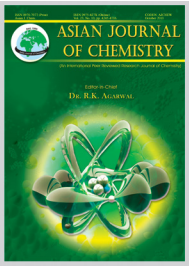
Asian Journal of Chemistry; Vol. 24, No. 11 (2012), 5349-5351

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Determination of Natural Occurrence of Ochratoxin A in Naked Oats of China by HPLC

YANG YANG[1], HONGXING ZHANG[1,*], JINGYU GAO[2], YUANHONG XIE[1], XIAOGONG WANG[3], HUI LIU[1] and TAO HAN[1]

[1]Beijing Key Laboratory of Agricultural Product Detection and Control of Spoilage Organisms and Pesticide Residue, Department of Food Science, Beijing University of Agriculture, Beijing 102206, P.R. China
[2]Beijing Entry-Exit Inspection and Quarantine Bureau of The Peoples Republic of China, Beijing 100621, P.R. China
[3]The National Research Center of Soybean Engineering and Technology, Northeast Agricultural University, Harbin 150030, P.R. China

*Corresponding author: Fax: +86 10 80799170; Tel: +86 10 80794124; E-mail: hxzhang511@163.com

(*Received*: 7 December 2011; *Accepted*: 16 June 2012) AJC-11633

Ochratoxin A is a ubiquitous mycotoxin produced by different species of *Aspergillus* and *Penicillium* fungi. It is widespread in food and feed and its occurrence has been reported in cereals, cereal-derived products, dried fruits and spices. The aim of our study was to investigate the presence of ochratoxin A in naked oats which was widely consumed and commercialized in China. The analytical methods used in this study involved the extraction of ochratoxin A by acidified toluene, immunoaffinity (IAC) clean-up and high performance liquid chromatography (HPLC) quantification with fluorescence detection. The limit of detection for ochratoxin A was 0.5 μg kg^{-1}. Levels and percentages of ochratoxin A contamination in different types of naked oats, 35 white oats, 56 upon dam naked oats and 48 Mongolia naked oats samples, were evaluated with incidences of 31, 23 and 33 %, respectively. The average of contamination by ochratoxin A found were 3.85, 2.33 and 2.38 μg kg^{-1}, respectively, for white oats, upon dam naked oats and Mongolia naked oats. Experimental results showed that the contamination percentages and levels in 2010 were lower than usual norms (5 μg kg^{-1}) established by the European commission in 2002.

Key Words: Ochratoxin A, Naked oats, High performance liquid chromatography.

INTRODUCTION

Ochratoxins were first isolated as a secondary metabolite of *Aspergillus ochraceus*[1,2]. Later researchers discovered that several other *Aspergillus*[3] and *Penicillium* spp.[4] had the capacity for producing ochratoxin A when the temperature, relative humidity and product moisture were favourable[5]. The family of ochratoxins consists of three members, including A, B and C, which differ slightly from each other in chemical structure. However, these differences have marked effects on their respective toxic potentials. Ochratoxin A is the most abundant and the most commonly detected among the three members, while it is the most toxic of the three members[6].

Ochratoxin A has been shown to possess nephrotoxic, carcinogenic, immunosuppressive and teratogenic properties. It has potential harm to animals and humans[7]. Human exposure to ochratoxin A has been clearly demonstrated by its detection in human blood, urine and food. Ochratoxin A was implicated in several human and animal pathologies such as the Balkan Endemic Nephropathy (BEN) and the Tunisian Chronic Interstitial Nephropathy (CIN) of unknown cause[8]. Based on the available toxicological data, the International Agency for Research on Cancer (IARC) has classified ochratoxin A as a potential carcinogen for humans[9].

Ochratoxin A can be frequently found in a variety of foods and beverages, including cereals, coffee, cocoa, spices, beer, wine, grape juice and dried fruits[10]. It is found mainly in cereal and cereal products in the human diet. This group of commodities has been reported to be the main contributors to ochratoxin A toxicity in exposure assessments carried out by the European Commission in 1997; Indeed, Devegowda *et al.*[11] reported that approximately 25 % of cereals consumed in the world were contaminated by mycotoxins.

The naked oats (*Avena nuda* L.) are important minor crops in semiarid regions of China. Because of their tolerance to infertile soils and arid, salty environmental conditions, these oats can be cultivated in a variety of regions[12]. The naked oats have high nutritional values. It can be used as both grain and forage crops. The naked oats is planted primarily in the semi-arid regions of northern China and mainly cultivated for grain. The naked oats represent a staple food for the northwest population of China and it's widely accepted as a healthy food.

Many studies have been carried out in the world with regard to the presence of ochratoxin A in cereals and cereal-based foods. There is less published data on the occurrence of ochratoxin A in the naked oats and their processed products were consumed in China. Therefore, the purpose of this paper

was to investigate the distribution of fungi with the potential to produce ochratoxin A in naked oats from the northwest (Shanxi province) and northern (Hebei and Inner Mongolia province) of China and assess the presence of ochratoxin A in the naked oats.

EXPERIMENTAL

A total of 139 naked oat samples, including 35 white oats samples, 56 upon dam naked oat samples and 48 Mongolia oat samples, were selected randomly from different oats-planted areas in Shanxi, Hebei and Inner Mongolia, during autumn season of 2010. All the samples were transported to the laboratory under ambient temperature and analyzed as soon as possible after collection, otherwise kept in polyethylene bag and stored at -20 ºC until used for analysis.

All solvents (acetonitrile, methanol and toluene) were purchased from Fisher Scientifics (Fisher chemicals HPLC, France) with HPLC grade. All reagents purchased from Sigma-Aldrich (Shanghai, China) were analytical grade. In all analytical steps, highly purified water generated by a Millipore Synergy 18S Ultra-Pure Water System from Millipore (Beijing, China) was used.

Preparation of standard solution: Ochratoxin A was purchased from Sigma-Aldrich (Shanghai, China) as a crystalline powder form. A stock solution (500 µg/mL) was prepared by dissolving 1 mg of ochratoxin A in 2 mL of toluene/acetic acid (99/1, v/v). The solution was left overnight at room temperature to ensure complete dissolution of the crystalline ochratoxin A. 200 µL of ochratoxin A standard stock solution was transferred to a 100 mL brown volumetric flask and was quickly diluted with toluene/acetic acid to obtain a working solution, 1 µg mL^{-1}. Standard working solution was stored at -20 ºC in the dark; the working solution was freshly prepared and held for less than 1 month.

Extraction and IAC clean-up: The methods used for ochratoxin A extraction from the oats samples and cleaning-up with IAC were according to the previously published methodology by Mario *et al.*[13].

HPLC conditions: Column (ODS2, 5 µm × 25 cm × 5 mm) temperature was adjusted to 35 ºC and a fluorescence detector was used to quantify ochratoxin A. Detection of ochratoxin A was carried out using 333 and 470 nm as wavelengths for excitation and emission, respectively. The mobile phase was acetonitrile/water/acetic acid (99/99/2, v/v/v) eluted at a flow rate of 1 mL min^{-1}. Ochratoxin A was identified by constant retention time and quantified by comparison with peak areas of standards in the mobile phase.

RESULTS AND DISCUSSION

Method validation: Prior to analysis of the samples, the analytical methods for ochratoxin A analysis were investigated for its suitability and parameters such as: selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ) and precision were validated.

To evaluate the selectivity, blank and spiked samples were processed according the adopted methods and the corresponding chromatograms were compared. HPLC-FLD conditions selected for analysis of ochratoxin A ensured a satisfactory selectivity as the analyte peaks were well separated and no interfering peaks in the retention times of analytes were observed. The chromatograms of the blank and the white oats sample with ochratoxin A at 1 µg kg^{-1} are shown in Fig. 1. The retention time was about 6.5 min for ochratoxin A and no peaks appeared at the retention time that can interfere in their concentration.

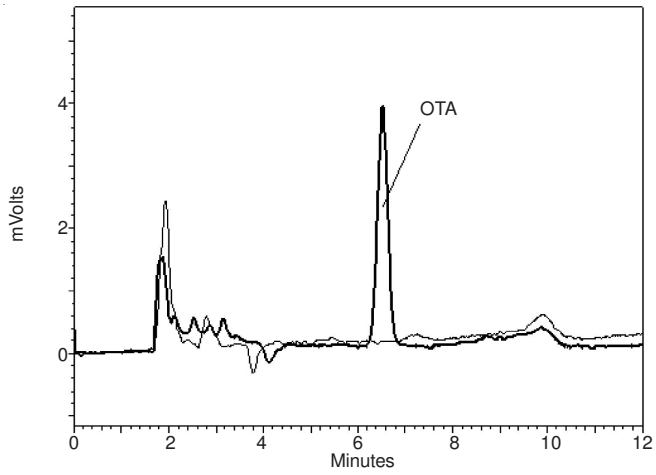


Fig. 1. Chromatogram of a blank white oat sample and an ochratoxin A naturally contaminated white oat sample of 1 µg/kg

Linearity was checked in the range of 0.5-25 µg kg^{-1} for ochratoxin A. The calibration curve showed a linear behaviour in the range with a determination coefficient (r^2) of 0.998. Sensitivity was estimated by the standard error method[14]. The limit of detection and limit of quantification of ochratoxin A in naked oats were determined by using the slope of the calibration curve and the standard error of the intercept. The limit of detection was calculated to be 0.50 µg kg^{-1} and the limit of quantification was 1.65 µg kg^{-1} of ochratoxin A in naked oat. The limit of quantification values were lower than the maximum levels fixed by current European regulations[15].

Occurrence of ochratoxin A in naked oats: Naked oats is one of the common cereals represent a staple food for the northwest China population. Therefore it has a high social, economic and nutritional relevance. Furthermore, they are usually planted in conditions which favour mould growth and mycotoxin production. Results of ochratoxin A occurrence in naked oats samples (white oats, upon dam oats and Mongolia oats) are shown in Table-1.

As shown in Table-1, ochratoxin A was detected in 40 samples representing 29 % of the whole samples and ochratoxin A was detected in 11 of the 35 (31 %) samples of white oats, in 13 of the 56 (23 %) samples of upon dam oats and in 16 of the 48 (33 %) samples of Mongolia oats. The mean concentrations of ochratoxin A found were 3.85 µg kg^{-1} in white oats, 2.33 µg kg^{-1} in upon dam oats and 2.38 µg kg^{-1} in Mongolia oats. The incidences of ochratoxin A positive samples of white oats in the range 0.5-2 and 2-5 µg kg^{-1} was 17.1 and 14.3 % (Table-2). The incidences of ochratoxin A positive samples of upon dam oats in the range 0.5-2 and 2-5 µg kg^{-1} was 14.3 and 8.9 %. The incidences of ochratoxin A positive samples of Mongolia oats in the range 0.5-2 and 2-5 µg kg^{-1} was 20.8 and 12.5 %.

TABLE-1
OCHRATOXIN A LEVELS IN THREE TYPES OF NAKED OATS IN CHINA

Oats samples	Number of samples	Positives samples	Incidence (%)	Range of ochratoxin A (μg kg^{-1})	Mean (μg kg^{-1})[b]	RSD (%)
White oats	35	11	31	< LOD[a]-4.72	3.85	3.6
Upon dam oats	56	13	23	< LOD -3.83	2.33	4.4
Mongolia oats	48	16	33	< LOD -4.26	2.38	6.8
Total	139	40	29	< LOD -4.72	3.32	4.6

[a]LOD, limit of detection, LOD for ochratoxin A = 0.50 μg kg^{-1}; [b]For calculations of mean, samples below the LOD are set to LOD/2.

TABLE-2
FREQUENCY OF OCHRATOXIN A IN THREE TYPES OF NAKED OATS IN CHINA

Ochratoxin A levels (μg kg^{-1})	White oats		Upon dam oats		Mongolia oats		Total	
	Number of samples	Frequency (%)	Number of samples	Frequency (%)	Number of samples	Frequency (%)	Number of samples	Frequency (%)
< 0.5	24	68.6	43	76.8	32	66.7	99	71.2
0.5-2.00	6	17.1	8	14.3	10	20.8	24	17.3
2.00-5.00	5	14.3	5	8.9	6	12.5	16	11.5
> 5.00	0	0	0	0	0	0	0	0
Total	35		56		48		139	

Conclusion

The high performance liquid chromatography with fluorescence detection was very useful for the ochratoxin A analysis in naked oats. The low costs, fastness, accuracy and repeatability for the analysis, makes of this method an important analytical tool for ochratoxin A contamination-control in oats.

Ochratoxin A and related pathologies have become a worldwide preoccupation and they raise serious economic and sanitary problems[16]. The present study concerned a large number of samples and covered all important naked oats habitat in china. In relation with these samples, no significant results were found. All samples were below the limit of 5 μg kg^{-1} by EU[17] for safe consumption. It could be explained chiefly by the climatic conditions especially humidity and temperature of northern and northwest China which are not in favour of the toxigenic fungi proliferation. Therefore, the contribution to intake of ochratoxin A for naked oats consumed in China could be considered to be rather small. Nevertheless, the general incidence of ochratoxin A contamination in naked oats meaning that it would be prudent to reduce exposure to ochratoxin A as much as possible. Additional studies are necessary to provide information about these aspects since they are also essential to determine their relative contribution to ochratoxin A risk, their distribution pattern in relation with different naked oats substrates and environmental conditions and to devise efficient strategies to prevent ochratoxin A entering into the naked oats and food chain. Concern to the regulatory aspect, China should have a regulation for ochratoxin A in naked oats as soon as possible.

ACKNOWLEDGEMENTS

The research was supported by National "863" Program Grant (No. SS2012AA101006) from the Ministry of Science and Technology of the People's Republic of China. The item of Beijing emphasis construction discipline and Natural Science fund of Heilongjiang province(No. B201120) and the program on Detection and Control of Spoilage Organisms and Pesticide Residues in Agricultural Products (No. PXM2012_014207_000011).

REFERENCES

1. L. Alvarez, A.G. Gil, O. Ezpeleta, J.A. Garcia-Jalon and A.L. de Cerain, *Food Chem. Toxicol.*, **42**, 825 (2004).
2. K.J. Van Der Merwe, P.S. Steyn, D.B.F. Scott and J.J. Theron, *Nature*, **205**, 1112 (1965).
3. Y. Horie, *Nippon Kingakukai Kaiko*, **36**, 73 (1995).
4. J.I. Pitt, R. Plestina, G. Shepard, M. Solfrizzo and P.J. Verger, Joint FAO/WHO Expert Committee on Food Additives (JECFA): Safety Evaluation of Certain Mycotoxins in Food, Food and Agriculture Organization, Rome, p. 281 (2001).
5. J. Blesa, H. Berrada, J. Soriano, J. Molto and J. Danes, *J. Chromatogr. A*, **1046**, 127 (2004).
6. S. Li, R.R. Marquardt and A.A. Frohlich, *J. Agric. Food Chem.*, **46**, 4307 (1998).
7. I. Rossella, C. Luca, L.P. Anna, V. Attilio and R. Luca, *Food Control*, **22**, 1905 (2011).
8. Z. Chiraz, A. Salwa, Z. Lazhar and B. Chayma, *Food Control*, **20**, 218 (2009).
9. IARC, IARC Monographs on the Evaluation of Carcinogenic Risks to Human, Some Traditional Herbal Medicine, Some Mycotoxins, Naphthalene and Styrene (Vol. 82), Lyon, France: IARC (2002).
10. European Food Safety Authority, Opinion of the Scientific Panel on Contaminants in the Food Chain on a Request from the Commission Related to Ochratoxin A in Food, EFSA J. 365, 1-56 (2006).
11. G. Devegowda, M. Raju and H. Swang, *Feedstuffs*, **70**, 12 (1998).
12. B. Wang and F.B. Song, *Ecol. Environ. Sci.*, **15**, 625 (2006) (in Chinese).
13. V. Mario, M. Katherine, S. Carolina, A. Mario, C. Victor, V. Ricardo and V. Orialis, *Food Control*, **20**, 631 (2009).
14. J.N. Miller and J.C. Miller, Editorial Practice, pp. 111-155 (2002).
15. European Commission, Commission Regulation 1881/2006 of December 19, 2006, Setting Maximum Levels for Certain Contaminants in Foodstuffs, *Official Journal of the European Communities*, **L364**, 16 (2006).
16. FAQ, Sampling Plan for Aflatoxins Analysis in Peanuts and Corn. FAO Food Nutrition Paper (p. 64). Rome, Italy: FAQ (1997).
17. European Commission, Commission Regulation (EC) No. 472/2002 of 12 March 2002 Amending Regulation (EC) No. 466/2001 Setting Maximum Levels for Certain Contaminants in Foodstuffs, *Official Journal of the European Communities*, **L75**, 18 (2002).