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Polymeric substances for the removal of ochratoxin A from red wine followed by computational modeling of the complexes formed

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ABSTRACT

Ochratoxin A (OTA) is a mycotoxin produced by filamentous-type fungi that contaminates a wide variety of foods and beverages such as wines. In these trials, we evaluated the capacity of the following polymers for the removal of OTA from acidic model solutions and red wine: polyvinylpolypyrrolidone (PVPP), resin of N-vinyl-2-pyrrolidinone with ethylene glycol dimethacrylate and triallyl isocyanurate (PVP-DEGMA-TAIC), and poly(acrylamide-co-ethylene glycol-dimethacrylate) (PA-EGDMA). In acidic model solution, PVP-DEGMA-TAIC and PA-EGDMA polymers removed up to 99.9% of OTA, but their trapping capacity was highly reduced by the presence of competing phenolic substances (i.e. gallic acid and 4-methylcathecol). In real red wine, PA-EGDMA polymer showed the most promising results, with more than 68.0% OTA removal and less than 14.0% reduction in total phenolic. Finally, computational chemistry analyses showed that the affinity between OTA and the polymers studied would be due to Van der Waals interactions.

1. Introduction

Ochratoxin A (OTA), is one of the most commonly occurring mycotoxins found in foods and beverages including cereals (Lee & Ryu, 2015), beer (Soto, Fernandez-Franzon, Ruiz, & Juan-García, 2014), coffee beans (Taniwaki, Teixeira, Teixeira, Copetti, & Lamanaka, 2014), spices (Ainiza, Jinap, & Sanny, 2015), grapes (Battilani, Giorni, Bertuzzi, Formenti, & Pietri, 2006), juices (Marino, Nostro, & Fiorentino, 2009) and wines (Vega et al., 2012; Khoury et al., 2006; Chiodini, Acherpenisse, & Bergwerff, 2006). This molecule is produced by filamentous fungi of the genus *Aspergillus and Penicillium*, and some of the main responsible species are *Aspergillus ochraceus* (Harris & Mantle, 2001), *Aspergillus niger* (Blumenthal, 2004), *Aspergillus carbonarius* (Horie, 1995), and *Penicillium verrucosum* (Geisen, Mayer, Karolewiez, & Farber, 2004).

Different biological studies have demonstrated that OTA is nephrotoxic, hepatotoxic, neurotoxic, immunotoxic and teratogenic (Marin-Kuan, Cavin, Delatour, & Schilter, 2008), and the International Agency for Research on Cancer has classified this mycotoxin as possibly carcinogenic for humans in the group 2B (IARC, 1993).

Numerous studies have reported the presence of OTA in wines from different counties (Rosa, Magnoli, Fraga, Dalcero, & Santana, 2004; Berente et al., 2005; Chiodini et al., 2006; Vega et al., 2012), thus possibly suggesting a worldwide occurrence of this problem (Quintela, Villarán, López de Armentia, & Elejalde, 2013). Red wines have higher concentrations of OTA than rosé and white wines, most likely due to the longer skin contact occurring during red wine processing, thus enhancing OTA extraction to the musts (Soto et al., 2014; Visconti, Pascale, & Centonze, 2001). The Regulatory Commission of the European Community has established that the level of OTA in commercial wines cannot exceed $2\,\mu g\,kg^{-1}$ (EC, 2006). However, many trade agreements require lower limits than those adopted by the regulation and some of these agreements require that OTA not exceed $0.5\,\mu g\,kg^{-1}$ (Solfrizzo, Avantaggiato, Panzarini, & Visconti, 2010).

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Several strategies have been proposed for the removal of OTA from wines, including biological, physical and chemical methods (Solfrizzo et al., 2010; Castellari, Versari, Fabiani, Parpinello, & Galassi, 2001; Quintela, Villarán, López de Armentia, & Elejalde, 2012; Piotrowska, Nowak, & Czyzowska, 2013), such as the use of fining agents like sodium bentonite, egg albumin, polyvinylpolypyrrolidone (PVPP), gelatin, chitin, chitosan, kaolinite, carbon, celite, silica gel, zeolite, cellulose, carrageenan, and pectin (Quintela et al., 2012; Castellari et al., 2001). Although some of them could be quite effective, they can also remove a variety of desired wine substances including volatiles and phenolic compounds (Quintela et al., 2012; Castellari et al., 2001; Piotrowska, Nowak, & Czyzowska, 2013).

Recently, the use of polymeric adsorbents has gained increased interest given that their structures can be synthetically modified to achieve molecules with more specificity in their trapping capabilities. For instance, in previous works we have analyzed the ability of various polymers to remove undesired substances in wine (Marican, Carrasco-Sánchez, John, Laurie, & Santos, 2014; Carrasco-Sánchez, John, Marican, Santos, & Laurie, 2015). Moreover, in a recently published work we tested PVPP, a resin of *N*-vinyl-2-pyrrolidinone with ethylene glycol dimethacrylate and triallyl isocyanurate (PVP-DEGMA-TAIC), and poly(acrylamide-co-ethylene glycol-dimethacrylate) (PA-EGDMA) for the removal of fumonisins B1 and B2 (FB1 and FB2) from red wines (Carrasco-Sánchez, Kreitman, Folch-Cano, Elias, & Laurie, 2017).

Given the positive results of these later trials, and the molecular characteristics of OTA, we hypothesize that these polymers, particularly PA-EGDMA would be good alternatives for the removal of OTA from red wines. Therefore, we investigated the ability of PVPP, PVP-DEGMA-TAIC and PA-EGDMA to remove OTA from model solutions and red wine. Additionally, we employed computational chemistry to determine the atomic level interactions underlying polymer–OTA complex formation.

2. Materials and methods

2.1. Reagents and solutions

Ochratoxin A (OTA) obtained from *Petromyces albertensis* (≥98.0%; crystalline powder), gallic acid (GA, ≥95.0%), and 4-methylcatechol (4-MC, ≥95.0%) were purchased as a form Sigma-Aldrich (St. Louis, MO, USA). Ethanol (≥99.5%), acetonitrile HPLC-grade (ACN, ≥98.0%), Folin-Ciocalteu's phenol reagent (≥99.5%; 2 N), L(+)-tartaric acid (≥99.5%), sodium hydroxide (≥99.0%), ultra-pure water for chromatography, and polytetrafluoroethylene (PTFE) membrane filters (0.45 μm) were acquired from Merck KGaA (Darmstadt, Germany). QuEChERS extraction kits (Salts: Sodium citrate 1 g, 99.9%; Disodium citrate sesquihydrate 0.5 g, 99.0%; Magnesium sulfate 4 g, ≥98.5% and Sodium chloride 1 g, ≥99.5%; and dispersive solid-phase extraction tubes, 900 mg $MgSO_4$ and 150 mg primary secondary amine (PSA) sorbent), were purchased from Agilent Technologies (Santa Clara, CA, USA).

2.1.1. Polymeric compounds

Polyvinylpolypyrrolidone (PVPP; Molecular weight 25,000–30,000) polymer was purchased from Sigma Aldrich (St. Louis, MO, USA). Resins of copolymerization of *N*-vinyl-2-pyrrolidinone with ethylene glycol dimethacrylate and triallyl isocyanurate (PVP–DEGMA–TAIC) were obtained as proposed by Zhao, Yan, Li, and Yan (2008). Poly (acrylamide-co-ethylene glycol-dimethacrylate) (PA-EGDMA) polymers were synthetized according to the methodology proposed by Lu et al. (2010).

2.1.2. Acidic model solution

The model solution used for the initial testing was made of a 12.5% ethanol solution (v/v), adjusted to pH 3.4 using tartaric acid (5.0 g L^{-1}) and a 1 mM solution of sodium hydroxide. Further trials were performed using a similar model solution containing also GA (1.0 g L^{-1}) and 4-MC (1.0 g L^{-1}) (Marican et al., 2014).

Table 1
Ochratoxin A (OTA) removal from an acidic hydroalcoholic solution using PVPP, PVP-DEGMA-TAIC and PA-EGDMA polymers. Results expressed as percentage of removal (%) ± standard deviation, SD (n = 3). *Same letters vertically indicates no statistically differences by Tukey HSD, at 95% level of confidence.

Polymer	Dose(mg mL^{-1})	OTARetention (%) ± SD		
		2 h	8 h	24 h
PVPP	5	6.16 ± 1.37a	12.7 ± 0.52a	22.7 ± 0.83a
	10	11.36 ± 0.40b	22.02 ± 0.14b	32.06 ± 0.19b
PVP-DEGMA-TAIC	5	99.9 ± 0.01c	99.9 ± 0.01c	99.9 ± 0.01c
	10	99.9 ± 0.01c	99.9 ± 0.01c	99.9 ± 0.01c
PA-EGDMA	5	99.9 ± 0.01c	99.9 ± 0.01c	99.9 ± 0.01c
	10	99.9 ± 0.01c	99.9 ± 0.01c	99.9 ± 0.01c

2.1.3. OTA standard

1000 μg of OTA was dissolved in 1.0 mL acetonitrile and left overnight at room temperature to ensure complete dissolution of the OTA crystals.

2.1.4. Working solution of OTA

100 mL of acid model solution was spiked with 500 μL of OTA standard (1000 μg mL^{-1}) up to a final concentration of 5000 μg L^{-1}. This concentration, higher than what is commonly found in wines, was used to test the retention capacity of the three different polymers. This solution was freshly prepared, kept refrigerated, and brought to room temperature before use.

2.2. Affinity between PVPP, PVP–DEGMA–TAIC and PA-EGDMA polymers towards ochratoxin A in acidic model solution

First, a screening study was conducted in order to determine the affinity of PVPP, PVP–DEGMA–TAIC and PA-EGDMA polymers towards OTA (5000 μg L^{-1}) in an acid model solution (described in Section 2.1.2), at different doses and contact times, based on our previous work (Carrasco-Sánchez et al., 2015, 2017).

The OTA removal assays were performed as follows: each dose of the polymers tested (5.0 or 10.0 mg) was individually weighed into 12 mL glass tubes, followed by the addition of 1.0 mL of acidic model solution, spiked with 5000 μg L^{-1} of OTA. The samples were mixed and agitated for 2, 8 and 24 h at room temperature (20 °C), using a rock motion agitator, operating at 100 rpm. Then, the samples were filtered through a 0.45 μm PTFE membrane and the concentration of free OTA was analyzed using high-performance liquid chromatography with fluorescence detector (HPLC-FLD), as explained later (See point 2.4.1).

An OTA positive control (5000 μg L^{-1} in acid model solution) in the absence of polymers, and a negative control (polymeric substances suspended in model solution without mycotoxin) were also incubated at the same time points to monitor the efficacy of the adsorbents in the binding OTA. These samples were also filtered with 0.45 μm PTFE membranes prior to their analyses. Moreover, all sets of samples and affinity experiments were carried out in triplicate.

2.3. Affinity of the polymers towards OTA in acid model solutions containing gallic acid and 4-methylcatecol

Given the results obtained in the screening study (data shown below), the concentration of the polymers tested was 2.5 and 5 mg mL^{-1}, with 8 h of contact time. The aim of this trial was to determine whether the removal capacity of OTA was affected by the presence of polyphenols. Therefore, gallic acid (1 g L^{-1}) and 4-