



Review

Mycotoxin Incidence of Ochratoxin A in Wine and Methods for its Control

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Abstract: Mycotoxins are toxic secondary metabolites produced under special conditions of moisture and temperature by aerobic, microscopic fungi that can colonize various foods from before harvesting up to consumer use. Contamination of wine by ochratoxin A has gained worldwide interest in recent years with the revelation of these toxins' effect on human health. Ochratoxin A, produced by *Aspergillus* species, poses the greatest threat to human health and is subject to government regulation in juices and beverages worldwide. This review provides an overview of the prevalence of ochratoxin A in wine and control methods, which help establish and carry out proper management strategies.

Keywords: mycotoxins; ochratoxin A; wine; quality; methods of detection; *Aspergillus*.

1. Introduction

Brews are among the many food product groups at risk of contamination by harmful mycotoxins [1]. These mycotoxins may have been formed in an agricultural product before brew manufacture or formed during manufacturing [2]. Mycotoxins are unlikely to be developed during storage of the finished product after manufacture because effective sterilization and storage conditions are almost always used to maximize shelf life [3]. Mycotoxin formation during manufacturing is a particular concern with fermented brews. Understanding the formation and fate of mycotoxins in manufactured foods is of interest because of the possible need to alter steps in the manufacturing processes to prevent the formation of mycotoxins or, more frequently, remove them. Most of the research regarding mycotoxins in brews has aimed to research ochratoxin A (OTA) in wine [4]. This mycotoxin will form the basis of the present review. However, there have been various less extensive studies on other mycotoxins in other food [5], such as aflatoxin M1 in milk [6] and in drinks from grain-derived trichothecenes like zearalenone in beer [7]. Ochratoxin A is a mycotoxin produced, under environmental conditions, by fungi belonging to several species of the genera *Aspergillus* and *Penicillium* [2,8]. Many studies have shown that this mycotoxin is frequently present in a series of products, particularly wine [9,10]. Ochratoxin A was first reported in South Africa as a secondary metabolite produced by a strain of *A. ochraceous* [11]. Ochratoxin A is nephrotoxic, hepatotoxic,

genotoxic, teratogenic and immunotoxic to animals and its carcinogenicity in rats and male mice is well-established [12]. It has been linked to Balkan Endemic Nephropathy and the development of human urinary tract tumors. The International Agency for Research on Cancer has classified OTA as a possible carcinogen to humans [13]. The Provisional Tolerable Weekly Intake (PTWI) recommendation for OTA of 100 ng/kg of average body weight, but the Scientific Committee on Food of the European Union has recommended a lower daily intake (5 ng/kg) [4].

Nevertheless, the Panel on Contaminants in the Food Chain (CONTAM) of the European Food Safety Authority recently derived a Tolerable Weekly Intake (TWI) of 120 ng/kg for OTA. Recent analyses of the dietary exposure of adult European consumers to OTA revealed that at present, the weekly exposure ranges from 15 to 60 ng/kg, including high consumers of foods containing OTA [14]. Given the description mentioned above of ochratoxin A toxic effects, it is essential to understand this toxin in wine, which will help establish and carry out proper management strategies. Therefore, this paper reviews the occurrence of OTA in wine and methods for managing this mycotoxin.

2. Incidence of Ochratoxin A in White, Red and Rose Wines in EU and Abroad

Wine is an important beverage in world trade [15]. France, Italy, Spain, and the US are the main producing countries, followed by Argentina, China, and Australia. France, Italy, and Spain are the main wine exporters [16]. After the first report on OTA's occurrence in wine, several surveys were conducted to assess the prevalence of this mycotoxin in wine and grape products. A total of 1,706 wine samples were analyzed and summarized, and two further surveys were also recently conducted in Spain. A focus of studies on OTA contamination of wines has been to identify what types of wine are most susceptible to contamination by the mycotoxin [17]. Suppose a particular type of grape or step in the manufacturing process could be associated with OTA contamination [18]. In that case, it might be possible to alter the process and type of product to avoid marketing contaminated products. Attention has focused on two areas: dessert wines and red wines. The rationale for focusing on dessert wines is that they are manufactured from grapes grown in warmer climates, which have a higher sugar content that allows optimal ethanol production while still leaving enough sugar to give the sweetness characteristic of dessert wines. Thus, grapes grown in warmer climates are more likely to be contaminated with mycotoxins [19], and OTA contaminating the fruit would be expected to contaminate wine made from it [20]. Red wines have been a focus of attention because the red color is extracted from red grapes' skins during the fermentation step. It was suspected that *Aspergillus* and *Penicillium* species that contaminated skins before harvest might produce OTA during the fermentation step. If this mechanism were shown to be an important source of OTA, it would be possible for vintners to avoid losses by preparing white wines from red grapes in cases when grapes had been identified on the vine as having skins contaminated with *Aspergillus* and *Penicillium* species. The first data on OTA occurrence in wine marketed in Spain, and most of the samples were domestic, but some of them were imported [21]. Dessert wines showed the highest incidence of contamination followed by rose, red and white wines, in that order. Further surveys have reported that OTA contamination in wine. The overall percentage of contaminated samples was 51.5%. The highest OTA concentration was 15.25 ng/mL in dessert wine, while in other wines, the highest levels were <4.5 ng/mL. Dessert wines are prone to contamination with OTA, as in the case of Spanish wines [19]. More than 66% of wine samples showed detectable OTA levels in Greece, and both red and sweet wines showed the highest levels [22]. More than 50% of the samples analyzed in Cyprus and Turkey, respectively, had detectable levels of the toxin [23]. In general, the OTA content of wines correlates with color, with a decrease from red to rose and white, as shown by several studies. Usually, red wines have higher OTA contamination levels than white wines, which may be due to the increased time of contact between berry skins and grape juice during the mashing stage. In Italy, wines have been extensively surveyed for this toxin. OTA incidence was higher in red wines (78.4%), followed by rose and dessert and white wines. The highest level (7.63 ng/mL) was found in red wine. In Germany, a 7.0 ng/mL value was found in Italian red wine exported to Germany [23]. In France, OTA was found in 29 samples of wines (0.01–0.27 ng/mL), but a value of 0.78 ng/mL was found in a French red wine exported to Germany [19]. In Portugal, OTA was not found in 64 domestic wines, but OTA was detected in five out

of 37 Portuguese wine samples. In wine samples from Brazil, OTA was detected in 24% of 42 and Argentina and Chile (0.02–0.07 ng/mL). Australian wines were included in several surveys. Most samples contained <0.05 ng/mL, and the highest level was 0.62 ng/mL. In South Africa OTA was detected the toxin in 24 local samples. The highest level (2.67 ng/mL) was found in noble wine, while in wine samples from Morocco presence of OTA was also detected [24]. The risk to public health related to the presence of OTA in wines from Fruška Gora was assessed based on 113 dry wines from vintages spanning 2011–2016, analyzed by immunoaffinity and liquid chromatography with fluorescence detection [25]. More than half of sampled wines (52.2%) was contained OTA in quantifiable amounts (>0.02 µg/L). Almost 64% of red wines had at least trace amounts of OTA, while the percentage of white and rose wines was considerably lower (42.6% and 36.4%, respectively). The maximum concentration of OTA (0.134 µg/L) was far below the regulatory limit. Occurrence and common OTA content between vintages were diverse, and the 2014 year's samples were particularly distinguished due to the highest average OTA amount (0.035 µg/L). For risk characterization, a margin of exposure approach was applied in respect of non-neoplastic and neoplastic effects [25]. Results revealed an exceptionally low risk to public health related to OTA exposure, regardless of wine consumption pattern. Thus, the potential health benefits of responsible wine consumption, related mostly to the prevention of cardiovascular diseases, outweigh the risk, providing a base for international recognition and promotion of the regional wines and wine tourism [15,26]. Some additional reports of OTA found in wine are presented in Table 1. Wine is considered the major source of OTA intake after cereals.

Table 1. Ochratoxin A levels reported in wine in different countries around the world.

| Country | Type of wine | OTA level (µg/L) |
|---------|------------------|------------------|
| Austria | Red, White | <0.01-0.02 |
| Germany | White | <0.003-0.006 |
| Hungary | Red, White | <0.024 |
| Morocco | White, Rose, Red | 0.028-3.24 |
| Italy | Red | <0.001-3.177 |
| Spain | Red, White | <0.05-4.24 |
| Taiwan | Red | <0.2-0.5 |
| Serbia | White, Rose | <0.03- 0.134 |

3. Control Methods for Ochratoxin A Detection

Ochratoxin A (Figure 1) detoxification strategies are classified depending on the type of treatment, physical, chemical, or microbiological, and their objective is to reduce or eliminate the OTA toxic effects by destroying, modifying, or absorbing this mycotoxin [2,27]. The ideal detoxification method would be easy to use and economical and would not generate toxic compounds or alter other food quality parameters such as nutrient content [28]. Thus, firstly, the effect of processing stages on the toxin reduction should be studied [2].

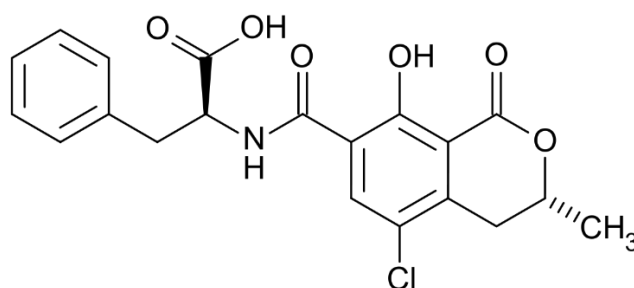


Figure 1. Structure of Ochratoxin A (*N*-[[*(3R)*-5-chloro-8-hydroxy-3-methyl-1-oxo-3,4-dihydro-1*H*-isochromen-7-yl]carbonyl]-*L*-phenylalanine) according to IUPAC nomenclature [29]

If this would not be possible, other additional treatments (physical, chemical, or microbiological) should be considered. In the European Union, dilution with non-contaminated foodstuffs is forbidden [30]. Initially, it was suggested that to avoid OTA, the external layers in contact with the producing fungi should be eliminated. However, in the case of grapes, this operation is complex and has been rejected. It has also been proposed to eliminate the products colonized by fungi from the manufacturing process. However, fungus absence does not imply the mycotoxin is gone, and the contamination might not be fully controlled [31]. Thermal treatments do not eliminate OTA [2]. A freezing (-20°C), defrost (26°C) process, and UV and gamma treatments can diminish the producing fungal conidia; however, only gamma radiation can destroy the OTA [32]. Ochratoxin A detoxification with chemical compounds such as activated charcoal, cholestyramine, sodium, and calcium aluminum silicates (mainly zeolites), bentonite, wood fragments or yeast has been tested. Some of these compounds were tested *in vivo*; however, their activity was not as high as expected except for the activated charcoal. Activated charcoal use, however, has been rejected due to the essential nutrient retention and animal poisoning [33]. Wine fining agents such as potassium caseinate or activated carbon have shown positive effects on OTA detoxification [34], but they have also damaged wine quality. A new insoluble vegetal fibre has been developed to adsorb the OTA present in liquid food products or, in the case of beer, present during the brewing step. Currently, the most promising adsorbent materials are modified zeolites [35]. Carboxypeptidase A is an enzyme capable of destroying OTA [36], and the use of toxigenic *A. niger* strains as carboxypeptidase sources has been suggested. Other enzymes obtained from *A. niger* strains and can efficiently degrade OTA are lipases, a crude enzyme preparation, and a metalloenzyme [37].

The study of Puvača et al. [38] aimed to investigate the influence of tea tree essential oil (*Melaleuca alternifolia*) on ochratoxin A synthesis by fungi. Commercially available tea tree essential oil was perched from Infinity Feed Company from Novi Sad, Serbia. GC-MS performed qualitative analyses of tea tree essential oil. The essential oil (7.5; 15.0 and 30.0 µg/ml) was evaluated by inhibiting the production of ochratoxin A by *A. niger*. HPLC quantified the toxin. The production of ochratoxin A was dependent on the incubation temperature, 20 and 30°C in the presence of the essential oil. Obtained values from 20°C showed a reduction in ochratoxin A synthesis ranged from 53.87 to 96.22% and 18.36 to 72.85% at 30°C, for the *A. niger*, respectively. Based on this research's obtained results, it was concluded that tea tree essential oil as a natural product could serve as a potential biocontrol agent against ochratoxin A contamination in feed [38].

A carboxypeptidase present in *Phaffia rhodozyma* can also degrade OTA up to 90% [39]. Moreover, certain bacteria belonging to *Streptococcus*, *Bifidobacterium*, *Lactobacillus*, *Butyribrio*, *Phenyllobacterium*, *Pleurotus*, *Saccharomyces*, *Bacillus* and *Acinetobacter* genera and certain fungi belonging to *Aspergillus* (*A. fumigatus*, *A. niger*, *A. carbonarius*, *A. japonicus*, *A. versicolor*, *A. wentii* and *A. ochraceus*), *Alternaria*, *Botrytis*, *Cladosporium*, *Phaffia*, *Penicillium* and *Rhizopus* (*R. stolonifer* and *R. oryzae*) genera, can degrade OTA *in vitro* up to more than 95%. Moreover, some of them have shown detoxifying properties in *in vivo* assays. Microbiological control of OTA is also vital during wine manufacturing [40]. Ochratoxin A content increases until the malolactic fermentation step preceding the bottling of wine. During this time, the mycotoxin level diminishes, probably due to its adsorption on the *Saccharomyces* yeast surface, its interaction with metabolites produced by yeast, or its degradation by the lactic bacteria still present in wine. Grape washing, cooking, and pressing can also promote the OTA content drop [41]. During vine fruit production, efficient drying and turning over are required because moisture and sugar content stimulates *Aspergillus* and OTA synthesis in these stages [42].

5. Conclusions

While studies investigating OTA's health effects have proved inconclusive, there is little doubt as to the potential danger inherent in the contamination of drinks and wine by this toxin. Past research has elucidated a great deal about OTA's chemical and biological nature, and many advances have been made in developing methods for detecting OTA. Research has also revealed several

potential control measures that may provide a basis for fully effective control measures in the future. Still, OTA contamination continues to affect wines. Thus, future research must continue to address the threat of OTA contamination in wine, emphasizing the following areas. Due to OTA toxicity and to assure human and animal health, this toxin should not be present in drinks, at least above the maximum permitted levels. Fungi that produce OTA grow best under certain environmental conditions. Factors that influence the production of OTA by *Aspergillus* and *Penicillium* include temperature, pH, and moisture. Eliminating the conditions necessary for fungal growth helps prevent the formation of OTA. Cleaning and disinfecting storage and transportation equipment to avoid cross-contamination by fungi can also help minimize OTA formation. Other microorganisms that can compete with OTA-producing organisms is another possible method of preventing OTA formation. However, this option carries its own, possibly harmful, implications, including concerns about human allergies and food adulteration. International cooperation for mycotoxin regulation in trading products or commodities is also needed. Countries should establish quality control limits for certain things intended for export or import. Producer countries would be stimulated to be aware of mycotoxin contamination in their exported susceptible commodities. Low-cost technologies for assessment, prevention, and control of environmental mycotoxins can be transferred from developed countries to developing ones.

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