International Journal of Food Microbiology 241 (2017) 181–190

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Influence of physical and chemical characteristics of wine grapes on the incidence of *Penicillium* and *Aspergillus* fungi in grapes and ochratoxin A in wines

Luísa Freire [a], Fabiana Reinis Franca Passamani [a], Ariela Betsy Thomas [a], Rita de Cássia Mirela Resende Nassur [b], Lais Mesquita Silva [a], Fabiano Narciso Paschoal [c], Giuliano Elias Pereira [b], Guilherme Prado [c], Luís Roberto Batista [a,*]

[a] Federal University of Lavras, Lavras, Brazil
[b] Empresa Brasileira de Pesquisa Agropecuária/EMBRAPA, Semiárido, Petrolina, PE, Brazil
[c] Fundação Ezequiel Dias, Belo Horizonte, MG, Brazil

ARTICLE INFO

Article history:
Received 21 June 2016
Received in revised form 30 September 2016
Accepted 20 October 2016
Available online 21 October 2016

Keywords:
A. carbonarius
Phenolic compounds
Mycotoxins
Grape varieties

ABSTRACT

The incidence of filamentous fungi and toxin levels in grapes and wines varies depending on the variety of grapes, the wine region, agricultural practices, weather conditions, the harvest and the winemaking process. In this sense, the objective of this study was to evaluate the diversity of *Aspergillus* and *Penicillium* fungi isolated from wine grapes of the semi-arid tropical region of Brazil, evaluate the presence of ochratoxin A (OTA) in the experimental wine and verify if there is a correlation between occurrence of these fungi and the physicochemical characteristics of the wine grapes grown in the region. For the isolation of fungi we used the direct plating technique. The presence of OTA in the experimental wine was detected by high-performance liquid chromatography. The species found were *Aspergillus niger*, *A. carbonarius*, *A. aculeatus*, *A. niger* Aggregate, *A. flavus*, *A. sojae*, *Penicillium sclerotiorum*, *P. citrinum*, *P. glabrum*, *P. decumbens*, *P. solitum* and *P. implicatum*. All isolates of *A. carbonarius* were OTA producers and all *P. citrinum* were citrinin producers. The highest concentration of OTA was found in red wine (0.29 μg/L). All species identified in this study, except *A. flavus*, showed a positive correlation with at least one physicochemical parameter assessed, highlighting the pectin content, total sugar, total acidity and phenolic compounds.

1. Introduction

A variety of grapevines are grown in wine producing regions, which results in high variability of physical and chemical characteristics of the grapes, and consequently of the wines produced (Abe et al., 2007). However, grapes may be susceptible to infection by filamentous fungi from the initial stages of maturation (Bau et al., 2005) and this is due to the ability of these microorganisms to produce an enzyme complex responsible for the degradation of specific substrates, and production of secondary metabolites and volatile substances (Medina et al., 2015). Moreover, the presence of mycotoxins in wine is due to contamination of grapes by fungi, which develop at pre-harvest or during the harvesting steps leading to vinification.

Among the contaminating microorganisms, there is greater concern about the mycotoxin-producing fungi, *Aspergillus*, the main producer genus in grapes (*Vitis vinifera* L.) (Rousseaux et al., 2014; Serra et al., 2006). Among the mycotoxins, ochratoxin A (OTA) is the main contaminant of grapes and wines (Lombaert et al., 2004; Rosa et al., 2004). This toxin has demonstrated neurotoxic (Sava et al., 2006), genotoxic (Tozlovanu et al., 2006), carcinogenic (Brown et al., 2007), mutagenic (Palma et al., 2007), teratogenic (Balasaheb et al., 2007) and immunosuppressive (Rossiello et al., 2008) effects in animals. Its occurrence in wine is due to the growth of filamentous fungi on the grapes and subsequent mycotoxin production, and it remains along the vinification process, being present in the final product.

Despite studies reporting that the incidence of these fungi and toxin levels vary depending on the grape variety, wine region, agricultural practices, weather conditions, harvest and the winemaking process, there is no accurate information on the correlation between the physicochemical characteristics of the grapes and the presence of *Aspergillus* and *Penicillium*.

In this sense, the objectives of this study were to evaluate the diversity of *Aspergillus* and *Penicillium* in wine grapes and correlate the occurrence of these fungi with the physicochemical characteristics of the grapes

* Corresponding author at: Department of Food Science, Federal University of Lavras, Campus Universitário, Lavras CEP. 37200-000, MG, Brazil.
E-mail address: luisrb@dca.ufla.br (L.R. Batista).

http://dx.doi.org/10.1016/j.ijfoodmicro.2016.10.027
0168-1605/

grown in the region, in addition to quantifying the presence of OTA in the experimental wine made from the same varieties studied.

2. Material and methods

2.1. Study area

Grape samples were collected in a semi-arid tropical region of Brazil (8°59′30″ S to 9°16′30″ S and 39°59′40″ W to 40°52′19″ W), between 362 and 422 m of altitude; in the municipalities of Lagoa Grande and Santa Maria da Boa Vista in Pernambuco (PE) and Casa Nova in Bahia (BA).

2.2. Sampling

Four samples of white grapes were collected (Muscat Italia, Muscat Canelli and Chenin Blanc) and seven samples of red grapes (Syrah, Touriga Nacional, Ruby Cabernet, Tempranillo and Barbera) in the final stages of maturation of the berries (harvest season), in the July/August/September 2014 harvest. For this, a diagonal transect was drawn along the vineyard and three bunches of grapes from three equidistant plants were collected, disregarding the ends.

2.3. Mycological analysis of grapes (Vitis vinifera L)

From each point (P1, P2, P3) 100 berries were selected and their surfaces were disinfected (alcohol 70% for 1 min, sodium hypochlorite for 30 s and 3 washes with distilled water) to remove incidental surface contaminants. After disinfection, the berries were plated on DRBC - Dichloran Rose Bengal Chloramphenicol medium (Merck, Darmstadt, Germany) as described by Samson et al. (2000). The plates were incubated at 25 °C for seven days. The results were expressed in percent of berries infected by filamentous fungi, according to Pitt and Hocking (1997).

2.4. Phenotypic identification of Aspergillus and Penicillium

From the pure cultures, the fungi were identified according to Klich (2002), Varga et al. (2011) and Pitt (2000). The strains were incubated on CYA - Czapek Dox Agar culture media (Sigma-Aldrich, St. Louis, MO) at 25 °C and 37 °C and MEA - Malt Extract Agar (Sigma-Aldrich, St. Louis, MO) at 25 °C. After seven days incubation, macroscopic and microscopic characteristics were observed.

2.5. Evaluation of mycotoxigenic potential of Aspergillus isolates

To evaluate the toxigenic potential we used the agar plug method on thin layer chromatography in which potentially toxigenic species of the section *Nigri* were inoculated on CYA (Sigma-Aldrich, St. Louis, MO), and the section *Flavi* in YES medium - Yeast Extract-Sucrose (yeast extract: 20.0 g; sucrose: 150 g; agar: 20.0 g; $ZnSO_4 \cdot 7H2O$: 0.1 g, $CuSO_4 \cdot 5H_2O$: 0.05 g, distilled water 1 L) for 7 days at 25 °C (Filtenborg and Frisvad, 1980). We used standard ochratoxin A and aflatoxin B1, B2, G1 and G2 (Sigma-Aldrich) solutions, thin layer chromatography plates (Merck-Silica gel 60, 20 × 20) and TEF - Toluene; ethyl acetate; formic acid 90% (60:30:10) as mobile phase. Confirmation of toxin production was performed under UV light at λ 366 nm in a Chromatovisor CAMAG (Uf-Betrachter). The isolates considered producers of these toxins presented an RF (retention factor), and a fluorescence spot similar to the standard.

2.6. Evaluation of citrinin production by Penicillium belonging to section Citrina

To assess the production of citrinin we used the Coconut Cream Agar Method. *Penicillium citrinum* isolates were inoculated on coconut agar culture medium (coconut milk cream: 400 g; agar: 12 g, distilled water: 400 mL) for 10 days at 25 °C as described by Mohamed et al. (2013). The confirmation for the production of citrinin was conducted under ultraviolet light at λ 366 nm in a Chromatovisor Camag (Uf-Betrachter). Isolates considered citrinin producers showed intense green-yellow fluorescence around the colony.

2.7. Physicochemical analysis of the grapes

We evaluated the weight and diameter of the berries in ten replicates. For weighing, an analytical scale (Mars AY220) was used. The diameter was measured in two perpendicular directions with a digital caliper (Lee Tools). The soluble solids, titratable acidity and pH were evaluated according to the methodology recommended by the AOAC (2012). The titratable acidity was expressed in grams of tartaric acid per 100 g of berry; the pH was measured with a digital potentiometer (Tecnal Tec-3MP) and total soluble solids with a digital refractometer (Atago PR-100) and expressed in % soluble solids. The total sugars were determined by the Antrona method (Dische, 1962) and expressed as g of glucose per 100 g of product. The total and soluble pectins were extracted according to the McCready and McComb (1952) technique and the determination performed according to the Bitter and Muir (1973) technique. The results were expressed in mg of galacturonic acid per 100 g of berry. The pectin solubilization percentage was calculated by dividing the obtained total pectin content by the soluble pectin content. The determination of antioxidant activity was carried out by DPPH method according to the methodology described by Rufino et al. (2007a), and the results expressed as a percentage of free radical scavenging (FRS %). The ABTS method, according to the methodology described by Rufino et al. (2007b), was also used and the results expressed in uM Trolox/g. For determining the color of the grapes we used a colorimeter (Minolta CR 400) in the CIE L* a* b* mode performing two measurements on opposite sides of each berry, analyzing five berries per replicate. The parameters Chroma (relation between the values of a* and b*, with which the actual sample color is obtained) and the Hue angle (angle between a* and b* indicating the sample color saturation) were also obtained. Total phenolics were determined using the Folin-Ciocalteu reagent in solution at concentration of 10% (v/v). The results were expressed in mg of gallic acid equivalents per 100 g of sample (Waterhouse, 2002). The total anthocyanin levels were quantified following the differential pH method proposed by Giusti and Wrolstad (2001). The results were expressed as mg equivalents of cyanidin-3-glucoside per 100 g of sample.

2.7.1. Phenolic compounds

Phenolic compounds [(+)-catechin, (−)-epicatechin, (−)-gallate epicatechin, (−)-gallate epigallocatechin, procyanidin A2, procyanidin B1, procyanidin B2, kaempferol-3-O-glycoside, rutin, quercetin, isorhamnetin-3-O-glycoside, myricetin, malvidin-3-O-glucoside, cyanidin-3-O-glycoside, delphinidin-3-O-glycosides, peonidin-3-O-glucoside, gallic acid, p-coumaric acid and trans-resveratrol] in the grapes were determined according to the methodology of Correa et al. (2012) adapted.

2.7.1.1. Reagents and solvents. The gallic acid standard was obtained from Chem (West Chester, USA). Kaempferol 3-O-glycoside, (+)-catechin, cyanidin-3-O-glucoside, (−)-epicatechin, (−)-gallate epicatechin, (−)-epigallocatechin gallate, isorhamnetin-3-O-glucoside, malvidin-3-O-glycoside, myricetin, delphinidin-3-O-glycoside, peonidin-3-O-glucoside, procyanidin A2, procyanidin B1, procyanidin B2, quercetin, trans-resveratrol and rutin were obtained from Extrasynthese (Genay,

France). p-Coumaric acid was purchased from Sigma (UK). The methanol, acetonitrile and phosphoric acid were supplied by the Merck Company (Germany), JT Baker (Phillipsburg, NJ) and Fluka (Switzerland), respectively.

2.7.1.2. Sample preparation. To obtain the extracts 100 g of berries were weighed in a beaker then the volume was completed to 200 mL with absolute ethanol. The berries were then homogenized at medium speed for 1 min (Philips Walita). Then the samples were shaken on a mechanical shaker, refrigerated for 1 h and transferred to a centrifuge at a rotation of 4500 rpm for 5 min, to sediment the solids. Continuing, 1.5 mL of the solution suspension were transferred to 2 mL Eppendorf tubes that were inserted into DVC-220660-N00 speed vac equipment (Mivac, Genevac Ltd - ipsw, Chengland) until the liquid was totally evaporated. Samples were resuspended with acetonitrile UV/HPLC to a volume of 1.5 mL, stirring the extracts after the process. The extract was then filtered through a 0.45 μm polypropylene filter for later chromatographic reading.

2.7.1.3. Quantification of phenolic compounds by high-performance liquid chromatography. Quantitation of the compounds was carried out using a waters liquid chromatograph (Alliance e2695) coupled to a diode-array (220, 320, 360 and 520 nm) and fluorescence (280 nm excitation and 360 nm emission) detectors using a Gemini-NX C18 column (150 mm × 4.60 mm × 3 μm) and Gemini-NX C18 pre-column (4.0 mm × 3.0 mm), both Phenomenex® (USA). The mobile phase consisted of 0.025 M potassium dihydrogen phosphate, adjusted to pH 2.05 with ortho-phosphoric acid (phase A), methanol (phase B) and acetonitrile (phase C). Injection volume was 10 uL of sample, furnace temperature 40 °C, flow rate 0.6 mL/min with a run time of 70 min. The equations of the calibration curves, coefficient of determination (R2), detection limits (DL) and quantification limits (QL) of each phenolic compound evaluated were: (+)-catechin (Y = 4.01e + 006 X–4.07e + 005, R2 0.99, DL 0.06 mg/L, QL 0.19 mg/L); (−)-epicatechin (Y = 1.61e + 007 X + 2.94e + 006, R2 0.99, DL 0.26 mg/L, QL 0.86 mg/L); (−)-epicatechin gallate (Y = 8.93e + 004 X + 8.55e + 003, R2 0.99, DL 0.13 mg/L, QL 0.42 mg/L); (−)-epigallocatechin gallate (Y = 9.64e + 004 X + 3.22e + 003, R2 0.99, DL 0.09 mg/L, QL 0.31 mg/L); procyanidin A2 (Y = 1.66e + 006 X + 5.87e + 005, R2 0.99, DL 0.23 mg/L, QL 0.76 mg/L); procyanidin B1 (Y = 1.17e + 005 X + 8.54e + 003, R2 0.99, DL 0.09 mg/L, QL 0.31 mg/L); procyanidin B2 (Y = 1.87e + 006 X + 4.44e + 004, R2 0.99, DL 0.27 mg/L, QL 0.89 mg/L); kaempferol 3-O-glucoside (Y = 2.80e + 004 X + 1.90e + 003, R2 0.99, DL 0.03 mg/L, QL 0.1 mg/L); rutin (Y = 2.77e + 004 X + 1.84e + 003, R2 0.99, DL 0.06 mg/L, QL 0.2 mg/L); quercetin (Y = 5.62e + 004 X + 1.70e + 002, R2 0.99, DL 0.06 mg/L, QL 0.18 mg/L); isorhamnetin-3-O-glucoside (Y = 3.06e + 004 X + 2.48e + 003, R2 0.99, DL 0.03 mg/L, QL 0.09 mg/L); myricetin (Y = 5.42e + 004 X - 4.14e + 003, R2 0.99, DL 0.03 mg/L, QL 0.11 mg/L); malvidin-3-O-glucoside (Y = 3.47e + 004 X + 1.46e + 004, R2 0.99, DL 0.27 mg/L, QL 0.89 mg/L); cyanidin-3-O-glucoside (Y = 6.42e + 004 X + 4.84e + 003, R2 0.99, DL 0.04 mg/L, QL 0.12 mg/L); delphinidin-3-O-glucoside (Y = 5.56e + 004 X + 6.28e + 003, R2 0.99, DL 0.09 mg/L, QL 0.3 mg/L); peonidin-3-O-glucoside (Y = 6.35e + 004 X + 4.12e + 003, R2 0.99, DL 0.04 mg/L, QL 0.13 mg/L); gallic acid (Y = 1.34e + 005 X + 5.31e + 004, R2 0.99, DL 0.12 mg/L, QL 0.41 mg/L); p-coumaric acid (Y = 1.09e + 005 X + 1.79e + 004, R2 0.99, DL 0.08 mg/L, QL 0.27 mg/L); trans-resveratrol (Y = 1.16e + 005 X + 4.02e + 003, R2 0.99, DL 0.03 mg/L, QL 0.09 mg/L). All samples were assayed in duplicate and the phenolic compound standard solutions were injected in triplicate.

2.8. Analysis of ochratoxin A in the experimental wines

The wines used in the experiment were made from red and white grape varieties collected for assessing the fungal contamination percentage and were produced by an oenology laboratory of Embrapa Semi-Arid Agriculture, Petrolina, Brazil. Quantification of OTA was performed by High Performance Liquid Chromatography (HPLC) with fluorescence detection as described in EN 14133/2003 (European Committee for Standardization, 2003).

2.8.1. Sample preparation and purification in immunoaffinity column

Initially, the samples were cooled to 4 °C for 12 h. From each sample, 40 mL were added to 40 mL of dilution solution (10 g polyethylene glycol 8000 and 50 g of sodium bicarbonate in 1000 mL of purified water (q.s.)) under mechanical stirring and homogenized in a shaker at medium speed for 30 min. This solution was subjected to vacuum filtration (2 mL/min) through a GFA membrane, then 40 mL of the filtrate was passed through an immunoaffinity column (Ochraprep, R-Biopharm Rhone Ltd) adapted to a Visiprep™ SPE Vacuum Manifold system (Sigma-Aldrich). The column was washed with 10 mL of wash solution (25 g sodium chloride) and 5 g of sodium bicarbonate in 1000 mL of purified water (q.s.) and then with 10 mL of purified water to remove non-specific residues. Subsequently 2 mL of methanol was added to the column to release the OTA bound to the antibody, repeating the procedure three times. The eluate obtained was evaporated by heating (± 50 °C) under a nitrogen atmosphere. This dry extract was reconstituted in 250 μL of the mobile phase (acetonitrile:methanol:aqueous acetic acid (35:35:30). 50 μL of the OTA standard solution and sample extracts were then injected into a liquid chromatograph. The stock OTA solution (Sigma-Aldrich) was prepared in toluene:acetic acid (99:1 v/v). The concentration was determined according to Association of Official Analytical Chemists - AOAC (1997) and verified in a UV spectrophotometer at 333 nm, with e = 5440 L/cm.mol.

2.8.2. Quantification by high-performance liquid chromatography

Quantification was conducted in a Shimatzu liquid chromatography system with a fluorescence detector (Model LC-10AD) at wavelengths of 333 and 476 nm for excitation and emission, respectively. We utilized a Shim-pack CLC-ODS RP-18 column (5 μm, 4.6 × 250 mm), preceded by Shim-pack ODS pre-column (5 μm, 4.6 × 25 mm), flow rate of 0.8 mL/min. Quantification of OTA in the samples was performed by constructing a calibration curve obtained by linear regression (y = 6935.3× + 913.169) since the coefficient of determination (R2) obtained was 0.99. The retention time was about 10 min. The detection limit was 0.001087 μg/L, and the quantification limit was 0.006254 μg/L. All samples were analyzed in duplicate and OTA standard solutions were injected in triplicates.

2.9. Statistical analyses

To assess the difference in *Penicillium* and *Aspergillus* occurrence frequency in the grape varieties and the presence of ochratoxin A in the wines, analysis of variance (ANOVA) with subsequent Tukey test were used. The analyses were performed using Sisvar software (Ferreira, 2011). To evaluate the effect of the physicochemical characteristics of the grapes on the incidence of *Penicillium* and *Aspergillus* fungi we used the Pearson correlation. The analyses were performed using R software (version 2.11.1, 2010).

3. Results and discussion

3.1. Percentage of contamination by Aspergillus and Penicillium in wine grapes

The varieties Touriga Nacional and Chenin Blanc had the highest percentage of contamination by *Aspergillus* species (100%), and the Touriga Nacional variety also had the highest percentage of contamination by *Penicillium* species (64.67%). There was no occurrence of *Penicillium* and *Aspergillus* in the Tempranillo variety (Fig. 1).

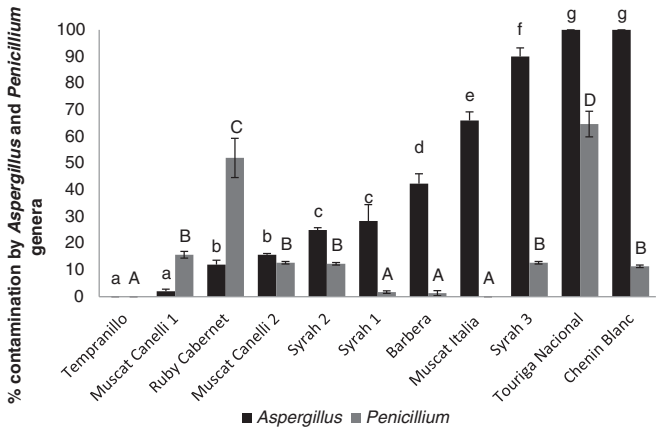

Fig. 1. Contamination percentage of wine grapes by *Aspergillus* and *Penicillium* fungi and their respective average colonization proportions. Different letters show statistically significant difference at $p < 0.05$, uppercase letters being for *Penicillium* and lowercase for *Aspergillus*.

The contamination by *Aspergillus* was higher than by *Penicillium* in most samples except Muscat Canelli 1 and Ruby Cabernet that showed the higher incidence of *Penicillium*. Kizis et al. (2014) evaluated the colonization of grapes by filamentous fungi from cultivation areas in Greece found *Aspergillus* as the most frequently isolated genus in all regions except in Macedonia where the *Alternaria* genus was the most commonly found. However, Serra et al. (2006) found a higher contamination by *Penicillium* when evaluating grapes from Portugal, which indicates the influence of geographical location on the incidence of fungi.

Samples belonging to varieties Syrah (samples 1, 2 and 3) and Muscat Canelli (samples 1 and 2) showed different contamination percentages, which demonstrates that despite the samples being the same variety, other factors contribute to contamination, such as climate conditions and cultivation practices. Setati et al. (2012) demonstrated that intra-vineyard variation may be greater than the inter-vineyard variation within the same harvest, presumably due to microclimatic influences on the grape microbiota. Passamani et al. (2012) observed that there is also a variation of the contamination of varieties among vineyards with the same geographical location due to agricultural practices.

3.2. Incidence of Aspergillus and Penicillium in wine grapes

Of the 466 identified fungi, 351 belong to the genus *Aspergillus* (75.3%), whereas only 115 belong to the genus *Penicillium* (24.7%). However, the diversity of *Penicillium* species was similar to that of *Aspergillus*. El Khoury et al. (2008) found similar results in Lebanese vineyards where 95.5% of the isolates belonged to the genus *Aspergillus*, whereas only 4.5% belonged to the genus *Penicillium*.

Aspergillus species found in the wine grapes of the region were *A. niger* (35.33%), *A. carbonarius* (33.90%), *A. aculeatus* (20.51%), *A. niger* aggregate (8.83%), *A. flavus* (1.14%) and *A. sojae* (0.28%). Section *Nigri* species represented 98.6% of the *Aspergillus* isolates, *A. niger* and *A. carbonarius* being the majority of the isolates identified.

These results are consistent with reports of Battilani et al. (2006), who found that *Aspergillus* Section *Nigri* were the most the frequently isolated species in hot and dry areas. Among these, *A. carbonarius* and *A. niger* are the most common species, representing 50 to 98.5% of *Aspergillus* isolates in grapes (Rousseaux et al., 2014).

The high incidence of *A. carbonarius* in the region of La Rioja in Argentina was attributed to semi-arid climate with low humidity and extremely hot summers (Chiotta et al., 2009). According to Lasram et al. (2010), *A. carbonarius* strains demonstrate different growth characteristics, with some isolates being able to develop a tolerance to the semi-

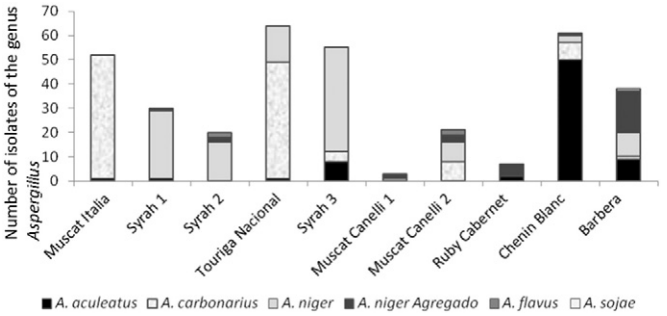

Fig. 2. *Aspergillus* species incidence in grape varieties.

arid climate, the climate found in the present study area, which could explain the high incidence of this species.

A. niger were dominant in 27.3% of the samples (Syrah 1, Syrah 2 and Syrah 3), being higher in Syrah 1 (93%). In contrast *A. flavus* was found in only 18.2% of the samples (Syrah 2 and Muscat Canelli 2) representing 10% of the isolates, and *A. sojae* was found only in the Barbera variety, representing 3% of the isolates (Fig. 2).

The highest incidence of *Aspergillus carbonarius* was in the varieties Muscat Italia and Touriga National, representing 98% and 75% of the isolates, respectively. Of all the *A. aculeatus*, 69.4% were isolated from the Chenin Blanc variety, it being the most prevalent species in this sample (82%).

A. niger Aggregate was present in 63.6% of the samples, the incidence varying from 0% (Muscat Italia, Touriga Nacional and Syrah 3) to 71% (Ruby Cabernet). It was also the most frequent species in Muscat Canelli 1 (67%) and Barbera (45%) samples. In Muscat Canelli 2, the highest incidence was of *A. carbonarius* (38%) and *A. niger* (38%).

The influence of the variety on the incidence of filamentous fungi in grapes was reported by Chiotta et al. (2009) who observed a higher percentage of *A. carbonarius* (50%) in Cabernet Sauvignon than in the Malbec and Syrah varieties in Argentine vineyards. Diaz et al. (2009) detected a higher contamination by *A. carbonarius* and *A. niger* in red varieties than in white varieties in Chilean vineyards. However, in a vineyard in Tunisia, there was no significant difference in the number of isolates of *Aspergillus* Section *Nigri* in Carignan (red) and Italia (white) varieties (Lasram et al., 2007). Similar results were observed in our study, in which the *A. carbonarius* incidence was significant, both in Muscat Italia (white) and Touriga Nacional (red) varieties.

The identified species of the genus *Penicillium* were *P. sclerotiorum* (44.4%), *P. citrinum* (20.0%), *P. glabrum* (18.3%), *P. decumbens* (13.0%), *P. implicatum* (2.6%) and *P. solitum* (1.7%). These results differ from those found in Portuguese vineyards, wherein the most common species of *Penicillium* were *P. brevicompactum*, *P. thomii* and *P. glabrum* (Serra et al., 2006).

In Syrah 3, Chenin Blanc and Barbera samples the species *P. citrinum*, *P. sclerotiorum* and *P. glabrum* represent 100% of isolates, respectively (Fig. 3). *P. decumbens* was present only in the Touriga Nacional variety, being the species with the highest incidence in this variety (50%).

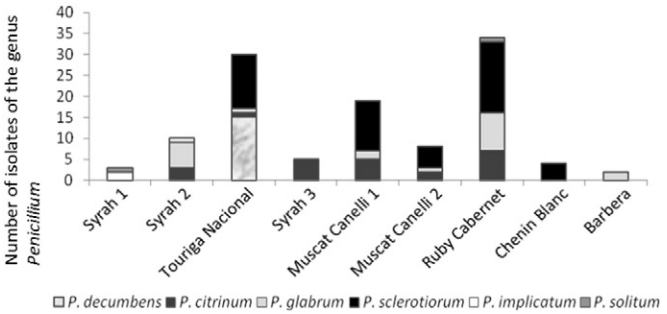

Fig. 3. *Penicillium* species incidence in grape varieties.

Most varieties were colonized by *P. sclerotiorum*, which was dominant in 33.3% of the analyzed samples, representing 63% of the isolates in the Muscat Canelli 1 and 2 varieties and 50% of the isolates in the Ruby Cabernet variety. *P. citrinum* was also present, however the distribution varied according to the variety. *P. glabrum* had the highest incidence in the Syrah 2 variety (67%) and *P. implicatum* in Syrah 1 (67%), it also being present in Syrah 2 (10%). Only Syrah 1 and Ruby Cabernet samples were contaminated by *P. solitum*, which accounted for 33% and 3% of the isolates, respectively.

P. chrysogenum was the most commonly isolated species in Argentina (Magnoli et al., 2003). In France and Portugal *P. brevicompactum* was the species with highest incidence (Sage et al., 2002; Serra et al., 2006). However, other studies have identified *P. expansum* as the most common species in Portuguese (Abrunhosa et al., 2001) and French (Bejaoui et al., 2006) vineyards. These species were not reported in our study, which demonstrates the difference in occurrence in different regions.

Studies in France demonstrated that the most frequently isolated species differ between crops and vineyards. Guérin et al. (2007) observed that the predominant species of *Penicillium* isolated differed among vineyards in 2004, with *P. expansum* in Bordeaux, Alsace and Val de Loire and *P. purpurescens* in Beaujolais and Burgundy. Diguta et al. (2011) identified *P. spinolusum* as the most frequently isolated species in the 2008 harvest in Burgundy. These studies demonstrate that the incidence of different species of fungi varies not only with the geographical location, but also with the harvest, climatic conditions and variety of grapes Passamani et al. (2012).

3.3. Mycotoxin production by species of Aspergillus and Penicillium

Of the total *Aspergillus* species identified, 346 were tested for ochratoxin A production capacity. Of these, 32.1% were toxin producers and identified as *A. carbonarius*, while *A. aculeatus*, *A. niger and A. niger* Aggregate species showed no potential for production of this toxin. *A. carbonarius* proved to be the only 100% producer species of ochratoxin A, which confirms that this is the main species responsible for the OTA presence in grapes (Magnoli et al., 2003; Battilani et al., 2006).

Our results corroborate the findings of Lasram et al. (2012a) in which 99.5% of ochratoxigenic isolates from Tunisia grapes were *A. carbonarius*, only 3.2% *A. niger* Aggregate and no uniseriate species. Kizis et al. (2014) also reported 98.3% of *A. carbonarius* and 1.6% of *A. niger* Aggregate isolated from grapes in Greece as OTA producers and no production from *A. ibericus* and *A. japonicus*.

No OTA producing *Aspergillus* species were found in the Syrah 1, Syrah 2, Muscat Canelli 1, Ruby Cabernet and Tempranillo samples, while 98.1% of the isolates from Muscat Italy and 75% from Touriga Nacional samples were OTA producers, these being identified as *A. carbonarius*.

The Syrah 3 (7.3%), Moscato Canelli 2 (42.1%), Chenin Blanc (11.5%) and Barbera (2.7%) samples were also contaminated by OTA producing *A. carbonarius*, but with lower incidence. These results demonstrate that besides the influence of cultivation practices, incidence of ochratoxigenic species can be influenced by the grape variety, even when located in the same region.

Battilani et al. (2004) evaluated the OTA production by toxigenic strains inoculated into 12 grape varieties and detected Bianco di Alessano, Pampanuto and 'Uva di Troia' varieties with low ochratoxin A levels, while Cabernet Sauvignon had the highest OTA level.

Muscat Canelli 1 and Ruby Cabernet samples had the highest contamination level by *Penicillium* species, which may have influenced in the absence of ochratoxigenic species. Some studies report that, in addition to the intrinsic and environmental factors, competitive microbiota are also an important factor in the contamination of grapes by potentially toxigenic species and OTA production. *A. japonicus*, *A. wentii*, *A. versicolor*, *A. clavatus* and some yeasts compete for space and nutrients, reducing the OTA concentration in grapes (Abrunhosa et al., 2001; Ponsone et al., 2012). Concomitantly, Kogkaki et al. (2015) demonstrated that *B. cinerea* could be an obstacle to *A. carbonarius* growth and OTA production in the field.

Only four *A. flavus* and one *A. sojae* were isolated from the wine grapes, which were not aflatoxin B1, B2, G1 and G2 producers. This confirms the low risk of this toxin in grapes of the region. Serra, Braga and Venancio (2005) found 27 *A. flavus* producers of aflatoxin B1 isolated from grapes in Portugal. In Lebanon, 43% of *A. flavus* were also producers (El Khoury et al., 2008). However, this species is not considered an ordinary member of grape mycobiota and studies conducted in Mediterranean countries have reported low incidence of these in the vineyards (Medina et al., 2005; Martinez-Culebras, Ramon, 2007).

All citrinin producing *Penicillium* isolates were *P. citrinum*. Although citrinin has demonstrated nephrotoxic effects, the presence of the toxin in wine is not of concern because of the low incidence of these species in grapes (Rousseaux et al., 2014). Of the 115 fungi of the *Penicillium* genus isolated from grapes, only 20% were *P. citrinum*, which shows a low risk of this toxin in grapes and their derivatives from the region.

3.4. Ochratoxin A occurrence in the experimental wines

Only 16.7% of red wine and 50% of white wine samples were positive for OTA presence, and the average concentration was 0.29 μg/L and 0.02 μg/L in red and white wines, respectively, red wines thus having greater contamination.

Remiro et al. (2013) found 100% of red wines from countries around the Mediterranean Sea contaminated by OTA. In Chinese wines, only 2.9% of white wines were found contaminated, whereas 57.1% of red wines showed OTA, with average concentrations of 0.07 μg/L and 0.8 μg/L, respectively (Zhang et al., 2013). According to Sarigiannis et al. (2014), these differences may be related to grape cultivation techniques, the microclimate and the different winemaking processes.

Table 1 demonstrates that the highest concentration of ochratoxin A was found in the wine made from the Touriga Nacional variety (0.29 μg/L), this also being the variety with highest contamination percentage by *Aspergillus* and *Penicillium* fungi. Moreover, this variety also showed high incidence of toxigenic isolates.

Despite the Muscat Italia variety having high contamination by OTA producer *A. carbonarius*, OTA was not detected in the wine made from this variety. Generally, white wines have lower OTA levels compared to red wines, as such, the presence of OTA in the wines obtained from Muscat Canelli variety can be possibly attributed to poor sample quality (Soto et al., 2014). Similar results were found in a study conducted by Pena et al. (2010), evaluating wines of different regions in Portugal, in which although 26% of the red wine samples and 12% of white wine samples were contaminated by OTA, only one white wine sample exceeded the level allowed by the European Union. However, in our study, none of the wines analyzed exceeded the maximum tolerable

Table 1
Ochratoxigenic species isolated from grapes and presence of ochratoxin A in experimental wines.

Variety	Number of toxigenic isolates (*A. carbonarius*)	OTA levels in wine (μg/L)*	
Muscat Italia	51	<DL	
Syrah 1	0	<QL	a
Ruby Cabernet	0	<QL	a
Chenin Blanc	7	<QL	a
Tempranillo	0	<QL	a
Barbera	1	<QL	a
Syrah 3	4	<QL	a
Muscat Canelli 1	0	0.02 ± 0	b
Muscat Canelli 2	8	0.03 ± 0.01	b
Touriga Nacional	48	0.29 ± 0.01	c

DL: detection limit (0.001087 μg/L).
QL: quantification limit (0.006254 μg/L).
* Different letters show significant statistical difference at $p < 0.05$.

OTA limit in wines proposed by the European Union (Commission of the European Communities - EC, 2006) and by Brazil, of 2 μg/kg (Brasil, 2011).

According to our results, the Brazilian wines have low concentrations compared with those of European origin in which the OTA content generally varies between 0.01 and 15.60 μg/L (Commission of the European Communities, 2002; Shundo et al., 2006; Terra et al., 2013). According to Terra et al. (2013) the low level of OTA can be related to the semi-arid climate of the region where higher temperatures are registered throughout the year, such temperatures can promote the growth of the species, but not toxin production.

3.5. Correlation between physical and chemical characteristics of wine grapes and incidence of Aspergillus and Penicillium

The antioxidant activity, total phenolic compounds, anthocyanins, total sugars, soluble and total pectin, color, total titratable acidity, total soluble solids and pH parameters, as well as berry weight and diameter, were assessed to establish a possible correlation between the grape varieties and filamentous fungi incidence (Table 2).

All *Aspergillus* species identified in this study, except *A. flavus*, were positively correlated ($p < 0.05$) with at least one evaluated variable (Table 3).

Aspergillus aculeatus demonstrated a positive correlation with the color parameter Chroma (0.55), which is the relation between the values of a* and b*, with which the actual sample color is obtained. *A. carbonarius* was positively correlated with the total pectin content (0.69). Positive correlation was also seen between the *A. niger* species and antioxidant activity measured by the ABTS method (0.76).

Aspergillus niger Aggregate and *A. sojae* presented a positive correlation with the total acidity content, 0.74 and 0.77, respectively. Moreover, a positive correlation was also observed between the *A. sojae* species and the color parameter a* (0.51) expressing the degree of variation between red and green (more negative a* = greener; more positive a* = redder). The presence of color shades with a higher red color contribution is commonly associated with a higher percentage of anthocyanins.

Of the *Penicillium* isolates, *P. decumbens*, *P. citrinum* and *P. sclerotiorum* correlated with physicochemical characteristics. There were no correlations for *P. glabrum*, *P. implicatum* and *P. solitum*.

P. decumbens was positively correlated with the total pectin content (0.66) and total soluble solids (0.50). *P. citrinum* showed a positive correlation with antioxidant activity measured by the ABTS method (0.54) and total sugars (0.53). Positive correlation between *P. sclerotiorum* and anthocyanin content was also found, this being the highest correlation observed (0.92).

Due to the great importance of phenolic compounds present in grapes, the content of 19 of the most important was assessed (Table 4).

The highest number of phenolic compounds was found in the Touriga Nacional variety, which was the most contaminated by *Penicillium* and *Aspergillus*.

All *Aspergillus* species identified in this study, except *A. flavus*, were positively correlated ($p < 0.05$) with at least one evaluated phenolic compound (Table 5). *Aspergillus aculeatus* had a positive correlation with the quercetin flavonol (0.57). *A. niger* was positively correlated with the flavanols (−)-epicatechin (0.75), procyanidin A2 (0.68) and procyanidin B2 (0.80); with the flavonols rutin (0.68) and isorhamnetin-3-O-glucoside (0.72) and the phenolic acid gallic acid (0.73).

Aspergillus sojae and *A. niger* Aggregate showed a positive correlation with the flavanol (−)-epigallocatechin gallate, 0.58 and 0.65, and the flavonol kaempferol-3-O-glucoside, 0.73 and 0.70, respectively. In turn, *A. carbonarius* showed a positive correlation with the flavanol (−)-epicatechin gallate (0.56).

All *Penicillium* species correlated with the phenolic compounds evaluated in this study. *Penicillium decumbens* demonstrated a higher number of correlations. It was positively correlated with the flavanols (−)-epicatechin (0.60), (−)-epicatechin gallate (0.98) and procyanidin A2 (0.54); flavonols quercetin (0,60) and isohamnetina-3-O-glucoside (0.54), the anthocyanins malvidin-3-O-glucoside (0.56) and peonidin-3-O-glucoside (0.78) and the phenolic acid p-coumaric (0.82). *P.citrinum* presented positive correlation only with anthocyanin delphinidin-3-O-glucoside (0.55).

A positive correlation was also observed between *P. glabrum* species and the anthocyanins delphinidin-3-O-glucoside (0.84) and malvidin-3-O-glucoside (0.63) and the flavanol (−)-epigallocatechin gallate (0.58). Positive correlation between *P. sclerotiorum* and delphinidin-3-O-glucoside (0.65) and malvidin-3-O-glucoside (0.54) anthocyanins was also found.

Table 2
Physicochemical characteristics evaluated in wine grape varieties from the semi-arid tropical region of Brazil.

Physical-chemical characteristics	Grape varieties (*Vitis vinifera* L.)[a]										
	Muscat Italia	Syrah 1	Syrah 2	Touriga Nacional	Syrah 3	Muscat Canelli 1	Muscat Canelli 2	Ruby Cabernet	Chenin Blanc	Tempranillo	Barbera
Antioxidant activity-DPPH (%FRS)	60.76	94.77	94.58	94.83	94.81	76.82	86.46	94.47	91.67	95.13	86.35
Antioxidant activity-ABTS (μM trolox/g)	131.97	1359.24	1271.17	1687.86	2638.90	334.30	707.94	1864.06	758.87	258.47	625.51
Total phenolic compounds (mg/100 g)	634.80	641.12	652.62	1128.79	817.56	308.02	460.23	879.51	437.85	981.57	606.84
Anthocyanins (mg/100 g)	ND	65.48	54.35	176.56	79.12	ND	ND	218.92	ND	91.14	88.48
Total sugars (mg/100 g)	21.41	34.90	35.06	27.53	28.74	37.56	28.27	33.28	27.83	21.36	26.37
Soluble pectin (mg/100 g)	15.68	76.14	61.54	157.10	95.26	78.90	119.46	114.19	133.05	153.20	166.34
Total pectin (mg/100 g)	664.40	491.88	396.77	885.42	505.22	306.46	507.45	429.78	528.18	590.47	716.74
Pectin solubilization (%)	2.36	15.49	15.51	17.74	18.86	25.74	23.55	26.57	25.21	25.97	23.21
L*	39.61	27.80	24.88	21.48	27.80	36.96	40.85	28.14	47.13	23.92	24.11
a*	2.48	0.14	0.24	3.36	0.14	−3.54	−3.23	−0.22	−2.75	2.95	6.52
b*	14.90	−0.48	−1.54	−3.27	−0.48	14.21	15.56	−1.14	22.47	−0.59	−3.43
Chroma	13.25	0.76	1.57	4.71	0.76	14.67	15.92	1.19	22.99	3.05	6.49
Hue	87.09	288.42	280.68	312.83	288.42	103.84	101.54	252.93	97.02	345.86	325.55
Total acidity (%)	1.12	0.84	0.83	0.84	0.84	0.84	1.39	0.76	0.97	0.99	1.69
Total soluble solids (%)	14.00	20.87	18.57	22.00	15.95	15.98	12.37	18.43	15.76	14.58	21.00
pH	3.50	3.98	3.74	3.90	3.98	3.83	3.74	3.86	3.88	4.02	3.30
Weight (g)	9.60	2.63	2.30	1.72	1.80	2.07	2.29	1.35	2.13	1.42	2.06
Diameter (mm)	21.48	13.54	12.11	9.60	12.25	13.40	12.70	11.79	11.22	11.69	13.97

L*: degree of brightness.
a*: degree of variation between red and green.
b*: degree of variation between blue and yellow.
[a] ND = not detected.

Table 3
Pearson correlation analysis correlating the physicochemical characteristics to the presence of species of *Aspergillus* and *Penicillium* genera in grapes (*Vitis vinifera* L.).

Physical-chemical characteristics	*A. aculeatus*	*A. carbonarius*	*A. niger*	*A. niger* Aggregate	*A. flavus*	*A. sojae*	*P. decumbens*	*P. citrinum*	*P. glabrum*	*P. sclerotiorum*	*P. implicatum*	*P. solitum*
Antioxidant activity-DPPH	0.11	−0.51	0.38	0.03	0.09	0.07	0.19	0.12	0.20	0.06	0.26	0.27
Antioxidant activity-ABTS	−0.02	−0.08	0.76*	0.11	0.02	0.16	0.28	0.54*	0.30	0.23	0.18	0.36
Total phenolic compounds	−0.23	0.36	0.28	0.09	0.18	0.06	0.58	0.10	0.18	0.22	0.02	0.19
Anthocyanins	0.04	0.46	0.17	0.12	0.25	0.05	0.47	0.54	0.55	0.92*	0.30	0.40
Total sugars	−0.14	−0.50	0.18	0.02	0.20	0.19	0.12	0.53*	0.46	0.35	0.46	0.42
Soluble pectin	0.28	−0.10	0.04	0.44	0.08	0.42	0.36	0.03	0.05	0.29	0.25	-0.04
Total pectin	0.07	0.69*	0.16	0.22	0.20	0.35	0.66*	0.42	0.26	0.02	0.17	-0.18
Pectin solubilization	0.28	−0.52	0.07	0.32	0.03	0.17	0.05	0.39	0.30	0.45	0.20	0.12
L*	0.49	0.03	0.40	0.26	0.06	0.29	0.39	0.14	0.31	0.05	0.26	-0.21
a*	−0.28	0.18	0.07	0.32	0.35	0.51*	0.21	0.48	0.15	0.36	0.11	-0.15
b*	0.48	0.11	0.46	0.30	0.07	0.31	0.30	0.18	0.36	0.02	0.31	-0.32
Chroma	0.55*	0.15	0.53	0.08	0.03	0.07	0.15	0.30	0.38	0.02	0.42	-0.45
Hue	−0.23	−0.12	0.48	0.29	0.09	0.32	0.29	0.07	0.22	0.04	0.29	0.25
Total acidity	0.08	0.05	0.12	0.74*	0.20	0.77*	0.16	0.36	0.18	0.28	0.23	-0.30
Total soluble solids	−0.07	0.04	0.32	0.38	0.25	0.40	0.50*	0.03	0.27	0.20	0.43	0.39
pH	0.08	−0.16	0.34	0.65	0.08	0.69	0.18	0.30	0.03	0.26	0.25	0.31
Weight	−0.17	0.31	0.32	0.24	0.15	0.12	0.16	0.39	0.31	0.36	0.11	-0.20
Diameter	−0.22	0.09	0.31	0.12	0.17	0.02	0.28	0.33	0.27	0.38	0.11	-0.15

L*: degree of brightness.
a*: degree of variation between red and green.
b*: degree of variation between blue and yellow.
* Significant correlation at $p < 0.05$.

Penicillium implicatum showed positive correlation with the flavonols rutin (0.54) and isorhamnetin-3-O-glucoside (0.61) and the stilbene trans-resveratrol (0.70), whereas *P. solitum* correlated only with the anthocyanin delphinidin-3-O-glucoside (0.60) and the stilbene trans-resveratrol (0.78).

Jiang, Shi and Zhu (2013) detected damaged berries and faster ochratoxin A accumulation in the Thompson Seedless variety, intermediate for Kyoho, and slower for Red Terra grapes. During the incubation period, a decrease in the sugars and soluble solids content in some varieties was observed, possibly consumed by *A. carbonarius*, and the increase of these components in other varieties, possibly due to the hydrolysis of polysaccharides (starch and pectin), which demonstrates the degradation ability of these fungi and the difference in susceptibility of the varieties.

Concomitantly, this study demonstrates the existence of a positive correlation between some physical and chemical characteristics of wine grape varieties and the incidence of fungi of the *Aspergillus* and *Penicillium* genera in them. Therefore, it follows that the higher the value of the correlated physicochemical parameter, the greater the species incidence.

Table 4
Concentration of phenolic compounds present in wine grape varieties of the semi-arid tropical region of Brazil.

Phenolic compounds (mg/L)	Grape varieties (*Vitis vinifera* L.)										
	Muscat Italia	Syrah 1	Syrah 2	Touriga Nacional	Syrah 3	Muscat Canelli 1	Muscat Canelli 2	Ruby Cabernet	Chenin Blanc	Tempranillo	Barbera
Flavanols											
(+)-Catechin	<QL	1.68	2.12	3.02	3.72	1.43	5.5	1.43	2.13	1.51	3.49
(−)-Epicatechin	<DL	1.19	0.97	2.84	2.9	<DL	<QL	1.03	<QL	<QL	1.46
(−)-Epicatechin gallate	<DL	0.44	<QL	3.68	0.47	<DL	<QL	0.52	<QL	<DL	<QL
(−)-Epigallocatechin gallate	<DL	0.35	1.14	1.84	0.59	<DL	<QL	2.11	<DL	0.33	2.39
Procyanidin A2	<DL	<QL	<QL	<QL	<QL	<DL	<DL	<DL	<DL	<DL	<DL
Procyanidin B1	0.84	0.88	0.94	2.32	1.35	<QL	1.01	2.25	0.79	7.22	2.3
Procyanidin B2	<DL	2.84	3.11	5.01	10.92	3.92	<DL	2.01	<DL	1.21	2.23
Flavonols											
Kaempferol 3-O-glucoside	<QL	0.91	0.1	0.2	0.36	<DL	0.31	0.32	0.62	<DL	1.27
Rutin	<DL	0.42	0.28	0.33	0.29	<DL	<QL	<QL	<DL	<DL	0.42
Quercetin	<DL	<DL	<DL	<QL	<DL	<DL	<DL	<DL	<QL	<DL	<DL
Isorhamnetin-3-O-glucoside	<DL	2.24	0.96	2.06	1.3	<DL	<QL	0.48	0.25	0.18	0.74
Myricetin	<DL	0.55	0.37	0.67	0.36	<DL	<QL	0.63	0.11	0.12	0.26
Anthocyanins											
Malvidin-3-O-glucoside	<DL	13.42	20.18	36.67	12.38	<DL	<DL	39.22	<DL	7.55	11.36
Cyanidin-3-O-glucoside	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Delphinidin-3-O-glucoside	<DL	1.44	3.73	5.12	0.99	<DL	<DL	12	<DL	1.1	1.79
Peonidin-3-O-glucoside	<DL	3	6.9	11.29	2.01	<DL	<DL	4.79	<DL	0.92	3.07
Phenolic acids											
Gallic acid	<DL	<QL	<DL	0.46	0.46	<QL	<QL	<DL	<DL	<QL	<QL
P-coumaric acid	<DL	<DL	0.44	0.71	<QL	<DL	<QL	<QL	<DL	<DL	<QL
Stilbenes											
Trans-resveratrol	<DL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL

DL: detection limit.
QL: quantification limit.

Table 5
Pearson correlation analysis correlating the phenolic compounds to the presence of species of *Aspergillus* and *Penicillium* genera in grapes (*Vitis vinifera* L.).

Phenolic compounds	*A. aculeatus*	*A. carbonarius*	*A. niger*	*A. niger* Aggregate	*A. flavus*	*A. sojae*	*P. decumbens*	*P. citrinum*	*P. glabrum*	*P. sclerotiorum*	*P. implicatum*	*P. solitum*
Flavanols												
(+)-Catechin	0.02	−0.18	0.39	0.28	0.49	0.25	0.15	0.04	−0.14	−0.05	−0.18	−0.28
(−)-Epicatechin	−0.08	0.16	0.75*	0.02	−0.18	0.12	0.60*	0.17	−0.06	0.09	0.00	−0.01
(−)-Epicatechin gallate	−0.14	0.56*	0.19	−0.13	−0.14	−0.06	0.98*	−0.07	−0.01	0.44	−0.07	−0.05
(−)-Epigallocatechin gallate	−0.19	−0.01	0.04	0.65*	−0.11	0.58*	0.38	0.21	0.58*	0.34	−0.10	0.23
Procyanidin A2	0.11	0.15	0.68*	−0.05	−0.08	0.03	0.54*	0.10	−0.22	0.06	−0.16	−0.23
Procyanidin B1	−0.19	−0.12	−0.23	0.01	−0.22	0.08	0.08	−0.23	−0.07	−0.11	−0.23	−0.07
Procyanidin B2	−0.13	−0.08	0.80*	−0.16	−0.22	−0.07	0.23	0.45	−0.08	0.00	0.00	−0.08
Flavonols												
Kaempferol 3-O-glucoside	0.34	−0.31	0.28	0.70*	−0.21	0.73*	−0.15	−0.33	−0.11	−0.27	0.30	0.29
Rutin	−0.17	−0.09	0.68*	0.41	−0.03	0.48	0.29	−0.16	0.03	−0.24	0.54*	0.27
Quercetin	0.57*	0.23	0.26	−0.03	−0.16	0.09	0.60*	−0.39	−0.23	0.01	0.14	−0.14
Isorhamnetin-3-O-glucoside	−0.16	0.11	0.72*	−0.09	−0.15	0.00	0.54*	−0.13	−0.09	−0.04	0.61*	0.37
Myricetin	−0.21	0.04	0.43	0.02	−0.13	−0.04	0.52	0.25	0.43	0.36	0.38	0.61
Anthocyanins												
Malvidin-3-O-glucoside	−0.28	0.11	0.16	0.07	−0.09	−0.03	0.56*	0.39	0.63*	0.54*	−0.09	0.47
Cyanidin-3-O-glucoside	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Delphinidin-3-O-glucoside	−0.22	−0.05	−0.12	0.14	−0.07	−0.05	0.25	0.55*	0.84*	0.65*	−0.02	0.60*
Peonidin-3-O-glucoside	−0.27	0.30	0.24	0.02	0.08	0.01	0.78*	0.09	0.39	0.35	0.18	0.14
Phenolic acids												
Gallic acid	−0.15	0.10	0.73*	0.15	−0.20	0.32	0.48	−0.19	−0.46	−0.18	0.12	−0.11
P-coumaric acid	−0.22	0.42	0.22	−0.04	0.28	0.00	0.82*	0.01	0.21	0.26	0.00	−0.23
Stilbenes												
Trans-resveratrol	−0.27	−0.11	0.49	−0.15	−0.02	−0.24	0.21	0.28	0.42	0.20	0.70*	0.78*

NA: no correlation.
* Significant correlation at $p < 0.05$.

We can infer that the total sugars content present in the Muscat Canelli 1 sample favors the incidence of *P. citrinum* species. This species is also related to the antioxidant activity in the Syrah 3 sample and Ruby Cabernet variety.

According to Magan (2007), mycotoxigenic fungi have a mechanism of adaptation to different environments, thus becoming good competitors. This is due to their ability to produce an enzyme complex responsible for degradation of specific substrates and production of secondary metabolites and volatile compounds (Medina et al., 2015).

Growth and metabolite production by filamentous fungi are usually a response to the physical-chemical conditions of the substrate in which they are inserted. In *in vitro* studies, production of extracellular enzymes by *P. janczewskii* was influenced by the carbon source, and the presence of fructose in the culture medium stimulated the p-fructofuranosidases enzyme release, which favored growth of this species (Pessoni et al., 2015).

The presence of the compounds (−)-epicatechin, (−)-epicatechin gallate, procyanidin A2, quercetin, isorhamnetin-3-O-glucoside, malvidin-3-O-glucoside, peonidin-3-O-glucoside, p-coumaric acid, the percentage of total soluble solids and total pectin content could have promoted the incidence of *P. decumbens* in the Touriga Nacional variety. The pectin content is also related to the *A. carbonarius* presence in this same variety and in Muscat Italia.

Species of *Aspergillus* are recognized as major producers of pectinase (Sandri et al., 2011), confirming the pectin degradation capacity by filamentous fungi, and hence their development in substrates with higher pectin contents. However, Teixeira et al. (2000), in an *in vitro* assay, described an inhibition in the pectinase production from *Aspergillus japonicus*, when the concentrations of glucose, sucrose and pectin were in excess.

The main contaminant group of the Barbera variety was *A. niger* Aggregate. Phenolic compounds (−)-epigallocatechin gallate, kaempferol-3-O-glycoside, and the total acidity may be related to such contamination. *A. sojae* was present only in this variety and also has correlation with these same parameters. Lorenzini et al. (2012) infected grapes with *Botrytis cinerea* and native grape strains and reported a decrease of tartaric acid due to degradation caused by the fungi present.

In a study evaluating the growth of *A. carbonarius* inoculated in grapes, Lasram et al. (2012b) observed a higher ochratoxin A production in grapes with higher acidity and lower sugar content, suggesting that the acids present in the grapes can promote fungal growth. In contrast, *in vitro* inhibition of *P. commune* by organic acids was reported by Zhang et al. (2005). These results confirm the different relationships between fungal species and the physicochemical characteristics of the substrate.

The Syrah 1 sample showed significant concentrations of isorhamnetin-3-O-glucoside, which may be associated with *P. implicatum* incidence in this variety. The highest incidence of *A. niger* occurred in the Syrah 3 sample and this variety showed high levels of phenolic compounds (−)-epicatechin and Procyanidin B2 and antioxidant activity.

The highest number of isolates of *A. aculeatus* was found in the Chenin Blanc variety (a white variety). This species showed a positive correlation with the Chroma color parameter, which is related to the color intensity. This result suggests the preference of *A. aculeatus* for white grapes.

The presence of delphinidin-3-O-glucoside compound in Ruby Cabernet variety may be related to the incidence of the *P. glabrum*, *P. citrinum*, *P. sclerotiorum* and *P. solitum* species in this variety. The anthocyanin content can also be associated with berry infection by *P. sclerotiorum*. The (−)-epigallocatechin gallate and malvidin-3-O-glucoside compounds may have influenced the growth of *P. glabrum* in this variety.

Phenolic compounds have a microbial growth inhibitory capacity (Li et al., 2007; Ortuño et al., 2006), however, such inhibition is closely related to the species and its concentration in the substrate. Wu et al. (2014) reported that growth inhibition of *A. niger* by polymethoxylated flavonoids was more efficient than that of *P. corylophilum*.

In evaluating the inhibition of ochratoxin A production using phenolic compounds, Palumbo et al. (2006) demonstrated a strong inhibition of OTA production by *A. albertensis* in the presence of catechin (87–94%) and gallic acid (57–87%). However, *A. melleus* produced from 5 to 8 times more OTA in the presence of catechin than in the control. These authors highlight that, although coffee and grape are antioxidant

sources, these substrates are also commonly colonized by *Penicillium* and *Aspergillus* species, indicating that the role of antioxidants and mycotoxin production in the fungal ecology is very complex.

Santos Júnior et al. (2014) observed growth inhibition of *A. ochraceus*, inoculated in coffee beans, by Guaijaverin, quercetin 3-O-α-rhamnopyranoside, quercetin 3-O-robinobioside and rutin phenolic compounds. Nevertheless, Tranchimand, and Brouant Iacazio (2010) reported the ability of species of the genera *Aspergillus* and *Penicillium* to metabolize flavonols, they being the only carbon source.

In this work, a positive correlation between various phenolic compounds and fungal species was observed, which leads us to believe that such species have the ability to metabolize these compounds. Moreover, the higher concentration of these compounds in the evaluated varieties may be related to the grape defence mechanism against stress caused by fungal colonization. These results demonstrate the effect of different varieties of grapes on incidence of *Aspergillus* and *Penicillium*. However, different correlations were observed for the same grape variety (Syrah and Muscat Canelli). This difference can be explained by the fact that the chemical composition does not depend only on the variety, but also on the plant quality, geographical origin, climatic conditions and environmental and technological factors (Martinez-Avila et al., 2012).

Acknowledgments

The authors thank the producers who allowed grape collection on their properties, Embrapa Semiárido and Funed for the aid in analysis execution, and the research support agencies FAPEMIG (CBB-APQ-04152-15) and CNPq (403438/2013-6) for financial support and scholarships concession.

References

Abe, L.T., Da Mota, R.V., Lajolo, F.M., Genovese, M.I., 2007. Compostos fenólicos e capacidade antioxidante de cultivares de uvas *Vitis labrusca* L. e *Vitis vinifera* L. Cienc. Tecnol. Aliment. 27 (2), 394–400.

Abrunhosa, L., Paterson, R.R.M., Kozakiewicz, Z., Lima, N., Venâncio, A., 2001. Mycotoxin production from fungi isolated from grapes. Lett. Appl. Microbiol. 32 (4), 240–242.

Association of Official Analytical Chemists, 1997. Official methods of the Association of the Agr. Chem. 16th ed. Washington, chapter 49, 37–38.

Association of Official Analytical Chemists, 2012. Official Methods of the Association of the Agr. Chem. 19th ed. p. 1410 (Washington).

Balasaheb, W.P., Sinha, N., Dwivedi, P., Sharma, A.K., 2007. Teratogenic effects of ochratoxin A and aflatoxin B1 alone and in combination on post-implantation rat embryos in culture. J. Turk. Ger. Gynecol. Assoc. 8 (4), 357–364.

Battilani, P., Barbano, C., Marin, S., Sanchis, V., Kozakiewicz, Z., Magan, N., 2006. Mapping of *Aspergillus* section *Nigri* in southern Europe and Israel based on geostatistical analysis. Int. J. Food Microbiol. 111, 72–82.

Battilani, P., Logrieco, A., Giorni, P., Cozzi, G., Bertuzzi, T., Pietri, A., 2004. Ochratoxin A production by *Aspergillus carbonarius* on some grape varieties grown in Italy. J. Sci. Food Agric. 84, 1736–1740.

Bau, M., Bragulat, M.R., Abarca, M.L., Minguez, S., Cabañes, F.J., 2005. Ochratoxigenic species from Spanish wine grapes. Int. J. Food Microbiol. 98, 125–130.

Bejaoui, H., Mathieu, F., Taillandier, P., Lebrihi, A., 2006. Black aspergilli and ochratoxin A production in French vineyards. Int. J. Food Microbiol. 111, 46–52.

Bitter, T., Muir, H.M., 1973. A modified uronic acid carbazole reaction. Anal. Biochem. 4 (4), 330–334.

Brasil, 2011. Resolução RDC n° 7, de 18 de fevereiro de 2011. Dispõe sobre limites máximos tolerados (LMT) para micotoxinas em alimentos. Brasília. available in: http://www.brasilsus.com.br/legislacoes/anvisa/107378-7.html (Acesso em: 22 set. 2015).

Brown, A.L., Odell, E.W., Mantle, P.G., 2007. DNA ploidy distribution in renal tumors induced in male rats by dietary ochratoxin. Exp. Toxicol. Pathol. 59 (2), 85–95.

Chiotta, M.L., Ponsone, M.L., Combina, M., Torres, A.M., Chulze, S.N., 2009. *Aspergillus* section *Nigri* species isolated from different wine-grape growing regions in Argentina. Int. J. Food Microbiol. 136, 137–141.

Commission of the European Communities, 2002. Assessment of Dietary Intake of Ochratoxin A by the Population of Eu Member States. Bruxellas, 2002 (Report of experts participating in Task 3.2.7).

Commission of the European Communities, 2006. Setting maximum levels for certain contaminants in foodstuffs, n° 1881/2006 of 19 Dec. 2006. Off. J. Eur. Union 364, 5–24 (Bruxelles).

Corrêa, L.C., et al., 2012. Desenvolvimento e validação de metodologia para a determinação de compostos fenólicos em vinhos brancos e tintos por cromatografia líquida de alta eficiência (CLAE). In: CONGRESSO LATINO-AMERICANO DE CROMATOGRAFIA E TÉCNICAS RELACIONADAS, 14., 2012, Florianópolis. Anais… Florianópolis: [s. n.]. p. 320.

Diaz, G.A., Torres, R., Veja, M., Latorre, B.A., 2009. Ochratoxigenic *Aspergillus* species on grapes from Chilean vineyards and *Aspergillus* threshold levels on grapes. Int. J. Food Microbiol. 133, 195–199.

Diguta, C.F., Vincent, B., Guilloux-Bénatier, M., Alexandre, H., Rousseaux, S., 2011. PCR ITS-RFLP: a useful method for identifying filamentous fungi isolates on grapes. Food Microbiol. 28, 1145–1154.

Dische, Z., 1962. General color reactions. In: Whistler, R.L., Wolfran, M.L. (Eds.), Carboydrates Chemistry 1. Academic, New York, pp. 477–512.

EL Khoury, A., Rizk, T., Lteif, R., Delia, M.L., Lebrihi, A., 2008. Fungal contamination and aflatoxin B1 and ochratoxin A in Lebanese wine–grapes and musts. Food Chem. Toxicol. 46 (6), 2244–2250.

European Committee for Standardization, EN 14133, 2003. Foodstuffs: Determination of Ochratoxin A in Wine and Beer, Hplc Method with Immunoaffinity Column Clean-up. p. 16 (Brussels).

Ferreira, D.F., 2011. Sisvar: a computer statistical analysis system. Ciênc. Agrotecnol. 35 (6), 1039–1042.

Filtenborg, O., Frisvad, J.C., 1980. A simple screening method for toxigenic moulds in pure cultures. Lesbensmittel-Wissnschaft Technologie. 13 (3), 128–130.

Giusti, M.M., Wrolstad, R.E., 2001. Anthocyanins: characterization and measurement with uv-visible spectroscopy. In: Wrolstad, R.E. (Ed.), Current Protocols in Food Analytical Chemistry. J. Wiley, New York, pp. 1–13 (cap. 1).

Guérin, L., Guyot, F., Vincent, B., Lempereur, V., 2007. Schémas épidémiologiques des déviations nommées goût moisi-terreux (GMT) dans différents vignobles. available in:. http://www.techniloire.com/documents/124963587/article45.pdf (Acesso em: 21 set. 2015).

Jiang, C., Shi, J., Zhu, C., 2013. Fruit spoilage and ochratoxin a production by *Aspergillus carbonarius* in the berries of different grape cultivars. Food Control 30, 93–100.

Kizis, D., Natskoulis, P., Nychas, G.J.E., Panagou, E.Z., 2014. Biodiversity and ITS-RFLP characterisation of *Aspergillus* section *Nigri* isolates in grape from four traditional grape-producing áreas in Greece. PLoS One 9 (4), e93923.

Klich, M.A., 2002. Identification of Common *Aspergillus* Species. CBS, Utrecht, pp. 2002–2116.

Kogkaki, E.A., Natskoulis, P.I., Magan, N., Panagou, E.Z., 2015. Effect of interaction between *Aspergillus carbonarius* and non-ochratoxigenic grape-associated fungal isolates on growth and ochratoxin A production at different water activities and temperatures. Food Microbiol. 46, 521–527.

Lasram, S., Belli, N., Chebil, S., Nahla, Z., Ahmed, M., Sanchis, V., Ghorbel, A., 2007. Occurrence of ochratoxigenic fungi and ochratoxin A in grapes from a Tunisian vineyard. Int. J. Food Microbiol. 114, 376–379.

Lasram, S., Oueslati, S., Valero, A., Marin, S., Ghorbel, A., Sanchis, V., 2010. Water activity and temperature effects on fungal growth and ochratoxin A production by ochratoxigenic *Aspergillus carbonarius* isolated from Tunisian grapes. J. Food Sci. 75 (2), 89–97.

Lasram, S., Oueslati, S., Mliki, A., Ghorbel, A., Silar, P., Chebil, S., 2012a. Ochratoxin A and ochratoxigenic black *Aspergillus* species in Tunisian grapes cultivated in different geographic áreas. Food Control 25, 5–80.

Lasram, S., Barketi, A., Mliki, A., Ghorbel, A., 2012b. Growth and ochratoxin A production by *Aspergillus carbonarius* at different pHs and grape maturation stages. Lett. Appl. Microbiol. 54, 418–424.

Li, S., Pan, M.H., Lai, C.S., Lo, C.Y., Dushenkov, S., Ho, C.T., 2007. Isolation and syntheses of polymethoxy flavones and hydroxylated polymethoxy flavones as inhibitors of HL-60 cell lines. Bioorg. Med. Chem. 15 (10), 3381–3389.

Lombaert, G.A., Pellaers, P., Neumann, G., Kitchen, D., Huzel, V., Trelka, R., Kotello, S., Scott, P.M., 2004. Ochratoxin A in dried vine fruits on the Canadian retail market. Food Addit. Contam. 22, 578–585.

Lorenzini, M., Azzolini, M., Tosi, E., Zapparoli, G., 2012. Postharvest grape infection of *Botrytis cinerea* and its interactions with other moulds under withering conditions to produce noble-rotten grapes. J. Appl. Microbiol. 114, 762–770.

Magan, N., 2007. Fungi in extreme environments. In: Kubicek, C.P., Druzhinina, I.S. (Eds.), Environmental and Microbial Relationships: The Mycota IV, 2nd ed. Springer, Berlin, pp. 85–103.

Magnoli, C., Violante, M., Combina, M., Palacio, G., Dalcero, 2003. Mycoflora and ochratoxin-producing strains of *Aspergillus* section *Nigri* in wine grapes in Argentina. Lett. Appl. Microbiol. 37, 179–184.

Martínez-Ávila, G.C., Aguilera-Carbó, A.F., Rodriguez-Herrera, R., Aguilar, C.N., 2012. Fungal enhancement of the antioxidant properties of grape waste. Ann. Microbiol. 62, 923–930.

Martínez-Culebras, P.V., Ramón, D., 2007. An ITS-RFLP method to identify black *Aspergillus* isolates responsible for OTA contamination in grapes and wine. Int. J. Food Microbiol. 113, 147–153.

Mccready, P.M., Mccomb, E.A., 1952. Extraction and determination of total pectic material. Anal. Chem. 24 (12), 1586–1588.

Medina, A., Schmidt-Heydt, M., Rodríguez, A., Parra, R., Geisen, R., Magan, N., 2015. Impacts of environmental stress on growth, secondary metabolite biosynthetic gene clusters and metabolite production of xerotolerant/xerophilic fungi. Curr. Genet. 61, 325–334.

Medina, A., Mateo, R., López-Ocaña, L., Valle-Algarra, F.M., Jiménez, M., 2005. Study of spanish grape mycobiota and ochratoxin A production by isolates of *Aspergillus tubingensis* and other members of *Aspergillus* section *Nigri*. Appl. Environ. Microbiol. 71 (8), 4696–4702 (Washington).

Mohamed, S., Flint, S., Palmer, J., Fletcher, G.C., Pitt, J.I., 2013. An extension of the coconut cream agar method to screen *Penicillium citrinum* isolates for citrinin production. J. Appl. Microbiol. 57, 214–219.

Ortuño, A., Báidez, A., Gómez, P., Arcas, M.C., Porras, I., García-Lidón, A., Del-Río, J.A., 2006. *Citrus paradisi* and *Citrus sinensis* flavonoids: their influence in the defence mechanism against *Penicillium digitatum*. Food Chem. 98 (2), 351–358.

Palma, N., Cinelli, S., Sapora, O., Wilson, S.H., Dogliotti, E., 2007. Ochratoxin A-induced mutagenesis in mammalian cells is consistent with the production of oxidative stress. Chem. Res. Toxicol. 20 (7), 1031–1037.

Palumbo, J.D., O'Keeffe, T.L., Mahoney, N.E., 2006. Inhibition of ochratoxin A production and growth of *Aspergillus* species by phenolic antioxidant compounds. Mycopathologia 164 (5), 351–358.
Pena, A., Cerejo, F., Silva, L.J.G., Lino, C.M., 2010. Ochratoxin A survey in Portuguese wine by LC–FD with direct injection. Talanta 82 (4), 1556–1561.
Passamani, F.R.F., Lopes, N.A., Pereira, G.E., Prado, G., Batista, L.R., 2012. *Aspergillus* section *Nigri* in grapes cultivated in the tropical winery region of Brazil. Food Public Health 2 (6), 276–280.
Pessoni, R.A.B., Tersarotto, C.C., Mateus, C.A.P., Zerlin, J.K., Simões, K., Figueiredo-Ribeiro, R.C.L., Braga, M.R., 2015. Fructose affecting morphology and inducing β-fructofuranosidases in *Penicillium janczewskii*. Spring Plus 4 (487), 1–11.
Pitt, J.I., 2000. A Laboratory Guide to Common *Penicillium* Species. Food Science Australia, Sydney, pp. 2000–2187.
Pitt, J.I., Hocking, A.D., 1997. Fungi and Food Spoilage. 2nd ed. Blackie Academic and Professional, London, p. 593.
Ponsone, M.L., Chiotta, M.L., Palazzini, J.M., Combina, M., Chulze, S., 2012. Control of ochratoxin A production in grapes. Toxins 4 (5), 364–372.
Remiro, R., Irigoyen, A., Gonzáles-Peñas, E., Lizarraga, E., Cerais, A.L., 2013. Levels of ochratoxins in Mediterranean wines. Food Control 32, 63–68.
Rosa, C.A., Magnoli, C.E., Fraga, M.E., Dalcero, A.M., Santana, D.M.N., 2004. Occurrence of ochratoxin A in wine and grape juice marketed in Rio de Janeiro, Brazil. Food Addit. Contam. 21, 358–364.
Rossiello, M.R., Rotunno, C., Coluccia, A., Carratù, M.R., Di Santo, A., Evangelista, V., Semeraro, N., Colucci, M., 2008. Ochratoxin A inhibits the production of tissue factor and plasminogen activator inhibitor-2 by human blood mononuclear cells: another potential mechanism of immune-suppression. Toxicol. Appl. Pharmacol. 229, 227–231.
Rousseaux, S., Diguta, C.F., Radoi-Matei, F., Alexandre, H., Guilloux-Bénatier, M., 2014. Non-botrytis grape-rotting fungi responsible for earthy and moldy off-flavors and mycotoxins. Food Microbiol. 38, 104–121.
Rufino, M.S.M., Alves, R.E., Brito, E.S., Morais, S.M., Sampaio, C.G., Pérez-Jiménez, J., Saura-Calixto, F.D., 2007a. Metodologia científica: determinação da atividade antioxidante total em frutas pela captura do radical livre DPPH. Embrapa, Brasília (Comunicado Técnico, 127).
Rufino, M.S.M., Alves, R.E., Brito, E.S., Morais, S.M., Sampaio, C.G., Pérez-Jiménez, J., Saura-Calixto, F.D., 2007b. Metodologia científica: determinação da atividade antioxidante total em frutas pela captura do radical livre ABTS. Embrapa, Brasília (Comunicado Técnico, 126).
Sage, L., Krivobok, S., Delbos, E., Seigle-Murandi, F., Creppy, E.E., 2002. Fungal flora and ochratoxin A production in grapes and musts from France. J. Agric. Food Chem. 50, 1306–1311.
Samson, A., Hoekstra, E.S., Frisvad, J.C., Filtenborg, O., 2000. Introduction to food-borne fungi. Centraalbureau voor schimmelcultures, Baarn and Delft 1995, 4th ed.
Sandri, I.G., Fontana, R.C., Barfknecht, D.M., Silveira, M.M., 2011. Clarification of fruit juices by fungal pectinases. LWT Food Sci. Technol. 44, 2217–2222.
Santos Júnior, H.M., Campos, V.A.C., Alves, D.S., Cavalheiro, A.J., Souza, L.P., Botelho, D.M.S., Chalfoun, S.M., Oliveira, D.F., 2014. Antifungal activity of flavonoids from *Heteropterys byrsonimifolia* and a commercial source against *Aspergillus ochraceus*: in silico interactions of these compounds with a protein kinase. Crop. Prot. 62, 107–114.
Sarigiannis, Y., Kapolos, J., Koliadima, A., Tsegenidis, T., Karaiskakis, G., 2014. Ochratoxin A levels in Greek retail wines. Food Control 42, 139–143.
Sava, V., Reunova, O., Velasquez, A., Harbison, R., Sánchez-Ramos, J., 2006. Acute neurotoxic effects of the fungal metabolite ochratoxin-A. Neurotoxicology 1 (27), 82–92.
Serra, R., Lourenço, A., Alípio, P., Venâncio, A., 2006. Influence of the region of origin on the mycobiota of grapes with emphasis on *Aspergillus* and *Penicillium* species. Mycol. Res. 110, 971–978.
Serra, R., Braga, A., Venâncio, A., 2005. Mycotoxin-producing and other fungi isolated from grapes for wine production, with particular emphasis on ochratoxin A. Res. Microbiol. 156, 515–521.
Setati, M.E., Jacobson, D., Andong, U.C., Bauer, F., 2012. The vineyard yeast microbiome, a mixed model microbial map. PLoS One 7 (12), 52609.
Shundo, L., Almeida, A.P., Alaburda, J., Ruvieri, V., Navas, S.A., Lamardo, L.C.A., Sabino, M., 2006. Ochratoxin A in wines and grape juices commercialized in the city of São Paulo, Brazil. Braz. J. Microbiol. 37 (4), 533–537.
Soto, J.B., Fernández-Franzón, M., Ruiz, M.J., Garcia, A.J., 2014. Presence of ochratoxin A (OTA) mycotoxin in alcoholic drinks from southern European countries: wine and beer. J. Agric. Food Chem. 62 (31), 7643–7651.
Teixeira, M.F.S., Lima Filho, J.L., Duran, N., 2000. Carbon sources effect on pectinase production from *Aspergillus japonicus* 586. Braz. J. Microbiol. 31, 286–290.
Terra, M.F., Prado, G., Pereira, G.E., Ematné, H.J., Batista, L.R., 2013. Detection of ochratoxin A in tropical wine and grape juice from Brazil. J. Sci. Food Agric. 93 (4), 890–994.
Tozlovanu, M., Faucet-Marquis, V., Pfohl-Leszkowicz, A., Manderville, R.A., 2006. Genotoxicity of the hydroquinone metabolite of ochratoxin A: structure-activity relationships for covalent DNA ad-duction. Chem. Res. Toxicol. 19, 1241–1247.
Tranchimand, S., Brouant, P., Iacazio, G., 2010. The rutin catabolic pathway with special emphasis on quercetinase. Biodegradation 21, 833–859.
Varga, J., Frisvad, J.C., Kocsubé, S., Brankovics, B., Tóth, B., Szigeti, G., Samson, R.A., 2011. New and revisited species in *Aspergillus* section *Nigri*. Stud. Mycol. 69 (1), 1–17.
Waterhouse, 2002. A. L. Polyphenolics: determination of total phenolics. In: Wrolstad, R.E., et al. (Eds.), Current Protocols in Food Analytical Chemistry. John Wiley & Sons, New York, pp. 1111–1118.
Wu, T., Cheng, D., He, M., Pan, S., Yao, X., Xu, X., 2014. Antifungal action and inhibitory mechanism of polymethoxylated flavones from *Citrus reticulata* Blanco peel against *Aspergillus niger*. Food Control 35, 354–359.
Zhang, X., Chen, L., Li, J., Zhu, B., Ma, L., 2013. Occurrence of ochratoxin A in Chinese wines: influence of local meteorological parameters. Eur. Food Res. Technol. 236, 277–283.
Zhang, Z., Dvir, O., Pesis, E., Pick, U., Lichter, A., 2005. Weak organic acids and inhibitors of pH homeostasis suppress growth of *Penicillium* infesting litchi fruits. J. Phytopathol. 153, 667–673.