., Lane, D. A., & Elejalde, E. (2012). Ochratoxin A removal from red wine by several oenological fining agents: Bentonite, egg albumin, allergen-free adsorbents, chitin and chitosan. *Food Additives & Contaminants, 29*, 1168–1174.

Ribéreau-Gayon, P., Peynaud, E., & Sudraud, P. (1982). *Science et Techniques du Vin. Tome* (Vol. 4). Paris: Dunod.

Silva, L. J. G., Rodrigues, A. P., Pereira, A. M. P. T., Lino, C. M., & Pena, A. (2019). Ochratoxin A in the Portuguese wine market, occurrence and risk assessment. *Food Additives & Contaminants, Part B Surveillance, 12*, 145–149.

Solfrizzo, M., Avantaggiato, G., Panzarini, G., & Visconti, A. (2010). Removal of ochratoxin A from contaminated red wines by repassage over grape pomaces. *Journal of Agricultural and Food Chemistry, 58*, 317–323.

Somers, T. C., & Evans, M. E. (1977). Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO_2, "Chemical age". *Journal of the Science of Food and Agriculture, 28*, 279–287.

Spagna, G., Barbagallo, R. N., & Pifferi, P. G. (2000). Fining treatments of white wines by means of polymeric adjuvants for their stabilization against browning. *Journal of Agricultural and Food Chemistry, 48*, 4619–4627.

Sun, X., Niu, Y., Ma, T., Xu, P., Huang, W., & Zhan, J. (2017). Determination, content analysis and removal efficiency of fining agents on ochratoxin A in Chinese wines. *Food Control, 73*, 382–392.

Ubeda, C., Hornedo-Ortega, R., Cerezo, A. B., & Troncoso, A. M. (2020). Chemical hazards in grapes and wine, climate change and challenges to face. *Food Chemistry, 314*, 126222.

Var, I., Kabak, B., & Erginkaya, Z. (2008). Reduction in ochratoxin A levels in white wine, following treatment with activated carbon and sodium bentonite. *Food Control, 19*, 592–598.

Vinzi, V. E., Chin, W. W., Henseler, J., & Wang, H. (2010). *Handbook of partial least squares*. Heidelberg: Concepts, Methods and Applications Springer-Verlag Berlin.

Visconti, A., Pascale, M., & Centonze, G. (1999). Determination of ochratoxin A in wine by means of immunoaffinity column clean-up and HPLC. *Journal of Chromatography A, 864*, 89–101.

Zimmerli, B., & Dick, R. (1996). Ochratoxin A in table wine and grape-juice: Occurrence and risk assessment. *Food Additives & Contaminants, 13*, 655–668.