

Review Paper

Ochratoxin: A Potent Carcinogen

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Abstract

Ochratoxins are hazardous mycotoxins in nature produced by type of species *Aspergillus ochraceus* and *Aspergillus penicillium*. Ochratoxins are the carcinogenic mycotoxins and these toxins may be present in different food entities, plants and animal products. It is placed in group 2B by IARC (the International Agency for Research on Cancer) and is an apparent carcinogen to human beings. Naturally, Ochratoxins, A, B and C are known but Ochratoxin A has been known as a potent nephrotoxic specie. This review introduces Ochratoxin, its presence in food commodities, its harmful effects and current methods for determining ochratoxins.

Keywords: Ochratoxins, Food toxin, Carcinogenic, Thin Layer Chromatography, High Performance Liquid Chromatography, Immuno assays.

Introduction

Ochratoxins- the secondary metabolites are produced by some species of fungus specifically penicillin, some moulds and aspergillus¹. These fungal species have particular requirements to grow i.e. temperature and humidity. There are four different types of ochratoxins i.e. A, B and C. The ochratoxin A is the most toxic than all other toxins. These toxins contain tetrogenic, mutagenic, hepatotoxic, nephrotoxic, and immunosuppressive properties. These hazardous properties of mycotoxins may badly affect human health on consuming infected food. The fungi are useful for the infestation of many crops, for preparation of beverages and other agricultural products that's why these mycotoxins have more chances to be ingested by humans and animals².

Chemical Structure of Ochratoxins

The biosynthesis of ochratoxin (an amide formed between a chlorinated dihydroisocoumarin and phenylalanine) is not completely understood. Ochratoxins are composed of 3, 4 dihydro-3 methylisocoumarin moiety which is linked by 7-carboxy group to L-B-phenylalanine with an amide bond^{3,4}. The ochratoxin synthesis depend upon some physiological factors i.e. temperature, moisture and substrates⁵.

Aspergillus species can synthesize ochratoxin A and ochratoxin B simultaneously. Some experiments performed with *A. ochraceus* have shown the simultaneous production of ochratoxin A and ochratoxin B and their yields and ratios depends upon the persisting culture conditions⁶. It has been also found that the amount of ochratoxin B production was

considerably lesser than ochratoxin A, but in some conditions the level of ochratoxin B can be compared with level of ochratoxin A. The reported production ratios for ochratoxin A and B (OTA: OTB) ranges from 2:1 to 34:1⁷. There are complex interactions found in many carbon sources, basal media and in nitrogenous sources which are found to be important⁸.

Some fermentation lab tests performed on *A. ochraceus*, the results obtained showed high yields (upto 10 mg/g) of ochratoxin A and B⁹. The intermediate metabolites OT α and OT β also formed which can be converted to their respective ochratoxins. The OT β is transferred to both ochratoxins i.e. A and B (14% and 19%, respectively). The OT α only converted in the ochratoxin A (4.9%)¹⁰.

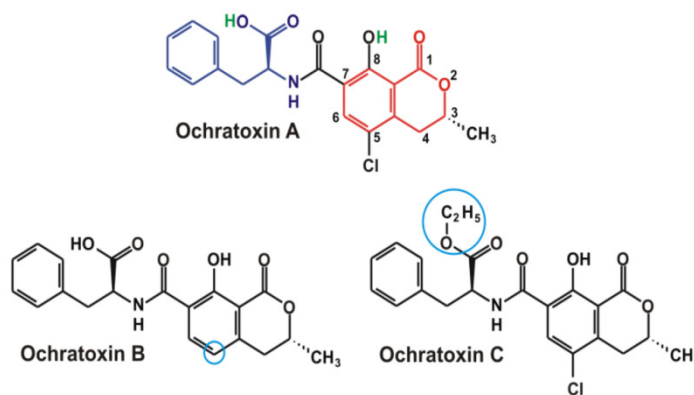
Source: www.google.com

Figure-1
General structures of Ochratoxins A-C¹¹

Contamination Level of Ochratoxins

Ochratoxins are found worldwide but OTA is the most discussed food contaminant due to its highly toxic nature. Sometimes in cases the contamination level of ochratoxin B is high as compare to OTA but OTB related information is very insufficient because it is found in complex with OTA in many food products and the OTA is mainly tested because of its toxicity¹².

A study was conducted to examine the ochratoxins. 681 different spice samples were examined and these showed the positive results for both ochratoxin A and ochratoxin B having the 21% and 10% values in samples respectively¹³. In another investigation, 57 red wines samples were examined and they found to have weak contamination below the legal limit value according to EU (ranging from 0.02 to 0.73 $\mu\text{g L}^{-1}$ and 0.04 to 0.66 $\mu\text{g L}^{-1}$ for ochratoxin A and ochratoxin B, correspondingly)¹⁴. OTA had 71.9% concentration in the sample and OTB had 64.9% concentration in sample¹⁵.

Hamilton and his co-workers examined the ratio of OTA, OTB and OTC in the food of poultry animals. The examined ratio for OTA, OTB and OTC was 90:8:2 respectively¹⁶. Humans exposure for toxins is possible by food and beverages. The estimated dietary exposure for OTA in humans is 15-60ng by weight per week. This value is estimated by EU for adults¹⁷. A recent biomonitoring study was conducted on Belgian human population for the determination of different 33 mycotoxins. 9 mycotoxins were identified by LC-MS/MS among them deoxynivalenol, ochratoxin A, citrinin and the associated metabolites were frequent in the detection. Citrinine and ochratoxin A were found in weak concentrations of 59% and 35% respectively. This showed that 1% of the examined population has crossed the tolerable level of ochratoxin A¹⁸.

Different Food Commodities having Ochratoxin A

Ochratoxin A is carcinogen which is produced by different fungal species. This toxin is more carcinogenic than other types of ochratoxins and food is the primary source for its exposure to humans. Grains and its derived products i.e. beer are more vulnerable for this toxin. Lab tests on animals confirmed the main targets for ochratoxin A i.e. liver and kidney. But the carcinogenic effect of ochratoxin A on humans has less evidence^{19,20}.

Meat and its products: The contamination of ochratoxin A in meat and its products had been the matter of great interest for researchers due to high consumption of different types of meat entities. There is much published data available on ochratoxin A contamination in the meat. In the starting years of research OTA was found an important contaminant in food and meat cannot be ignored because it's a main contributor in human's diet. The mean level of ochratoxin A contamination in meat was 0.2 mg/kg. Pig and its meat products have been found to be the

most contaminated with OTA specifically blood kidney and liver were found more contaminated²¹.

The OTA contamination rate in chicken is higher in Pakistan as compared to other countries. 41% of Pakistani broiler samples were examined which provided different mean values of OTA. OTA mean values for wings had 1.39 ± 0.78 ng/g, chests had 0.28 ± 0.79 ng/g, legs had 1.12 ± 0.19 ng/g, and liver had 2.21 ± 0.43 ng/g.²²

Cereals and products: The cereal foodstuffs together with different bakery products had the maximum OTA concentration of 2.14ng/g. Research revealed that OTA in different cereal products showed different concentrations. The sponge biscuits had 2.14 ng/g amount of OTA, sweet biscuits had 1.69 ng/g and muesli had 1.44 ng/g OTA²³. This information is comparable with the study conducted by European countries. A recent Spanish study was conducted for ochratoxin presence in samples which gave the mean values for the OTA: 2.8% and 0.728ng/g.

Ochratoxin A in corn based breakfast cereals was detected upto 25% and 0.293ng/g and in wheat based breakfast cereals it was 12.9% and 0.283 ng/g²⁴. Another Lebanese study was conducted in which cereal products exhibited the highest concentration of OTA which is 2.844ng/g specifically in biscuits and croissants. 69% breakfast samples were examined in France having the OTA concentration of 0.2- 8.8ng/g. The highest concentration of OTA was found in those samples which had dry fruits and bran²⁵.

Nuts: The average value for OTA was 0.34 ± 0.19 ng/g found in fresh chestnuts with highest concentration of 6.44ng/g. The 68% of OTA was occurred in chestnut flour²⁶. Tiger nuts having range of OTA contamination was 1.2-37 ng/g²⁷. 85.7% peanuts had OTA contamination with the mean value of 3.7ng/g and its highest value was 4.9ng/g²⁸. According to Lebanese study seed, nuts, dates and olives had low concentration of OTA with the mean value of 0.078ng/g²⁹.

Spices: The overall mean value for OTA in spices is 1.2mg/kg and the highest value of OTA is 24mg/kg. The OTA contaminated spices are nutmegs, paprika, chilies and pepper. But the information related to the spices is very limited. In Spain, chilies and paprika were examined. The results showed that 99% paprika samples having ochratoxin A with LOQ-281 ng/g range and 100% samples of chilies having OTA range of 0.62-44.6ng/g³⁰. It has also been found that the crushed chilies contain more OTA contamination as compare to powder form³¹.

Beer: In a study conducted 39% samples of beer were found contaminated with OTA. The beer samples had the mean value of OTA 0.065 ng/g and maximum value was 0.26 ng/g³². In many studies it has been observed that mean values of OTA were less than 0.070 ng/mL except the beer samples of Korea, Scotland, and Belgian³³.

Rice: In a study conducted on rice samples marketed in Morocco, it was found that 26% of rice samples were tainted with OTA and the contamination range was 0.08–47ng/g and the calculated average value was 3.5ng/g. According to these results their daily intake was also calculated which was 0.32 ng/kg bw/day³⁴. Same study was conducted in Vietnam and the researchers found that 35% rice samples are contaminated with OTA having average value 0.75 ng/g and the maximum value 2.75 ng/g. It had also been found that OTA contamination was elevated in arid season as compared to wet season³⁵.

Methods for the Detection of Ochratoxin

Ochratoxin A was found in 1965 for the first time³⁶. The other types of ochratoxins such as B, C OT α and OT β are now discovered by using improved detection methods³⁷.

A detection, differentiation and separation of ochratoxins are done by using various methods. Some of these methods are more expensive to use but immunological techniques are less expensive and they can easily detect the presence of ochratoxin in food entities. Immunological methods cannot differentiate between different kinds of ochratoxins which is a main disadvantage of these methods. Commonly a detection of OTA is done by immunological methods³⁸. The analytical methods for the analysis of ochratoxins are TLC and HPLC combined with MS.

Thin Layer Chromatography (TLC): Mainly the technique used for the study of mycotoxins such as aflatoxins and ochratoxins is TLC. It is less expensive and can handle a huge sample size³⁹. A qualitative and semi-qualitative analysis can easily be done by TLC. This is possible due to its ability to identify the target compounds under UV spectral analysis⁴⁰. A prior preparation and purification of samples is the main necessity for analysis of ochratoxins by TLC. Usually, silica gel columns are used for the purification for samples.

High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS): In modern epoch HPLC-MS is commonly used for the detection of ochratoxin. The use of HPLC mainly depends on the physical and chemical structures of ochratoxins. Mainly two columns, normal phase and reverse phase are used to separate and purify ochratoxins. A selection of columns depends on the polarity of ochratoxins. Pretreatment of samples is done by mini columns and the preparation of mycotoxin standard is done by large scale preparative columns⁴¹. The use of UV and fluorescence detectors (FD) helps in the detection of mycotoxins by HPLC. Fluorescence detectors, detects the chromophore. A large number of mycotoxins naturally have chromophore molecules and easily be identified by HPLC-FD⁴².

OTA analysis at industry levels is done by HPLC standard method. First step in HPLC technique is sample clean up and separation. After separation, ochratoxins are analyzed under FD

or MS⁴³. A popular combination of columns which are used in HPLC is reverse phase column and acidic mobile phase. An acidic mobile phase consists of orthophosphoric acid.

A major advantage of HPLC is a high quality separation and its ability to build an association with fluorescence detectors and mass spectrometry. Different compounds can simply be detected in one sample by HPLC. It is easily automated which gives a main benefit above additional techniques, such as TLC and ELISA⁴⁴.

Immunoassay Formats: The immunoassay formats for the detection of ochratoxins are ELISA, sandwich ELISA and Fluorescent Immunoassay (FIA).

In ELISA enzymes are used instead of radionuclides. A colored product is formed, when these enzymes combines with their substrates. Many types of enzymes are present which are used in ELISA but ALP (Alkaline Phosphatase) and HRP (Horse Radish Peroxide) are mostly used⁴⁵. Plastic reaction tubes are used as solid support and coating of reagents is done in these tubes. Microtiter plates can also be used as solid support on which the immunochemical procedure can easily be accomplished. To shorten a time of reaction, a step of centrifugation is avoided.

ELISA may be designed in various formats. The simplest configurations are direct competitive ELISA and indirect competitive ELISA. In direct competitive ELISA (dcELISA), an antibody is coated on the walls of plastic reaction tubes. After this, a standard/sample is added and incubation is done. Later on, an enzyme reaction is performed and analysis of ochratoxin is done with Spectrophotometry. In indirect competitive ELISA (icELISA), the hapten usually attached to a carrier protein, coated on solid support and standard/sample is added. Remaining steps are as same as in dcELISA. OTA can easily be detected and quantified in bodily fluids and tissues, food, raw products and beverages by ELISA.

Sandwich ELISA is used for greater target compounds which have numerous epitopes. In this process a coating of one antibody is done on one epitope of the compound. Second antibody is elevated for another epitope which is present on the target compound. Third antibody can also be used in the same manner. A labelling of second and third antibodies is done for the detection process. Mainly, sandwich ELISA is used to detect numerous molds such as *Fusarium*, *Penicillium* and *Aspergillus*⁴⁶.

A sandwich ELISA scheme was developed to identify numerous molds (*Penicillium*, *Aspergillus* and *Fusarium*) concurrently using monoclonal antibodies against the extracellular polysaccharide of these species. Remarkably, a sandwich ELISA is defined by means of two monoclonal antibodies and is more sensitive process⁴⁷.

In FIA, a labelling of tracer is done with fluorofore. These fluorofores have the ability to excite at wide range and emit the larger wavelengths. An extraordinary microtiter plates are used in this process and background fluorescence is avoided. A main type of FIA is Time-Resolved Fluorescent Immunoassay (TR-FIA) which is used to get more sensitive and specific results⁴⁸.

Conclusion

Ochratoxins are very harmful toxins which may spoil foods and then deteriorate health of humans and animals. Safety of foodstuff is of great concern and to avoid ochratoxin contamination quality assurance and quality control of food entities is very necessary. Moisture and temperature are the factors which must be controlled to avoid ochratoxin growth. There is a dire need to make strategies for the reduction of mycotoxin moulds in food. Monitoring and measurement by modern methods and techniques are necessary for the check and balance of production and contamination of food stuffs with health hazardous ochratoxins.

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