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# Removal of Ochratoxin A from Contaminated Red Wines by Repassage over Grape Pomaces

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Ochratoxin A contamination of red wines might be quite severe in certain high-risk regions and vintages, thus requiring corrective measures to fulfill acceptable standards for human consumption. This work proposes an innovative and environmentally friendly corrective measure to reduce ochratoxin A levels by repassage of contaminated musts or wines over grape pomaces having no or little ochratoxin A contamination. Grape pomaces have a high affinity for ochratoxin A and have been shown to remove ochratoxin A from must and wine during vinification. Time course experiments showed that ochratoxin A adsorption by pomaces is a rapid process, reaching equilibrium in less than 10 h, and is not affected by the tested toxin concentrations. Repassage of wine from Primitivo grapes spiked with 2−10 μg/kg ochratoxin A over pomaces obtained from the same grapes removed up to 65% ochratoxin A within 24 h. Similar results (50−65% ochratoxin A reduction) were obtained with Primitivo or Negroamaro wines repassed over pomaces from different grape varieties including white grapes (Malvasia, Greco di Tufo) and red grapes (Sangiovese, Aglianico). Grape pomaces maintained a good efficacy in removing ochratoxin A after being reused four times. Unlike other enological fining agents, the use of grape pomaces to adsorb ochratoxin A from red wines of the same grape variety (Primitivo) did not affect relevant wine quality parameters, including color intensity and health-promoting phenolic content (*trans*-resveratrol, quercetin, total polyphenols). These quality parameters were instead positively or negatively affected when contaminated wines were repassed over grape pomaces from other grape varieties, the effect being related to the intrinsic characteristics of the pomace variety. The proposed decontamination procedure can be applied in a modern winery provided that contaminated grapes are identified early and processed separately from uncontaminated grapes.

**KEYWORDS: Ochratoxin A; must; red wines; grape pomaces; repassage; adsorption; decontamination; polyphenols.**

## INTRODUCTION

Ochratoxin A is a mycotoxin produced by several species of *Aspergillus* and *Penicillium* naturally occurring in a variety of food commodities prior to harvest or more commonly during storage. Ochratoxin A has been shown to be carcinogenic, nephrotoxic, teratogenic, and immunotoxic to animals and is suspected to be involved in the pathogenesis of the Balkan Endemic Nephropathy and tumors of the upper urinary tract in humans (*1*). The occurrence of ochratoxin A has been reported in wine, grape juice, and vine fruits worldwide, and risk assessment studies have been performed to estimate the relevant human intake (*1*−*3*). In 2002, an assessment of dietary intake of ochratoxin A by the population of the EU Member States concluded that wine contributes 13% of the mean European total dietary intake making it the second most important contributor, cereals and cereal products contributing the most (50%) (*3*). Accumulation of ochratoxin A in grape originates in the vineyard and is caused mainly by *Aspergillus carbonarius*, a fungus developing on grape berries especially after veraison and increasing with ripening until harvest (*2*). The level of ochratoxin A varies considerably among various types of wines and vine products, regions and vintages. It is found more frequently and in greater quantities in red wines than in rosé or white ones (*4*), and wines from the Mediterranean basin appear to be more contaminated than those produced in other European regions with cooler climates (*2*). Since the vintage of 2006, with the adoption of Regulation CE 123/05, the level of ochratoxin A in commercial wines cannot exceed 2 μg/kg (*5*, *6*). However, many trade agreements require lower limits than those adopted by the Regulation and some of these agreements require that ochratoxin A not exceed 0.5 μg/kg.

Several approaches have been tested to reduce the occurrence of ochratoxin A in grape and to remove the toxin during and after vinification. Prevention strategies to control ochratoxin A in the vineyard include the use of biocontrol agents and fungicides

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against *A. carbonarius*, insecticides against *Lobesia botrana*, a pest that promotes *A. carbonarius* infection in vineyards (*2*, *7*−*9*). However they cannot completely prevent the ochratoxin A problem, and severe contamination of wine can occur especially for some susceptible grape varieties in certain high risk regions or vintages with climatic conditions conducive to *A. carbonarius* infection. Therefore corrective actions are necessary and should be adopted in winery. Several fining agents have been tested for their ability to remove ochratoxin A from contaminated musts or wines, with enological charcoal showing the highest adsorption capacity for ochratoxin A (*2*, *10*, *11*). Charcoal for enological use has been proposed as an acceptable practice for still wines (*12*), and the Code of practice for the prevention and reduction of ochratoxin A contamination in wine foresees the use of the lowest possible and most effective doses of enological charcoal (*13*). However it is well-known that the efficacy of charcoal in ochratoxin A removal is directly related to the reduction in quality parameters of the treated wines including the polyphenol content (*2*, *10*). For example, red wine-induced production of nitric oxide in human healthy peripheral blood mononuclear cells has been shown to drop dramatically when the wine is deprived of polyphenols through addition of charcoal (*14*). Polyphenolic compounds can exert numerous beneficial biological activities including antiatherogenic activities and endothelium vasorelaxation via increased synthesis of nitric oxide (*15*). *trans*-Resveratrol and quercetin, belonging to stilbene and flavonol chemical groups, respectively, occur frequently and at high levels in red wines and exert several beneficial biological actions including protection of low-density lipoprotein cholesterol against oxidation and promotion of endothelial vasorelaxation (*16*, *17*). A positive correlation between ochratoxin A and total stilbene levels was found in red wines highly susceptible to ochratoxin A contamination, suggesting that toxic levels of ochratoxin A may be, to some extent, counterbalanced by the beneficial effects of resveratrol derivatives (*18*). Studies on the fate of ochratoxin A during vinification of red grape demonstrated that 95% of ochratoxin A originally present in grape remains adsorbed to grape pomaces (*2*, *19*). High levels of ochratoxin A have been found in red grape pomaces obtained in 2005 (up to 468 $\mu$g/kg), a vintage particularly conducive to *A. carbonarius* infection, which confirms the high affinity of grape pomaces for ochratoxin A (*20*). Grape pomace is the press residue remaining when grapes are processed for wine-making and consists of pressed skins, disrupted cells from the grape pulp, and seeds.

The purpose of this study was to develop an environmentally friendly corrective measure to remove ochratoxin A from contaminated red wine, without affecting wine quality parameters, using grape pomaces from uncontaminated grapes. The proposed decontamination procedure has been applied at both laboratory and industrial scale to red wines of grape varieties, such as Primitivo and Negroamaro, quite susceptible to infection by *Aspergillus carbonarius* and ochratoxin A accumulation (*21*).

## MATERIALS AND METHODS

**Reagents and Materials.** All chemicals used were of analytical grade unless otherwise stated. All solvents (HPLC grade) and chemicals were purchased from Mallinckrodt Baker (Milan, Italy). Water was of Milli-Q quality (Millipore, Bedford, MA). Ochratoxin A, quercetin, *trans*-resveratrol, poly(ethylene glycol) (PEG 8000), and sodium hydrogen carbonate ($NaHCO_3$, ACS grade) were purchased from Sigma-Aldrich (Milan, Italy). OchraTest immunoaffinity columns were purchased from Vicam (Watertown, MA). Ochratoxin A stock solutions (1 mg/mL) were prepared by dissolving the solid standard in toluene/acetic acid (99:1, v/v) for HPLC calibration or in 2.3% (w/v) aqueous $NaHCO_3$ for spiking purposes. For HPLC calibration, aliquots of ochratoxin A stock solution, previously evaporated to dryness under nitrogen stream, were dissolved in an adequate amount of HPLC mobile phase, whereas for spiking purposes, adequate amounts of the stock solution were mixed with a mixture of must or wine (previously centrifuged) and distilled water (50:50, v/v). Stock solutions of quercetin or *trans*-resveratrol (5 mg/mL) were prepared in methanol and stored at −20 °C in the darkness after elimination of oxygen with a nitrogen stream to avoid oxidation of phenolic compounds. For calibration purposes working solutions containing both quercetin and *trans*-resveratrol in the range 5.0−0.25 $\mu$g/mL were prepared by diluting stock solutions with the HPLC mobile phase.

**Analysis of Ochratoxin A, Phenolic Compounds, and Other Quality Parameters in Wines.** Ochratoxin A was determined in wines (after centrifugation) by the AOAC official method for the analysis of ochratoxin A in wine (*4*). In brief, centrifuged wines were diluted with a water solution containing PEG (1%) and $NaHCO_3$ (5%), mixed, filtered, and cleaned-up by OchraTest immunoaffinity columns. Ochratoxin A was eluted with methanol and quantified by reversed-phase HPLC with fluorometric detector (FLD) with excitation and emission wavelengths set at 333 nm ($\lambda_{ex}$) and 460 nm ($\lambda_{em}$), respectively. Ochratoxin A in grape pomaces was determined by using a method developed in our laboratory specifically for this matrix (*20*). Briefly, pomace samples were dried at 50 °C for 48 h, finely ground and extracted with a mixture of acetonitrile/water (60:40 v/v). An aliquot of the filtered extract was diluted with a water solution containing PEG (1%) and $NaHCO_3$ (5%), filtered, purified by immunoaffinity column cleanup and analyzed by HPLC-FLD. Quercetin and *trans*-resveratrol were analyzed by HPLC with a UV−visible diode array detector (DAD), by direct injection of filtered (0.45-$\mu$m membrane filters, Millipore, Milford, MA) wine samples as described by Careri et al. (*22*). Chromatograms were recorded with DAD wavelengths set at the absorbance maxima of *trans*-resveratrol (307 nm) and quercetin (370 nm). The latter were identified by checking peak purity and comparing retention times and UV−vis spectra, in the 220−450 nm range, with those of the relevant standard compounds.

The HPLC apparatus was an Agilent 1100 series equipped with a G1312A binary pump, G1313A autosampler, G1316A column thermostat set at 30 °C, G1315B UV−visible DAD, G1321A spectrofluorometric detector, and Agilent Chemstation G2170AA Windows 2000 operating system (Agilent, Waldbronn, Germany). The column used was a 150 × 4.6 mm, 5 $\mu$m, Symmetry $C_{18}$ (Waters, Milford, MA) with a 3 mm i.d., 0.45 $\mu$m pore size guard filter (Rheodyne, Cotati, CA).

A Tekno apparatus (Chema, Latina, Italy) was used for the analysis of total polyphenols, alcohol, volatile acidity, and color intensity in wines. In this paper, the term must is used when the alcohol content is lower than 1%, while wine is used for must partially or completely fermented.

**Repassage of Red Wines over Grape Pomaces.** *Time Course of Ochratoxin A Adsorption by Uncontaminated Grape Pomaces*. Red grapes of the early maturing Cardinale variety, harvested in Adelfia (Apulia, Italy), were used to assess the time course of the adsorption of ochratoxin A onto pomaces. The selection of this early maturing variety (end of July) for this preliminary experiment allowed us to plan within the same harvesting season the following repassage experiments using late maturing varieties (middle to end of September) of ochratoxin A susceptible red grapes. Eight 1.1 kg grape portions (1−8) were manually destemmed, crushed, and placed in small plastic basins. Four portions (5−8) were artificially contaminated with ochratoxin A by adding 10 mL of a mixture of must (previously centrifuged) and ochratoxin A stock solutions (50:50, v/v) to obtain 5, 10, 32, 70 $\mu$g/kg ochratoxin A levels in must, respectively. Each portion was mixed and left to macerate/ferment at room temperature (25−30 °C) for 6 days. After fermentation, 5 mL aliquots of each wine were submitted to ochratoxin A analysis to assess ochratoxin A concentrations in spiked samples (5−8) and confirm ochratoxin A absence in the four control samples (1−4). Then each sample of crushed grape was devatted and pressed by using a mod. PM20 Agrolmacchine small press for fruit (Agrolmacchine, Taurianova, Italy) to separate pomaces from wine. Pomaces of samples 1−4 were used to decontaminate wines derived from samples 5−8, while the remaining materials (pomaces from samples 5−8 and wines from samples 1−4) were discarded. Pressed pomaces from samples 1−4 were individually placed in small plastic basins, and ochratoxin A contaminated wines obtained from samples 5−8, respectively, were added and left in contact for 28 h by mixing three times at regular intervals. For the above and the other

repassage experiments described herein, the amounts of pomaces and wine used were those corresponding to the amounts derived from processed grapes. To assess the time course of the ochratoxin A adsorption by pomaces, 5-mL aliquots of wine were collected after 2, 8, 21, and 28 h. For ochratoxin A analysis wine samples were centrifuged at 4000 rpm for 15 min, and then 4 mL of supernatants was analyzed as described above. Ochratoxin A reduction (%) achieved by repassage over pomaces was calculated by the difference in ochratoxin A concentration in wine before and after repassage.

*Repassage of Wine over Pomaces of the Same Red Grape Variety*. Repassage experiments with wines and pomaces of the same red grape variety were carried out in a winery by using four lots of about 25 kg of Primitivo grapes produced in Manduria (Apulia, Italy). Vinification trials were carried out at Consorzio Produttori Vini e Mosti (Manduria, Italy) employing the technology currently used in that winery. Briefly, grapes were destemmed and crushed by a Cantinetta stalk remover (Zambelli Enotech, Camisano Vicentino, Italy), supplemented with 0.1 g/kg grape of potassium metabisulfite, 0.5 g/kg grape of active dry yeast commercial *Saccharomyces cerevisiae* Blastosil Gran Cru FR 95, (Perdomini, Verona, Italy), and 0.3 g/kg grape of fermentation activator Actibiol, (Perdomini, Verona, Italy). The mixtures were placed into 50 L plastic tanks and left to macerate/ferment for 5 days at room temperature (25−30 °C) and then devatted as described above to separate wine from the pomaces. Pomaces of lots 1−3 were weighed and placed in three plastic tanks while pomace of lot 4 was discarded. Wines of lots 1−4 were weighed (ca. 20 kg each) and artificially contaminated to obtain ochratoxin A levels of 2, 5, 10, and 5 μg/kg, respectively, and then homogenized by mixing for 1 h. Afterward, wine of lots 1−3 were added to the relevant pomaces in 50 L plastic tanks and left at room temperature for 24 h by mixing three times at regular intervals to allow ochratoxin A adsorption onto pomaces. After 24 h, bulks were re-devatted as described above, and each sample of wine and pressed pomace was weighed. Samples of wine for ochratoxin A analysis were taken before and after spiking and after repassage over pomaces. Samples of pressed pomaces for ochratoxin A analysis were taken before and after repassage. Samples of wine for quality parameter analyses were taken before and after repassage.

To evaluate the capacity of the same grape pomaces to decontaminate several lots of contaminated wine, the exhausted pomaces of lot 2 were consecutively used. In particular, an aliquot (corresponding to one-third) of pomaces collected from lot 2 after the first repassage was placed in a 15 L plastic tank and treated three times consecutively (24 h as described above) with aliquots (corresponding to one-third) of contaminated wine of lot 4 (spiked at 5 μg/kg ochratoxin A). After each repassage period wines were collected and submitted to ochratoxin A analysis to calculate the relevant ochratoxin A percent reduction.

*Repassage over Pomaces at Industrial Scale*. The proposed decontamination procedure was also tested at industrial scale by performing maceration/fermentation of crushed grapes in 50 ton vertical cylindrical tanks. Two tanks out of the five tanks available at the Consorzio Produttori Vini e Mosti (Manduria, Italy) were selected after ochratoxin A analysis of wine. Since the wine of all tanks were contaminated by ochratoxin A, the tanks with higher (1.44 ± 0.04 μg/kg) and lower (0.83 ± 0.05 μg/kg) ochratoxin A concentrations were selected for the experiment. In particular, the wine with lower ochratoxin A content was drained off and dripping pomaces were left in the tank. Then the wine with higher ochratoxin A content was drained off and pumped in the tank containing dripping pomaces. Wine was circulated within the tank for 24 h by repassing of wine over the pomaces (pumping wine from bottom to upper part of the tank three times). After 24 h, aliquots of wine were sampled and analyzed for ochratoxin A.

*Repassage of Wine over Uncontaminated Pomaces Obtained from Different Grape Varieties*. This experiment was performed to confirm the adsorption capacity of different pomaces and evaluate the change of quality parameters when pomaces from different varieties are used to decontaminate Primitivo and Negroamaro wines. Primitivo wine (80 kg) was prepared as described above, whereas 80 kg of Negroamaro wine was provided by the winery Cantele (Guagnano, Apulia, Italy). Primitivo and Negroamaro wines were spiked with 400 μg of ochratoxin A and well mixed for 1 h. Clean (ochratoxin A free) pomaces of two red grape varieties (Sangiovese and Aglianico) and two white grape varieties (Malvasia and Greco di Tufo) were obtained as described above with the difference for white grapes that pressing was performed soon after destemming and crushing. For each grape variety 50−60 kg of grapes, produced in Conversano (Apulia, Italy), were used.

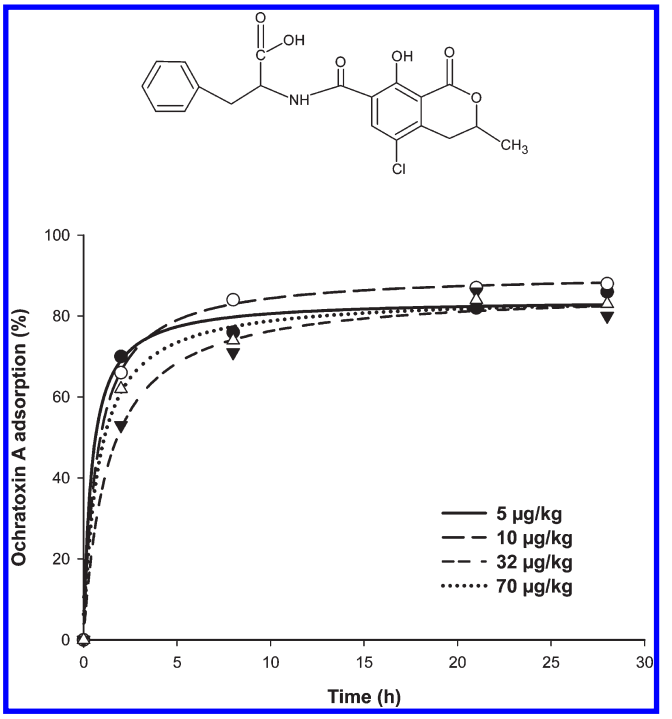


**Figure 1.** Chemical structure of ochratoxin A and time course of toxin adsorption from Cardinale wines with different ochratoxin A levels (5, 10, 32, 70 μg/kg) using pomaces from Cardinale grapes.

Four aliquots (15 kg each) of contaminated Primitivo and Negroamaro wines were decontaminated on pomaces obtained from Sangiovese, Aglianico, Malvasia, and Greco di Tufo, following the procedure described above (same ratio wine/pomaces, 24 h contact, mixing three times at regular interval). Ochratoxin A analyses were performed on wine samples taken before and after ochratoxin A spiking and after repassage as well as on pomace samples before and after repassage. Wine analyses for quality parameters were performed before and after repassage.

**Statistical Data Analysis.** Statistical analyses were completed using the GraphPad Instat statistical software package (Instat, San Diego, CA). Statistical significance was set at $p < 0.05$ (paired $t$ test, two-tailed $p$ values).

## RESULTS AND DISCUSSION

**Time Course of Ochratoxin A Adsorption by Uncontaminated Grape Pomaces.** Results of the repassage experiments to investigate the time course of ochratoxin A adsorption from wine to grape pomaces are reported in **Figure 1**. Ochratoxin A adsorption by grape pomaces reached equilibrium (i.e., a steady distribution of ochratoxin A content between wine and pomaces) in less than 10 h and was not affected by the toxin concentration in the wine. A slight difference in the percentage of adsorption, depending on the initial toxin concentration, was only observed at the beginning of the trials, after 2 h contact between pomaces and wine, when relative toxin adsorption (ranging from 53% to 70%) in wines containing lower toxin levels was higher than that in wines with high toxin levels. After 24 h, above 80% ochratoxin A adsorption was recorded for all tested toxin levels (ranging from 5 to 70 μg/kg). A contact time of 24 h was then used for successive adsorption/repassage experiments. The natural ratio between pomaces and wine of crushed grapes was also maintained for the other decontamination experiments described herein.

**Removal of Ochratoxin A from Red Grape Wines by Repassage of Wines over Pomaces from the Same Grape Variety.** For these

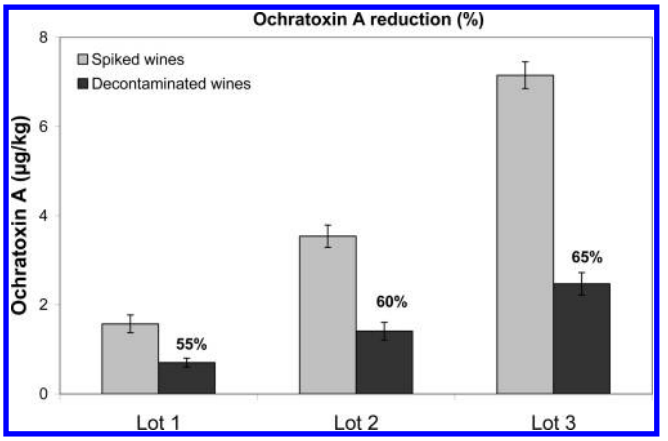


**Figure 2.** Removal of ochratoxin A from Primitivo wine samples (18.9 kg) spiked at different ochratoxin A levels (2, 5, 10 $\mu$g/kg in lots 1, 2 and 3, respectively) after 24 h repassage over pomaces (3.2 kg) from Primitivo grapes.

**Table 1.** Mass Balance of Ochratoxin A during Repassage of Primitivo Wine over Pomaces of Primitivo

| parameters | lot 1 | lot 2 | lot 3 |
|---|---|---|---|
| ochratoxin A spiking concentration ($\mu$g/kg) | 2.0 | 5.0 | 10.0 |
| ochratoxin A added to wine ($\mu$g)[a] | 39.5 | 95.5 | 190.5 |
| endogeneous ochratoxin A level in pomaces ($\mu$g/kg) | 4.1 | 4.3 | 4.4 |
| endogeneous ochratoxin A in pomaces ($\mu$g) | 2.9 | 2.6 | 3.5 |
| weight of wine after repassage (kg) | 18.7 | 19.1 | 18.1 |
| ochratoxin A concentration in wine after repassage ($\mu$g/kg) | 0.7 | 1.4 | 2.5 |
| ochratoxin A in wine after repassage ($\mu$g) A | 13.1 | 26.9 | 44.6 |
| weight of dry exhausted pomaces after repassage (kg) | 0.7 | 0.6 | 0.8 |
| ochratoxin A level in dry exhausted pomaces ($\mu$g/kg) | 42.0 | 90.3 | 158.7 |
| ochratoxin A in dry exhausted pomaces ($\mu$g) B | 29.4 | 54.2 | 127.0 |
| total ochratoxin A ($\mu$g) (A + B) | 42.5 | 81.1 | 171.6 |
| ochratoxin A recovered with respect to the initial amount (%) | 100 | 83 | 88 |

[a] The endogenous ochratoxin A content of wine (1.5 $\mu$g for each Lot weighing 18.9 kg) was included.

experiments, ochratoxin A was added to wine samples after separating wine from pomaces (devatting). Results obtained with Primitivo wine samples spiked at three different ochratoxin A levels are shown in **Figure 2**. After 24 h contact with pomaces, ochratoxin A content in wines decreased by 55−65% ($p < 0.001$) with respect to the levels measured in spiked samples. Ochratoxin A reduction due to repassage over pomaces was calculated by comparing ochratoxin A concentrations in wine measured before and after repassage. The actual ochratoxin A concentration measured by HPLC analysis of spiked samples, other than the spiking levels, was used to calculate the effective toxin removal obtained by repassage over pomaces. In fact, just devatted wine contains suspended biomass that can adsorb part of the spiked toxin giving the actual toxin concentration in spiked wine lower than the spiking level. Since nearly uncontaminated (blank) wine samples containing suspended biomass were used for decontamination experiments described in this paper the amount of ochratoxin A adsorbed by the biomass was not ascribed to the repassage over clean pomaces (see **Figures 2** and **4**). Results of ochratoxin A mass balance for this experiment (**Table 1**) show that the amounts of ochratoxin A removed from wine were recovered almost completely in the exhausted pomaces, which contained 83−100% of ochratoxin A present in wine before repassage. In particular, ochratoxin A level of 158 $\mu$g/kg compared with the endogenous 4.4 $\mu$g/kg was found in exhausted pomaces analyzed after repassage of wine spiked at 10 $\mu$g/kg

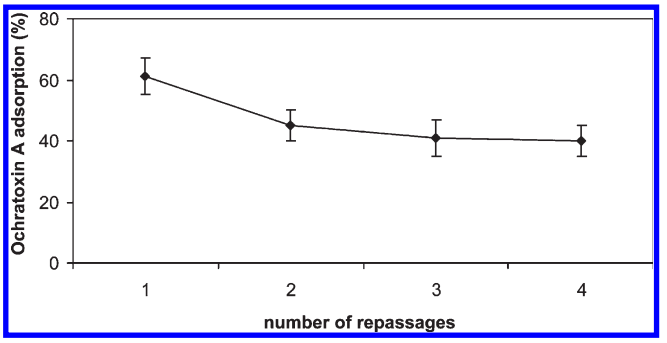


**Figure 3.** Ochratoxin A removal from four aliquots of Primitivo wine spiked at 5 $\mu$g/kg ochratoxin A after consecutive 24 h repassages over pomaces by reusage of the same grape pomaces.

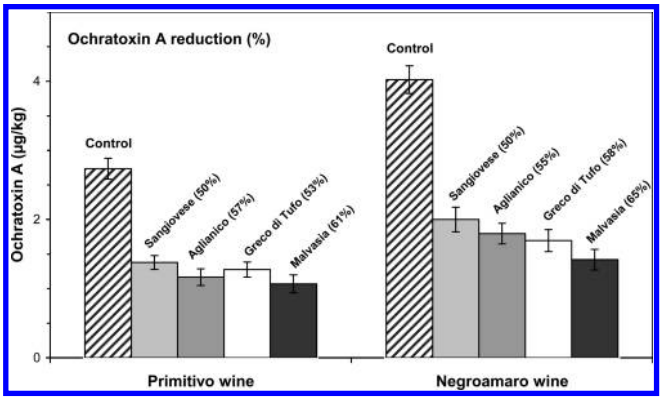


**Figure 4.** Removal of ochratoxin A in Primitivo and Negroamaro wines spiked at 5 $\mu$g/kg ochratoxin A by repassage of wines over uncontaminated pomaces from Sangiovese, Aglianico, Greco di Tufo, or Malvasia. Pomaces and wine were left in contact for 24 h.

ochratoxin A. Although grape bunches used in this experiment were manually selected to avoid using contaminated bunches, low levels of ochratoxin A were found in Primitivo wine (0.08 $\mu$g/kg) and pomaces (4.4 $\mu$g/kg). This is not surprising because sometimes bunches without visible symptoms can also contain ochratoxin A (*23*). The endogenous ochratoxin A content of wine and pomaces were considered for mass balance calculations.

Results shown in **Figure 3** demonstrate that pomaces can be reused to remove a significant amount of ochratoxin A from four aliquots of wine spiked at 5 $\mu$g/kg ochratoxin A and consecutively repassed over the same pomaces. In particular, ochratoxin A reduction ranged from 61% (1st repassage) to 40% (4th repassage).

The efficacy of repassage in removing ochratoxin A from wine was also proven at industrial level by using pomaces from less contaminated grapes in tanks of 50 ton capacity. Repassage of wine containing 1.44 $\mu$g/kg ochratoxin A for 24 h over dripping pomaces derived from wine containing 0.83 $\mu$g/kg ochratoxin A led to a 40% decrease of toxin concentration, namely, from $1.44 \pm 0.04$ $\mu$g/kg to $0.87 \pm 0.03$ $\mu$g/kg. These results demonstrate that repassage provides a good decontamination also when applied to naturally contaminated wine. Moreover, these results demonstrate that pomaces from wines containing less than 1 $\mu$g/kg ochratoxin A can be used to significantly reduce toxin levels in wine containing more than 1 $\mu$g/kg ochratoxin A.

**Removal of Ochratoxin A in Red Grape Wine by Repassage over Pomaces Obtained from Different Grape Varieties.** The results on the efficacy of repassage to remove ochratoxin A from wine by using pomaces from different varieties are shown in **Figure 4**. Uncontaminated pomaces of Sangiovese and Aglianico (red grape) or Greco di Tufo and Malvasia (white grape) were tested

on artificially contaminated Primitivo and Negroamaro wines. Pomaces obtained from any of the tested grape varieties significantly reduced ochratoxin A levels in both Primitivo and Negroamaro wines with respect to controls ($p < 0.001$). No significant difference in ochratoxin A reduction was found between pomaces from red and white grape varieties. Ochratoxin A content decreased by 50% to 61% in Primitivo wines and by 50% to 65% in Negroamaro wines, pomaces of Malvasia grapes being slightly more effective (**Figure 4**). As for the previous experiment the mass balance of ochratoxin A before and after these repassage experiments confirmed that the amount of ochratoxin A removed from Primitivo or Negroamaro wines was found in the exhausted pomace samples (data not shown). Although Primitivo and Negroamaro wines were spiked at the same ochratoxin A levels (5 $\mu$g/kg), the ochratoxin A concentrations as measured by HPLC before repassage over pomaces were different. In particular, the measured ochratoxin A concentration in Negroamaro wine was higher than that in Primitivo wine. This difference is not surprising and can be explained by the fact that Primitivo wine was particularly rich in suspended biomass since it was just devatted (wine separated from pomaces), whereas Negroamaro wine was nearly free of suspended biomass since it was devatted and successively racked (siphon the wine off the sediments). As reported above, the suspended biomass can adsorb part of the spiked ochratoxin A, which explain the lower measured toxin concentration in Primitivo wine compared with Negroamaro wine. Also for these experiments the amount of the toxin adsorbed by the biomass before repassage was not considered for calculation of toxin removal obtained with the repassage over clean pomaces (see **Figure 4**).

**Table 2.** Quality Parameters of Primitivo Wine before and after Repassage over Primitivo Pomaces[a]

| quality parameters | before repassage | after 24 h repassage |
|---|---|---|
| *trans*-resveratrol ($\mu$g/L) | 64 [a] | 62 [a] |
| quercetin ($\mu$g/L) | 354 [a] | 429 [b] |
| total polyphenols (mg/L) | 1225 [a] | 1243 [a] |
| alcohol content (g/100 mL) | 11.48 [a] | 11.43 [a] |
| volatile acidity (g/L) | 0.24 [a] | 0.28 [a] |
| color intensity (420 nm+520 nm) | 5.7 [a] | 5.7 [a] |
| tonality (420 nm/520 nm) | 0.68 [a] | 0.68 [a] |

[a] Different letters between columns indicate significant difference ($p < 0.05$). Values are means of three replicate experiments.

**Effect of Repassage on Quality Parameters of Wines.** The basic parameters describing wine quality have been measured before and after each experiment to assess the relevant effect of the repassage over pomaces. In addition to total polyphenols, resveratrol and quercetin were considered in this study because of their relevant occurrence in red wines and their health-promoting properties (*16*, *17*, *21*). The analytical data relevant to Primitivo wine before and after the repassage over Primitivo pomaces are reported in **Table 2**. Statistical analysis of the results shows that repassage did not affect negatively any of the tested quality parameters, while a significant increase of quercetin was observed in wine resulting in a quality improvement.

Quality parameters of Primitivo and Negroamaro wines before and after repassage over Sangiovese, Aglianico, Malvasia, and Greco di Tufo pomaces are reported in **Table 3**. As expected, the use of pomaces derived from grape varieties different from the wine to be decontaminated produced a significant modification of quality parameters depending on the intrinsic characteristics of the pomaces used for the repassage. As shown in **Table 3**, the repassage significantly affected most of the wine quality parameters of treated wines. For Primitivo wine, positive and negative effects were observed for 63% and 29% of the cases, respectively, whereas for Negroamaro the observed effects were negative in most cases. From these data, it can be concluded that for decontamination of Negroamaro wine the use of pomaces from the same variety should be preferred, whereas for Primitivo wine, the use of pomaces deriving from different grape varieties could be considered taking into account that some quality parameters can be affected.

Our findings demonstrate that ochratoxin A can be effectively removed from contaminated wine by repassage of wine over uncontaminated pomaces obtained from the same grape variety or from different grape varieties. The repassage of wine over pomaces from the same variety did not affect quality parameters of treated wine. Pomaces obtained from grape varieties different from the wine to be decontaminated can also be used bearing in mind that their intrinsic characteristics can modify (positively or negatively) quality parameters of the treated wine. This is the first report on the use of grape pomaces as a corrective measure to reduce ochratoxin A contamination in must/wine. The process

**Table 3.** Quality Parameters of Primitivo and Negroamaro Wines before and after Repassage (24 h) over Uncontaminated Pomaces of Sangiovese, Aglianico (red grapes), Malvasia, and Greco di Tufo (white grapes)

| | | after repassage over pomaces | | | |
|---|---|---|---|---|---|
| quality parameters | before repassage | Sangiovese | Aglianico | Malvasia | Greco di Tufo |
| | | Primitivo wine | | | |
| *trans*-resveratrol ($\mu$g/L) | 540 | 450[a] | 320[a] | 650[a] | 1050[a] |
| quercetin ($\mu$g/L) | 1450 | 2910[a] | 1630[a] | 7090[a] | 6760[a] |
| total polyphenols (mg/L) | 516 | 590[a] | 639[a] | 467[a] | 570[a] |
| alcohol content (g/100 mL) | 10.23 | 10.33 | 10.00[a] | 9.78[a] | 10.17 |
| volatile acidity (g/L) | 0.20 | 0.15[a] | 0.15[a] | 0.13[a] | 0.12[a] |
| color intensity (420 nm+520 nm) | 4.0 | 5.9[a] | 5.4[a] | 3.2[a] | 3.2[a] |
| | | Negroamaro wine | | | |
| *trans*-resveratrol ($\mu$g/L) | 930 | 640[a] | 580[a] | 480[a] | 810[a] |
| quercetin ($\mu$g/L) | 8420 | 4780[a] | 4210[a] | 7240[a] | 10450[a] |
| total polyphenols (mg/L) | 1522 | 1585 | 1167[a] | 1265[a] | 1208[a] |
| alcohol content (g/100 mL) | 14.23 | 14.02 | 13.71[a] | 11.62[a] | 11.90[a] |
| volatile acidity (g/L) | 0.18 | 0.18 | 0.18 | 0.18 | 0.21 |
| color intensity (420 nm+520 nm) | 12.1 | 13.6[a] | 11.5[a] | 8.3[a] | 9.6[a] |

[a] Significantly different ($p < 0.05$) from control (Primitivo or Negroamaro wine before repassage). Values are means of three replicate measurements.

can be easily performed at industrial scale by using equipment already available in wineries and can be applied to both musts and wines. On the other hand, the technique of repassing wine over pomaces, known as "ripasso", is commonly used for the production of some high-quality Italian wines such as Valpolicella. The "ripasso" technique consists of adding the skins of just-pressed dried grapes (e.g., Amarone) to regular wines (e.g., Valpolicella) and letting them to referment for 15−20 days to lend extra richness and depth. Nowadays, there are over 60 "ripasso" Italian wines on the market, which are made by a number of producers, often using their own variations of this basic method. However to our knowledge this technique has never been reported to remove ochratoxin A from contaminated wines, and no data are available on the toxin content before and after repassage both in the wines and in the pomaces.

The proposed procedure can be applied for ochratoxin A decontamination in a modern winery provided that contaminated grapes are identified early and processed (destemmed/crushed and macerated) separately from ochratoxin A-free or low contaminated grapes. Ochratoxin A analysis can be easily and quickly performed within the winery by using a rapid test kit commercially available. The process would imply that contaminated and clean lots are identified and devatted at least 24 h before the maceration end and that contaminated wine is repassed over clean pomaces for 24 h. The results reported in this paper were obtained by using fresh pomaces; therefore no negative consequences were observed on the wine after repassage. Storage of pomaces to be used for repassage purposes should be carefully conducted in order to prevent oxidative reactions that could negatively affect wine during repassage.

We are aware that in high risk areas and in certain seasons it may not be easy to find clean pomace to be used for decontamination purposes. However it is well-known that in several geographical areas ochratoxin A risk is low and the pomaces therein produced could be effectively used for decontamination purposes. This means that ochratoxin A contaminated must/wine produced in high-risk regions (generally associated with warm and humid climates and early maturing crops) can be decontaminated in wineries located in regions with higher availability of pomaces with no or little ochratoxin A contamination. Another possibility for negative vintage, that is, with heavy ochratoxin A contamination, could be the storage of ochratoxin A contaminated wine lots until vintages with clean pomaces from the same grape variety become available. In this regard, the additional advantage of the proposed procedure allowing grape pomaces to be reused for several repassages to reduce ochratoxin A content in several contaminated must/wine samples coming from either different locations or vintages should be pointed out.

The decontamination procedure proposed herein is a useful and environmentally friendly technique that can be used by wineries to fulfill both the EU Regulation and private trading standards without affecting quality parameters of musts and wines.

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