Ochratoxin A removal during the main steps of Wine Making

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Abstract

A few years ago, the presence of ochratoxin A (OTA) was reported for the first time in wine. Grape juice is usually more contaminated than wines, and red grape products are more contaminated than white ones. This knowledge has led researchers to conclude that grape processing could contribute to a reduction in the content of this mycotoxin in grape products, such as the case of wines.

This study presents the effect of the more common vinification steps on the fate of the mycotoxin during wine making. Grapes with a content of OTA ranging from 0.43 to 7.48 µg/Kg were used for vinification. These grapes were obtained by inoculating recently harvested grapes with an ochratoxin A producing *Aspergillus*. It was found that after alcoholic fermentation just about 31.8% of the OTA initially present in grapes remained in the wine. After racking, this amount decreased to 10.9 %, and, after malolactic fermentation, to 8.1%. Also, it was found that OTA was present in higher amounts in spent fractions from wine making, such as the lees obtained after fermentation or the sediment obtained after racking. After malolactic fermentation, the most common eonological fining agents were able to decrease even more the content of OTA in the final wine. Vinification assays with eonological enzymes commonly used in wine making industry were also done. Based on this data, we concluded that this reduction is associated with the mycotoxin removal by adsorption into solid wastes or fining agents, and not due to any degradation of ochratoxin A into other compounds.

The authors gratefully acknowledge the support of the EC, Quality of Life Program (QOL), Key Action 1 (KA1) on Food, Nutrition and Health; contract number QLK1-CT-2001-01761 - Wine-Ochra Risk; and the support of the INIAP - Instituto Nacional de Investigação Agrária e das Pescas, through the Program AGRO, contract number 255. Authors would also like to acknowledge the Estação Vitivinícola Amândio Galhano. Abrunhosa, L. was supported by grant SFRH/BD/11228/2003 from Fundação para a Ciência e Tecnologia.

Keywords: ochratoxin A, wine, review

1. Introduction

A few years ago, Zimmerli and Dick (1996) reported the presence of ochratoxin A (OTA), a mycotoxin with carcinogenic properties, in wines. Since then, surveys conducted in different countries have revealed the presence of OTA in wine (Otteneder and Majerus, 2000). In Portuguese wines, OTA has been found in Vinho Verde and Port Wine, although at lower levels than the recommended limit (Ratola *et al.*, 2004).

The fungi reported as OTA producers belong to the genera *Aspergillus* and *Penicillium*. The species mainly responsible for the production of OTA in grapes is *A. carbonarius*; however, other OTA-producing fungi, such as strains of *A. niger* aggregate and *A. ochraceus*, have also been isolated from grapes (Serra *et al.*, 2003; Serra *et al.*, in press).

As has already been mentioned for other undesirable compounds, such as pesticides (Cabras and Angioni, 2000), grape processing reduces the level of OTA, with grape juice

usually being more contaminated than wines, and red juices or red wines more contaminated than white ones. Furthermore, grape products originating from southern Europe and North Africa (Mediterranean climates), are more affected than those originating from the more temperate regions of central Europe (Otteneder and Majerus, 2000). The higher levels of OTA in red wines over white were interpreted as a consequence of the differences in the wine processing for both types. Furthermore, a stronger presence of OTA-producing fungi in grapes grown in southern Europe was considered the probable cause for the higher incidence of OTA in these regions. However, a direct correlation has not yet been established between these two parameters.

Several physical, chemical or microbiological methods have been proposed to remove mycotoxins from foods and feeds, but few of these have practical application. The best solution for this purpose is considered to be detoxification by biodegradation, since it is possible to remove mycotoxins without using harmful chemicals and without significant losses in nutritive value or palatability of decontaminated food and feed (Bata and Lasztity, 1999). In wines, methods to remove OTA have also been tried. The utilization of chemical adjuvants has been the method more studied so far (Fernandes *et al.*, in press; Rousseau and Blateyron, 2002; Castellari *et al.*, 2001). However, these studies showed that most of these chemical adjuvants have little effect on the removal of OTA, at the dosages currently employed in wine production. Active charcoal was the most effective, but only at relatively high dosages, which causes severe damages in organoleptic quality of wine. Microbiological or enzymatic methods could also be, in the future, an attractive solution in wine technology but its applicability has not yet been established, being some solutions under experimental evaluation (Abrunhosa *et al.*, 2002).

The fate of ochratoxin A throughout the major steps in vinification is reviewed in this study.

2. Material and methods

2.1 Grapes

Grapes were collected in EVAG - Estação Vitivinícola Amândio Galhano, a research and experimentation centre from Vinho Verde Portuguese wine-producing region. The regional two most representative varieties of grapes were collected: Vinhão (red) and Loureiro (white). The red variety was employed in all experiments, while the white variety was only used for those experiments where chemical adjuvants were employed to aid clarification.

2.2. Contamination of grapes with OTA

The natural content of OTA in collected grapes was checked, and, since it was not detected, grapes were inoculated with the ochratoxin A producing *Aspergillus carbonarius* strain MUM 03.59, isolated from grape must and preserved in the MUM culture collection.

Grapes were sprayed with a spore suspension (10^3 spores ml^{-1}), and incubated in controlled temperature chambers at 25 ºC for 3 to 6 days, to achieve different levels of OTA contamination.

2.3. Vinification Experiments

Several microvinification trials were performed employing the different technological solutions that are currently in use for the production of Vinho Verde. These trials started with the whole grape and were pursued until clarified wine was obtained as described below. The number of trials that were performed was dependent on the amount of available grapes.

Vinho Verde vinification trials started by crushing grapes, yielding a mixture of a liquid (the must) and solid phase (pomace: skins and seeds). Just after crushing and before fermentation, a sulphur dioxide-generating agent was added, only when fermentation was

to be done with selected commercial yeast – *Saccharomyces cerevisiae* strain QA23. Fermentation of must was done in the presence of pomace, which is usually the case in red vinification. Following alcoholic fermentation, racking and malo-lactic fermentation was done, as described in Fernandes and co-workers (in press).

Microvinification trials with eonological commercial enzymes (Lallzyme CB; Lallzyme MMX, Lallzyme CH and Lallzyme β) were also done. They were used as recommended by manufacturer in a typical white vinification: grapes were crushed, enzyme was added, and the mixture was allowed to macerate overnight.

2.4. OTA analysis

OTA clean up from samples - The content of ochratoxin A was determined for all samples using Ochratest immunoaffinity columns (Vicam, Boston, USA). However, the method employed was dependent on the nature of the sample. Liquid samples, as clarified musts or wines, were analysed according to the method of Visconti *et al.* (1999). Solid ones, as whole grapes or liquids with suspended solids, were analysed according to Serra *et al.* (2004).

OTA detection by HPLC - The samples were analysed using a reverse-phase HPLC equipped with a Jasco FP-920 fluorescence detector (330 nm excitation and 460 nm emission wavelengths). Chromatographic separations were performed on a C18 column (ODS2, 4.6 mm x 250 mm, 5 µm), fitted with a pre-column with the same stationary phase. The mobile phase consisted of an isocratic program as follows: acetonitrile:water:acetic acid (99:99:2, v/v), with a flow rate of 1.0 ml/min and the injection volume was 100 µl.

The OTA standard was supplied by Sigma (St. Louis, USA). Samples were taken as positive for OTA presence if they yielded a peak at a retention time similar to the OTA standard peak (approximately 12 min), with a height five times higher than the baseline noise.

3. Results and discussion

3.1 Grape crushing

After crushing, OTA content was distributed between the must and the pomace. Based on eight independent experiments, the concentration of OTA in grape juice is 59 ± 14% of initial total concentration in grapes.

3.2 Must fermentation

After fermentation, the concentration of OTA in wine is lower than in the original must. After a material balance (Table 1), it was found that OTA present in wine represents 31.8% of the amount in the “whole grapes”. Nevertheless, most of the OTA present in the fermentation vessel was detected in the lees (50.4 ± 10.3 %). A material balance performed in the fermentation vessel showed that almost all the OTA present in the grapes was recovered in wine and in lees (82.4% ± 14.4). Although this does not represent all the OTA entering the vessel, the difference from 100% could be due to the uncertainty of the results of OTA determination.

3.3 Wine clarification

The settling of wine after alcoholic fermentation contributes to a further reduction in OTA content. After clarification (racking), the amount of OTA in wine was only about 11% of its initial value. The sediment recovered from the settling vessel constitutes only a small mass fraction; however its OTA content represents about 18 % of the OTA in the original grapes.

3.4 Malolactic fermentation

The fate of OTA during malolactic fermentation was also investigated (Table 1). At this stage a reduction in OTA concentration in wine also occurred. This reduction was not as

significant as in alcoholic fermentation and may be due, at this stage, to adsorption to the biomass, based on the 99.4% recovery of OTA. An overall reduction of OTA of about 3% (in relation to the whole grape) was achieved during malolactic fermentation.

Table 1. Recoveries of OTA content throughout the vinification experiment in relation to its content in grapes.

Vinification trial	OTA recovery (%)							Total recovery in each trial
	In grape	After alcoholic fermentation		After wine clarification		After malolactic fermentation		
		wine	lees	wine	sediment	wine	lees	
A	100	22.3	45.3	8.5	16.1	5.6	3.6	70.6
B		44.5	46.6	10.6	30.2	--	--	87.5
C		34.9	55.2	10.7	19.5	10.6	1.9	87.2
		32.3	70.9	14.1	15.0	9.4	3.0	98.3
		37.8	57.9	11.9	16.3	6.7	3.3	84.2
D		25.9	40.7	11.8	10.8	--	--	63.3
		31.1	44.1	10.0	17.8	--	--	71.8
		25.5	42.5	9.7	15.3	--	--	67.5
Mean overall recovery		31.8 ± 7.3	50.4 ± 10.3	10.9 ± 1.7	17.6 ± 5.7	8.1 ± 2.3	3.0 ± 0.74	78.2 ± 12.2
Mean recovery in each step		82.4 ± 14.4		91.0 ± 10.3		99.4 ± 15.6		

3.5 Clarification aided by fining agents

The use of chemical adjuvants to aid the clarification of wines contributes to a further reduction in OTA (Fernandes *et al.*, in press). However, this approach may not solve the problem by itself, since it may reduce the value of the final wine by removing colour, aroma, flavour or other characteristics of wines. Control samples, clarified without the use of these products, showed a degree of OTA removal comparable to wines clarified with a chemical adjuvant, with some agents completely ineffective when compared to the control sample clarification (as was the case for PVPP).

3.6 Effects of eonological commercial enzymes

Preliminary data of trials performed with some commercial enzymes indicate that some may have the ability to hydrolyze ochratoxin A. Confirmation of these results is in progress.

4. Conclusions

Experimental data collected in the last few years contribute to the understanding of those observations reported by Otteneder and Majerus (2000) stating that white wines are less contaminated than red ones and that grape juices are more contaminated than wines. In fact, the distribution of OTA between must and pomaces after crushing, explains the lower OTA content in white musts, and the fate of OTA after fermentation explains the lower OTA content in wines compared to juices.

This enables us to conclude that a reduction in OTA takes place mainly during fermentation, and that such reduction is due to its adsorption onto the solid phase, since no evidence of OTA degradation into other compounds, due to the metabolic activity of the

yeast, was observed. However, the use of enzymes in must clarification prior to fermentation may also contribute to a reduction in OTA, this time due to degradation.

Of greater concern was the finding that by-products from vinification with high levels of OTA are generated. Further use of these by-products should take into consideration the risk of contamination by this mycotoxin.

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