Influence of several oenological fining agents on ochratoxin A removal

Filipa Carvalho[a], *António Inês*[a], *Fernando Milheiro Nunes*[b], *Luís Filipe-Ribeiro*[a], *Luís Abrunhosa*[c], *Fernanda Cosme*[a]*

[a] Institute for Biotechnology and Bioengineering, Centre of Genomics and Biotechnology, (IBB/CGB-UTAD), University of Trás-os-Montes and Alto Douro, School of Life Sciences and Environment, Vila Real, Portugal
[b] Chemical Research Centre - Vila Real (CQ-VR), Chemistry Department, University of Trás-os-Montes and Alto Douro, School of Life Sciences and Environment, Vila Real, Portugal
[c] CEB-Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal
*fcosme@utad.pt

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ABSTRACT

In Europe, wine is estimated to be the second source, after cereals, of ochratoxin A (OTA), one of the most important mycotoxin found in food and feed products [1]. Its chemical structure consists of a chlorine-containing dihydro-isocoumarin linked through the 7-carboxyl group to L-β-phenylalanine. In wine, this fungal metabolite represents a severe risk for consumer health. According to the European Commission Regulation (EC) No. 123/2005 the maximum limit for OTA in wine is 2 µg/Kg [2]. Therefore, it is important to prevent and control their occurrence in wines. With the purpose to remove this toxin, several chemical, microbiological and physical methods were described in the literature [1, 3, 4]. Consequently, the aim of this work is to understand the interaction of different types of fining agents on OTA removal from wine. To evaluate their effectiveness, eleven commercial fining agents, including mineral, synthetic, animal and vegetable proteins were used to get new approaches on OTA removal from wine. Trials were performed in wines added of OTA. Most effective fining agent in removing OTA was a commercial formulation that contains activated carbon, a well-known adsorbent of mycotoxins. Reductions between 10 and 30% were also obtained with potassium caseinate, yeast cell walls and pea protein. However, with bentonites, carboxymethylcellulose (CMC), polyvinylpolypyrrolidone (PVPP) and chitosan no considerable reduction of OTA was verify. Also, the influence of these fining agents on the physicochemical wine characteristics, namely wine color, total phenolic compounds, flavonoids and non-flavonoids were studied.

Final results could provide important information to the wine industry to select treatments based on fining agents to remove OTA, in order to reduce toxicity and consequently to improve wine safety and preserving wine quality.

1.INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by certain molds that occur naturally in agri-food products worldwide. The most relevant to the health safety of foods are aflatoxins, ochratoxin A, patulin, fumonisin, zearalenone and deoxynivalenol, which presence in foods is regulated. These mycotoxins are mainly produced by species belonging to the genera *Aspergillus*, *Penicillium* and *Fusarium*, being toxic to humans and animals when ingested in small quantities. They may be carcinogenic, mutagenic, teratogenic, cytotoxic, neurotoxic, nephrotoxic, immunosuppressive and estrogenic. Ochratoxin A (OTA) is one of the most relevant mycotoxins. It can be found, among others, in cereals, wine, coffee, cocoa, dried fruits, grape juice and raisins. Its chemical structure is a dihydro-isocoumarin connected at the 7-carboxy group to a molecule of L-β-phenylalanine via an amide bond. The presence of OTA in wine is a serious risk to consumer health. For example, in Europe, after the cereals, it is estimated that the wine is the second major dietary source of this mycotoxin [1]. According to the Regulation No. 123/2005 of the European Commission (EC), the maximum limit for OTA in wine is 2 μg/kg [2]. Therefore, it is important to prevent and control its occurrence in this product. To control OTA in food, various chemical, physical and microbiological methods described in the literature may be used [1, 3, 4]. In the particular case of the wine, liable technologies to be applied are more limited. Currently, the use of adsorbents is the technology most used.

2.MATERIAL AND METHODS

2.1. Fining experiments

Eleven commercial oenological products with different characteristics (sodium bentonite - B1, calcium bentonite - B2; potassium caseinate - C; carboxymethylcellulose - CMC1 and CMC2; chitosan - Q; polyvinylpolypyrrolidone - PVPP; pea protein - PE; mannoproteins - MP1 and MP2; mixture composed by gelatin, bentonite and activated carbon - MIX) were applied using the average dose as recommended by the manufacturer in order to assess their ability to remove OTA artificially supplemented (at a final concentration of 10 μg/L) in a white wine with the following characteristics: alcohol content 10.4% (v/v); density 0.9917 (g/cm^3); pH 3.14; total acidity 6.8 (g/L of tartaric acid); volatile acidity 0.16 (g/l of acetic acid) analyzed according to OIV methods [5].

2.2. OTA analysis

After the wine fining, the supernatant was centrifuged at 4000 rpm for 15 min. Then, 2 mL of the supernatant were collected and added of an equal volume of acetonitrile/methanol/acetic acid (78:20:2 v/v/v). Also, the solid fractions obtained after fining, were centrifuged at 4000 rpm for 15 min, the resulting supernatant discarded, and the pellet extracted with 1 mL of the above solution and 1 mL of H_2O. After 12 hours, the extracts were filtered through a syringe filter with porosity of 0.45 μm and stored at 4 °C until analyzed by HPLC with fluorescence

detection. The chromatographic separation was performed on a C18 reversed-phase YMC-Pack ODS-AQ analytical column (250 x 4.6 mm I.D., 5 mm), fitted with a pre-column with the same stationary phase. The samples were eluted at a flow rate of 1 mL/min during 20 min with a mobile phase consisting of water/acetonitrile/acetic acid (99:99:2 v/v/v). The injection volume was 50 µl and parameters for detection: λ_{exc} = 333 nm, λ_{em} = 460 nm and gain = 1000. The OTA retention time was approximately 16 min. The OTA concentration in the samples was determined by comparison of peak areas with a calibration curve made with standards of OTA (Sigma-Aldrich).

2.3 Analysis of wines physicochemical parameters

After fining, color at 420 nm [5], phenolic compounds [6], flavonoids and non-flavonoids [7] and browning potential [8] were also analyzed.

3.RESULTS AND DISCUSSION

The most effective oenological product in ochratoxin A removal (≅ 80%) was a commercial mixture containing activated carbon, a known mycotoxin adsorbent. Reductions of mycotoxin from 10 to 30% were also obtained in the samples treated with potassium caseinate, pea protein and mannoproteins. Bentonites, carboxymethylcellulose, polyvinylpolypyrrolidone and chitosan did not produce a considerable reduction in wine OTA (Figure 1).

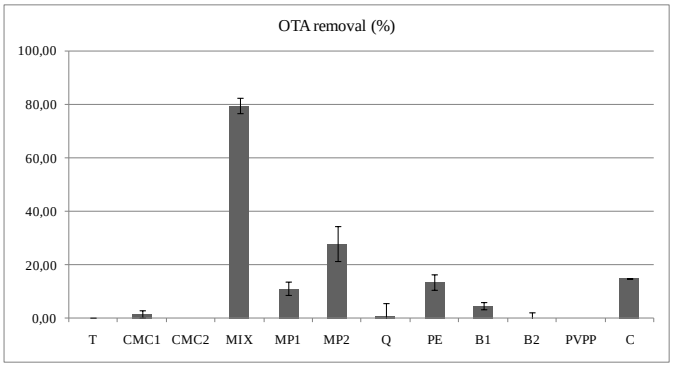
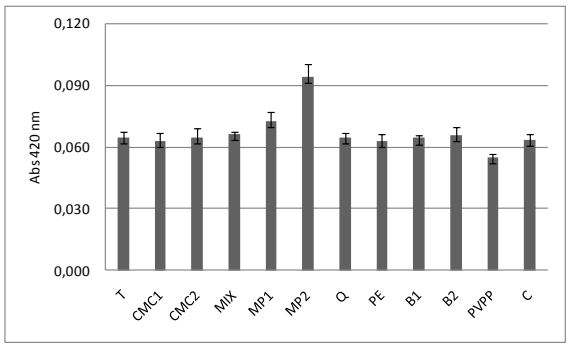
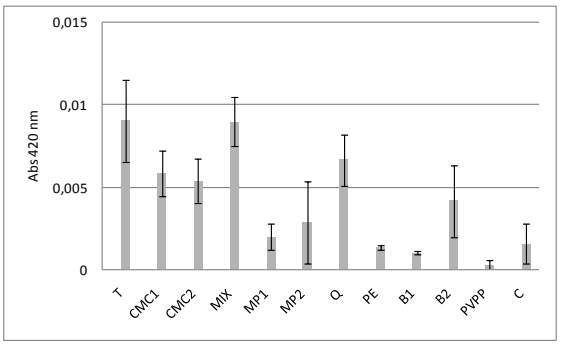


Figure 1. OTA removal efficiency (%) obtained for the evaluated oenological products.

The impact of oenological products on the physicochemical characteristics of the wine, in particular color (Figure 2A), browning potential (Figure 2B), and total phenolic compounds, flavonoids and non-flavonoids (Figure 2C) were also studied. Considering the oenological products that performed better in removing OTA, it was found that the color of the wine was not altered by the application of MIX, PE and C. However, concerning to browning potential, the MIX was not efficient, while the PE and C were effective in reducing wine browning potential.



2A



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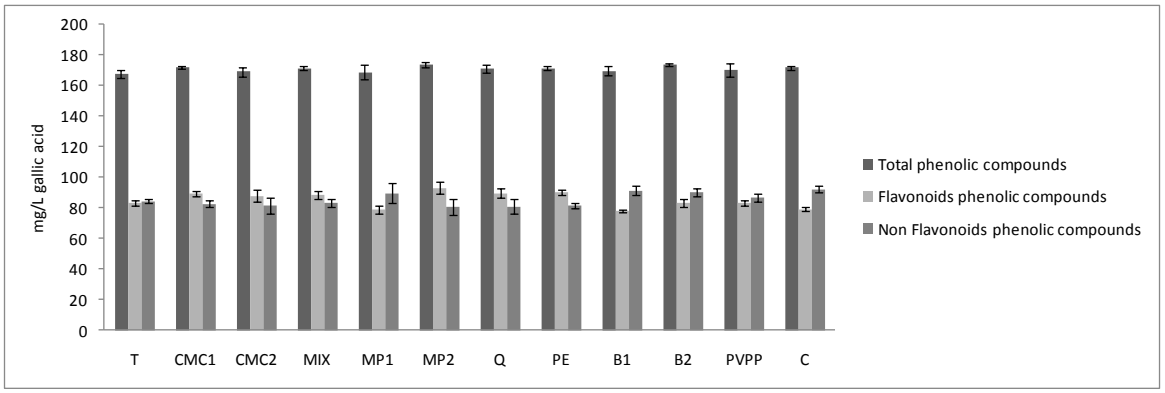

2C

Figure 2. Effects of the evaluated oenological products on: (2A) color, (2B) browning potential, and (2C) phenolic composition of white wine.

4.CONCLUSIONS

These results may provide useful information for winemakers, namely in the selection of the most appropriate enological product for OTA removal, reducing the toxicity and simultaneously enhancing food safety and wine quality.

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