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Ochratoxin in Cocoa, Health Risks and Methods of Detoxification

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ABSTRACT

Ochratoxin a is a potent mycotoxin with nephrotoxic, teratogenic, immunosuppressive and carcinogenic properties. Ochratoxin production by various species and strains of Aspergillus and Penicillium has been associated with cocoa. Cocoa is a cash crop produced in many countries of the world with about 71% of the world production from West African countries. Cocoa and its fermented derivatives are utilized in various food and pharmaceutical companies for a range of products. Ochratoxin A producers are widely distributed in various geographical locations with species of Aspergillus dominating the tropical region and species of Penicillium, the temperate climates of the world. The OTA production by these fungi is favored by high water activity level, poor plant health and physical damage to plant. Seasonal variations, cultivation practice, phytosanitary conditions, harvesting, fermentation and transportation conditions influence growth and ochratoxin a production by OTA fungi. Methods applied for ochratoxin A control include inhibition of growth of OTA fungi and subsequent ochratoxin A production, OTA detoxification using physical and chemical methods, antibiotics and essential oils, or the application of biocontrol agents. Involved in biotransformation of OTA are carboxypeptidases, chymotrypsins, proteases and lipases. The OTA is transformed to ochratoxin α, L-β-phenylalanine or other less harmful substances. The current paper highlights the incidence of ochratoxin A (OTA) in cocoa with available methods of decontamination.

Key words: Ochratoxin A, fungi, mycotoxin, cocoa, toxicity, decontamination

INTRODUCTION

Ochratoxin A ($C_2OH_{18}ClNO_6$) is a mycotoxin produced by species of only two genera *Aspergillus* and *Penicillium*. It was first isolated from *A. ochraceus* in South Africa (Van Der Merwe *et al*., 1965). The OTA is the most important and most commonly occurring of a group of structurally related compounds. Ochratoxin A consists of a polyketide-derived dihydroiso-coumarin moiety linked through the 12-carboxy group to phenylalanine (Fig. 1, 2). There are several analogues of OTA such as ochratoxin B and ochratoxin C (Table 1). Ochratoxin B and C are not chlorinated and are less toxic (Luster *et al*., 1987; Weidenborner, 2001). The OTA is the major compound found as a natural contaminant of plant material. The role of OTA in the producing fungus is not yet known. Cocoa is a perennial tree crop of the humid tropics grown frequently under forest sheds and a cocoa field has an economic life of about 25-30 years. In West Africa including Nigeria, it is mostly grown under extensive management system by smallholders. Cocoa contamination by ochratoxigenic fungi is mostly encountered during fermentation of its powder to various products at various stages.

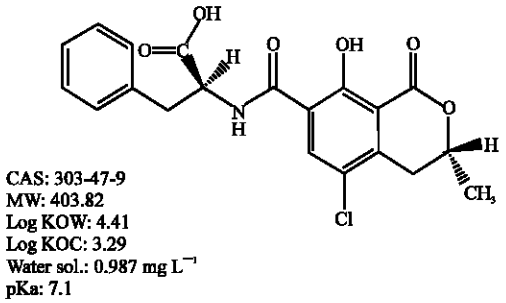

Fig. 1: Chemical structure, partitioning constant and water solubility of Ochratoxin A, (Dall'Asta *et al*., 2008; Hussein Brasel, 2001; Mortensen *et al*., 2006)

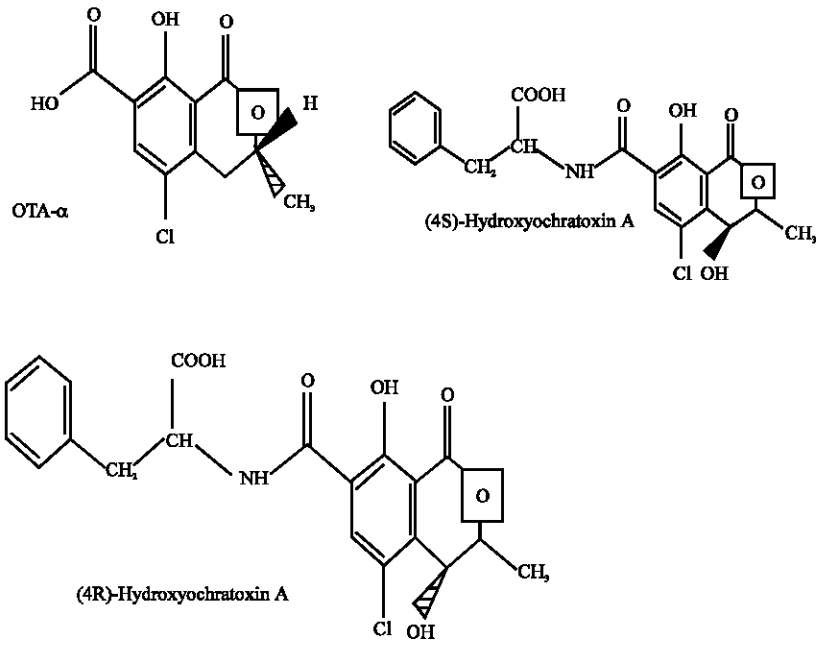

Fig. 2: Structures of major Ochratoxin A metabolites, (Khoury and Atoui, 2010; Brien and Dietrich, 2005)

OTA producers are ubiquitous and are found as spoilage agents of a wide range of food including cereals, legumes, fruits, coffee, cocoa, spices and their derivatives (Abarca *et al*., 2003; Jabbari *et al*., 2007). Ingestion of contaminated feed stuff by non-ruminant animals and poultry maintains a steady supply of OTA contaminated poultry, meat and their products (Centre for Food Safety, 2006; Rosa *et al*., 2009). As a result, OTA is detectable in blood serum of the majority, if not all human population in various concentrations (Schwerdt *et al*., 2007, 2009). OTA is very stable with a serum half life of 35 days (Petzinger and Zielgler, 2000) leading to average blood concentrations between 0.5 and 1 nmol L^{-1} (Assaf *et al*., 2004; Skaug, 2003). Susceptibility of cells to OTA is often specie, sex and dose dependent (O`Brien *et al*., 2001).

Contamination of agricultural products consistently varies depending on microbial species and varieties, agricultural practices, climatic conditions, storage and processing techniques amongst other things (Counil *et al*., 2006). There are existing regulations in some regions on accepted level

Table 1: Characteristic composition of ochratoxin A derived metabolites (Khoury and Atoui, 2010)

Name	R1	R2	R3	R4	R5
Narural ochratoxins					
Ochratxin A	Phenylalanine	Cl	H	H	H
Ochratxin B	Phenylalanine	H	H	H	H
Ochratxin C	Ethyl-ester, phenylalnine	Cl	H	H	H
Ochratxin A Methyl-ester	Methyl-ester, phemylanine	Cl	H	H	H
Ochratxin B Methyl-ester	Methyl-ester, phemylanine	H	H	H	H
Ochratxin B Eethyl-ester	Ethyl-ester, phenylalanine	H	H	H	H
Ochratxin α	OH	Cl	H	H	H
Ochratxin β	OH	H	H	H	H
4-R-Hydroxyochratixn A	Phenylalanine	Cl	H	OH	H
4-s-Hydroxyochratixn A	Phenylalanine	Cl	OH	H	H
10-Hydroxyochratixn A	Phenylalanine	Cl	H	H	OH
Tyrosine analog of OTA	Tyrosine	Cl	H	H	H
Serine analog og OTA	Serine	Cl	H	H	H
Hydroxyproline analog of OTA	Hydroxypoline	Cl	H	H	H
Lysine analog of OTA	Lysine	Cl	H	H	H
Synthetic ochratoxins					
d-Ochratoxin A	d-phenylalanine	Cl	H	H	H
Ochratoxin A Ethyl amid	Ethyl amid, phenylalanine	Cl	H	H	H
O-methyl Ochratoxin A	Phenylalanine $OHCH_3$ on C-8	Cl	H	H	H

of OTA in certain foods, in daily and weekly intake of this toxin (Goryacheva *et al*., 2006), while some are yet to take such steps. OTA have elicited great concern and have been previously and extensively reviewed (Pohland *et al*., 1992; Khoury and Atoui, 2010).

OTA: Health risks: OTA in the blood is distributed to the kidney, liver, muscle and adipose tissue (Gareis and Scheuer, 2000). The main target is the renal proximal tubule where it exerts cytotoxic and carcinogenic effects. They specifically target kidney (nephropathy) and the developing nervous system where they produce both acute and chronic lesions (Giray *et al*., 2009). The nephrotoxic, immunogenic, carcinogenic and teratogenic effects of OTA in lab animals and human cell lines is well documented (Bragulat *et al*., 2008a, b). The estimated tolerable dosage of OT Ain humans is at 0.2 to 4.2 ng kg^{-1} b.wt. based on NTP caricinogenicity study in rats. The OTA has been implicated as the causative agent of Balkan endemic nephropathy (Atoui *et al*., 2006), urothelial tumors in the Balkan State and Egypt (Wafa *et al*., 1998), chronic karyomegalic interstitial snephropathy and chronic interstitial nephropathy in Tunisia (Maaroufi *et al*., 1999). OTA was reported to induce iron deficiency anaemia. Based on some available information, the International Agency for Research on Cancer (IARC) classified ochratoxin A as possible human carcinogen (Group 2B) (IARC, 1993).

OTA inhibits mitochondrial respiration causing a depletion of ATP and induces oxidative damage by enhancing lipid peroxidation (Kamp *et al*., 2005; Gautier *et al*., 2001; Rahimtula *et al*., 1988). A resultant impairment of the endoplasmatic reticulum membrane further leads to impaired calcium homeostasis (Gekle *et al*., 2005). Inhibition of protein synthesis through competition with the enzyme responsible for the production of phenylalanine-tRNA is part of the cellular effects of OTA (Petroulakis and Wang, 2002; Marin-Kuan *et al*., 2008). The OTA dependent induction of apoptosis *in vivo* and *in vitro* in kidney of rats and mice has been reported (Gekle *et al*., 2005; Brien and Dietrich, 2005; Petrik *et al*., 2003; Sauvant *et al*., 2005; Seegers *et al*., 1994).

Pigs are most sensitive to the nephrotoxicity by OTA (Bragulat *et al.*, 2008b). Fowls, dogs, rabbits, horses and cattle are also susceptible (Centre for Food Safety, 2006). These farm animals are exposed to ochratoxin A, given that their feed constitute of grains, legumes and other supplements equally contaminated by ochratoxin producing fungi or their toxin.

A study using human cell lines in primary culture, showed that prolonged intake of minute quantities of OTA affected kidney cells and fibroblasts. Sava *et al.* (2006) reported that chronic exposure in mice may destroy certain brain functions and possibly lead to Parkinsonism. OTA leads to a modification of host immune function by alteration of the size of vital immune organs and number of immune cells within tissue (Al-Anati and Petzinger, 2006). Low concentrations of OTA in the body compromises the host immune system and adversely affects host response to infectious agents (Alvarez *et al.*, 2004; Ferrante *et al.*, 2008).

The threat which OTA poses to animal and human health may be heightened by the possible synergistic effect which fungal toxins are thought to exert in affected cells; or incases where other nephrotoxic and/or carcinogenic substances are present in the cell (Speijers and Speijers, 2004; Stoev *et al.*, 2002; Weber *et al.*, 2005).

Fungal ochratoxin producers: A number of Aspergillus species have been implicated in ochratoxin production. Members of Aspergillus subgenus *Circumdati* reported to produce OTA include *A. ochraceus*, *A. cretensis*, *A. flocculosus*, *A. pseudoelegans*, *A. roseoglobulosus*, *A. sclerotiorum*, *A. steynii*, *A. sulphureus*, *A. westerdijkiae*. Other members of section *Circumdati sensu stricto* are *A. auricimus*, *A. elegans*, *A. insulicola*, *A. melleus*, *A. ostianus*, *A. petrakii*, *A. sulphureus* and *Neoppetromyces muricatus*. The OTA producers among *Aspergillus* section *Nigri* include *A. carbonarius*, *A. lacticoffeatus*, *A. niger*, *A. slerotioniger*, *A. tubingensis*, *A. aculeatus*, *A. nigri* aggregates. Members amongst *Aspergillus* section *Flavi* include *Petromyces albertensis* and *Petromyces alliaceu* (Frisvad *et al.*, 2004; Perrone *et al.*, 2007).

Penicillium verrucosum and *P. nordicum* are the Penicillium strains widely reported as ochratoxin A producers. These organisms are slow growing species belonging to the series Verrucosa, subgenus Penicillium. Both are quite similar but can be differentiated based on differences in secondary metabolite profiles, colony reverse color on yeast extract sucrose agar (YES) and habitat preference (Bragulat *et al.*, 2008a; Larsen *et al.*, 2001). *Penicillium verrucosum* is mostly found on plant substrates contaminating plants in the field prior to harvest in temperate and cold climates (Lund and Frisvad, 2003). *P. nordicum* produce less ochratoxin compared to *P. verrucosum* and are mostly isolated from meat and meat products or from cheese (Larsen *et al.*, 2001). The Penicillium species grow at temperatures below 30°C and can synthesize OTA at a temperature as low as 5°C (Centre for Food Safety, 2006). *Penicillium cyclopium*, *P. viridicatum*, *P. chrysogenum*, *P. brevicompactum*, *P. crustosum*, *P. olsonii*, *P. oxalicum* and a few other species of the genus Penicillium have also been reported to produce OTA (Rosa *et al.*, 2006; Vega *et al.*, 2006).

Predisposing conditions for OTA production in food: The presence of OTA synthesizing fungi in food does not necessarily translate to OTA production. OTA production by ochratoxigenic fungi is only possible at certain environmental conditions such temperature, water activity level, pH, oxygen availability and presence of certain metal ions (Astoreca *et al.*, 2007; Amezqueta *et al.*, 2009; Kokkonen *et al.*, 2005). These parameters vary between different Penicillium or Aspergillus strains (Aziz and Moussa, 1997; O`Callaghan *et al.*, 2006). Available data indicate that OTA

production is stimulated by a high water activity level (Belli *et al.*, 2007a; Pardo *et al.*, 2004; Kapetanakou *et al.*, 2009; Tassou *et al.*, 2007; Valero *et al.*, 2006). Production of OTA by *A. carbonarius* is highly water activity dependent unlike A. ochraceus sp., which produce OTA even at low water activity levels (Kapetanakou *et al.*, 2009). The OTA limit for all ochratoxin producers may be around $0.81\alpha_w$ to $0.83\alpha_w$ (Lindblad *et al.*, 2004; Cairns-Fuller *et al.*, 2005). The impact of pH on OTA secretion by OTA producing strain is not known (Kapetanakou *et al.*, 2009). The composition of substrate determines rate of OTA production (Pardo *et al.*, 2006), sucrose and glucose promoted OTA synthesis (Muhlencoert *et al.*, 2004). OTA production in Aspergillus generally occurs in the range 20 to 30°C, at temperatures a little below optimal temperature for their growth (Belli *et al.*, 2004; O'Callaghan *et al.*, 2003; Pardo *et al.*, 2004, 2006; Tassou *et al.*, 2007; Valero *et al.*, 2006). The Penicillium species grow at temperatures below 30°C and can synthesize OTA at a temperature as low as 5°C (Centre for Food Safety, 2006). Other factors which predispose a crop to OTA include physical damage or poor plant health (Amezqueta *et al.*, 2009). However, it is still not clear why OTA production in a given food is strain specific or the reason for its ochratoxin production (Pardo *et al.*, 2004).

Available results indicate that OTA adsorbs to soil organic matter and is rapidly degraded especially in planted soil due to its abundant microflora (Mortensen *et al.*, 2006) reducing the risk of OTA being washed into the underground water environment.

OTA in cocoa: Cocoa is a cash crop produced in many countries of the world with around 71% of the world production from West Africa (Codex Alimenatarius Commission, 2008). Cocoa is mostly fermented to produce cocoa powder used for numerous products in the food and pharmaceutical industries. Though over two third of world cocoa production is in Africa, European countries are the major consumers with a figure of 41.1% reported for the year 2006 (Codex Alimentarius Commission, 2008). Ochratoxin is the major mycotoxin occurring in cocoa (Mounjouenpou *et al.*, 2007). OTA and OTA producing fungi are thought to be present in all stages of cocoa production though a greater percentage is associated with cocoa bean shell (Amezqueta *et al.*, 2005).

Dongo *et al.* (2008) reported the presence of OTA in 54 out of 59 samples tested from Nigerian ready to be sold cocoa with concentrations ranging between 1.0 and 277.5 μg kg^{-1}. Evaluation of cocoa samples from ports of Cote d`Ivoire, showed that 23 out of 147 samples from Abidjan and 10 out of 151 samples at San Pedro had over 2.0 μg kg^{-1} of OTA (Codex Alimentarius, 2008). Similarly, sampling of 21 cocoa and cocoa products in Brazil yielded 123 Aspergillus toxigenic isolates from 42.9% of the samples. All the *Aspergillus carbonarius* and *A. ochraceus* isolates were ochratoxigenic while only 18.2% of *A. niger* isolates were OTA positive. Based on the reports of the investigation carried out by the Federal Agency for Food Safety, Belgium in 2006, 5 out of 13 cocoa beans samples had OTA levels above 0.4 μg kg^{-1} (Codex Alimentarius Commission, 2008). Investigation of OTA presence in cocoa, cocoa bean, cake, nib, powder, shell, butter, chocolate and chocolate cream from different countries was positive for 40% of samples, cocoa had a high concentration of OTA, while OTA levels were low for cocoa products (Bonvehi, 2004). Studies carried out by Amezqueta *et al.* (2005) showed that by carefully removing cocoa shell, 20 samples out of the 22 tested had over 65% reduction of OTA concentration indicating that OTA is predominantly on the shell. Tabata *et al.* (2008) investigated the occurrence of OTA in 157 samples consisting of cereal, fruit, coffee and cocoa products from Japan. Highest incidence of OTA was found in cocoa powder (10/12) and in the third place was cocoa with OTA present in 5 out of 8 samples, co-occurrence of OTA and aflatoxins was found to occur in cocoa products. Result of

a sampling of retail chocolates in Japan for OTA in 2005 was positive for all the 41 samples; levels in 20 were above 0.20 μg kg^{-1}, 17 samples were below 0.20 μg kg^{-1} while 14 samples had OTA below 0.10 μg kg^{-1} (Codex Alimentarius Commission, 2008). A project sponsored by CAOBISCO/ECA/FCC in 1999 to reduce impact of OTA in cocoa showed a mean OTA level of 1 μg kg^{-1} for cocoa samples tested as opposed to 0.26 μg kg^{-1} for dark chocolate which had highest value of OTA amongst the derivatives, indicating that only low levels of OTA are present in 'ready to consume' cocoa products, (Codex Alimentarius, 2008). A study on cocoa powder from different sources marketed in Italy indicated that 22% of samples tested exceeded the European limit for cocoa, 50% of samples had OTA level above 0.22-0.77 μg kg^{-1} (Tafuri *et al.*, 2004). According to the assessment of dietary intake of Ochratoxin A by the EU population, 81.3% of the cocoa derived products were OTA positive with contamination levels from 0.01 to 3.8 μg kg^{-1} (Miraglia and Brera, 2002). In 1997 and 1998 Ministry of Agriculture, Fisheries and Food (MAFF) presented a result on cocoa powder samples in which 19/20 and 20/20 samples, respectively were OTA positive. Contamination levels had a mean value of 0.68 for 1997 and 1.67 for 1998 (MAFF, 1999). Burdaspal and Legarda (2003) reported the occurrence of OTA in 296 samples of different types of chocolate and cocoa powder purchased, in the following order; from 16 different countries. Results showed that though 99.7% of the samples were contaminated, OTA levels were low leading to the conclusion that consumption of cocoa and its associated products only contributes a minor fraction to the OTA tolerable daily intake. The SCOOP Task 3.2.7. findings indicated that cocoa contributed only 5% of total OTA intake (Miraglia and Brera, 2002) while data from Center for Food Safety, Food and Environmental Health Department (FEHD) of Hong Kong showed that chocolates contributed 6% of DTI (Centre for Food Safety, 2006).

Levels of cocoa contamination has been related to their seasonal variations, cultivation practice, phytosanitrary conditions such as damage of cocoa pods based on pest, physically wounded, rotten and mummified. Other factors include harvesting and fermentation conditions with prompt removal of cocoa shell playing a positive role (Mounjouenpou *et al.*, 2007). A work carried out by Sanchez-Hervas *et al.* (2008) showed that the predominant fungal contaminants of the cocoa beans are members of the genus Aspergillus belonging to section Nigri and Flavi, 49.2% of the black Aspergilli isolated were ochratoxigenic. Only 2 ochratoxigenic species of Aspergilli were found amongst 86 isolates from Ghana, 16 from Nigeria and 13 from Cote d`Ivoire obtained during cocoa processing (Codex Alimentarius, 2008). Mounjouenpou *et al.* (2008) worked on the fungal population of cocoa during post-harvest practices. *A. fumigatus*, *A. tamari*, *A. versicolor*, *P. sclerotium*, *P. paneum* and *P. crustosum* isolated did not produce OTA, OTA producing strains isolated were predominantly *A. carbonarius* (100%) and to a lesser extent *A. niger* (70%).

Ochratoxin decontamination

Physical and chemical methods: Drying of fresh farm products in a clean, dry environment with mechanical driers as opposed to sun (Suarez-Quiroz *et al.*, 2005; Valero *et al.*, 2007) in small batches or piles to avoid prolonged drying (Lopez-Garcia *et al.*, 2008) ensures reduction in post harvest mold growth and OTA production or recontamination of products. OTA level in wheat bread and artificially contaminated barley meal significantly decreased with application of extrusion cooking at very high temperatures (Scudamore *et al.*, 2004).

Altering the atmosphere during grain storage with CO_2 was reported for OTA detoxification. However, the relative success of this method varies between ochratoxigenic species (Magan and Aldred, 2007). According to Pateraki *et al.* (2007) low levels (100 m, 250 mg L^{-1}) of sodium

metabisulphite solution led to increased OTA production by *A. carbonarius* while about 750-1000 mg L^{-1} totally inhibited mycelial growth and OTA production irrespective of water activity level. Field application of cyprodinil and fludioxonil at veraison and 21 days before harvest dramatically reduced the growth and contamination of grapes by the black Aspergilli prior to harvest (Belli *et al*., 2007b).

Natamycin, a natural polyene which acts by compromising the fungal membrane functions leading to leakage of ions and electrolytes has been reported as an effective agent in checking the growth of ochratoxigenic fungi (Medina *et al*., 2007). Amezqueta *et al*. (2008) reported the use of sodium carbonate and sodium bicarbonate at different conditions of pressure, temperature and time for decontamination of ochratoxin A present on cocoa shells. Aqueous potassium carbonate (2%) at 1,000 lb/in2 at 90°C for 10 min gave the highest decontamination value.

It is noteworthy that use of inadequate amounts of fungicides create stress conditions which could stimulate ochratoxin production in some species (Arroyo *et al*., 2005; Magan and Aldred, 2007). Fluazinam, procymidone, carbendazim had stimulating effects on OTA production of *A. carbonarius* (Medina *et al*., 2007; Battilani and Pietri, 2002). Bleve *et al*. (2006) reported that some fungicides containing sulphur enhanced OTA production.

Studies have been carried out on use of adsorbents materials such as activated charcoal, cholestyramine, activated carbon, potassium caseinate, sodium and calcium aluminum silicates, bentonites and wood fragments with variable results. Though use of charcoal was relatively effective compared to others, poor product quality and animal poisoning makes it unfavorable (Amezqueta *et al*., 2009; Gambuti *et al*., 2005). Modified zeolites and insoluble vegetable fibers have also been reported as effective adsorbents for OTA decontamination of food (Dakovic *et al*., 2005; Tomasevic-Canovic *et al*., 2003).

Biotransformation: A wide range of microorganisms are able to transform OTA to ochratoxin alpha or other less harmful substances. These organisms produce intra and extracellular enzymes which have the ability of degrading ochratoxin. Enzymes involved in biotransformation of OTA include carboxypeptidase, chymotrypsin, protease and lipase (JECFA, 2001; Stander *et al*., 2001). Microbial agents producing these agents have been tested as biocontrol agents for the reduction of growth and suppression of OTA production by OTA fungi. Table 2 shows a list of various microorganisms reported to suppress microbial growth or OTA production by OTA fungi.

Fuchs *et al*. (2008) tested OTA detoxification using 30 different strains of lactic acid bacteria, *L. acidophilus* strain (VM12) had 90% efficiency when viable cells were used, heat treated cells were less effective. Petchkongkaew *et al*. (2008) reported the isolation of an OTA detoxifying strain of *Bacillus licheniformis* from fermented soybean. OTA detoxifying strains of *Streptococcus salivarius, Bifidobacterium bifidum, Lactobacillus delbrueckii* and yoghurt bacteria were isolated from milk samples (Skrinjar *et al*., 1996).

Bacterial flora of the mammalian gastrointestinal tract possesses OTA degrading abilities. *Butyrivibrio fibrisolvens* a rumen bacteria was antagonistic to ochratoxin production (Westlake *et al*., 1987). Madhyastha *et al*. (1992) reported the degradation of OTA by bacteria isolated from the caecum and large intestine of rats. Akiyama *et al*. (1997) reported the detoxification of OTA by microbial flora of the human intestine. Ruminant animals are thought to be relatively free from OTA as OTA detoxifying organisms within their intestines or stomachs efficiently degrades OTA.

Yeasts capable of detoxifying OTA have been reported (Cecchini *et al*., 2006; Masoud and Kaltoft, 2006; Peteri *et al*. (2007). Bejaoui *et al*. (2004) reported the detoxification of OTA by

Table 2: Antagonistic activity of some microorganism against OTA or OTA producers

Target	Control Agent	Reference
A. carbonarius	*Aureobasidium pullulans*	Dimakopoulou *et al.* (2008)
A. carbonarius	*Alternaria alternate* and*Candida hebarum*, *Penicillium decumbens* and *P. janthinellum*, *Trichoderma harzianum* and *P. decumbens*	Valero *et al.* (2006)
A. carbonarius	*Issatchenkia terricola, Issatchenkia orientalis*	Bleve *et al.* (2006)
A. niger	*Metschnikowia pulcherrima, C. incommunis* *Kluyveromyces thermotolerans*	
OTA fungi	Rumen bacteria- *Butyrivibrio fibrisolvens*	Westlake *et al.* (1987)
A. carbonarius	*Auereobasidium pullulans*	De Felice *et al.* (2008)
A. ochraceus	*Pichia anomala, Pichia kluyveri*, *Hanseniaspora uvarium*	Masoud and Kaltoft (2006)
OTA	Black Aspergilli: A. niger aggregate, A. *carbonarius, A. japonicas*	Bejaoui *et al.* (2006)
OTA	*Rhizopus stolonifer, R. microspores*, *R. homothalicus, R. oryzae*	Varga *et al.* (2005)
OTA	Asthaxanthin producing yeast *Phaffia rhodozyma, Xanthophyllomyces dendrorhous*	Peteri *et al.* (2007)
OTA	*L. acidophilus* (VM12)	Fuchs *et al.* (2008)
OTA	*Bacillus licheniformis*	Petchkongkaew *et al.* (2008)
OTA	*Streptococcus salivarius*, Bifidobacterium, *L. delbrueckii*, yoghurt bacteria	Skrinjar *et al.* (1996)
OTA	Bacteria from the caecum and large of rats	Madhyastha *et al.* (1992)
OTA	Microbial flora of human intestine	Akiyama *et al.* (1997)
OTA	*S. cerevisiae, S. bayanus*	Bejaoui *et al.* (2004)
OTA	*Pleurotus ostratus*	Engelhardt (2002)
OTA	*Phenylobacterium* sp.	Wegst and Lingens (1983)
OTA	Alternaria, Cladosporium, Trichoderma	Abrunhosa *et al.* (2002)

Saccharomyces cerevisiae and *S. bayanus* in synthetic grape medium. Dimakopoulou *et al.* (2008) isolated 21 yeasts strains out of which 17 strongly inhibited the growth of wine contaminating ochratoxigenic *Aspergillus carbonarius*. *Aureobasidium pullulans* had the most inhibitory effect. De Felice *et al.*, 2008 isolated four strains of *Aureobasidium pullulans*, AU14-3-1, AU18-3B, AU34-2 and LS 30 with a negative effect on growth and OTA production of *A. carbonarius* coupled with detoxifying abilities. Major products of detoxification were ochratoxin α and L-β-phenylalanine. Out of 144 yeast isolates from epiphytic flora associated with grape berries, 6 were selected by Bleve *et al.* (2006) for their relatively high antagonistic properties against OTA producing strains. Amongst these isolates were *Issatchenikia orientalis, Metschnikowia pulcherrima, Kluyveromyces thermotolerans, Issatchenkia terricola* and *Candida incommunis*. Peteri *et al.* (2007) reported that astaxanthin-producing yeast strains, *Phaffia rdodozyma* and *Xanthophyllomyces dendrorhous* converted ochratoxin A to ochratoxin β, an action which was probably made possible by the production of a metallo carboxypeptidase., However, heat treated cells of *P. rhodozyma* was found to be more effective than viable cells. *Pleurotus ostratus* (Engelhardt, 2002) and *Phenylobacterium* sp., (Wegst and Lingens, 1983) were reported to have ochratoxin detoxifying activities. Cultivation or co-cultivation of strains of *Hanseniaspora uvarum*, *Pichia anomala* and *P. kluyveri* with ochratoxigenic *A. ochraceus* inhibited the growth and/or production of OTA to certain degrees (Masoud and Kaltoft, 2006). Mold isolates from grapes

including Alternaria, Cladosporium and Trichoderma efficiently degraded OTA (Abrunhosa *et al.*, 2002). Varga *et al.* (2005) reported the isolation of fungi belonging to the genus *Rhizopus* which capably degraded over 95% of OTA samples in a period of 16 days.

The OTA detoxifying ability has been reported for some atoxigenic *Aspergillus niger*, *A. niger aggregate* and *A. fumigatus* strains (Valero *et al.*, 2007a, b; Varga *et al.*, 2000). Atoxigenic *A. clavatus*, *A. ochraceus*, *A. versicolor* and *A. wentii* species with a high OTA degrading ability were isolated from grapes (Abrunhosa *et al.*, 2002). A total of 76 strains of filamentous fungi from grapes were tested out of which 51 degraded OTA to varying certain degrees. Members of the *Aspergillus* section *Nigr*i species including *A. niger* and *A. japonicus* isolated from French grapes were reported to be capable of degrading OTA (Bejaoui *et al.*, 2006). Growth was optimal on synthetic grape medium (SGM) for most of the 40 isolates tested while yeast extract broth favored OTA degradation.

Efficacy of biological control agents vary among species (Bleve *et al.*, 2006) depending on concentration of pathogen and environmental factors (Shtienberg and Elad, 2002), combining compatible treatments may be more effective for ochratoxin control in some cases (Elmer and Reglinski, 2006).

Essential oils and antioxidants: A number of agents including plant extracts and chemicals have been tested for their ability to detoxify ochratoxin. As shown in Table 3, available results indicate that essential oils such as oregano, bay, clove, cinnamon oil and thyme inhibited growth of some *A. niger* sp., (Aldred *et al.*, 2008). Cinnamon leaf, clove bud, thyme controlled the growth and ochratoxin production by *P. verrucosum* and *A. westerdijikiae* sp. *in vitro* (Cairns and Magan, 2003; Hope *et al.*, 2003). Using artificially contaminated cocoa powder and beverage, Aroyeun and Adegoke (2007) showed that *Afranmomum danielli* essential oils and their aqueous extracts possess detoxifying abilities against ochratoxigenic fungi. Treating kunu-zaki (a drink made from millet) with Daniellin™ totally degraded OTA present from millet and inhibited re-contamination of product for a period of 5 days (Adegoke *et al.*, 2007).

Antioxidants with antagonistic properties towards ochratoxin have been reported. Phenolic antioxidants butylated hydroxytoluene (BHT) affected growth and ochratoxin production of four *Aspergillus* section *nigri* strains (2 strains of *A. niger aggregate*, *A. carbonarius* and *A. aculeatus*) at different concentrations (Barberis *et al.*, 2009). Fanelli *et al.* (2003) reported that antioxidants reseveratrol and butylated hydroxyanisole (BHA) effectively inhibited OTA production of

Table 3: Chemical agents reported to suppress the growth of OTA producing fungi

Target	Control agent	Reference
A. niger	Oregano, bay, clove, cinnamon oil, thyme	Aldred *et al.* (2008)
P. verrucosum,	cinnamon leave, clove, thyme	Cairns and Magan (2003)
A. westerdijikiae		Hope *et al.* (2003)
OTA fungi	Danielli from *Afranmomum danielli*	Aroyeun and Adegoke (2007)
A. niger strains	Butylated hydroxy toluene (BHT)	Barberis *et al.* (2009)
P. verrucosum	Reservatrol and butylated hydroxyanisole (BHA)	Fanelli *et al.* (2003)
OTA fungi	Paraben (p-hydroxybenzoic acid	Aldred *et al.* (2008)
Aldred	derivatives), reservatrol	
Ochratoxigenic	Gallic acid, protocatechuic acid, vanillic	Palumbo *et al.* (2007)
	spergilli acid, chlorogenic acid, 4-hydroxybenzoic acid	
A. carbonarius	Flavonoid and non flavonoid compounds	Romero *et al.* (2009)

P. verrucosum at different water activity levels. Antioxidants such as paraben (p-hydroxybenzoic acid derivatives), reserveatrol were used to control ochratoxigenic fungal strains (Aldred *et al.*, 2008). Palumbo *et al.* (2007) tested the effect of antioxidants (gallic acid, vanillic acid, protocatechuic acid, 4-hydroxybenzoic acid, catechin, caffeic acid, chlorogenic acid) on the OTA production of 12 strains of ochratoxigenic Aspergilli. Vanillic acid and 4-hydroxybenzoic acid were most inhibitory to both fungal growth and OTA production, though the *A. ochraceus* strain present was not inhibited by any of the antioxidants. Variations observed between strains were thought to be due to both ecological and environmental factors. Romero *et al.* (2009) have reported the inhibition of the growth and ochratoxin A biosynthesis in *Aspergillus carbonarius* by flavonoid and nonflavonoid compounds. They observed that the growth of the fungus was totally inhibited at concentration of 500 mg mL^{-1} by all the phenolic compounds evaluated. The study showed a significant reduction in ochratoxin A production in the presence of all the phenolic compounds used in the study.

Scientist are currently working on identifying genes and enzymes responsible for ochratoxin A detoxification in some microorganisms for genetic engineering of these substances into organisms which are useful/harmless in certain foods (Varga *et al.*, 2005).

CONCLUSION

Given the possible health risks which intake of OTA poses to man, there is a dire need to reduce daily intake of ochratoxin to the barest minimum. There is need for more research directed towards the effect of chronic OTA intake on the human adults and acute intake on infants and children. In depth research need to be done to determine factors which promote OTA for a given region and climate. Awareness should be raised amongst the players in these areas to equip farmers and processors with the knowledge necessary to reduce fungal contamination of food either by infection or intoxication. This is imperative as characteristics of OTA producing strains vary greatly based on a wide range of factors. Good Agricultural Practice (GAP), including adequate pre- and post harvest practices should be maintained. Farmers and food processors should adhere to existing laws by regulatory bodies to minimize risk of OTA contamination of food. If possible, harvest of farm crops should be delayed to periods when climatic conditions are not conducive for growth and OTA production by fungi. Prompt and efficient drying is necessary for products like cocoa to avoid prolonged moist conditions which encourage the growth of molds. Proper and adequate storage facilities need to be employed to ensure that products do not get re-contaminated during transport and processing. Adequate concentrations of correct biological or chemical control agents should be applied to avoid stimulation of OTA production by such treatments. Biostimulation of fermentation environment by addition of OTA degrading strains of bacteria and fungi may be necessary to facilitate increased decontamination of OTA during processing of some foods. Information about pathway for OTA synthesis and conditions of OTA degradation will help in finding novel methods for OTA decontamination in food. Genetic engineering of plants could be carried out to produce varieties of crops which are resistant to ochratoxigenic species of fungi or insects which expose the crops to easier accessibility by ochratoxigenic fungi.

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