Ochratoxin A in Cereal Products, Potential Hazards and Prevention strategies: A Review

Majid Majeed, Ali Asghar, Muhammad Atif Randhawa, Aamir Shehzad, Muhammad Sohaib and Abdullah

National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan

Corresponding author: arbabakhtar13@gmail.com

ABSTRACT

Mycotoxins are microbial agents which cause food or feed-borne intoxications. Mycotoxins are undesirable compounds often found in cereal grains and forages. Production of mycotoxins is dependent on environmental conditions and agricultural practices. Moisture level ranges between 13% to 18% and temperatures between 20 C and 30 C can increase fungal growth rate during growing seasons as well as during transport and storage. Extensively, mycotoxins are aflatoxins (Afs), ochratoxin A (OTA), Fusarium and Patulin. Ochratoxin is naturally occurring mycotoxin produced by fungi Aspergillus and Penicillium. Family of ochratoxin consist of three member ochratoxin A, B and ochratoxin C. Ochratoxin A is most abundant and most toxic produced mainly by Aspergillus ochraceus, A. carbonarius and Penicillium verrucosum. Penicillium verrucosum mainly produced the ochratoxin A in cereals. A. ochraceus is responsible for ochratoxin A production in coffee, grapes and spices. Conclusively, findings of some researchers related to the mycotoxins, their production and concentration especially ochratoxin A in different cereal products and effect of processing on the ochratoxin A contents and its health implication on human health have been reviewed.

Key Words: Mycotoxins, Ochratoxins A, Toxicity, Health implications, Preventive measures

INTRODUCTION

Mycotoxins are natural food and feed contaminants, mainly produced by moulds of genera Aspergillus, Penicillium and Fusarium. The number of mycotoxins known to exert toxic effect on human and animal health is constantly increasing as well as the legislative provisions taken to control their presence in food and feed. Extensively considered mycotoxins are aflatoxins (AFs), ochratoxin A (OTA), Fusarium toxins and patulin (Miraglia and Brera, 2000). A valuable review on the most prominent aspects of mycotoxins is given by the CAST Report (CAST, 2003). They can cause acute and chronic toxic effects in animals and can be transferred into products, or may affect human health directly. They are relatively small molecules with highly diverse chemical structures and biological activity. The production of mycotoxins is not essential for the fungal growth or reproduction, but could be a “virulence factor” for some plant diseases and act against other microorganisms and higher organisms (Puschner, 2002). Plant stressors such as draught or over-irritation, insect damage and pesticide exposure result in a higher susceptibility to fungal infection, whereas the production of mycotoxins may be due to stress or altered conditions for the fungus. Mycotoxin production does not only depend on the genotype of a certain strain alone, but also on a range of environmental factors including humidity, temperature, water activity, processing-errors and insect damage, which have an influence on fungal growth and metabolism (CAST, 2003). Most mycotoxins are stable chemical compounds and cannot be destroyed by processing and heat treatment of feed and foodstuffs. When the fungal metabolites present in foods in sufficiently high levels, they can have toxic effects, either acute (for example, liver or kidney deterioration), chronic (for example, liver cancer), mutagenic, or teratogenic.
The resulting symptoms range from skin irritation to immunosuppression, birth defects, neurotoxicity, and death (Murphy et al., 2006). Problems associated with mycotoxin contamination and the economic losses resulting will continue to be seen in food and agriculture industries. Therefore, it can be predicted that food and feed are always contaminated with toxins to a greater or less extent, and with increasing accuracy of analysis (lower detection limits) toxins can be detected in more and more cases.

Ochratoxins

Ochratoxins are mycotoxins produced by several species of Aspergillus and Penicillium. Among different types of ochratoxins, ochratoxin A is most important, other being the methyl and ethyl ester of OTA which is also known as ochratoxin C (OTC), ochratoxin B (OTB), its methyl and ethyl ester and ochratoxin α. Structurally all the ochratoxins comprise of polyketide-derived dihydroisocoumarin moiety which is linked via the 7-carboxy group to L-β-phenylalanine by an amide bond. Most economically important form of ochratoxin, while OTB and OTC are less important and less common. The empirical formula of OTA is C20H18O6NCl and the molecular weight is 403.82. It is crystalline solid, white in color, slightly soluble in water but highly soluble in the polar organic solvents. OTA is also soluble in the aqueous sodium bicarbonate solution. Ochratoxin A is mainly produced by Penicillium verrucosum and several species of Aspergillus. Ochratoxin A (OA) has nephrotoxic and immunosuppressive properties (Murphy et al., 2006).

Ochratoxins are widely spread metabolites mainly produced by some toxigenic species of fungi as Aspergillus and Penicillium (Sedmikova et al., 2001; Bayman et al., 2002). Major OTA-producing fungus in northern Europe is P. verrucosum, more important in warmer climatic zones is A. ochraceus (Cairns-Fuller et al., 2005). More Aspergillus species have been found to produce OTA, for example A. sulphureus, A. melleus, A. sclerotiorum, A. alliaceus, (Bayman and Baker, 2006), A. albertensis, A. lanosus (Palumbo et al., 2007). OTA is important because of the contamination of ochratoxin A of agricultural products including cereals and grains and influence chronic effect on human exposure (Dehelean, 2011). Natural occurrence in maize and maize-based products is a worldwide problem of OTA contamination (Duarte et al., 2010). Maize kernels are a good substrate for mould infection and production of mycotoxins harmful to both humans and animals. A. niger is commonly isolated from maize (Shah et al., 2010) and a high incidence of A. carbonarius has been also reported (Alborch et al., 2011). Both species are the main source of ochratoxin in corn and other food products in both subtropical zones and tropical of the world (Palencia et al., 2010). There is highest contamination of OAT in cereal grains, and to a lesser extent in grapes, wine, dried vine fruits and grape juice (Clark and Snedeker, 2006).

Production of ochratoxin A

Production of ochratoxins is influenced by various factors including medium composition, temperature and water activity. The fungi responsible for the production of OTA in the cool and temperate regions are mainly Penicillium verrucosum or P. nordicum. P. nordicum is found in the cheese and meat products while P. verrucosum contaminates the cereal crops. For the production of OTA in the tropical and semitropical regions Aspergillus...
ochraceus also referred to as A. allutaceus var. allutaceus, is mainly responsible and other species of Aspergillus i.e. A. niger var. niger (Biffi et al., 2004).

OTA occurrence in cereal grains

There are multifactorial differences in OTA contamination between cereals. Therefore, it is very difficult to establish relationship between OTA content and individual factor, and therefore it is very hard to expect with all confidence that each type of cereals have the OTA content Gonzalez-Osnaya et al. (2007). Garcia et al. (2009), showed association with the early development of predictive mycotoxicology, which have very high importance of food spoilage prevention. Eskola (2002) studied that occurrence and production of ochratoxin A in grains of cereal is largely depends upon the condition of the grain at time of harvest, drying conditions and the storage facilities. Scudamore et al. (2003) demonstrated that in the particular circumstances of Western and Northern Europe, Canada and other moderate zones, cereals have high possibility for the production of ochratoxin A because of cereal have high moisture content, sometimes more than 20%. The production of ochratoxin A in grains is due to inadequate aeration or long term storage without adequate drying (Uysal et al., 2009). Shah et al. (2010) Studied aflatoxin B1 and ochratoxin A and Proimate analysis of maize from Swat Valley, KPK of Pakistan and reported that Upper and Lower Regions, showed AFB1 concentration from zero to 30.92 ppm with average values of 14.94 and 16.22 ppm. Ochratoxin A contamination level was in the range of < 0.001 to 7.32 ppm. Juan et al. (2007) demonstrated that whole-grain cereal samples have high concentration OTA as compared to samples of non-whole-grain cereals (33% versus 14%). Organic whole-grain rye sample showed maximum value which is (27 ng/g). Only seeds or whole grain cereals is important because surface of the grain is site where most of fungi are present, so there are high chances of contamination on the surface of grain is expected.

Jaun et al. (2007) collected 61 bread samples of bread from Portugal which were analyzed for ochratoxin A determination. For the determination Liquid Chromatography with Fluorescence Detection was used. Detection limits and quantification were 0.03 and 0.09 ng/g by LC-MS/MS and 0.015 and 0.03 ng/g, using LC–FD. Presence of ochratoxin A in wheat bread is 12.9% and in maize bread 70%. Maize bread showed the highest ochratoxin A level and only one sample surpassed the maximum limits of European legislation which is established for ochratoxin A in cereal products. A. carbonarius grows at rather lower temperatures than A. niger and produces the large number of spores with temperature at 30 centigrade. They also have ability to grow at reduced moisture content with maximum germination occurs at 0.85 aw at 25 and at the temperature 30 degree centigrade. A. niger is commonly present in warm climates, because it grows optimally at the relatively high temperatures of 35-37 centigrade. (Magnoli et al., 2007). It is important to mention within few days A. niger isolates are able to produce OTA in commercially grown crops (JECFA, 2008).

OTA levels in contaminated food

Apart from measuring OTA in human fluids and tissues, exposure can also be estimated by measuring OTA levels in contaminated food that may have been consumed. Studies on some foods show that there are differences between the contamination level of different batches of food, and even within the batches, the mycotoxin might not be homogeneously distributed but be restricted to a small part of the batches (Speijers, 2001). Furthermore, the occurrence of mycotoxins can fluctuate considerably in time. Sometimes the mycotoxin concentration can be high for a certain episode, whereas for another it might be negligible low. It is difficult to compare OTA levels.
between countries or between types of food, as data on the occurrence of OTA in food and beverages are not available for many commodities in many countries, and the data that are available are often out of date and/or incomplete. The consumption data used were mainly based on intake in Europe. The European Commission (2000) calculated and summarised intake figures for OTA. The total mean intake of OTA for Europe was estimated to be 3.7 ng/kg body weights per day, assuming a body weight of 60 kg.

**Table 1: Relative contribution of different food categories to human OTA exposure**

<table>
<thead>
<tr>
<th>Food category</th>
<th>OTA level (μg/kg)</th>
<th>Intake (g)</th>
<th>Daily intake of OTA (ng/kg body weight/day)</th>
<th>% of total intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereals</td>
<td>0.94</td>
<td>230</td>
<td>3.58</td>
<td>57.8</td>
</tr>
<tr>
<td>Wine</td>
<td>0.32</td>
<td>240</td>
<td>1.23</td>
<td>20.8</td>
</tr>
<tr>
<td>Grape juice</td>
<td>0.39</td>
<td>69</td>
<td>0.44</td>
<td>7.3</td>
</tr>
<tr>
<td>Coffee</td>
<td>0.76</td>
<td>24</td>
<td>0.30</td>
<td>5.1</td>
</tr>
<tr>
<td>Pork meat</td>
<td>0.17</td>
<td>76</td>
<td>0.21</td>
<td>3.5</td>
</tr>
<tr>
<td>Beer</td>
<td>0.023</td>
<td>260</td>
<td>0.09</td>
<td>1.6</td>
</tr>
<tr>
<td>Dry fruits</td>
<td>2.2</td>
<td>2.3</td>
<td>0.08</td>
<td>1.1</td>
</tr>
<tr>
<td>Pulses</td>
<td>0.19</td>
<td>25</td>
<td>0.08</td>
<td>1.1</td>
</tr>
<tr>
<td>Cocoa</td>
<td>0.55</td>
<td>6.3</td>
<td>0.06</td>
<td>0.8</td>
</tr>
<tr>
<td>Poultry</td>
<td>0.041</td>
<td>53</td>
<td>0.06</td>
<td>0.8</td>
</tr>
</tbody>
</table>

**Human exposure**

Mycotoxins can affect human and animal health, as mentioned before. In general, animals are directly exposed to mycotoxins through the consumption of mouldy feedstuff. Human exposure can be via one of two routes; direct exposure due to the consumption of mouldy plant products, or indirect exposure through the consumption of contaminated animal products, containing residual amounts of the mycotoxin ingested by the food producing animals (Boutrif and Bessy, 2001). However, animal derived food products contribute to a lesser extent to human OTA exposure, with the exception of babies and infants, due to their high consumption of milk and milk products, and their specific metabolism (Kuiper-Goodman, 1998).

**Toxicity and health implications**

**Metabolism**

OAT is absorbed through gastrointestinal tract. Ochratoxin A is absorbed from the stomach due to its acidic properties in most species. Ochratoxin A is circulated through blood, mainly to the kidneys, and at lower concentrations to the muscle, fat and liver, with a proportion metabolised into the non-toxic metabolite and other less toxic metabolites. Ochratoxin A has a long serum half-life in non-ruminant animals and in humans (72-120 h in pigs, 840 h in a human subject). Cows and sheep (ruminant animals) are normally resistant to the toxic effect of OTA because before the absorption into blood it is hydrolyzed into the metabolites of non-toxic substances in the stomachs by protozoa. (Kiessling et al., 1984).

**Acute toxicity**

The acute toxicity of OAT is generally low, although large species differences and sensitivity are seen with oral LD50 values ranging widely in different species. Oral LD50 values has been demonstrated to range from 1 mg/kg bw in pigs, 0.2 mg/kg bw in dogs 3.3, 46-58 mg/kg bw in mouse and mg/kg bw in chicken, and. Effects of acute poisoning such as multifocal haemorrhages in various organs and fibrin. At present, there are no documented cases of acute toxicity reported in human.
Chronic toxicity

The chronic effects of ochratoxin A are of great importance. It has been shown to be hepatotoxic, nephrotoxic, teratogenic and immunotoxic to species of animals and carcinogenic to different species of animals.

Neurotoxicity

It has been shown that the administration of OTA during the gestation period in rats encouraged malformations in different portion of the central nervous system. In the same way, Soleas et al. reported that OTA can cause certain lesions as well as damage at the cerebral system. Thus, OTA seems to be highly toxic for the nervous cells and able to reach at any time the neural tissue.

Teratogenicity

OTA is a potent teratogen to laboratory animals. It cross the placenta during the gestation period and store in fetal tissue causing morphological anomalies. The mechanism has not been clearly defined and may involve an indirect effect through maternal action and/or a direct effect on the developing conceptus. Thus, intensity of malformations depends on the gestative period and route of administration.

Immunotoxicity

OTA presents a powerful immunosuppressor effect under the certain conditions, Necroses of lymphoid tissues which shows there high sensitivity to the OTA. OTA seems to play a role in the inhibition of the peripherals T and B lymphocytes proliferation and stops the production of interleukin 2 and its receptors. Moreover, it stops the production of killer cells as well as the production of interferon. The administration of OTA to many animal species causes variable effects on the osseous marrow and immunity response. Thus, this molecule is considered to be the origin of:

- Regression of the thymus
- Lymphopenia,
- Suppression of the immunity response.

Following these results, OTA is clearly taken as an important immune-suppressor agent.

Carcinogenesis

OTA is reasonably expected to be a human carcinogen based evidence of carcinogenicity in experimental animals. When this molecule was administered in the diet, hepatocellular tumors, renal cell tumors, hepatomas, and hyperplastic hepatic nodules were observed in male mice.

Prevention or management of Mycotoxins Contamination

For preventing fungous growth on grains, you should dry them fast and wholly and maintain them on dry place. For preventing OTA forming by A. ochraceus, activity of the grain by moisture would reduce to less than 0.8. The most influential method of grains storing includes; steaming, airing. Sealed storing and controlled atmosphere especially in tropical and mid tropical region in which insect damage is critical problem. Controlled atmosphere storing is based on applying atmosphere by low oxygen or high density of dioxide carbon. Using improved atmosphere to control insect helps to the control of fungus.

Improved Farm management

Research on pre-harvest OAT contamination has revealed that on favorable conditions fungus can attack on plants in the field and therefore, result OAT contamination. Certain commodities such as peanuts, corn and cottonseed growing under stress condition are vulnerable for fungus attack. To prevent pre-harvest mycotoxin contamination, good management practices, such as using sound, fungus free seeds for planting, controlling insects and plant disease and controlling irrigation practices,
should be implemented. Furthermore, mycotoxin contamination can also be minimized by using proper adjustment and operation of harvesting equipment during crop harvesting (CAST, 2003).

**Temperature**

The use of proper temperature management during storage could prevent mycotoxins contamination in food or food products. To avoid mycotoxin contamination during transportation containers or sacks must be free from any contamination before taking mature products. The food overripe, damaged, fermented or fallen onto the soil must be discarded and eliminated even off the field, as they are likely to have high mycotoxin levels or to harbor mycotoxin-producing molds which could be rapidly propagate (Perez de Obanos, *et al.*, 2005). Blend with poor quality products or with other commodities is also unadvisable (Lopez-Garcia, *et al.*, 2008). The food and food products have to be homogeneously and fastly dried under clean and environmentally controlled conditions. In the case of tropical crops, it is very important not to store fresh products (FAO, 2006). Piles have to be turned over in order to promote their aeration and to prevent mycotoxigenic mold development (Kouadio *et al.*, 2006). The FAO/WHO/UNEP, 1999c has recommended that carrying out a fast drying and, in general, under a moisture level of 10%. In coffee, it has been demonstrated that sun drying, less expensive than mechanical dryers, entails greater risks of mycotoxin contamination (Suarez-Quiroz *et al.*, 2005). Moreover, Lopez-Garcia *et al.*, 2008 have pointed out the importance of using small driers to avoid long periods of wet products storage.

**Adsorbing Gages**

For many years modified atmospheres or alternative gases have been examined for the medium and long term storage of cereal grain destined for food/feed. While fungi involved In biodeterioration of grain are considered to be obligate aerobes, many are actually microaerophilic, being able to survive and grow in niches where other species cannot grow and thus dominate specialized grain ecosystems. In many cases decreasing O2 by 0.14% is required before growth can be substantially reduced. Increasing CO2 to N2 50% is required for inhibition of mycelial growth. Some fungi, e.g. *P. roqueforti*, are able to grow and infect grain at 80% CO2 provided at least 4% O2 is present. The use of integrated post-harvest systems for prevention of deterioration entails modifying O2 and CO2 simultaneously and the use of (O2 free) N2. The tolerance to low O2 and high CO2 is also influenced by interactions with grain type and water availability. The drier the grain, the more effective the treatment. Modified atmosphere storage is used for control of both moulds and insects in moist stored grain. Regimes sufficient for moulds may not however be effective against some storage insects, which can survive and grow over a wider equilibrium relative humidity range. Modified atmosphere storage has been examined for the storage of moist grain especially for animal feed. Studies with *P. verrucosum* and *A. ochraceus* with up to 50% CO2 suggest that spore germination is not markedly affected, although germ tube extension and hence colonization is significantly inhibited by 50 to 75% CO2, especially at 0.90 to 0.995 aw for both *P. verrucosum* and *A. ochraceus* (Cairns-Fuller *et al.*, 2005). Growth and OTA production were highest in air, followed by 25 and 50% CO2 regardless of the aw level tested on wheat grain. Generally, CO2 and aw together cause an enhanced inhibitory effect, although this was not synergistic.

**Removing Ochratoxin**

There is much strategy for reducing OTA level. These methods are used for eliminating or reducing OTA level. These different technology are ranked according physical, chemical and biologically, microbiologically methods. Ideal toxin method is easy and expensive and don’t
produce toxin compound or don’t change quality parameters of material (Hundhausen et al., 2005).

Physical Methods

Physical methods include division, sorting, purification; peeling, peeling procedure’s aim is removing the most contaminated one. They include using materials as additive food in which absorbed through OTA, hence reduce biologic frequency (Riley and Norred, 1999).

Chemical Methods

These methods require compounds for removing . We use ammonium, alkali hydrolyze, bisulfites and ozone in some procedures they has been reported as an effective compound for eliminating OTA and other mycotoxins. Although some chemical residue may remain, we don’t study there is reducing on taste and quality of cared foodstuff (Riley and Norred, 1999).

Microbiologic Methods

By the aids of microorganism for decomposing, absorb or changing OTA, to remove toxin from contaminated products or when eating mycotoxin .Carboxypeptidase A could damage OTA. Using a toxigenic A. niger strain has been suggested as carboxypeptidase source (Varga et al., 2000). Other enzymes in which get from A. niger and damage OTA includes: lipase (Stander et al, 2000), enzymatic crude and metalloenzyme. We have one carboxypeptidase from phaffia rhodozyma in which damage more than 90% of OTA (Abrunhosa et al., 2007).

Conclusion

Ochratoxin contamination cause serious health disparities. Therefore, keeping toxins in low levels in foodstuff is of great importance. To minimize contamination, foodstuff should be kept below 14% moisture level. As, these are moderately stable compounds therefore cooking has no effect on the reduction of mycotoxin level. To control this emerging problem it is compulsarily needed to pass some regulations to decrease mycotoxigenic moulds in food. To control mycotoxins growth at stores by controlling the moisture contents and preventing the cereals from damage. Its imperative to reduce mould contamination by opting the international standards for safe storage of food and feed; because, the contaminated products endanger human health seriously.

REFERENCE


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