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Removal of fumonisin B_1 and B_2 from model solutions and red wine using polymeric substances

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ABSTRACT

Fumonisins are a group of mycotoxins found in various foods whose consumption is known to be harmful for human health. In this study, we evaluated the ability of three polymers (Polyvinylpolypyrrolidone, 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) to remove fumonisin B_1 (FB1) and fumonisin B_2 (FB2) from model solutions and red wine. Various polymer concentrations (1, 5 and 10 mg mL^{-1}) and contact times (2, 8 and 24 h) were tested, with all polymers exhibiting fumonisin removal capacities (monitored by LC-MS). The impact of all polymers on polyphenol removal was also assessed. PA-EGDMA showed to be the most promising polymer, removing 71% and 95% of FB_1, and FB_2, respectively, with only a 22.2% reduction in total phenolics.

1. Introduction

Fumonisins are mycotoxins consisting of a 19- to 20-carbon aminopolyhydroxyalkyl chain that is diesterified with propane-1,2,3-tricarboxylic acid groups (tricarballylic acid) (Pietri & Bertuzzi, 2012). To date, four groups of fumonisins have been identified and are referred to as A, B, C, and P series (Bezuidenhout et al., 1988; Rheeder, Marasas, & Vismer, 2002). Among them, fumonisins B1 (FB_1) (Fig. 1A), B2 (FB_2) (Fig. 1B) and B3 (FB_3) are the most abundant (Shephard, Thiel, Stockenstrom, & Sydenham, 1996).

Fumonisins have been found to be hepatotoxic and nephrotoxic in various animals (Haschek, Gumprecht, Smith, Tumbleson, & Constable, 2001; Voss, Smith, & Haschek, 2007; Wild & Gong, 2010). They have also been associated with the occurrence of human esophageal cancer (Gelderblom, Kriek, Marasas, & Thiel, 1991), and for that reason the International Agency for Research on Cancer has categorized FB_1 as a group 2B carcinogenic substance (IARC, 2002). Therefore, the World Health Organization (WHO) has recommended a provisional maximum tolerable daily intake of 2 μg kg^{-1} body/weight/day for FB_1, FB_2 and FB_3, independently or combined (WHO, 2002).

To date, fumonisins are found mainly in corn-based foods (Sydenham, Shephard, Thiel, Marasas, & Stockenstrom, 1991); however, FB_2 has been detected in grapes, raisins, must, and wines in recent years (Knudsen, Mogensen, Larsen, & Nielsen, 2010; Logrieco et al., 2009; Mogensen, Frisvad, Thrane, & Nielsen, 2010a). The fungus *Aspergillus niger*, commonly found in grains, and periodically on the surfaces of grapes (Hocking, Leong, Kazi, Emmett, & Scott, 2007), has been associated with the production of FB_2, FB_4 and FB_6 (Abrunhosa, Calado, & Venancio, 2011; Frisvad, Smedsgaard, Samson, Larsen, & Thrane, 2007; Logrieco, Ferracane, Visconti, & Ritieni, 2010; Mogensen et al., 2010a). FB_2 represents approximately 73% of the total fumonisins produced by *A. niger* (Varga et al., 2010).

One of the first studies to report fumonisins in wine analyzed 77 samples from 13 different countries, 23% of which tested positive for FB_2 in the range of 1–25 μg L^{-1} (Mogensen, Larsen, & Nielsen, 2010b). Another study focusing on the presence of FB_2 and FB_4 in 51 Italian wines (45 red wines, five white wines, one rosé) found FB_2 in nine red samples ranging from 0.4 to 2.4 ng mL^{-1}, yet no FB_4 was detected (Logrieco et al., 2010). More recently, FB_1, FB_2, and FB_3 were found in red or white wines (Tamura, Takahashi, Uyama, & Mochizuki, 2012).

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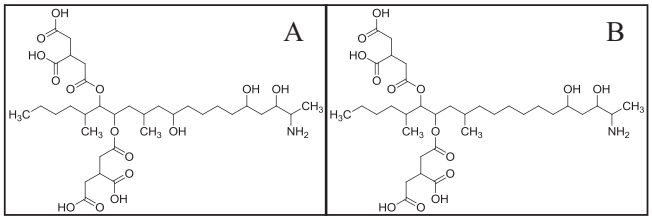

Fig. 1. Chemical structures of (A) fumonisin B_1 and (B) fumonisin B_2.

Given the potential significance of mycotoxins in wine quality, the aim of this study was to test the ability of three polymeric substances (PVPP, PVP-DEGMA-TAIC and PA-EGDMA), acting as clarifying agents, for the selective removal of FB_1 and FB_2 from wine.

2. Materials and methods

2.1. Solvents and reagents

Fumonisin B1 (FB_1) (96%) was obtained from Acros Organic (New Jersey, USA). Fumonisin B2 (FB_2) (⩾96% from *Fusarium moniliforme*), gallic acid (GA) (97.5–102.5%) and 4-methylcatecol (4-MC) (⩾95%) were obtained from Sigma-Aldrich, Co. (St. Louis, USA). For the mass spectrometry (MS) analyses, the following reagents were used: acetonitrile (ACN) (LC-MS grade) (⩾99.9%) and water (LC-MS grade) were purchased from Avantor performance materials, Inc. (USA), methanol (LC/MS grade) was obtained from Honeywell International Inc. (MI, USA) and formic acid Optima® LC/MS grade was purchased from Fisher Scientific (New Jersey, USA). Milli-Q (Millipore (Bedford, USA) water (18.2 mΩ) was used to prepare model wine solutions. Sodium carbonate anhydrous (>99.5%) was purchased from Mallinckrodt Baker, Inc. (Phillipsburg, USA), and ethanol (⩾99.5%) was purchased from Merck (Darmstadt, Germany).

2.1.1. Polymers

The polymers used in this study were PVPP polymers, resins of copolymerization of *N*-vinyl-2-pyrrolidinone with ethylene glycol dimethacrylate and triallyl isocyanurate (PVP–DEGMA–TAIC) (Zhao, Yan, Li, & Yan, 2008) and poly(acrylamide-co-ethylene glycol-methacrylate) (PA-EGDMA) polymers. PVP–DEGMA–TAIC and PA-EGDMA polymers were synthetized according to the methodology proposed by Zhao et al. (2008) and Lu et al. (2010), respectively. The polymers PVP-DEGMA-TAIC and PA-EGDMA were originally used to produce decaffeinated green tea.

2.1.2. Fumonisin stock solutions

Two separate stock solutions of FB_1 and FB_2 were prepared by dissolving each mycotoxin into methanol to achieve a 1 g L^{-1} solution, after which they were stored in the dark at −20 °C until required.

2.2. Detection and quantification of fumonisin B_1 and fumonisin B_2 with LC-MS

2.2.1. Fragmentation patterns of fumonisins

MS/MS fragmentation was performed using a Micromass Quattro Micro API triple quadrupole mass spectrometer (Waters, Millford, MA, USA) with electrospray ionization operating in positive ion mode. The parameters used for the mass spectrometer in all experiments were as follows: capillary voltage, 4.0 kV (positive mode); source block temperature, 120 °C; desolvation temperature, 350 °C; desolvation gas flow rate, 650 L h^{-1}; cone gas flow rate, 50 L h^{-1}; cone voltage 40 V and different collision energies (between 30 and 50 eV) were applied to perform fragmentation. Each mycotoxin was directly injected and analyzed in full-scan mode in order to select the optimal precursor ions for the study of fragmentation. For this, 10 μL of fumonisin stock solution (1 mg mL^{-1}), were dissolved in 990 μL of ACN with 0.1% formic acid, and analyzed in scan mode (*m*/*z* 100–1000). Analyses were performed in positive ion mode using multiple reaction monitoring, MRM. FB_1 showed a main ion at *m*/*z* 722.6 and FB_2 at *m*/*z* 706.6. The collision energy (CE) of 50 V was chosen because it produced the greatest dissociation of the precursor ions, generating the daughter ions *m*/*z* 334.0 and *m*/*z* 336.0, respectively for FB_1 and FB_2. In contrast to the CE of 30 V, which generated the ions *m*/*z* 528.0 and *m*/*z* 512.0.

2.2.2. LC-MS conditions

Sample analysis was performed using an Shimadzu series 10 AVvp interfaced with a Micro mass spectrometer (Waters, Milford, MA, USA). The HPLC system consisted of a binary pump (G1312A), a degasser (G1322A), an autosampler (G1313A), a column compartment (G1316A), and a diode array UV−vis detector (G1315A). An injection volume of 10 μL was used, and samples were separated on an Ascentis Express C18 column (2.1 mm × 150 mm, 2.7 μm) (Supelco) at 35 °C. The mobile phase was maintained at a flow rate of 0.2 mL min^{-1} using a binary solvent system of (A) water and (B) 0.1% formic acid in acetonitrile. The elution gradient started at 100% B (0–15 min), linearly increased to 20% B and 80% A (16–25 min). Analytes were detected using an inline triple quadrupole MS spectrometer equipped with a LockSpray dual exact mass ionization source inlet system, consisting of two electrospray probes for the co-introduction of the analyte and lock mass reference compound. The source was operated in ESI+ mode with the following conditions: source temperature, 120 °C; desolvation temperature, 350 °C; capillary voltage, 4.0 kV; cone voltage, 40 V. An internal reference, reserpine (100 mg/L delivered at 50 μL/min), was used to obtain exact mass measurements. The reference spray was sampled at an interval of 10 s. Accurate mass measurement was conducted in MS mode (ranging from 100 to 1000 Da) with a scan time of 1.0 s. The structural information of analytes was obtained in MS/MS mode with a collision energy of 50 V.

MRM was performed as described above using the following precursor-to-fragment transitions: *m*/*z* 722.6 → 334.0 for FB_1, and *m*/*z* 706.6 → 336.0 for FB_2. Two MS/MS transitions were acquired for each mycotoxin. For each compound, the most abundant MRM transition was used for quantification while the other was used for confirmation. The MassLynx V4.1 software (Waters) was used for data acquisition and processing.

In all cases, samples were filtered through 0.45 μm PTFE syringe filters of 13 mm diameter prior to analysis (VWR International USA).

2.2.3. Experimental samples

Model solution: 500 mL of a 12% ethanol solution containing 5 g L^{-1} of tartaric acid, adjusted to pH 3.25 with 0.1 M sodium hydroxide was prepared (Dallas, Ricardo-da-Silva, & Laureano, 1996). One hundred mL of this solution was spiked with FB_1 and FB_2 to a concentration of 2 mg L^{-1} each.

Red wine samples: A commercial bottle of Cabernet Sauvignon, 2013, from Maule valley (Chile), pH 3.24 without detectable presence of fumonisin (as previously tested) was spiked with 2 mg L^{-1} of each mycotoxin (FB_1 and FB_2).

2.2.3.1. Calibration curves. Linearity between analyte concentration and MS detector response was evaluated for each sample (model