Fate of Ochratoxin A during Vinification of Semillon and Shiraz Grapes

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Semillon and Shiraz grapes containing ochratoxin A (OA) were obtained by inoculation of bunches on the vine with *Aspergillus carbonarius.* Citric acid content was greater in the inoculated grapes than in healthy grapes. Samples were collected throughout vinification of these grapes and the OA content was quantified using a stable isotope dilution liquid chromatographic−tandem mass spectrometric method. The mass of processed and waste streams during vinification was also noted. Reduction in the amount of OA in juice and wine occurred at every solid−liquid separation stage. The OA concentration (μg/kg) in white and red wine after racking was 4% and 9%, respectively, of that in crushed grapes. This corresponds to 1% and 6% of the total OA content that was initially present in the inoculated grapes. The OA content was divided between solid and liquid phases at each stage of vinification. OA did not appear to be transformed either chemically or biologically by yeast during fermentation, rather was discarded with the marc, juice lees, and gross lees.

KEYWORDS: Ochratoxin A; grapes; wine; *Aspergillus carbonarius*; stable isotope dilution; mass transfer

INTRODUCTION

The presence of ochratoxin A (OA), a nephrotoxin and possible human carcinogen, in wine is thought to result from infection of grapes on the vine by toxigenic black Aspergillus species, in particular, *Aspergillus carbonarius* (*1*, *2*). OA production in grapes ceases at the commencement of processing, typically a sterilization step in industrial juice and wine production (*3*). Hence, the concentration of OA in the finished wine is a function of the initial concentration in the grapes and the effect of processing. Processes that reduce OA content can be classified into two groups, physical removal and transformation of the molecule (degradation).

Physical removal of OA involves first removing the site at which OA has been produced, for example, the removal of visibly moldy berries from table grapes. A strong association between OA and the berry skin (*4*) would suggest that a relatively small proportion of OA remains in the finished beverage. A second aspect of physical removal of OA is the partitioning of the toxin between solid and liquid phases during processing. In microvinification trials with grapes artificially contaminated with OA, the greatest reductions resulted from solid−liquid separation steps, such as pressing the juice or wine from the skins or decanting the wine from precipitated solids (*5*, *6*). Many of the solids present in grape juice appear to have an affinity for OA, as OA decreased when such solids precipitated (*3*).

Little is known about the degradation of OA by wine yeasts during fermentation, though this has been demonstrated during beer fermentation (*7*). Decreases in OA during wine fermentation may be affected by choice of yeast strain (*8*). Such decreases are thought to be due to binding of OA to yeast cells, rather than biological transformation by the yeasts because no transformation products have been identified. The fate of radiolabeled OA during fermentation of grape juice supports this hypothesis (*9*). The addition of sulfur dioxide has little effect on OA content (*3*, *10*).

Legislation by the European Union to limit the allowable amount of OA in wine to 2 μg/kg (*11*) requires oenologists to have a better understanding of the fate of OA during vinification so that wine, which consistently falls below that limit, can be produced. The European studies noted above have typically examined vinification of artificially contaminated red grapes, or focused only on selected aspects of vinification. This paper is the first to compare the effects of white and red grape vinification, according to current Australian oenological practice, on OA concentration and content where OA contamination occurred on the vine as a result of infection with *A. carbonarius*. The aim of this trial was to monitor the mass and OA content of both processed and waste streams at key stages during the

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entire vinification process and, thus, to determine if OA was removed by physical processes or by transformation of the molecule.

MATERIALS AND METHODS

Vineyard Site. Difficulties reported by other authors in obtaining sufficient naturally contaminated grapes for vinification (*5, 6*) were overcome by inoculation of grapes on the vine with *A. carbonarius*, to simulate natural contamination. The trial site comprised single rows of Semillon and Shiraz vines in the Hunter Valley, New South Wales, Australia, during January and February 2004. The vines were over 25 years old, trained onto horizontal wires and under drip irrigation.

Preparation of Spore Suspension. A spore suspension of two OA-producing strains of *A. carbonarius* (FRR 5682 and FRR 5683, Food Science Australia Culture Collection, North Ryde, New South Wales, Australia; isolated from the trial vineyard) was prepared as described previously (*12*). The concentration of spores was determined to be ca. 10^7 spores/mL using a haemocytometer.

Generation of Ochratoxin A-Contaminated Grapes. Inoculation of grapes with *A. carbonarius* was timed to allow an incubation period of 7−10 days before harvest of grapes at commercial maturity. A syringe was filled with *A. carbonarius* spore suspension and slight pressure was continuously applied on the plunger, to ensure that the tip of the needle was coated with spores. Greater than 90% of berries were punctured in each bunch. Procymidone was applied to the Semillon vines 1 day after inoculation, as part of the grower's standard fungicide program; otherwise, no fungicides were applied between inoculation and harvest. Semillon bunches were harvested at commercial maturity (ca. 16 °Brix) 9 days after inoculation. Ripening of Shiraz grapes was slower than predicted; hence, commercial maturity (ca. 21 °Brix) was attained and grapes were harvested 15 days after inoculation. Harvested grapes were chilled at 4 °C before processing through a crusher/destemmer (Winery Supplies, Knoxfield, Victoria, Australia) at 1 tonne/h. Uninoculated bunches were harvested at the same time for comparison.

White Vinification. Crushed grapes (must) were divided into nine approximately homogeneous, 3.5 kg portions, which were subsequently treated with 50 ppm SO_2. Musts were pressed through 50% shade cloth in a hydraulic press (S. Stowe & Sons, Bristol, U.K.). The cloudy juice was held at 1 °C for 4 days to allow precipitation of the sediment, after which the clarified juice was siphoned from the surface.

The clarified juice from the replicates was pooled and the titratable acidity adjusted to 6.5−7.0 g/L by the addition of tartaric acid (Fermtech, Queensland, Australia). Diammonium phosphate (DAP; 0.5 g/L; Sigma, MO) and *Saccharomyces cerevisiae* QA23 (0.2 g of dry yeast/L; Lallemand, Plympton, South Australia, Australia) were added to the juice. Fermentation of 1.8 L aliquots was conducted at 15 °C in four replicate glass vessels fitted with rubber stoppers containing water traps and was deemed complete when the concentration of reducing sugars was below 0.1% (Clinitest tablets, Bayer Australia Ltd., Pymble, New South Wales, Australia). Malolactic fermentation was not conducted. The wine was racked and 50 ppm SO_2 added.

Red Vinification. The must was divided into approximately homogeneous, 4 kg portions, which were subsequently treated with 50 ppm SO_2. The titratable acidity of the juice was adjusted to 6.5 g/L. DAP (0.5 g/L) and *S. cerevisiae* D254 (0.3 g of dry yeast/L; Lallemand, Plympton, South Australia, Australia) were added to the must. Fermentation was conducted at 25−30 °C in food-grade plastic buckets fitted with lids containing water traps and with mixing three times daily. Eight replicate fermentations were conducted. After fermentation for 4 days, the must was pressed as described for white vinification. The resulting wine was held in glass bottles at 25 °C, as above, until fermentation was deemed complete (concentration of reducing sugars less than 0.25%; Clinitest tablets, Bayer Australia Ltd., Pymble, New South Wales, Australia). Malolactic fermentation was not conducted. The wine was racked and 50 ppm SO_2 added.

Analysis of Total Soluble Solids and Acidity. Total soluble solids present in juice immediately after crushing were assessed using a refractometer (Atago PR-32, Tokyo, Japan) and titratable acidity was assessed by titration to pH 8.2 (*13*). The presence and concentration of organic acids in the juice was determined using HPLC (*14*) by the Analytical Services Group at the Australian Wine Research Institute, Glen Osmond, South Australia, Australia. Juice from uninoculated grapes was also assessed.

Sampling during Vinification. Samples of must (crushed grapes) were collected so that an estimate of the total OA initially present in these samples could be determined. After pressing, samples of juice/wine and marc (skins and seeds) were collected. Similarly, after racking (decanting) from the gross lees (precipitated yeasts upon completion of fermentation), samples of the racked wine and gross lees were collected. In addition, during Semillon juice clarification, cloudy juice, clarified juice, and juice lees were collected. Samples were also collected at various stages during fermentation of the Semillon juice, after the vessels had been mixed to resuspend any precipitated yeasts. Samples were stored at −20 °C before analysis for OA.

Extraction of Ochratoxin A from Grapes and Vinification Products. Water was added to crushed grapes and marc (3 and 9 parts w/w, respectively) and the resultant samples were then mixed for 2 min using a blender (Philips HR2835/AB). In the case of juice lees and gross lees, the samples were diluted with water (1:2 sample to water, w/w) before processing.

After the addition of the internal standard, [$^{13}C_{20}$]OA (1 µg/mL in methanol, 250 µL), the diluted sample (15 g of grapes or marc, 10 g of juice lees, gross lees or wine) was vortexed and then allowed to equilibrate at 4 °C. After 12 h, methanol (5 mL/15 g or 1.5 mL/10 g of sample) and hydrochloric acid (10.5 M, 3−5 drops) were added to the sample. After mixing, the resulting acidified sample was centrifuged (2500 rpm, 15 min) and the supernatant was passed through a C18 solid-phase extraction cartridge (900 mg Maxi-Clean, Alltech, Deerfield, IL), which had been conditioned with acetonitrile (5 mL) and water (5 mL), at a flow of ca. 5−10 mL/min using a vacuum manifold. Aqueous methanol (1:9 methanol/water, v/v, respectively, 10 mL) was then passed through the cartridge, after which it was removed from the vacuum manifold and connected in series with an aminopropyl cartridge (200 mg Extract-Clean, Alltech, Deerfield, IL) using an appropriate adaptor (Alltech, p/n 210705). OA was eluted from the C18 cartridge in methanol (10 mL) and adsorbed onto the aminopropyl cartridge. The C18 cartridge was removed, and the analyte was eluted from the aminopropyl cartridge in acidified 35% ethyl acetate in cyclohexane (0.75:34.25:65 formic acid/ethyl acetate/cyclohexane, respectively). The solvent was evaporated to dryness using a hot block (50 °C) and a stream of nitrogen. The resulting residue was dissolved in 0.1% formic acid in 50% aqueous methanol (1 mL, v/v).

Quantification of Ochratoxin A by Liquid Chromatography−Tandem Mass Spectrometry. An aliquot (20 µL) of the above extract was chromatographed on a Ultracarb (30) C18 2.0 × 50 mm, 5 µm column (Phenomenex, Torrance, CA), which was kept at 30 °C in an oven. The mobile phase, methanol/water/formic acid (85:4.985:0.015, v/v/v, respectively) was pumped through the column at a rate of 250 µL/min. Under these conditions, OA and its internal standard eluted from the column in ca. 1.1 min. The column effluent was diverted to waste for the first 30 s and then switched to the detector for the next 60 s. Detection of OA and its internal standard was achieved by monitoring the daughter ions arising from the collision-induced dissociation of the parent ions of OA ($m/z = 404$, M + 1) and [$^{13}C_{20}$]-OA ($m/z = 424$) and the corresponding sodium adducts ($m/z = 426$ and 446, respectively). Argon (1 mTorr) was used to fragment these ions, and in the case of OA and its corresponding sodium adduct the daughter ions $m/z = 239$ and 261 were produced by applying collision energies of 22 and 26 V, respectively, to the parent ions. Similarly, applying the same collision energies to the parent ions of the internal standard produced the corresponding daughter ions with m/z values of 259 and 281. A scan width of 0.01 amu and a dwell time of 100 ms were used to monitor the daughter ions. The resolution of both quadrupoles was set to 0.7 amu fwhm. The positive parent ions of OA and its internal standard, [$^{13}C_{20}$]OA, were produced using an electrospray ion source and capillary voltage of 4.0 kV. The temperature of the ion capillary transfer tube was set to 300 °C and nitrogen at a flow of 25 L/h was used as the sheath gas. Other parameters such as source CID and tube lens were optimized using a 2.5 µg/mL solution of OA in 50% aqueous methanol containing 0.15% formic acid (v/v).

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Calibration. A stock solution of OA was prepared by dissolving the crystalline material in HPLC-grade methanol. UV spectrophotometry was used to calculate the concentration of this solution by assuming the extinction coefficient of OA in methanol to be 6640 (*15*). All solutions were stored at −20 °C until required.

A set of eight working standards in 50% aqueous methanol was made with the concentrations 2.56, 6.42, 12.9, 25.7, 64.2, 128, 193, and 321 μg/L. Calibration standards (including a blank) for the LC-MS/MS calibration curve were prepared by adding 250 ng (in 250 μL of methanol) of internal standard to each of the working standards (1 mL). A calibration curve was constructed by plotting the amount, in nanograms, of OA against the response (area) of the target ions, $m/z =$ 238 and 261, of the analyte and its sodium adduct (respectively) relative to the response of the corresponding target ions of the internal standard. The calibration curves for the analyte ($y = 0.0025x + 0.0578$) and its corresponding sodium adduct ($y = 0.0026x + 0.0613$, $r^2 = 0.9999$) were linear over this range.

The detection limit of the method was better than 0.05 ng/g and the limit of quantification was better than 0.2 ng/g. The imprecision (CV%) of the method was determined by the analysis of a naturally contaminated sample as a set of five replicates and was calculated to be <2%. Due to the isotopic purity of the internal standard, the accuracy of the method was determined by the standard addition technique (one level of addition) using a reference material (port wine) that was known to contain OA (3 μg/kg). The agreement between the calculated amount of OA in the samples and the experimentally determined value was better than 96%. Based on its precision, accuracy, and repeatability (>97%), the method was deemed to be fit for the purpose of this study. The internal standard, [$^{13}C_{20}$]OA, compensated for the differing recoveries from the various matrixes. This internal standard was biosynthesized using uniformly ^{13}C-labeled glucose and a high OA-producing strain of *Aspergillus ochraceus* (FRR 3846, Food Science Australia culture collection, North Ryde) in a liquid culture medium. The product was isolated by liquid−liquid extraction and purified by flash chromatography on silica. The product was characterized by UV spectroscopy, thin-layer chromatography, and tandem mass spectrometry, which revealed its isotopic purity to be 94%. A more detailed description of the method and the biosynthesis of the internal standard will be reported elsewhere.

Statistical Analysis. Where amenable to statistical analysis, data were examined by ANOVA (Genstat, 6th Edition, Lawes Agricultural Trust, Rothamsted, U.K.). In the absence of significant higher order interactions, means were compared according to Tukey's test of honestly significant difference.

RESULTS AND DISCUSSION

Berries inoculated with *A. carbonarius* were more discolored and shriveled than uninoculated berries. Upon crushing, it was observed that the berry pulp was macerated because of fungal growth. This was particularly noticeable for Shiraz must, where the juice of inoculated berries was heavily pigmented compared with that of uninoculated fruit. Members of *Aspergillus* section *Nigri* are potent producers of pectinase (*16*); thus, pectolytic activity by *A. carbonarius* may explain the maceration. Loss of water associated with shriveling of inoculated berries increased the total soluble solids compared with uninoculated fruit (**Table 1**). All values for titratable acidity and organic acids were similar to the range found in grapes at harvest (*17, 18*). However, inoculated fruit displayed increased titratable acidity compared with uninoculated fruit. The increases in malic and tartaric acid (ca. 60% and 14%, respectively) could be associated with berry shriveling of inoculated fruit. However, citric acid increased by a far greater amount, exceeding 450% of the concentration in uninoculated fruit. This increase cannot be attributed to berry shriveling alone, suggesting that either fungal infection induced citric acid formation in the berries or that *A. carbonarius* was the direct source of the citric acid. Strains of *A. niger* are used in the commercial production of citric acid

Table 1. Effect of *Aspergillus carbonarius* Infection on Total Soluble Solids and Organic Acid Profile of Semillon and Shiraz Juice at Harvest

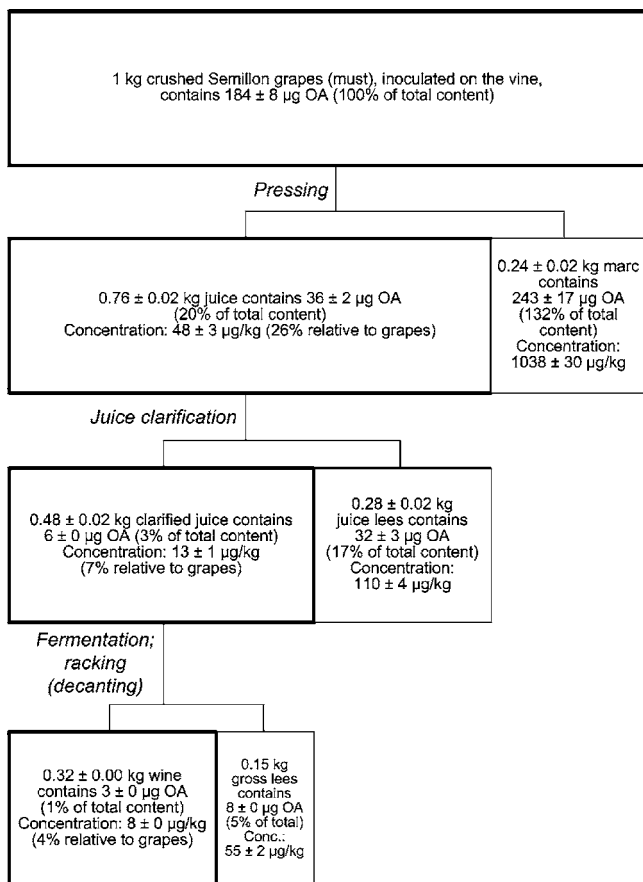| compositional parameter[a]	Semillon		Shiraz	
	uninoculated	inoculated[b]	uninoculated	inoculated[c]
total soluble solids (°Brix)	15.8	19.0	20.8	25.4
titratable acidity (g/L)	4.7	7.3	5.1	8.2
malic acid (g/L)	1.1	1.8	0.5	0.8
tartaric acid (g/L)	3.6	4.1	6.1	7.2
citric acid (g/L)	0.2	0.9	0.2	1.5

[a] Single determination of a bulk sample. [b] Inoculated with *A. carbonarius* 9 days before commercial maturity (harvest), as indicated by uninoculated grapes. [c] Inoculated with *A. carbonarius* 15 days before commercial maturity (harvest), as indicated by uninoculated grapes.

(*16*), and it is probable that *A. carbonarius*, a closely related species, also produces this organic acid.

Uninoculated grapes were not vinified in this trial or assayed for OA because the toxin was not detected (our unpublished data) in a previous trial with uninoculated grapes from this vineyard (*19*). During vinification of inoculated grapes, the extent of pressing and racking mirrored commercial practice as closely as possible. Semillon and Shiraz marc contained 57% and 52% moisture, respectively. Semillon juice lees contained 85% moisture, and Semillon and Shiraz gross lees contained 94% and 87% moisture, respectively.

Data for the total amount of OA in product and waste streams are presented in **Figures 1** and **2**, expressed as vinification of 1 kg of must. Reduction in OA content occurred at each of the solid−liquid separation stages. Furthermore, the greatest reduction occurred during pressing, with additional reductions upon racking from juice and gross lees. OA was significantly different ($P < 0.001$) between the solid and liquid phases at each separation stage, and preferentially partitioned into the solid-phase waste streams (marc and lees), although these phases represented only a small proportion by mass. For example, during clarification of white juice, juice lees comprised about one-third of the mass, yet contained 5-fold more OA (32 μg) than clarified juice (6 μg of OA). Generally, the amount of OA entering a particular stage was conserved and divided between the solid and liquid phases at the next separation step. The amount of OA in marc (87−132% of total content) was overestimated because of the error associated with diluting the marc samples so that the OA concentration was within a suitable range for analysis. When marc was diluted 10-fold with water and homogenized, the heavier seed particles settled immediately, whereas lighter skin particles remained suspended in the homogenate. Consequently, a 15 g sample of homogenate was biased toward sampling the skins, which often contain more OA than pulp (*4*), and biased against sampling the seeds, which are normally sterile (*20*) and thus unlikely to contain OA. Overall, the trends in **Figures 1** and **2** suggested that OA was not chemically or biologically transformed during vinification; rather, the reduction in OA occurred when it was discarded with the solid waste stream.

The concentration of OA in wine after the first racking, relative to the initial concentration in crushed grapes, was greater in red (9%) than in white (4%) wine. These values are similar to those previously reported for red wines (6−17% (*5, 6*); 13% (*19*)) and white wines (4%; (*19*)), although higher relative concentrations for white wines have also been noted (11−33%, (*5, 6*)). Similar values obtained by Leong et al. (*19*) during vinification of grapes of initial OA concentration 2−114 μg/kg suggest that reduction in OA concentration of above 80% is

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Figure 1. Vinification of Semillon grapes (1 kg), showing mass transfer, ochratoxin A content, and concentration (± standard error of the mean of four or more replicate vessels). Box sizes denote the relative masses of processed (bold lines) and waste streams.
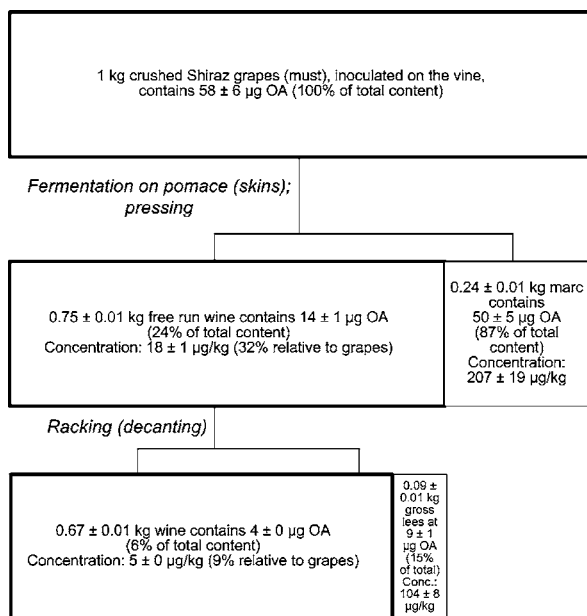
Figure 2. Vinification of Shiraz grapes (1 kg), showing mass transfer, ochratoxin A content, and concentration (± standard error of the mean of eight replicate vessels). Box sizes denote the relative masses of processed (bold lines) and waste streams.

common during vinification, regardless of the initial concentration in grapes.

The greater retention of OA during red compared with white vinification was noted after two processes: pressing (24% cf.
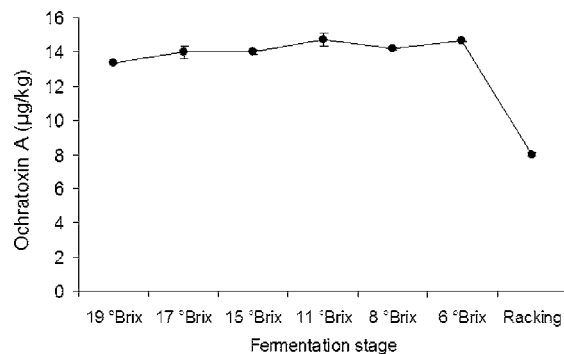
Figure 3. Ochratoxin A in juice and suspended yeasts during fermentation of Semillon juice. Error bars denote the standard error of the mean of four replicate vessels.

20% of the total OA content in red and white grapes, respectively) and racking (6% in red vinification cf. 1% in white vinification). This may be partly attributed to two key differences during red and white vinification. In white vinification, grapes are pressed before fermentation and, hence, in the absence of alcohol, OA may be poorly partitioned in the liquid phase and may bind more effectively to grape solids. White vinification also has an additional juice clarification step, when such solids are allowed to settle and the juice decanted. A proportion of mass was lost at this stage so that the final mass of white wine was half that of red wine. Nevertheless, this white juice clarification step reduced OA to 3% of the total content, with a further reduction to 1% achieved by binding to yeast during fermentation. This two-stage reduction is not present during red vinification. Rather, grape solids, grape skins, and yeasts compete to bind OA during red grape fermentation. The OA concentration in red juice before fermentation (grape solids removed) was similar to that of wine after fermentation (grape solids and yeasts removed) (*21*). Thus, increased yeast biomass during red fermentation was not able to confer the additional reduction in OA observed in white fermentation. Pressing of white grapes before fermentation, coupled with white juice clarification, were likely reasons for the greater reduction of OA in white relative to red wine. The overall trend for decreased incidence of OA contamination in white compared with red wine has also been attributed to the growth of white cultivars in cooler regions with decreased incidence of *Aspergillus* contamination (*1*, *22*).

The OA concentration was unchanged during fermentation of Semillon juice, indicating that the toxin was not metabolized by the yeast (**Figure 3**). OA concentration was significantly reduced ($P < 0.05$) only upon racking from the gross lees. In contrast, Lataste et al. (*9*) reported a linear decrease in OA concentration during juice fermentation; however, this appears to result from binding of OA to the increasing yeast biomass, rather than from biological transformation of the molecule. In the current study, this biomass was included in the sampling during fermentation and subsequent analysis; thus, the OA concentration was constant, and decreased only after the yeast cells precipitated and the wine was decanted from the gross lees. Recovery of OA from the gross lees (primarily yeast cells; **Figure 1**) further supports the finding that OA is not biologically transformed during grape juice fermentation, as also reported by other authors (*8*, *9*).

Minimizing infection of grapes by toxigenic strains of *A. carbonarius* or *A. niger* is critical for reducing the incidence and concentration of OA in wine. In addition to producing the toxin, these fungi may cause berry shriveling, increasing the total soluble solids of berries, and also increasing citric acid

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content; all are factors which may alter wine quality. The concentration of OA in the finished wine is much less than that in the grapes, as most of the toxin is removed during vinification through binding to the marc and lees. The binding of OA to the increased yeast biomass during fermentation appears to be an important stage in the reduction of OA. This stage is not present in grape juice production, which may contribute to the greater contamination of grape juice than wine (*23−25*). Differences in red and white vinification, in particular, the additional juice clarification stage during white vinification, may explain the greater OA contamination observed in red than in white wine reported in this study, and also noted in surveys of wines in Europe (*1*). Further research on the nature of OA binding to precipitated grape constituents and yeasts during various stages of vinification may lead to practices to enhance removal of OA. Of greater concern is the potential use of waste streams, such as rachides after crushing or grape marc, for production of grape alcohol, extraction of tannins, or extraction of grapeseed oil. OA is relatively heat-stable (*3, 26*) and little is known about the fate of OA during these processes.

SAFETY

OA is a nephrotoxin and a potential human carcinogen. Protective clothing was worn when handling OA extracts and standards. Glassware was decontaminated in bleach solution.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Bellí, N.; Marín, S.; Sanchis, V.; Ramos, A. J. Ochratoxin A (OTA) in wines, musts and grape juices: occurrence, regulations and methods of analysis. *Food Sci. Technol. Int.* **2002**, *8*, 325−335.

(2) Abarca, M. L.; Accensi, F.; Cano, J.; Cabañes, F. J. Taxonomy and significance of black aspergilli. *Antonie Leeuwenhoek* **2004**, *86*, 33−49.

(3) Roset, M. Survey on ochratoxin A in grape juice. *Fruit Process.* **2003**, *13*, 167−172.

(4) Battilani, P.; Giorni, P.; Bertuzzi, T.; Pietri, A. Preliminary results on ochratoxin A production by fungi invading grape berries. In *11th Congress of Mediterranean Phytopathological Union and 3rd Congress of Sociedade Portuguesa de Fitopatologia*, Evora, Portugal, 2001; pp 120−122.

(5) Fernandes, A.; Ratola, N.; Cerdeira, A.; Alves, A.; Venâncio, A. Fate of ochratoxin A during vinification. *Am. J. Enol. Vitic.* **2006**, submitted for publication.

(6) Fernandes, A.; Venâncio, A.; Moura, F.; Garrido, J.; Cerdeira, A. Fate of ochratoxin A during a vinification trial. *Asp. App. Biol.* **2003**, *68*, 73−80.

(7) Baxter, D. E.; Shielding, I. R.; Kelly, B. Behavior of ochratoxin A in brewing. *J. Am. Soc. Brew. Chem.* **2001**, *59*, 98−100.

(8) Bejaoui, H.; Mathieu, F.; Taillandier, P.; Lebrihi, A. Ochratoxin A removal in synthetic and natural grape juices by selected oenological *Saccharomyces* strains. *J. Appl. Microbiol.* **2004**, *97*, 1038−1044.

(9) Lataste, C.; Guérin, L.; Solanet, D.; Berger, J. L.; Cottereau, P.; Molot, B. Évolution de la contamination en ochratoxine A: du vignoble Français au vin. *Prog. Agric. Vitic.* **2004**, *121*, 57−64.

(10) Ratola, N.; Abade, E.; Simões, T.; Venâncio, A.; Alves, A. Evolution of ochratoxin A content from must to wine in Port Wine microvinification. *Anal. Bioanal. Chem.* **2005**, *382*, 405−411.

(11) European Commission. Commission Regulation (EC) No 123/2005 of 26 January 2005 amending Regulation (EC) No 466/2001 as regards ochratoxin A. *Off. J. Eur. Union* **2005**, *L25*, 3−5.

(12) Leong, S. L.; Hocking, A. D.; Scott, E. S. Effects of water activity and temperature on the survival of *Aspergillus carbonarius* spores *in vitro. Lett. Appl. Microbiol.* **2006**, *42*, 326−330.

(13) Iland, P.; Ewart, A.; Sitters, J.; Markides, A.; Bruer, N. *Techniques for chemical analysis and quality monitoring during winemaking*; Patrick Iland Wine Promotions: Campbelltown, SA, Australia, 2000.

(14) Frayne, R. F. Direct analysis of the major organic components in grape must and wine using high performance liquid chromatography. *Am. J. Enol. Vitic.* **1986**, *37*, 281−287.

(15) Valenta, H. Chromatographic methods for the determination of ochratoxin A in animal and human tissues and fluids. *J. Chromatogr. A* **1998**, *815*, 75−92.

(16) Raper, K. B.; Fennell, D. I. *The Genus Aspergillus*; The Williams & Wilkins Company: Baltimore, MD, 1965.

(17) Diakou, P.; Moing, A.; Svanella, L.; Ollat, N.; Rolin, D. B.; Gaudillere, M.; Gaudillere, J. P. Biochemical comparison of two grape varieties differing in juice acidity. *Aust. J. Grape Wine Res.* **1997**, *3*, 117−126.

(18) Zoecklein, B. W.; Fugelsang, K. C.; Gump, B. H.; Nury, F. S. *Wine Analysis and Production*; Aspen Publishers: Gaithersburg, MD, 1999.

(19) Leong, S. L.; Hocking, A. D.; Pitt, J. I.; Kazi, B. A.; Emmett, R. W.; Scott, E. S. Black *Aspergillus* species in Australian vineyards: from soil to ochratoxin A in wine. In *Advances in Food Mycology*; Hocking, A. D., Pitt, J. I., Samson, R. A., Thrane, U., Eds; Springer: New York, 2006; pp 153−171.

(20) Bau, M.; Bragulat, M. R.; Abarca, M. L.; Minguez, S.; Cabañes, F. J. Ochratoxigenic species from Spanish wine grapes. *Int. J. Food Microbiol.* **2005**, *98*, 125−130.

(21) Leong, S. L.; Hocking, A. D.; Scott, E. S. The effect of juice clarification, static or rotary fermentation and fining on ochratoxin A in wine. *Aust. J. Grape Wine Res.* **2006**, in press.

(22) Battilani, P.; Barbano, C.; Marín, S.; Sanchis, V.; Kozakiewicz, Z.; Magan, N. Mapping of *Aspergillus* section *Nigri* in Southern Europe and Israel based on geostatistical analysis. *Int. J. Food Microbiol.* **2006**, in press.

(23) Majerus, P.; Otteneder, H. Nachweis und Vorkommen von Ochratoxin A in Wein und Traubsaft. *Deutsche Lebensmittel-Rundschau* **1996**, *92*, 388−390.

(24) Ministry for Agriculture, Fisheries and Food. *1998 Survey of retail products for ochratoxin A*; Ministry for Agriculture, Fisheries and Food: London, UK, 1999.

(25) Zimmerli, B.; Dick, R. Ochratoxin A in table wine and grape-juice: occurrence and risk assessment. *Food Add. Contam.* **1996**, *13*, 655−668.

(26) Arici, M.; Gümüs, T.; Karah, F. The fate of ochratoxin A during the Pekmez production from mouldy grapes. *Food Control* **2004**, *15*, 597−600.