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OCHRATOXIN A-PRODUCING FUNGI IN GRAPES AND THEIR CONTROL BY BIOLOGICAL AGENTS - A REVIEW

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Abstract

The dependence on antagonistic microbes, particularly yeasts to prevent the possible occurrence of Ochratoxin A (OTA) and its degradation is a promising and friendly solution to attain safe food and consumer confidence. The article aims at reviewing the current literature on OTA removal by combination of yeasts and other exogenous agents, as well as the mechanisms of action and the factors affecting OTA degradation process. It is important to acknowledge that attempts to avoid/reduce the use of harmful chemicals without compromising the loss of quantity and quality or palatability of decontaminated food are commendable. Besides, development of reliable and rapid research outcomes may be considered a paramount stride toward commercialization of technologies. Indeed, current knowledge on yeasts demonstrates their ability to remove ochratoxin A (OTA) through viable cells, cell walls and cell wall extracts as major mechanisms of action. The OTA removal may differ from yeast strain to yeast strain. Additional factors such as pH, moisture, temperature and water activity, influence OTA production and OTA detoxification by microbial control agents. Lastly, molecular studies to affirm the interactions between enhanced antagonistic yeasts, OTA-producing fungi and OTA degradation/detoxification will be important due to the pursuance of providing safe food and consumer confidence

Keywords: ochratoxin A (OTA), biocontrol agent, combination, degradation, mycotoxin

Introduction

Ochratoxin A (OTA) is among the most studied mycotoxins due to its occurrence and toxicity in foods worldwide. Apparently, it is one of the most important mycotoxins alongside aflatoxins (AFs), fumonisins, trichothecenes, zearelenone (ZEN) and patulin (PAT), which are mostly found in foods (Mahunu et al., 2015). The fungi that produce the OTA are tolerant to hot and humid climates, which favour their growth and infection process. The contamination of foodstuffs by OTA leads to deterioration of the marketable quality and account for significant economic losses. OTA poses a potential risk to human health based on its slow chemical degradation qualities. It has been reported that OTA is nephrotoxic and hepatotoxic mycotoxin, causing kidney and liver cancer in mice and rats (Joint and Additives, 1983). The nephrotoxicity occurrence in monogastric animals such as porcine nephropathy is widely documented, which results in significant economic losses in the swine and poultry industries. Again, OTA has been linked to Balkan Endemic Nephropathy and the

development of urinary tract tumors in humans (Marquardt and Frohlich, 1992). Genotoxic, immunotoxic, teratogenic and neurotoxic in laboratory animals are associated with OTA contamination (Shundo et al., 2006). It was reported in 1993 that OTA was classified as a possible human carcinogen (group 2B) by the International Agency for Research on Cancer as experimental studies proved the evidence of OTA carcinogenicity in animals (Bellí et al., 2005). Due to the serious health risk associated with excessive ingestion of OTA contaminated foods, various maximum permissible limits (MPL) (Table 1) in foods and other products have been established and documented by the European Union (De Curtis et al., 2012).

Therefore, this review focused on mycotoxins OTA-producing fungi in grapes, the occurrence of OTA in grapes and derived products, improvement of the control efficacy with biocontrol agents and the mechanisms of action during biocontrol activities were also discussed.

Table 1: Maximum permissible limits of OTA in some foodstuffs

Foodstuffs	**Maximum levels (µg/kg)**
Unprocessed cereals	5.0
All products derived from unprocessed cereals, including processed cereal products and cereals intended for direct human consumption	3.0
Dried vine fruit (currants, raisins and sultanas)	10.0
Roasted coffee beans and ground roasted coffee, excluding soluble coffee	5.0
Grape juice, concentrated grape juice as reconstituted, grape nectar, grape must and concentrated grape must as reconstituted, intended for direct human consumption	2.0

De Curtis et al. (2012)

Ochratoxin A and its occurrence in fruits and derived products

OTA is a phenylalanyl derivative of a substituted isocoumarin (R)-N-[5-chloro-3,4-dihydro-8-hydroxy-3-methyl1-oxo-1H-2-benzopyran-7-y1)-carbonyl]-L-phenylalanine (Marin et al., 2013) as shown in Fig. 1. It is a product from hydrolysis of OTA as a result of the lack of the phenylalanine molecule (Marin et al., 2013). OTAs are metabolites of *Aspergillus ochraceus* and *Penicillium verrucosum* mainly, in tropical and temperate regions respectively (Mantle et al., 2009). However, apart from *nordicum* and *verrucosum* of the, *Penicillium genus,* most species from the genus Aspergillus are mostly responsible for OTA in food. This genus may have more than 100 species, with a complex taxonomy under continuous revision.

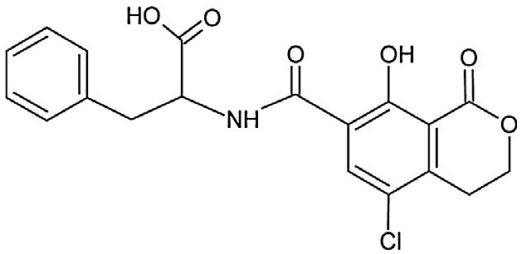

Fig. 1. Molecular structure of ochratoxin A (Marin et al., 2013)

Due to the strong toxicity of OTA, there is a great concern about its occurrence in many commodities such as cereal grains, grapes, wine, grape juice, dried vine fruits, coffee, legumes, beer, nuts, cocoa

and spices (Bayman and Baker, 2006). After cereals, grapes and its derived products are known to be the second most susceptible to OTA contamination (Zhang et al., 2016). As a matter of fact, the OTA infected grapes are considered the major source of wine contamination. Further study also suggested that the occurrence of OTA in wines is related to the growth of OTA-producing fungi (*Aspergillus carbonarius* and other black aspergillius) on grapes during cultivation. Reports have it that OTA concentration in beverages and their substrata vary widely. According to a report by Bizaj et al. (2009), OTA toxicity was detected in grapes and grape products such as grape juice (< 3-311 ng/L) and wine (< 3-388 ng/L) in 1996. The same authors reported on studies of OTA detections in various samples; between 0- 13.08 ng/g in grape berries, up to 9.8 ng/L in grape juice and up to 7.63 ng/mL in wines.

OTA production has been linked to induction by the physiochemical properties of the substrate. Stratakou and Fels-Klerx (2010) indicate that the most imperative factors that impact on OTA contamination of grapes and wine include temperature and relative humidity in the month before harvesting the berries, the type of wine (maceration), and the proportion of berries damage before vinification. Generally, the possible development of molds producing OTA depends on climatic conditions and it is more frequent in areas with tropical climates (Belajová and Rauová, 2007)**.** It was also found that climatic and geographic differences determine the decay caused by fungi and possible OTA contamination of grapes (Varga and Kozakiewicz, 2006). For instance, OTA production by *A. carbonarius* at water activity (a_w) of 0.80 has been reported (Ribeiro et al., 2006). Cairns-Fuller et al. (2005) have also reported high levels of OTA production by *P. verrucosum* at 0.93–0.98 a_w at 10–25 °C, while production by *A. ochraceus* at 0.98 while 0.99 a_w was indicated by Pardo et al. (2004). Earlier studies indicated significant levels of OTA production by *P. verrucosum* can occur at a_w as low as 0.86 and at 4°C (Sweeney and Dobson, 1998). Recently, it was reported that wines coming from southern regions of Europe showed increased levels OTA content (Cicoňová et al., 2010). OTA production by *A. ochraceus* occurs between 12 °C and 37 °C with an optimum at 31 °C (Sweeney and Dobson, 1998). Optimal temperature of 30 °C has also been reported (Pardo et al., 2004).

Furthermore, *Penicillium* and *Aspergillus* spp. are opportunistic pathogens known to be responsible for several disorders in various plants (Varga et al., 2004). Mostly, *Aspergillus* spp. may occur in damaged grapes during ripening and subsequently form OTA in grapes after harvest (Visconti et al., 2008). The concentrations of OTA tend to increase with the grape maturity (Rousseau, 2004). Damage of berry is the major factor that contributes to the development of disease and OTA accumulation in berries. Actually, the higher the punctures on the berries, the higher the possibility of molds contamination (Belajová and Rauová, 2007). Such damage may be caused by birds, insects, infection by other fungi, or rain (Visconti et al., 2008). It was found that *A. carbonarius* is a very invasive species which can penetrate berries even without skin damage (Battilani et al., 2006). This also implies that grape varieties with thin and fragile skins may suffer contamination much easier (Belajová and Rauová, 2007), while some may exhibit more susceptibility to *Aspergillus* bunch rots than others (Cicoňová et al., 2010).

Control of Ochratoxin A

Though prevention of OTA contamination at source or prevention of the growth of mycotoxigenic fungi does not constitute sufficient control, yet it is recommended as the first approach. Several combinations of biocontrol agents have been confirmed to be an effective method to improve control of postharvest pathogens on the fruits (Arrebola et al., 2010). The potential of two or more biocontrol agents applied together have shown better control of OTA-producing pathogens than the antagonist alone. The biocontrol efficacies of *Aureobasidium pullulans* against rots caused by *B.*

cinerea on sweet cherries (Ippolito et al., 2005) have been improved effectively by the use of sodium bicarbonate (SBC). The integration of *Hanseniaspora uvarum* with salicylic acid (2 mmol L^{-1}) or with sodium bicarbonate (2% w/v) both significantly reduced the decay incidence caused by *Botrytis cinerea* (gray mold) in grapes (Fig. 2).

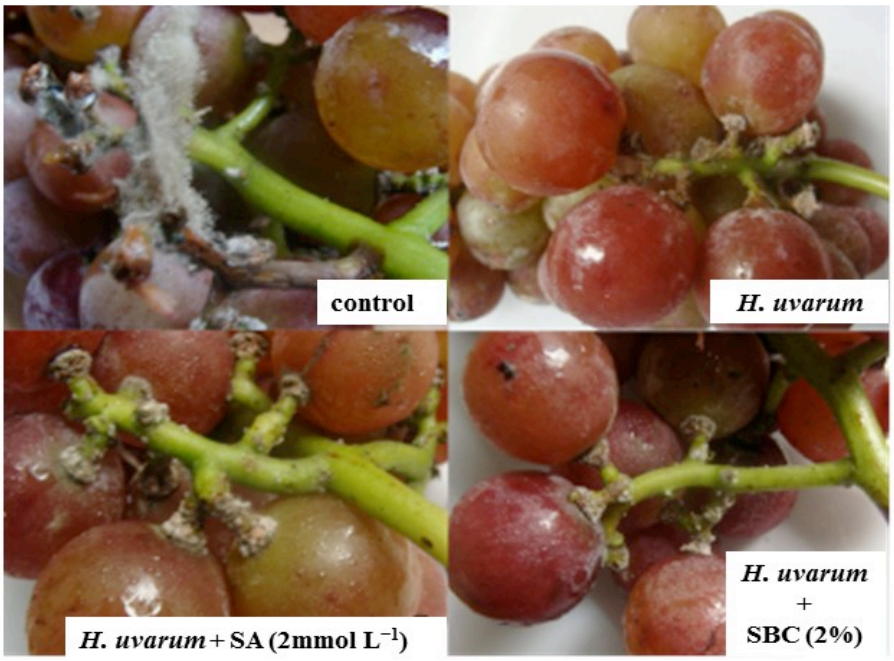

Fig. 2. Effects of different treatment on gray mold of grapes after 90days of cold storage; control (untreated fruit showed visible infections); *Hanseniaspora uvarum*; *H. uvarum* combined with 2mmol L^{-1} salicylic acid (SA); and *H. uvarum* combined with 2% sodium bicarbonate (SBC).

Photo obtained from Qin et al. (2015).

It has been reported that OTA contamination at preharvest or during the postharvest stages maybe very difficult to avoid; therefore, various approaches have been used to decontaminate and/or detoxify contaminants to essentially prevent or lessen the detrimental effects on health (Kabak, Dobson & Var, 2006). These approaches have been grouped as follows:

1) the detoxification of mycotoxins that exist in foods, and

2) inhibition of mycotoxin absorption in the gastrointestinal tract (thus, the use of specific materials that absorb mycotoxins by avoiding or limiting their bioavailability in the gastrointestinal tract).

Decontamination/detoxification measures using physical separation and physical, chemical and biological inactivation and/or removal of the toxin play a vital role in the industry (Kabak, Dobson & Var, 2006). By this, the decontamination process which is aimed at reducing the toxic and commercial impact of mycotoxins must meet certain basic criteria as previously reported by Grosso et al. (2003):

(1) it must destroy, deactivate or eliminate the mycotoxins in foods;
(2) it must not have residual effects by producing or leaving toxic and/or carcinogenic residues in the final products (Fig. 3);
(3) it should not modify significantly the nutritional and technological properties of the product;
(4) it must be capable of inhibiting fungal spores and mycelia in order to

avoid new toxin that may form under favorable conditions; and

(5) it has to be technically and economically viable.

Postharvest contamination of food by ochratoxigenic fungi and sequential production of OTA will occur, if environmental conditions are favorable (Cicoňová, Laciaková, & Máté, 2010). Indeed, it is difficult to eliminate PAT grapes and other fruits, when contamination occurs. The process of decontamination is complex and expensive; because high temperatures are required for decomposition (Barkai-Golan & Paster, 2008), which could alter nutritive and the palatability of the final product (Dong et al., 2010). It has been recognized that the use of chemical and physical methods to eliminate or minimize OTA contaminants in foods has not achieved satisfactory results and further poses serious challenges as recently reported by Droby et al. (2016), while the physical methods including filtration or adsorption could alter sensorial attributes (color, taste and aroma) and other desirable properties (Grazioli et al., 2006). As a stable compound, OTA is not destroyed through common food preparation procedures; instead it requires very high temperatures (above 250 ^{o}C) for several minutes to reduce its concentration (Boudra et al., 1995). Actually, several studies have been reported on the cumulative and residual effects on human life, environment, and development of resistance by toxigenic fungi and other major plant pathogens (Mahunu et al., 2015; Sharma et al., 2012). Also, treatment with fungicides will not completely prevent pathogen infection (Chen et al., 2012). Therefore, among all the strategies applied during preharvest (e.g. field management, use of biological and chemical materials), harvest management, postharvest strategies (e.g. improving drying and storage conditions), prevention of grapes from fungal infection and subsequent OTA contamination is preferred (Bellí et al., 2006; Leong et al., 2006).

Thorough studies on the interaction effects of yeasts and molds on the growth of ochratoxigenic microorganisms, the control of *Aspergillus* infections and OTA contents by the use of some microorganisms have been promising. In line with this, some yeasts (*Issatchenkia orientalis*, *Metschnikowia pulcherrima*, *Issatchenkia terricola*, and *Candida incommunis*) isolated from grapes have been identified to reduce the ability of *A. carbonarius* and *A. niger* to infect grape berry (Bleve et al., 2006). Among these yeasts, *I. orientalis* isolates showed the best antagonistic biocontrol ability. Again, treatment with *Trichoderma* (T.) *harzianum* was able to suppress *A. ochraceus* and *A. niger* grown as combined cultures on potato dextrose agar (PDA) medium (Gachomo and Kotchoni, 2008). *Trichoderma* spp. are strong opportunistic invaders, fast growing prolific producers of spores and powerful antibiotic producers (Harman, 2006). The production of gliotoxin (antifungal antibiotics substance) by *Trichoderma viride* was toxic to both *Rhizoctonia solani* and *Sclerotinia americana*, which eventually inhibited their growth. Further, all isolates of *Trichoderma* spp. and *Gliocladium* spp. studied inhibited *P. chrysogenum* growth on PDA medium (Abou-Zeid, Altalhi, & El-Fattah, 2008). The combination of different fungi (T. harzianum, *Al. alternata*, *Cladosporium* (Cl.) *herbarum*, *Eurotium* (E.) *amstelodami*, *P. janthinellum*, *P. decumbens*, and *Candida* spp.) in culture treatments against the growth of grape-associated fungi (*A. carbonarius*, *A. niger*, and *A. japonicus*) on medium has been significantly effective. The results of the above study indicated that at 0.97 a_w, the growth rates of *Aspergillus* spp. showed visible inhibition by *T. harzianum*. In the same study, the growth of *A. niger* was reduced by almost all molds. It was found that *Candida* spp. rather stimulated the growth of *Aspergillus* spp. At water activity of 0.92, *Penicillium* spp. and *Cl. herbarum* stimulated the growth of OTA-producing fungi (*A. carbonarius*) (Valero et al., 2007).

Additionally, two strains of *A. pullulans* (LS30 and AU34-2) were able to degrade OTA to less toxic levels in both synthetic medium and fresh grape must (De Curtis et al., 2012). In an on-farm trial, the use of *A. pullulans* Y-1 (phyllosphere yeast) reduced sour rot disease caused by *A. carbonarius* in grapes by 99% in the year 2005 and 90% in 2006 (Dimakopoulou et al., 2008). Also, native yeast strain *Kluyveromyces thermotolerans* effectively controlled *A. niger* and *A. carbonarius* in grape vineyards in Argentina and further reduction of both growth and OTA accumulation by 50% (Ponsone et al., 2011). Even though, some strains produced effective control of the growth of OTA-producing fungi but could not remove the toxin already produced (Zhang et al., 2016).

Interestingly, plant substances such as essential oils (EOs) have also showed capability in prevention of fungal growth and OTA production. EOs are a group of secondary metabolites that play an important role in plant defense (Prins, Vieira, & Freitas, 2010; Taiz and Zeiger, 2004). Several EOs such as those derived from garlic, cinnamon, thyme, oregano, clove, basil, coriander, citrus peel, eucalyptus, ginger, rosemary, and peppermint have been reported to contain antimicrobial properties (Hyldgaard, Mygind, & Meyer, 2012). They exhibit broad-range of antibacterial properties as well as insecticidal antiparasitic, antiviral, antifungal and antioxidant properties (Brenes and Roura, 2010; Silva et al., 2011). Largely, EOs and their components are considered to be relatively safe, with wide consumer acceptability, which intensifies the need for exploring their potential multi-purpose uses (Ormancey, Sisalli & Coutiere, 2001; Sawamura, 2000) such as in postharvest diseases control. For instance, EOs of thyme and anise (500 ppm), cinnamon (1000 ppm) and spearmint (2000 ppm) were found to inhibit the growth of *A. ochraceus* (Cicoňová, Laciaková, & Máté, 2010)**.** Nguefack et al. (2009) reported that thyme essential oil exhibited high fungicidal effect on *A. ochraceus* and *P. verrucosum*. Cinnamon essential oil was also found to be highly effective against *A. niger* (Singh, Maurya, & Catalan, 2007), while garlic bulb extract completely inhibited the growth of *A. ochraceus* (Reddy, Reddy, & Muralidharan, 2007).

Mechanisms of OTA degradation

The mechanism of action of OTA degradation is an important component of the biocontrol systems, which helps to explain how the efficacy of biological control agents (BCAs) can be improved for broad and persistent effects against mycotoxins. The interactions between microbial species (pathogen and antagonists) influence the density of ochratoxigenic fungi and production of OTA in medium. The relative capabilities of each microbial species over the other will produce the controlling effects or mycotoxin contamination. According to Irtwange (2006), after harvest the combat of pathogens can be accomplished through the inoculation of microbial antagonists culture by spraying or dipping fruits in solutions. Also, it has been reported that the production of compounds by some actinimycetes strains can suppress mycotoxins in postharvest storage; thus by blocking the biosynthesis path way of mycotoxins (Medeiros et al., 2012). The yeast *Trichosporon mycotoxinivorans* detoxified OTA through the cleavage of the phenylalanine moiety to produce the derivative OTAα. Probably, due to the fact that *T. mycotoxinivorans* can be freeze-dried, fermented and stabilized without losing its efficacy. In other words, its response in postharvest treatments for OTA detoxification appears to be viable (Zhang et al., 2016).

Certainly, the application of BCAs must involve the simultaneous harmonization of several mechanisms in order to make it almost impossible for pathogen survivability (Limón and Codón, 2004). Fungi causing decay are mostly sensitive to the lack of nutrients, which leads to starvation as the most common cause of death of microorganisms. As a result, biological control of plant fungal pathogens can be achieved through competition for limiting nutrients in the biocontrol environment (Chet, Inbar, & Hadar, 1997). It was found that uptake of iron is vital for viability of most filamentous fungi.

Therefore, when iron starvation occurs most fungi excrete low-molecular-weight ferric-iron specific chelators to mobilize environmental iron (Eisendle et al., 2004). As the nutrient concentration decreases, biocontrol becomes more effective by competing for both colonization and nutrients. In this regard, an in-depth knowledge of the mechanisms of action of BCAs will improve the use of biocontrol methods. By identifying the genes and traits involved in the processes of colonization during BCA-pathogen-host interactions will also lead to more efficient application of strains (Limón and Codón, 2004). According to Zhao et al. (2013), *B. cinerea* in apple wounds 6 days after incubation at 20 ^{o}C increased the intracellular trehalose content (5 mg mL^{-1}) of *Pichia caribbica*; thus enhanced the activity of *P. caribbica* to improve disease resistance of apple and inhibit the growth of the hyphae of pathogen in apple wounds directly (Fig. 3). Probably, the attachment to pathogen cell walls and prevention of the proliferation of pathogen in apple wounds are mechanisms by which antagonistic yeast controls post-harvest diseases of apples (Qin et al., 2015). Furthermore, studies on the interaction between *A. ochraceus* strain 3. 44212 and *Bacillus subtilis* CW 12 in mix culture was used to affirm the mechanism of controlling the OTA-producing fungi growth (Shi et al., 2014). The results clearly showed that *B. subtilis* CW 12 has the potential as a biological agent to control OTA levels in crops; thus, the hyphae growth was disrupted by the presence of antagonistic microbe in contrast with the control samples.

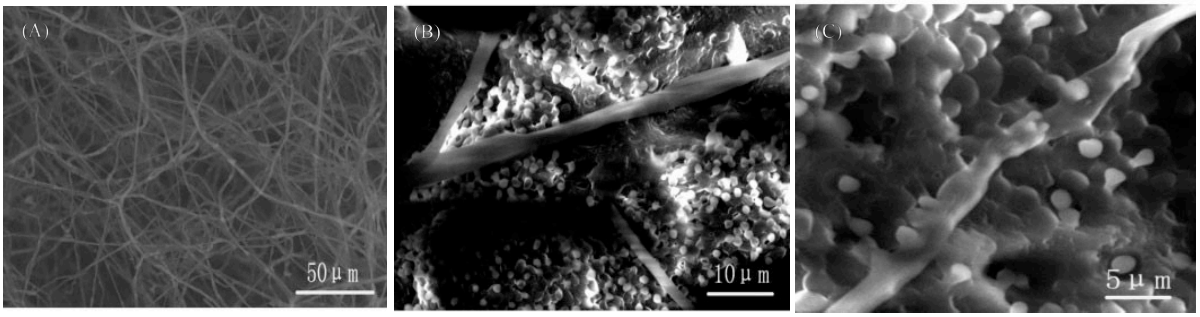

Fig. 3. Scanning electron micrographs showing effect of interaction between antagonistic microbe integrated with exogenous compound on the hyphae of decay causing fungi in apple wounds 6 days after incubation at 20 ^{o}C. Treatments: (A) sterile distilled water, (B) *P. caribbica* harvested from NYDB and (C) *P. caribbica* harvested from NYDB amended with trehalose at 5 mg mL^{-1} (Zhao et al., 2013).

The main methods of OTA degradation by BCAs can be grouped into two;

1) the hydrolysis of the OTA amide bond to a non-harmful OTAα and $_{L}$-β-phenylalanine (Fig. 4), and
2) the hydrolysis of the lactone ring causing lactone OTA form to open. Some degree of success have been achieved by the use of various types of lactic acid producing bacteria and yeasts to detoxify OTA in wines (Zhang et al., 2016).

Pediococcus parvulus (UTAD 473) isolated from Douro wine successfully detoxified OTA during malolactic fermentation, because some of the strains have probiotic structures (Abrunhosa et al., 2014). Besides, biodegradation capability of probiotic strains was established, when *lactobacilli* and *bacilli* were able to degrade OTA and trichothecenes (Štyriak et al., 2001). Similarly, probiotic strains of *S. cerevisiae* can bind effectively to most aflatoxins (Kabak, 2010; Pizzolitto et al., 2012). Probiotic yeasts or products containing cell wall of yeast or other additives are also applied to oppose mycotoxicosis in livestock (Pizzolitto et al., 2012). However, there is another facet of yeast–mycotoxin interactions that is relatively understudied (Pfliegler, Pusztahelyi, & Pócsi, 2015). Further *in vivo* investigations to explore the influence of more probiotic bacteria/ yeasts to bind mycotoxin; thus, to reduce exposure

of humans and/or animals to this highly toxic contaminant are essential.

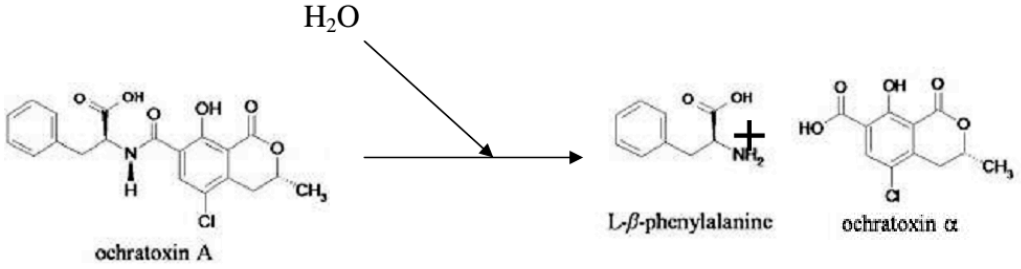

Fig. 4. Conversion of OTA in L- β-phenylalanine and OTα (Hathout and Aly, 2014)

There are available reports on some lactic acid producing bacteria that have the ability to detoxify OTA through adsorption by their cell walls in juice. They include *Oenococcus oeni* RM11 and *L. plantarum* CECT 748T (Del Prete et al., 2007). Polysaccharides and peptidoglycan are believe to be involved in this mechanism. *Lactobacillus acidophilus* reduced OTA by 97% in grape juice (Hathout and Aly, 2014) and their report revealed that OTA was detoxified via cleavage of the peptide bond; thus, leading to the release of phenylalanine.

Biodegradation and a variety of decontamination measures of OTA by yeasts, yeast cell walls and yeasts cell wall extracts have been reported (Caridi et al., 2006). Previous reports indicate that significant diversity has been noted among yeasts for their parietal absorption activity, where the outermost layer of the cell wall contain a chemical composition that differs from yeast to yeast, with the absorption being considered as strain dependent) (Bizaj et al., 2009). Recently, it was reported that, viable cells, non-viable cells and cell wall products of probiotic yeasts with extraordinary adsorption capability are proficient of decreasing bioavailability of toxins in food (Pfliegler, Pusztahelyi, & Pócsi, 2015). The effective absorption capability is due to the fact that yeast species are highly diverse in cell wall composition. Yeast *b*-D-glucans, glucomannans and mannan-oligosaccharide cell wall components have been demonstrated to be responsible for the adsorption of mycotoxins (Pfliegler, Pusztahelyi, & Pócsi, 2015; Yiannikouris et al., 2006)**.** Reports indicate that the proportions of OTA removal may range from 11% to 45% in yeast pepton medium depending on the strain and the method employed. The absorption of OTA by *S. cerevisiae* strain treated with heat was rapid and complete degradation within 5 min, while it persisted up to 2 h in grape juice (Bejaoui et al., 2004). Other yeasts have the ability to produce satisfactory effects on toxin producing pathogen and OTA itself. More than 90% of OTA was degraded by *Phaffia rhodozyma* (Péteri et al., 2007), while *Aureobasidium pullulans* degraded OTA in grape must (De Curtis et al., 2012) and yeast *Botrytis cinerea* in grape-like synthetic medium (Valero et al., 2008).

Piotrowska, Nowak, & Czyzowska (2013) reported that viable yeasts treatment alone was able to adsorb 35% of the OTA, whereas heat combined with yeast cells and acid-treated yeasts cells were able to detoxify 90.80% and 73% of the OTA content in wine respectively. From the results, it implies that heat can alter the surface structure of the cell, by denaturing the proteins and forming Millard reaction products. Regarding acid-treated yeast, the acid might have affected the polysaccharides through the release of monomers, which fragmented into aldehydes following the breaking of the glycosidic bonds. It was also seen that excreted products stimulated higher adsorption sites than viable yeast cells (Piotrowska, Nowak, & Czyzowska, 2013). Generally, biosorption is based on a set of physical and chemical mechanisms, resulting in the immobilization of solute on the microbial cell wall, which relies on metabolism (Ringot et al., 2007). Therefore, further studies on

yeast strains with potential dual purpose as a functional starter culture may be considered.

The use of microbiological-binding agents to remove OTA has been a hot spot in mycotoxin research. Recently, antagonistic yeasts and some lactic acid producing bacteria have shown great prospect for detoxifying OTA without any effect on the organoleptic and functional characteristics of products, as compared to chemical and some physical methods; which have effects on the nutritional and functional properties (Petruzzi et al., 2014). It was found that the potential of wine yeasts to adsorb OTA is genetically controlled and it is a polygenic inheritable trait (Caridi et al., 2012). These inherent characteristics when exploited further can serve as breeding strategies to develop more effective mycotoxin-adsorbing strains.

Furthermore, a study on mechanisms of OTA degradation indicated that different macromolecules (*β*-D-glucans and mannoproteins) that exist in the cell walls of *S. cerevisiae* are biomass that act as adsorbent materials (Ringot et al., 2007). *S. cerevisiae* is made up of an inner cell wall consist of chitin and *β*-1, 3-glucan (forming 50-60% of the cell wall dry weight) and outer layer (consist of *β*-1, 6-glucan heavily glycosylated with mannoproteins). The mannoproteins are partly polar constituents that are discharged during alcohol fermentation process, particularly at the end. With regard to wine pH, mannoproteins establish polar and non-polar interactions with OTA, when they are negatively charged (Ringot et al., 2007). It was found that though OTA cannot be biologically altered by yeasts during fermentation, but it is adsorbed onto the biomass mainly composed of mannoproteins and glucans (Hocking et al., 2007)._ENREF_50

The studies by Piotrowska, Nowak, & Czyzowska (2013) indicated adsorption of OTA onto some *Lactobacillus* and *Lactococcus* strains. In their experiment, OTA content was reduced by all strains, but strains of *Lactobacillus* caused the highest decrease of >50% of the initial concentration. It was also found that both viable and nonviable cells of *Lb. rhamnosus* were able to reduce OTA content (Turbic et al., 2002). A similar experiment (Shi et al., 2014) found that viable cells bound 66.6% of initial OTA (6 µg/mL) after 24 h *in vitro* incubation which is lower than 87.9% from those of autoclaved cells. However, OTA adsorption by *B. subtilis* CW 14 was similar to that of *Saccharomyces* and *Lactobacillus* reported by Shi et al. (2014). All these degradation mechanisms are identified to be based on physical adsorption to the cell wall of the microorganism.

It was reported that *Aspergillus* spp. isolated from grapes and belonging to *Aspergillus* section *Nigri* have shown OTA degradability (Abrunhosa et al., 2014)**.** The removal of OTA by conidia of *A. niger*, *A. carbonarius*, and A. *japonicus* was demonstrated in grape juice (Bejaoui et al., 2006). Notably, although *A. carbonarius* known as OTA-producer, it was able to provide effective detoxification of OTA in grape juice. Compared to *A. carbonarius, A. niger* under favorable conditions was found to be more suitable OTA detoxification and less toxicogenic (Hocking et al., 2007). Actually, various *A. niger* enzymes are considered GRAS (generally recognized as safe) by the United States Food and Drug Administration. Generally, *A. niger* is regarded as a safe microorganism and is one of the most important microorganisms used in biotechnology, which is used for enzymes and citric acid production (Cicoňová et al., 2010). Further studies on *A. niger* is required to establish the degradation pathway or the mechanisms involved.

Factors affecting OTA degradation

Physicochemical factors affect the survival of BCAs and their interaction with pathogen and host (Lahlali et al., 2011). Moreso, factors such as temperature, water activity, ripening, ethanol concentration and pH, either individually or together influence activities of mycotoxins production and their degradation in foods. According to Abarca et al. (2001), these conditions favour ochratoxigenic *Aspergillus* spp as the main OTA-producing fungi than *Penicillium* spp. Various results on the effects of temperature on OTA-

producing fungi have been reported and the highest OTA production was temperature between 15-20°C for *A. carbonarius* and 20-25°C for *A. niger* (Esteban et al., 2004). Furthermore, it was noticed that *A. niger* reached its highest growth stage and it was able to tolerate temperatures greater than 37°C, while *A. carbonarius* can grow faster at temperature of 30°C (Battilani et al., 2003).

In addition, the growth rate increased with increasing a_w (with highest growth rate at 0.95 and 0.99 a_w) (Bellí et al., 2005)**.** In a similar study, ochratoxigenic *A. carbonarius* strain A1102 was totally controlled or inhibited by two strains of the yeast-like fungus (*A. pullulans* AU34-2 and LS30 and *Metschnikowia pulcherrima* LS16) at 20°C and 60% relative humidity (De Curtis et al., 2012). According to Petruzzi et al. (2014), who investigated the effects of *S. cerevisiae* strains treatments on OTA degradation; it degraded more at 30°C, pH 3.0 and 5% ethanol. It was stated that the kind of substrates, the type of strains, flocculence, toxin concentration, cell dimension and cell sedimentation kinetics are additional factors that influence OTA detoxification by microbial antagonists (Petruzzi et al., 2014). It was also reported that adsorption of OTA is affected by pH due to the different adsorption mechanisms used by different microorganisms, and cell wall composition (Huwig et al., 2001).

Conclusion

Since the consumption of fresh fruits, particularly grapes and its products is increasing, the prevention of decay caused by OTA-producing fungi and detoxification of OTA itself is necessary to ensure a free product. From the fore going, it appears some significant progress has been made toward the use of biological agents to control/detoxify mycotoxins in fruits. To this extent, antagonistic yeasts represents very promising potential biological material, considering their important role in the biocontrol systems of disease process and their maximum capacity to remove OTA. OTA degradation by BCAs is achieved through conversion into non-toxic compounds or binding of the toxic compounds in the cells walls of the microbial agents. The removal of OTA involves adsorption processes mediated by constituents of efficacious yeast strains. Besides, knowledge of the mechanisms of action of combinations of BCAs is important to establish effective treatment procedures. Further molecular studies to affirm the interactions between enhanced antagonistic yeasts, OTA-producing fungi and OTA degradation/ detoxification will be important due to the pursuance of providing safe food and consumer confidence.

References

Abarca, M., Accensi, F., Bragulat, M., Cabanes, F. (2001). Ochratoxin A-producing black aspergilli in dried vine fruits. ***Book of Abstracts Bioactive Fungal Metabolites Impact and Exploitation*** 38.

Abou-Zeid, A., Altalhi, A., El-Fattah, A. (2008). Fungal control of pathogenic fungi isolated from some wild plants in Taif Governorate, Saudi Arabia. ***Malaysian Journal of Microbiology*** 4: 30-39.

Abrunhosa, L., Inês, A., Rodrigues, A.I., Guimarães, A., Pereira, V.L., Parpot, P., Mendes-Faia, A., Venâncio, A. (2014). Biodegradation of ochratoxin A by *Pediococcus parvulus* isolated from Douro wines. ***International Journal of Food Microbiology*** 188: 45-52.

Arrebola, E., Sivakumar, D., Bacigalupo, R., Korsten, L. (2010). Combined application of antagonist *Bacillus amyloliquefaciens* and essential oils for the control of peach postharvest diseases. ***Crop Protection*** 29: 369-377.

Barkai-Golan, R., Paster, N. (2008). Mouldy fruits and vegetables as a source of mycotoxins: part 1. ***World Mycotoxin Journal*** 1: 147-159.

Battilani, P., Giorni, P., Pietri, A. (2003). Epidemiology of toxin-producing fungi and ochratoxin A occurrence in grape, In

Epidemiology of Mycotoxin Producing Fungi. Springer, pp. 715-722.

Battilani, P., Magan, N., Logrieco, A. (2006). European research on ochratoxin A in grapes and wine. ***International Journal of Food Microbiology*** 111: S2-S4.

Bayman, P., Baker, J.L. (2006). Ochratoxins: a global perspective. ***Mycopathologia*** 162: 215-223.

Bejaoui, H., Mathieu, F., Taillandier, P., Lebrihi, A. (2004). Ochratoxin A removal in synthetic and natural grape juices by selected oenological Saccharomyces strains. ***Journal of Applied Microbiology*** 97: 1038-1044.

Bejaoui, H., Mathieu, F., Taillandier, P., Lebrihi, A. (2006). Black aspergilli and ochratoxin A production in French vineyards. ***International Journal of Food Microbiology*** 111: S46-S52.

Belajová, E., Rauová, D. (2007). Determination of ochratoxin A and its occurrence in wines of Slovakian retail. ***Journal of Food and Nutrition Research*** (Slovak Republic).

Bellí, N., Marín, S., Sanchis, V., Ramos, A.J. (2006). Impact of fungicides on Aspergillus carbonarius growth and ochratoxin A production on synthetic grape-like medium and on grapes. ***Food Additives and Contaminants*** 23: 1021-1029.

Bellí, N., Ramos, A., Coronas, I., Sanchis, V., Marín, S. (2005). *Aspergillus carbonarius* growth and ochratoxin A production on a synthetic grape medium in relation to environmental factors. ***Journal of Applied Microbiology*** 98: 839-844.

Bizaj, E., Mavri, J., Cus, F., Raspor, A. (2009). Removal of ochratoxin A in *Saccharomyces cerevisiae* liquid cultures. ***S Afr J Enol Vitic*** 30: 151-155.

Bleve, G., Grieco, F., Cozzi, G., Logrieco, A., Visconti, A. (2006). Isolation of epiphytic yeasts with potential for biocontrol of *Aspergillus carbonarius* and *A. niger* on grape. ***International Journal of Food Microbiology*** 108: 204-209.

Boudra, H., Le Bars, P., Le Bars, J. (1995). Thermostability of Ochratoxin A in wheat under two moisture conditions. ***Applied and Environmental Microbiology*** 61: 1156-1158.

Brenes, A., Roura, E. (2010). Essential oils in poultry nutrition: Main effects and modes of action. ***Animal Feed Science and Technology*** 158: 1-14.

Cairns-Fuller, V., Aldred, D., Magan, N. (2005). Water, temperature and gas composition interactions affect growth and ochratoxin A production by isolates of *Penicillium verrucosum* on wheat grain. ***Journal of Applied Microbiology*** 99: 1215-1221.

Caridi, A., Galvano, F., Tafuri, A., Ritieni, A. (2006). In-vitro screening of *Saccharomyces* strains for ochratoxin A removal from liquid medium. ***Annals of Microbiology*** 56: 135-137.

Caridi, A., Sidari, R., Pulvirenti, A., Meca, G., Ritieni, A. (2012). Ochratoxin A adsorption phenotype: An inheritable yeast trait. ***The Journal of General and Applied Microbiology*** 58: 225-233.

Chen, X., Li, J., Zhang, L., Xu, X., Wang, A., Yang, Y. (2012). Control of postharvest radish decay using a *Cryptococcus albidus* yeast coating formulation. ***Crop Protection*** 41: 88-95.

Chet, I., Inbar, J., Hadar, I. (1997). Fungal antagonists and mycoparasites. T***he Mycota IV: Environmental and microbial relationships***. Berlin: Springer-Verlag, 165-184.

Cicoňová, P., Laciaková, A., Máté, D. (2010). Prevention of ochratoxin A contamination of food and ochratoxin A detoxification by microorganisms-a review. ***Czech Journal of Food Sciences*** 28: 465-474.

De Curtis, F., de Felice, D.V., Ianiri, G., De Cicco, V., Castoria, R. (2012). Environmental factors affect the activity of biocontrol agents against ochratoxigenic *Aspergillus*

carbonarius on wine grape. ***International Journal of Food Microbiology*** 159: 17-24.

Del Prete, V., Rodriguez, H., Carrascosa, A.V., de las Rivas, B., Garcia-Moruno, E., Munoz, R. (2007). *In vitro* removal of ochratoxin A by wine lactic acid bacteria. J***ournal of Food Protection***® 70: 2155-2160.

Dimakopoulou, M., Tjamos, S.E., Antoniou, P.P., Pietri, A., Battilani, P., Avramidis, N., Markakis, E.A., Tjamos, E.C., (2008). Phyllosphere grapevine yeast *Aureobasidium pullulans* reduces *Aspergillus carbonarius* (sour rot) incidence in wine-producing vineyards in Greece. ***Biological Control*** 46: 158-165.

Dong, J., Wan, G., Liang, Z. (2010). Accumulation of salicylic acid-induced phenolic compounds and raised activities of secondary metabolic and antioxidative enzymes in *Salvia miltiorrhiza* cell culture. ***Journal of Biotechnology*** 148: 99-104.

Droby, S., Wisniewski, M., Teixidó, N., Spadaro, D., Jijakli, M.H. (2016). The science, development, and commercialization of postharvest biocontrol products. Postharvest Biology and Technology.

Eisendle, M., Oberegger, H., Buttinger, R., Illmer, P., Haas, H. (2004). Biosynthesis and uptake of siderophores is controlled by the PacC-mediated ambient-pH regulatory system in Aspergillus nidulans. ***Eukaryotic Cell*** 3: 561-563.

Esteban, A., Abarca, M.L., Bragulat, M.R., Cabañes, F.J. (2004). Effects of temperature and incubation time on production of ochratoxin A by black aspergilli. R***esearch in Microbiology*** 155: 861-866.

Gachomo, E.W., Kotchoni, S.O. (2008). The use of *Trichoderma harzianum* and *T. viride* as potential biocontrol agents against peanut microflora and their effectiveness in reducing aflatoxin contamination of infected kernels. ***Biotechnology*** 7: 439-447.

Grazioli, B., Fumi, M.D., Silva, A. (2006). The role of processing on ochratoxin A content in Italian must and wine: A study on naturally contaminated grapes. International ***Journal of Food Microbiology*** 111: S93-S96.

Grosso, F., Saıd, S., Mabrouk, I., Fremy, J., Castegnaro, M., Jemmali, M., Dragacci, S. (2003). New data on the occurrence of ochratoxin A in human sera from patients affected or not by renal diseases in Tunisia. ***Food and Chemical Toxicology*** 41: 1133-1140.

Harman, G.E. (2006). Overview of Mechanisms and Uses of *Trichoderma* spp. ***Phytopathology*** 96:190-194.

Hathout, A.S., Aly, S.E. (2014). Biological detoxification of mycotoxins: a review. ***Annals of Microbiology*** 64: 905-919.

Hocking, A.D., Su-lin, L.L., Kazi, B.A., Emmett, R.W., Scott, E.S., (2007). Fungi and mycotoxins in vineyards and grape products. ***International Journal of Food Microbiology*** 119: 84-88.

Huwig, A., Freimund, S., Käppeli, O., Dutler, H. (2001). Mycotoxin detoxication of animal feed by different adsorbents. ***Toxicology Letters*** 122: 179-188.

Hyldgaard, M., Mygind, T., Meyer, R.L. (2012). Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. ***Frontiers in Microbiology*** 3.

Ippolito, A., Schena, L., Pentimone, I., Nigro, F. (2005). Control of postharvest rots of sweet cherries by pre- and postharvest applications of *Aureobasidium pullulans* in combination with calcium chloride or sodium bicarbonate. ***Postharvest Biology and Technology*** 36: 245-252.

Irtwange, S. (2006). Application of biological control agents in pre-and postharvest operations.

Joint, F., Additives, W.E.C.o.F., 1983. Toxicological evaluation of certain food additives and contaminants.

Kabak, B. (2010). Prevention and Management of Mycotoxins in Food and Feed, In: Rai, M.,

Varma, A. (Eds.), ***Mycotoxins in Food, Feed and Bioweapons.*** Heidelberg, Berlin: Springer pp. 201-227.

Kabak, B., Dobson, A.D., Var, I.l. (2006). Strategies to prevent mycotoxin contamination of food and animal feed: a review. ***Critical Reviews in Food Science and Nutrition*** 46: 593-619.

Lahlali, R., Hamadi, Y., Jijakli, M.H. (2011). Efficacy assessment of *Pichia guilliermondii* strain Z1, a new biocontrol agent, against citrus blue mould in Morocco under the influence of temperature and relative humidity. ***Biological Control*** 56: 217-224.

Leong, S.-l.L., Hocking, A., Pitt, J., Kazi, B., Emmett, R., Scott, E.S. (2006). Australian research on ochratoxigenic fungi and ochratoxin A. ***International Journal of Food Microbiology*** 111: S10-S17.

Limón, M.C., Codón, A.C. (2004). Biocontrol mechanisms of Trichoderma strains. ***International Microbiology*** 7: 249-260.

Mahunu, G.K., Zhang, H., Yang, Q., Li, C., Zheng, X. (2015). Biological Control of Patulin by Antagonistic Yeast: A case study and possible model. ***Critical Reviews In Microbiology***, 1-13.

Mantle, P.G., Faucet-Marquis, V., Manderville, R.A., Squillaci, B., Pfohl-Leszkowicz, A. (2009). Structures of covalent adducts between DNA and ochratoxin A: A new factor in debate about genotoxicity and human risk assessment. ***Chemical Research in Toxicology*** 23 (1): 89-98.

Marin, S., Ramos, A., Cano-Sancho, G., Sanchis, V. (2013). Mycotoxins: Occurrence, toxicology, and exposure assessment. ***Food and Chemical Toxicology*** 60: 218-237.

Marquardt, R.R., Frohlich, A.A. (1992). A review of recent advances in understanding ochratoxicosis. ***Journal of Animal Science*** 70 (12): 3968-3988.

Medeiros, F.H.V.d., Martins, S.J., Zucchi, T.D., Melo, I.S.d., Batista, L.R., Machado, J. (2012). Biological control of mycotoxin-producing molds. ***Ciência e Agrotecnologia*** 36 (5): 483-497.

Nguefack, J., Dongmo, J.L., Dakole, C., Leth, V., Vismer, H., Torp, J., Guemdjom, E., Mbeffo, M., Tamgue, O., Fotio, D. (2009). Food preservative potential of essential oils and fractions from *Cymbopogon citratus*, *Ocimum gratissimum* and *Thymus vulgaris* against mycotoxigenic fungi. ***International Journal of Food Microbiology*** 131(2): 151-156.

Ormancey, X., Sisalli, S., Coutiere, P. (2001). Formulation of essential oils in functional perfumery. ***Parfums, Cosmetiques, Actualites*** 157 (1): 30-40.

Pardo, E., Marın, S., Sanchis, V., Ramos, A. (2004). Prediction of fungal growth and ochratoxin A production by *Aspergillus ochraceus* on irradiated barley grain as influenced by temperature and water activity. ***International Journal of Food Microbiology*** 95 (1): 79-88.

Péteri, Z., Téren, J., Vágvölgyi, C., Varga, J., 2007. Ochratoxin degradation and adsorption caused by astaxanthin-producing yeasts. ***Food Microbiology*** 24 (3): 205-210.

Petruzzi, L., Bevilacqua, A., Baiano, A., Beneduce, L., Corbo, M.R., Sinigaglia, M. (2014). Study of *Saccharomyces cerevisiae* W13 as a functional starter for the removal of ochratoxin A. ***Food Control*** 35 (1): 373-377.

Pfliegler, W.P., Pusztahelyi, T., Pócsi, I. (2015). Mycotoxins – prevention and decontamination by yeasts. ***Journal of Basic Microbiology*** 55 (7): 805-818.

Piotrowska, M., Nowak, A., Czyzowska, A. (2013). Removal of ochratoxin A by wine *Saccharomyces cerevisiae* strains. ***European Food Research and Technology*** 236 (3): 441-447.

Pizzolitto, R.P., Armando, M.R., Combina, M., Cavaglieri, L.R., Dalcero, A.M., Salvano, M.A., 2012. Evaluation of *Saccharomyces cerevisiae* strains as probiotic agent with

aflatoxin B1 adsorption ability for use in poultry feedstuffs. ***Journal of Environmental Science and Health, Part B*** 47 (10): 933-941.

Ponsone, M.L., Chiotta, M.L., Combina, M., Dalcero, A., Chulze, S. (2011). Biocontrol as a strategy to reduce the impact of ochratoxin A and Aspergillus section Nigri in grapes. ***International Journal of Food Microbiology*** 151 (1): 70-77.

Prins, C.L., Vieira, I.J., Freitas, S.P. (2010). Growth regulators and essential oil production. ***Brazilian Journal of Plant Physiology*** 22 (2): 91-102.

Qin, X., Xiao, H., Xue, C., Yu, Z., Yang, R., Cai, Z., Si, L. (2015). Biocontrol of gray mold in grapes with the yeast *Hanseniaspora uvarum* alone and in combination with salicylic acid or sodium bicarbonate. P***ostharvest Biology and Technology*** 100: 160-167.

Reddy, K., Reddy, C., Muralidharan, K. (2007). Exploration of ochratoxin A contamination and its management in rice. ***American. Journal of Plant Physiology*** 2 (3): 206-213.

Ribeiro, J., Cavaglieri, L., Fraga, M., Direito, G., Dalcero, A., Rosa, C., 2006. Influence of water activity, temperature and time on mycotoxins production on barley rootlets. ***Letters in Applied Microbiology*** 42 (2): 179-184.

Ringot, D., Lerzy, B., Chaplain, K., Bonhoure, J.-P., Auclair, E., Larondelle, Y. (2007). In vitro biosorption of ochratoxin A on the yeast industry by-products: Comparison of isotherm models. ***Bioresource Technology*** 98 (9): 1812-1821.

Rousseau, J. (2004). Ochratoxin A in wines: current knowledge first part: factors favouring its emergence in vineyards and wines. ***Vinidea. Net Wine Internet Technical Journal*** 5.

Sawamura, M. (2000). Volatile components of essential oils of the citrus genus. ***Recent Research Developments in Agricultural & Food Chemistry*** 4 (1): 131-164.

Sharma, N., Sharma, S., Prabha, B. (2012). Postharvest Biocontrol – New Concepts and Application, In: Venkateswarlu, B., Shanker, K.A., Shanker, C., Maheswari, M. (Eds.), ***Crop Stress and its Management: Perspectives and Strategies***. Dordrecht, Netherlands:Springer pp. 497-515.

Shi, L., Liang, Z., Li, J., Hao, J., Xu, Y., Huang, K., Tian, J., He, X., Xu, W. (2014). Ochratoxin A biocontrol and biodegradation by *Bacillus subtilis* CW 14. ***Journal of the Science of Food and Agriculture*** 94 (9): 1879-1885.

Shundo, L., Almeida, A.P.d., Alaburda, J., Ruvieri, V., Navas, S.A., Lamardo, L.C., Sabino, M. (2006). Ochratoxin A in wines and grape juices commercialized in the city of São Paulo, Brazil. ***Brazilian Journal of Microbiology*** 37 (4): 533-537.

Silva, F., Ferreira, S., Duarte, A., Mendonça, D.I., Domingues, F.C. (2011). Antifungal activity of *Coriandrum sativum* essential oil, its mode of action against *Candida* species and potential synergism with amphotericin B. ***Phytomedicine*** 19 (1): 42-47.

Singh, G., Maurya, S., Catalan, C.A., 2007. A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. ***Food and Chemical Toxicology*** 45 (9): 1650-1661.

Stratakou, I., Fels-Klerx, H.v.d. (2010). Mycotoxins in grapes and wine in Europe: occurrence, factors affecting the occurrence and related toxicological effects. ***World Mycotoxin Journal*** 3: 283-300.

Štyriak, I., Conková, E., Kmec, V., Böhm, J., Razzazi, E. (2001). The use of yeast for microbial degradation of some selected mycotoxins. ***Mycotoxin Research*** 17 (1): 24-27.

Sweeney, M.J., Dobson, A.D. (1998). Mycotoxin production by *Aspergillus*, *Fusarium* and *Penicillium* species. ***International Journal of Food Microbiology*** 43 (3): 141-158.

Taiz, L., Zeiger, E. (2004). Metabólitos secundários e defesa vegetal. Fisiologia Vegetal. 3ª. Ed. Porto Alegre, RS. Editora Artimed, 309-332.

Turbic, A., Ahokas, J., Haskard, C. (2002). Selective *in vitro* binding of dietary mutagens, individually or in combination, by lactic acid bacteria. ***Food Additives & Contaminants*** 19:144-152.

Valero, A., Marín, S., Ramos, A.J., Sanchis, V. (2008). Survey: Ochratoxin A in European special wines. ***Food Chemistry*** 108 (2): 593-599.

Valero, A., Sanchis, V., Ramos, A.J., Marín, S. (2007). Studies on the interaction between grape-associated filamentous fungi on a synthetic medium. ***International Journal of Food Microbiology*** 113 (3): 271-276.

Varga, J., Juhász, Á., Kevei, F., Kozakiewicz, Z. (2004). Molecular diversity of agriculturally important *Aspergillus* species, Molecular Diversity and PCR-detection of Toxigenic *Fusarium* Species and Ochratoxigenic Fungi. Berlin: Springer, pp. 627-640.

Varga, J., Kozakiewicz, Z. (2006). Ochratoxin A in grapes and grape-derived products. T***rends in Food Science & Technology*** 17 (2): 72-81.

Visconti, A., Perrone, G., Cozzi, G., Solfrizzo, M. (2008). Managing ochratoxin A risk in the grape-wine food chain. ***Food Additives and Contaminants*** 25 (2): 193-202.

Yiannikouris, A., André, G., Poughon, L., François, J., Dussap, C.-G., Jeminet, G., Bertin, G., Jouany, J.-P. (2006). Chemical and conformational study of the interactions involved in mycotoxin complexation with β-D-glucans. ***Biomacromolecules*** 7 (4): 1147-1155.

Zhang, H., Apaliya, M.T., Mahunu, G.K., Chen, L., Li, W. (2016). Control of ochratoxin A-producing fungi in grape berry by microbial antagonists: A review. ***Trends in Food Science & Technology*** 51: 88-97.

Zhao, L., Zhang, H., Lin, H., Zhang, X., Ren, X. (2013). Effect of trehalose on the biocontrol efficacy of *Pichia caribbica* against post-harvest grey mould and blue mould decay of apples. ***Pest Management Science*** 69 (8): 983-989.