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Short communication

Occurrence and daily intake of ochratoxin A of organic and non-organic rice and rice products

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Abstract

Ochratoxin A (OTA) was extracted from 84 rice samples and rice products by using accelerated solvent extraction (ASE) and analysed with liquid chromatography coupled with fluorescence detection. Samples were collected from rice cultivars, local markets and supermarkets; 64 were of non-organic and 20 of organic production. 7.8% of non-organic samples had OTA levels from 4.3 to 27.3 μg/kg and in 30% of organic samples was detected the presence of this mycotoxin varying from 1.0 to 7.1 μg/kg. OTA presence was confirmed by methyl-ester derivatization.

Rice and rice products labelled with denomination of origin (DO) were not detected OTA due to the fact that its production has implemented food safety measures such as good agricultural practices (GAPs), good manufacturing practices (GMPs), and the hazard analysis and critical control point (HACCP) system. Estimated daily intake of OTA was 0.17 ng/kg b.w./day. This value reflects that the analysed samples have a minimal contribution to toxicological risk.

Keywords: Rice; Rice products; Non-organic; Organic; Estimated daily intake

1. Introduction

Rice (*Oryza sativa*) is the main food of half of our planet (FAO, 2004). Rice cultivation has been carried out in all regions that have the necessary warmth and abundant moisture ideal to its growth, mainly subtropical rather than hot or cold weather conditions. In Spain, moisture and temperature are critical factors in both natural contamination and mycotoxin production, especially in some regions like the "Albufera" of Valencia, a natural lake close to the Mediterranean Sea, "Calasparra" and "Delta del Ebro" where rice is grown and labeled with a Denomination of Origin (DO), which contributes to food safety and food control (Council Regulation, 1991; MAPA, 2004).

These conditions are adequate to the growth of different mycotoxigenic moulds as are *Penicillium verrucosum*, *Aspergillus ochraceus* and *A. carbonarius* that are producers of ochratoxin A (OTA) in different countries and at varying concentrations (Albert and Gauchi, 2002; Ramakrishna et al., 1996; Trung et al., 2001; Pardo et al., 2004; Arroyo et al., 2005; Kokkonen et al., 2005). This mycotoxin has been experimentally shown to be teratogenic, a potent renal carcinogen, immunosuppressive, an enzyme inhibitor, has effects on lipid peroxidation and has been implicated in Balkan nephropathy in humans, it is listed as a possible carcinogen of group 2B by the International Agency for Research on Cancer (IARC, 1993). European Union regulates OTA level in cereals and cereal products with maximum residue levels (MRLs) that cannot be greater than 5 and 3 μg/kg, respectively (Commission Regulation, 2005). Generally, methods of analysis for OTA include the usual basic steps applied in mycotoxins methodology such as extraction, cleanup and determination, sometimes followed by confirmation of identity (Miraglia et al., 2004).

The objective of this study was to obtain data on the occurrence of OTA from organic and non-organic rice and rice products that are obtained from rice cultivars, local markets and supermarkets. Furthermore, it helped to evaluate its potential contribution to the dietary OTA exposure of consumers of these products.

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2. Materials and methods

2.1. Chemical and reagents

OTA crystalline material was purchased from Sigma (St. Louis, MO, USA). A stock standard solution of OTA at 500 μg/mL in methanol was prepared and kept wrapped in aluminum foil at −20 °C, due to OTA gradually breaks down under UV light. OTA working solutions were prepared by diluting in the same solvent and stored in glass stopped tubes at −20 °C. Deionised water (<8 MΩ cm resistivity) was obtained using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Liquid chromatography (HPLC) grade methanol was supplied by Merck (Darmstadt, Germany). Formic acid was obtained from Scharlau (Barcelona, Spain).

2.2. Samples

Rice samples were collected from rice cultivars and local markets and supermarkets. All samples were stored in sealed plastic bags and kept at room temperature in a dark and dry place. The samples were divided with a subsample divider. A 200 g subsample was milled and collected in a plastic bag and then stored in the same conditions until analysis.

2.3. Extraction procedure

An automated Dionex ASE 200 system (Dionex Co., Sunnyville, CA) was used for OTA extractions. Stainless steel extraction cells were sealed at one end with circular cellulose filters of 1.98 cm diameter (Dionex Co.). Milled rice (5 g) was placed into an 11 mL extraction cell and extracted with methanol at 1500 psi of pressure, temperature 40 °C, 5 min of static time, 50% flush volume, 60 s of purge time, one cycle and 11 mL cell size. The total extraction time was around 12 min. OTA was extracted with 15 mL methanol and the extract was concentrated using a Büchi Rotavapor R-200 until a volume of 5 mL was reached. The eluate was evaporated at 55 °C with a gentle stream of nitrogen and reconstituted with methanol to a final volume of 0.5 mL. This final solution was degassed for 5 min using a Branson 5200 (Branson Ultrasonic Corp., CT, USA) ultrasonic bath and then injected into the liquid chromatographer (LC) coupled on fluorescence detection (FD).

2.4. Liquid chromatography analysis

A Shimadzu (Kyoto, Japan) SCL-6A system LC equipped with two LC-6A pumps, a Rheodyne Model 7125 injector (20 μl loop) and a SRF-535 fluorescence detector was used. A LC phenomenex column Luna C_{18} (5 μm) (150 × 4.6 mm I.D.) was employed with a mobile phase consisting of methanol–formic acid 0.1M (70:30 v/v) at a flow rate of 0.7 ml/min. Detection of OTA was carried out using 334 and 464 nm as wavelengths for excitation and emission, respectively (Blesa et al., 2004). The limit of detection (LOD) (s/n 3:1) and limit of quantification (LOQ) (s/n 10:1) were 0.01 and 0.03 μg/kg, respectively. Recoveries obtained with the proposed method in samples with a level of OTA of 5 μg/kg were over 90%; such recoveries were considered as valid for analysing residues of ochratoxin A in foods (Commission Directive, 2002/27/CE).

2.5. Confirmation procedure

Identity of OTA was confirmed by methyl ester formation according to Zimmerli and Dick (1995), briefly this technique consists in adding 2.5 mL of methanol and 0.1 mL of concentrated hydrochloric acid to the OTA residue, the vial is closed and kept overnight at room temperature. The reaction mixture is evaporated to dryness and the residue re-dissolved in mobile phase. Then 20 μL are analysed using liquid chromatography coupled with fluorescence detection.

3. Results and discussion

3.1. Occurrence of OTA in rice and rice products

The results of non-organic rice and rice products are shown in Table 1. OTA was present in 5 out of 63 samples analysed; these included puffed, white, wild rice, wild rice products and rice from rice cultivars. All positive samples exceeded the maximum permitted levels in the European Union, which cannot be greater than 5 and 3 μg/kg for cereals and cereal products, respectively. Fig. 1 shows the chromatogram of OTA found in a puffed rice sample.

The highest value of OTA is obtained from the rice sample obtained directly from the cultivars; rice in this stage is called “paddy rice” and it is the whole grain taken off the plant at harvest. White rice grain that is normally consumed makes up less than three-quarters of the weight of a paddy rice grain, which also includes the hull and bran. Taking the latter under consideration, one could explain the high OTA concentration found in this sample given that in the hulls is where the highest

Table 1
Occurrence of ochratoxin A in non-organic rice and rice products

Sample	Country	Incidence	Range of OTA (ng/g)
Basmati rice	Holland	0/1	Nd
Basmati rice	Spain	0/2	Nd
Bomba rice	Spain	0/5	Nd
Brown rice	Spain	0/6	Nd
Parboiled rice	Spain	0/9	Nd
Puffed rice	Spain	1/2	7.3
Red rice	Spain	0/1	Nd
Rice[a]	Spain	1/1	27.3
Rice flour	Spain	0/2	Nd
Semolina	Spain	0/1	Nd
White rice	Spain	1/31	4.3
White rice	UK	0/1	Nd
Wild rice	Spain	1/1	21.0
Wild rice product[b]	Spain	1/1	7.3
Total		5/64	4.3–27.3

Nd=not detected (below the quantification limit).
[a] Rice samples from rice cultivars used for non-organic rice products.
[b] Including wild rice (20%)+white rice (70%)+vegetables (10%).

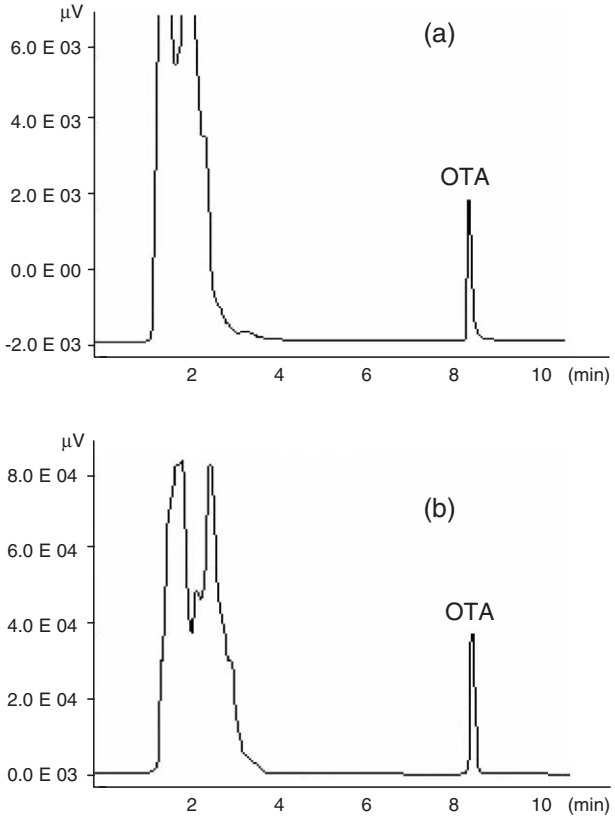

Fig. 1. LC-FD chromatograms obtained after ASE extraction: (a) rice sample fortified at 5 µg/kg of OTA and (b) positive rice sample (puffed rice) containing 4.3 µg/kg of OTA.

concentration of mycotoxins and mycotoxigenic moulds is found for most cereals. Once the grain is processed into rice products, a decrease of OTA contamination can be expected.

It has been reported that rice is naturally contaminated with *A. ochraceus* (Begun and Samajpati, 2000; Trung et al., 2001). Park et al. (2005) found that the fungal species most commonly present in rice are *Aspergillus* (e.g. *A. ochraceus*), *Penicillum* (e.g. *P. verrucosum*) and *Fusarium*. Since this product is not consumed directly, elaboration process could reduce the concentration of OTA. However, Ha et al. (1979) and Mheen et al. (1982) report that the dominant genus during storage of milled and paddy rice is *Aspergillus.*

In the other hand, wild rice has a higher OTA concentration than the conventional rice. Wild rice is a tall aquatic plant (*Zizania aquatica*) of the family *Gramineae* (grass family), of a genus separate from common rice (*O. sativa*). Wild rice is usually harvested at very high moisture levels (35–50 %) and is held for 7 to 14 days under these conditions to allow fermentation to take place, after completion of fermentation and before parching and hulling there are mould counts up to 83×10^3 CFU/g of rice, as well as bacteria and yeast. Therefore, it is very likely that mycotoxin-producing moulds could be also present and produce important quantities of OTA during this period (Lindenfelser et al., 1978), even though OTA concentration may possibly diminish with rice processing, final concentration could reach important levels, as is the case of wild rice samples analysed.

According to the study of Zhai et al. (1994, 2001), in wild rice, the protein and amino acid content is two times higher than that of white rice. Furthermore, essential amino acid values for white rice were lower than those of wild rice. Medina et al. (2004) suggested a positive correlation between protein content of grains and OTA level. In agreement with this finding and with our results, it may be deduced that wild rice is a better substrate than white rice for the characterization of OTA-producing *A. ochraceus* strains. Bacon et al. (1975) stated that 25% of glutamic acid is utilized for the synthesis of OTA. Serra and Escola (1997) hypothesized that stimulation of OTA production is probably due to the high free amino acid content in the substrate. This hypothesis can be supported by our results.

Non-organic samples had been labeled with a denomination of origin (DO) from “Calasparra”, “Valencia” and “Delta del Ebro” regions. In general, it can be said that the occurrence of OTA in this kind of products is low due to the fact that several food safety and quality standards as good agricultural practices (GAPs), good manufacturing practices (GMPs), and the hazard analysis and critical control point (HACCP) system must be followed (Council Regulation, 1991).

Table 2 reflects the occurrence of OTA in organic rice and rice products. Rice flour and rice cake exceeded the European legislated MRL, which is 3 µg/kg of OTA in cereal products. Brown and puffed brown rice values of OTA were below the maximum permitted levels for the European Union.

From results presented in Tables 1 and 2, it can be observed that the incidence of OTA in organic rice and rice products is higher than in conventional rice and rice products. This may be a consequence of organic production since it involves varied cultivation practices, limited use of non-synthetic fertilizers, pesticides, fungicides, conditioners of low solubility and prohibits the use of genetically modified organisms and/or any products derived from such organisms, giving a wider margin for probable fungi proliferation and mycotoxin production.

Miraglia and Brera (2002) reported that 4 out of 22 rice samples contained OTA with a mean concentration of 0.11 µg/kg and in none of the rice samples from Uruguay and United

Table 2
Occurrence of ochratoxin A in organic rice and rice products

Sample	Country	Incidence	Range of OTA (ng/g)
Basmati rice	India	0/1	Nd
Brown rice	Italy	0/1	Nd
Brown rice	Thailand	0/1	Nd
Brown rice	Spain	1/6	1.0
Puffed brown rice	Spain	1/1	2.8
Red rice	Spain	0/1	Nd
Rice cake	Italy	1/1	4.0
Rice cake	Spain	2/3	3.6–7.1
Rice flour	Spain	1/1	3.3
Rice “gofio”	Spain	0/1	Nd
Semolina	Spain	0/1	Nd
Sweet brown rice	Italy	0/1	Nd
White rice	Spain	0/1	Nd
Total		6/20	1.0–7.1

Nd=not detected (below the quantification limit).

Kingdom was detected the presence of this mycotoxin. Scudamore et al. (1997) showed that OTA occurred in 3 out of 40 rice samples from UK with a mean concentration between 1 and 19 μg/kg. Furthermore, in 25 Vietnamese rice samples were contaminated with OTA at 21.3 and 26.2 μg/kg (Trung et al., 2001). Abdelhamid (1990) detected the occurrence of OTA in 33% of rice germs and rice germ cake with an average value of 577 and 4 μg/kg, respectively. Blesa et al. (2004) did not detect the presence of OTA in none of the rice samples studied.

3.2. Estimated daily intake of OTA

On the other hand, a study of risk assessment of OTA was carried out. In Spain, an average adult (70 kg) consumes around 16.4 g of rice and rice products per day (Ministerio de Agricultura, Pesca y Alimentación, 2004). The mean OTA content of food measured in this study was 0.74 μg/kg, excluding the rice sample from rice cultivars that was used (Table 1), since it is not consumed directly. OTA estimated daily intake was 0.17 ng/kg b.w./day. This value is considerably lower than the provisional tolerable daily intakes that are 14 ng OTA/kg b.w./day established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2001) and 5 ng OTA/kg b.w./day according to the Scientific Committee on Food of the European Commission (1998).

Based on the data exposed in this paper, it could be stated that OTA present in rice and rice products contributes with 1.2% and 3.4%, respectively, to the level considered at risk for human health caused by OTA exposure in Spain alone. Even when these percentages might seem low, the accumulation of OTA in the organism should also be considered when evaluating the risk of exposure, for the reason that OTA once in the stomach, it is quickly resorbed into the blood where it binds to plasma proteins in relatively high proportion in humans, pigs and monkeys. There is a considerable inter-species variation with regard to the biological half time that affects the residues. For example, for pigs are 88–140 h and 4.1 h for chickens (Hagelberg et al., 1989). This last statement along with the fact that rice is the staple food for more than half of the world population; that in Asia alone, more than 2000 million people obtain 60% to 70% of their calories from rice and its products; it is the most rapidly growing source of food in Africa, and that it is of significant importance to food security in an increasing number of low-income food-deficit countries, increase the importance of evaluating OTA occurrence in this cereal.

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