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Ochratoxin A Burdens in Rice from Lagos Markets, Nigeria

[1]O.O. Ayejuyo, [2]A.B. Williams and [3]T.F. Imafidon
[1]Department of Chemistry, University of Lagos, Lagos, Nigeria
[2]Department of Chemistry, Covenant University, Ota, Ogun State, Nigeria
[3]Department of Chemistry, Lagos State University, Ojo, Lagos, Nigeria

Abstract: Twenty five different brands of rice from Lagos markets in Nigeria were sampled and assessed for Ochratoxin A (OTA) contamination using High Performance Liquid Chromatography (HPLC). Samples were homogenized by grinding and extracted with 12.5 mL of 0.1 M phosphoric acid and 125 mL of methylene chloride. The extracts were cleaned-up with a syringe packed with acid washed Celite 545 column fitted to a solid phase extraction vacuum manifold and the analyte was eluted with methylene chloride/formic acid. The extract was pre-concentrated prior to HPLC analysis. Result indicated that OTA level in pic rice was non-detectable. Twenty four of the twenty five brands of rice analyzed contained trace levels of OTA, the content of which ranged from 0.01 ng g^{-1} in miss lily parboiled rice to maximum concentration of 2.18 ng g^{-1} in rice king. The mean concentration was 0.34 ng g^{-1}. The low level of contamination evident from the study suggested that consumption of rice available in Lagos markets was relatively safe. Results indicated that the levels of OTA in the rice samples were within acceptable limits. Recovery of Ochratoxin A in selected samples on spiking ranged from 84.6-92.5%, the mean recovery being 89.8%.

Key words: Ochratoxin A, rice, Lagos markets, mycotoxin, toxigenic moulds

INTRODUCTION

Ochratoxin A (OTA) is a mycotoxin. It is an innately fluorescent compound and detection during analysis is usually based on its fluorescence (Pena *et al*., 2005). Mycotoxins are secondary metabolites of fungi which evoke pathological changes in man and animals and are contaminants of various foods and foodstuffs such as cereals, dried fish and pig meat. OTA results from moulds that grow in these food materials. The toxigenic moulds that contaminate grains produce them while the crop is growing in the field, during storage, during processing and handling or after the finished food products have been packaged for sale and consumption. Mycotoxin contamination is favoured by stress factors during plant growth, late harvesting of crops, high ambient humidity preventing thorough drying, unscientific storage practices and lack of awareness (Bankole and Adebanjo, 2003). Important factors needed for fungal growth and mycotoxin production are oxygen, temperature and water activity. Some fungi could also grow under anaerobic conditions.

Mycotoxins contamination vary from year to year and from one geographical region to another. The safety of food for human and animal consumption should be of concern. Mycotoxins are low molecular weight toxic secondary metabolites of fungal origin, which when ingested, inhaled or absorbed through the skin cause lowered performance, sickness or death in humans and animals. Mycotoxicosis is the pathological abnormality of ingesting toxin-contaminated foods (Richard, 2000a). There has been a growing concern about their health implications. OTA has been implicated as immunosuppressive, teratogenic, mutagenic, genotoxic, nephrotoxic and possibly carcinogenic

Corresponding Author: A.B. Williams, Department of Chemistry, Covenant University, P.M.B. 1023, Ota, Ogun State, Nigeria Tel: +234-803-6706086

(Finn, 2003; Bankole and Adebanjo, 2003; IARC, 1993). It is a kidney toxin and is suspected as the causative agent of a human disease, balkan endemic nephropathy that affects the kidneys with tumours. If its concentration is sufficiently high there may be damage to the liver as well. It is a carcinogen in rats and mice and is regulated in some European countries (FAO, 1995). OTA levels accumulate in the blood and tissues of humans and animals and have been linked to problems in animal husbandry ranging from poor feed conversion, low weight gain, digestive distress, reproductive deficiency, tumor development, lack of immunity and death. In certain areas of the world, human diseases are attributed to them. Therefore, most countries have regulatory levels for their occurrence in certain commodities and require all grains to be tested for them.

Nigeria has tropical climate with an all year round high ambient temperature and relative humidity that provide optimal condition for the growth of toxigenic moulds. There are also poorly developed processing and storage facilities. Initial growth of fungi in grains could form sufficient moisture from metabolism to allow for further growth and mycotoxin formation. Although hundreds of mycotoxins are known, relatively few are recognized as serious contaminants of food. Among these are aflatoxins, fumonisins, zearalenone, patulins and ochratoxins. The only proven way to determine if grains or foods contain mycotoxins is to test for them. However, one might suspect contamination if certain signs appear in the grain. Such signs include visually discoloured kernel, musty odour and kernels with lighter weight than usual, blanched kernels or wrinkled kernels. The fungus that produce them may be absent from a sample of the grain due to a number of reasons such as death. However, they will remain unless chemically removed. Processes such as ammoniation can remove them from a commodity (Miller, 1993). Mycotoxins continue to be formed in stored commodities and products when the fungus is viable and the conditions for their formation including moisture and temperature are appropriate. Available literature in Nigeria show occurrences of mycotoxins in various food products (Ogunbanwo *et al*., 2005; Essien, 2000).

Fungi that produce toxins in food are classified into field and storage fungi based on their ecological requirements for growth (Bankole, 1994). Field fungi requires grain moisture above 20% in cereals and often cause diseases and toxin production before harvest. The storage fungi usually grow in grains with moisture content in equilibrium with 70-90% relative humidity, which corresponds to less than 18% moisture content in cereals and the most important genera are Aspergillus and Penicillium. *Penicillium verrucosum* is the toxigenic mould for ochratoxins. The primary producers of ochratoxin A are *Aspergillus ochraceous* and *Penicillium verrucosum*. Ochratoxin may have a long half-life in humans. It is a colourless, crystalline compound that is soluble in polar organic solvents and dilute sodium bicarbonate solution and slightly soluble in water. It has a melting point of about 90°C when crystallized from benzene as a solvate. Non-solvated crystals of melting point 169°C have been obtained from xylene which are suitable for X-ray structural analysis. Ochratoxin A is optically active and its important chemical reaction is esterification of the carboxyl group. Inadequate data are available on the presence of OTA in rice in Nigeria. The purpose of this study is to determine the levels of contamination of Ochratoxin A in different brands of rice from Lagos markets in Nigeria.

MATERIALS AND METHODS

Twenty five different brands of rice were collected in 2003 from Lagos markets in Nigeria and assessed for OTA using High Performance Liquid Chromatography (HPLC). Stratified random sampling was carried out to obtain 5 kg samples from stored material and from the open markets. Samples were later quartered to obtain representative samples of laboratory size (Pineiro *et al*., 1996). OTA was extracted from ground rice with methylene chloride. Interferences were removed with hexane and the toxin that was eluted with methylene chloride/formic acid was determined using HPLC-fluorescent detection method (Richard, 2000b). The lowest limit of detection was 0.01 ng g^{-1}.

Operating HPLC system conditions were set. Mobile phase: acetonitrile/water (45/54/1); pump: Waters 510 HPLC pump set at 1 mL min^{-1} flow rate; column: waters Novapak C18; 3.9×150 mm, 5 μm preceded by a waters C18, 4×20 mm, 4 μm column guard; injection system: Rheodyne 7125 with 100 μL loop; detector: waters 474 scanning fluorescence detector set at 333 and 460 nm excitation and emission wavelengths, respectively. Injection sequence was mobile phase, OTA standard (retention time = 5.3) and then samples.

RESULTS AND DISCUSSION

The results as shown in Table 1 indicate that OTA level in pic rice was non-detectable. Twenty four of the twenty five brands of rice analyzed contained trace levels of OTA, the content of which ranged from 0.01 ng g^{-1} in miss lily parboiled rice to maximum concentration of 2.18 ng g^{+} in rice king. The mean concentration was 0.34 ng g^{-1} while the median was 0.16 ng g^{-1}. Stallion rice contained 0.66 ng g^{-1} while the commonly consumed tomato brands ranged from 0.05 ng g^{-1} in tomato star rice to 1.14 ng g^{-1} in tomato unique. Table 2 shows result of recovery test on selected rice samples. Recovery of Ochratoxin A in selected samples on spiking ranged from 84.6-92.5%, the mean recovery being 89.8%.

This confirms that the methodology for this study was consistent with internationally acceptable standard. The Standard Deviation (SD) was 4.5% while the Coefficient of Variation (CV) was 5.01%. The European Union Scientific Committee on food endorsed the maximum level of 5 ng g^{-1} for OTA in raw cereals (WHO, 1996). Table 1 shows that the levels of Ochratoxin A in the rice samples were

Table 1: Amount of Ochratoxin A detected in samples of rice in Lagos

Sample code	Name of sample	Ochratoxin A detected (ng g^{-1})
001	Siamese rice	0.45
002	Louis cater rice	0.41
003	Miss lily rice	0.16
004	Sunflower rice	0.15
005	Toyin tomato premium rice	0.07
006	Joy rice	0.05
007	Red tomato rice	0.62
008	Champion rice	0.15
009	Wazobia rice	0.05
010	Tomato star rice	0.05
011	Dama parboiled rice	0.04
012	Miss lily parboiled rice	0.01
013	Tilda pure basmatic rice	0.05
014	American type thai rice	0.05
015	Tomato hart rice	0.16
016	Rice king	2.18
017	Rice star	0.62
018	Tomato unique parboiled rice	0.21
019	Great African rice	0.05
020	Red tomato Amina style rice	0.28
021	Stallion rice	0.66
022	Pic rice	ND
023	Top rice	0.41
024	Tomato unique	1.14
025	Sona rice	0.58

ND: Not Detected

Table 2: Result of recovery test on selected rice samples

Sample code	Name of samples	Recovery (%)
001	Siamese rice	92.2
022	Pic rice	92.5
025	Sona rice	84.6

within acceptable limits. The low level of contamination evident from the study suggests that consumption of rice available in Lagos market was relatively safe. However, there should be consistent analyses of OTA in rice samples as new batches of the existed products and new products are being imported into the country from time to time. It was observed from the chromatogram that Ochratoxin A had a retention time of about 7 min for the entire reference standard and the samples that contain it. The chromatograms of reference standards had little or no interference whereas many peaks came before the peak of interest when samples were injected into the High Performance Liquid Chromatography (HPLC) system.

Foods contaminated by OTA have long been considered to be a risk for humans and animals. In the study of Ochratoxin A in rice cultivars after inoculation of *Penicillium verrucosum* (Axberg *et al*., 1998), the natural occurrence of ochratoxin A in grain samples of 23 rice cultivars was in the range 0.01-1.0 ng g^{-1}. During incubation, OTA was accumulated in all cultivars. Significant varietal differences in its accumulation were observed ($p<0.0001$). OTA has also been determined in Portuguese rice where 42 rice samples were assessed using high performance liquid chromatography with fluorescence detection (Pena *et al*., 2005). The presence of OTA was detected in six samples at concentrations ranging from 0.09-3.52 ng g^{-1} while recovery from rice samples spiked at 0.05 ng g^{-1} was 92%. The levels of OTA in these studies are comparable with present findings.

Humidity and water activity encourage the growth of the Ochratoxin mould, which may result in its build up in the samples and environment leading to a rise in the level of OTA. Rapid drying of agricultural products is recommended since mycotoxin contamination is mainly traced to non-maintenance of stored products at safe moisture content. Dry grains are devoid of moulds because the moisture level required for their growth is lacking. Regulatory bodies should be encouraged to test every batch of rice entering Nigeria before they are released into the markets. Good Agricultural Practices (GAP) including proper harvesting, storage and transportation conditions should be strictly adhered to. Careful sorting and processing may further reduce the level of contamination. Further research work should also be carried out on development of fungus resistance plant species and biological control agents which may be useful in controlling Ochratoxin A.

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