Article

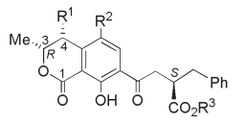
The Quantitation of Ochratoxin A in Foodstuffs Sold in New Zealand

Darren A. Saunders,[a] Shirley Jones,[a] and Rudolf Schmitt[b]

[a]Institute of Environmental Science and Research (ESR) Ltd, 27 Creyke Road, Ilam, Christchurch and [b]University of Zurich, Switzerland (e-mail: *darren.saunders@esr.cri.nz*)

Introduction

Ochratoxins are naturally occurring mycotoxins produced primarily by *Aspergillus ochraceus* and *Penicillium verrucosum*.[1] Ochratoxin A (OA; **1**) is the most abundant and the most toxic of the mycotoxin group that also includes ochratoxins B (**2**) and C (**3**), **4**, the 4-hydroxy derivative of **1**, and methyl esters of ochratoxins A, B, and C where R^3 = Me.[2] Ochratoxin A occurs in plants such as cereals (mainly wheat, oats and barley), beans, nuts, coffee and cocoa beans, dried fruits, spices, and wine. It has also been found in the kidney's of pigs fed with contaminated feed;[3,4] it is nephrotoxic, hepatotoxic, carcinogenic, teratogenic, genotoxic, and immunotoxic.[4,5]



1, ochratoxin A: $R^1 = R^3 = H$; $R^2 = Cl$
2, ochratoxin B: $R^1 = R^2 = R^3 = H$
3, ochratoxin C: $R^1 = H$; $R^2 = Cl$; $R^3 = Et$
4, $R^1 = OH$; $R^2 = Cl$; $R^3 = H$

The Joint UN Food and Agriculture Organization and World Health Organization Expert Committee on Food Additives (JECFA) has proposed a provisional maximum tolerable weekly intake of 0.1 µg/kg body weight for **1**; the European Union recently adopted a limit of 2.0 µg/kg for it in red and white wines.[5] Of the spices, paprika and chilli are particularly prone to contamination. In general, the limit adopted by the international spice trade for **1** is in the range 10-20 µg/kg.[2] The European Union has proposed tolerance levels for **1** of 1 µg/kg for infant foods and 5 µg/kg for other foods. Other countries with legislation applying to 1 have regulatory limits ranging from 1 to 50 µg/kg.[4,6] Little information is available regarding the presence of **1** in NZ foodstuffs, although ESR undertook a survey of 160 selected foods and wines including coffee, cereals, grains, dried fruit, and pate in 2000.[7]

During 2007 we extended this study to examine a range of NZ foodstuffs employing an improved detection method for **1** that is based upon HPLC analysis. Hereafter this is referred to as the 2007 survey.

Experimental

The amount of ochratoxin A (**1**) in a variety of foodstuffs including cereals, dried fruits, spices, and wines was determined using an High-Performance Liquid Chromatographic (HPLC) method that was adapted primarily from that of Lobeau *et al*.[8] After establishing the validity of the analytical procedure, a small survey of the levels of **1** in foodstuffs known to be prone to OA contamination, and available locally (except for wines that were purchased in Auckland), was conducted (see Table 2).

Method - sample extraction

A sample of the foodstuff (15 g) was blended with 150 mL of 4:1 MeOH/aqueous $NaHCO_3$ (3% w/v). The mixture was then filtered through Whatman No 4 filter paper and a 10 mL aliquot of the filtrate was removed and diluted to 50 mL with 0.01 M phosphate buffered saline (pH 7.4) containing casein (0.1% w/v). This solution was then passed through an OchraTest immunoaffinity column (Vicam USA; #G1034) under gravity. The column was subsequently washed with two 5 mL aliquots of deionised water.

Ochratoxin A (**1**) was eluted from the immunoaffinity column with methanol (4 mL) which was concentrated to dryness at 50°C (reduced pressure) and the residue made up to 1 mL with the HPLC mobile phase.

HPLC analysis

Column: 220 x 4.6 mm i.d. Applied Biosystems RP-18, 5 µm bead diam. with a 15 x 3.2 mm i.d column. RP-18, 7 µm bead diam. guard column; mobile phase: MeCN (47%), aq.AcOH (53%; 1% v/v); flow rate: 1.5 mL/min; detection: fluorescence λ_{ex} = 333 nm, λ_{em} = 460 nm; injection volume: 50 µL

Linearity and reproducibility

The ochratoxin standard curve was linear over the range 0.1–50 µg/L, all $r^2 > 0.99$. The limit of detection (LOD) in samples was <0.1 µg/kg (chromatographic peak to baseline noise ratio: 3:1). The limit of quantification (LOQ) in samples was <0.5 µg/kg (ratio chromatographic peak to baseline noise 10:1); spiked recoveries were > 80% over the range 0.1 – 50 µg/L ochratoxin regardless of matrix investigated.

Table 1. Repeatability

Matrix	OA spike Conc (µg/kg)	No repeats	[OA] found (µg/kg)	CV[a] (%)
Wine	5	5	4.6	4.3
Bread	2	5	2	10.5
Bread	10	5	10	8.4
Paprika (authentic)	43.2	5	43.2	7.6

[a]Coefficient of variation

Results

Table 2. Ochratoxin A (1) in local and imported foodstuffs purchased in Christchurch.

Food Matrix	Total samples	+ve samples	% +ve	Range OA (1)/µg/kg
Cereals	**23**	**7**	**30**	**0.11-2.85**
Extruded	13	3		0.20-1.12
Muesli	9	4		0.11-2.85
Weetbix	1	0		
Coffee	**8**	**7**	**88**	**<0.1-1.02**
Dried Fruits	**10**	**3**	**30**	**0.28-1.02**
Apricots	2			
Dates	2	1		1.02
Figs	2			
Prunes	1			
Raisins	3	2		0.28-0.74
Spices	**10**	**10**	**100**	**0.23-50.60**
Chilli	4	4		0.23-39.91
Nutmeg	2	2		4.26-23.52
Paprika	4	4		13.28-50.6
Wines[a]	**21**	**1**	**5**	**1.25**

[a]Red and white wines both foreign and domestic

Discussion

The levels of ochratoxin A (**1**) in the majority of foodstuffs, *i.e.* bread, cereals, dried fruit, and coffee were below 5 0 µg/kg, while all the wines (foreign and domestic) had levels below the European Union 2 0 µg//L regulatory limit.

As with the 2000 survey, the 2007 investigation found the highest percentage of positive samples to be from coffee. In 2000 this comprised all samples (n = 21; 100%) with the amount falling in the range 0.2-2.7 µg/kg. The 2007 survey showed seven of eight samples to be positive (88%) but with a lower content range of 0.05-1.02 µg/kg. In 2000 the highest levels of **1** occurred in dried fruit (0.1 to 22 µg/kg; n = 10) whereas in 2007 the range was 0.28-1.02. The presence of **1** in cereals differed little from those found in 2000 (0.1-0.77 µg/kg; n = 22; 38% positive). However, the levels in some of the spice samples in this 2007 survey exceeded the recommended allowable concentrations used by the spice industry, especially for chilli and paprika. In addition, baby foods that tend to have lower regulatory limits set for **1** were not investigated. The relatively high levels of **1** in some cereals suggest further work in this area to be prudent.

It is known, at least in regard to paprika, that low moisture content (<11%) and water activity (<0.75) are crucial in preventing mould growth.[2] Water activity (a_w) is a measurement of the energy status of the water in a system. It indicates how tightly water is bound within a substance and is measured on a scale from 0 to 1.0. Pure water has an a_w value of 1.0 and saturated aqueous sodium chloride 0.76. Water activity is a useful parameter in determining food quality and safety as most moulds, yeasts, and bacteria cannot grow in products with an a_w of < 0.7. Water activity of foodstuffs can be determined easily and quickly using a water activity meter.[9] Further investigation of the relationship between water activity, moisture content, and the levels of **1** in spices may result in a simple and inexpensive means of ensuring that these levels are kept within acceptable limits.

Acknowledgements

The authors thank both Valerie Orchard and Dr Stephen On (ESR) for their support and encouragement. This study was funded from MoRST (Ministry of Research, Science & Technology) capability funding.

References

1. Sibinda, L.; De Saeger, S.; Van Peteghem, C. *J. Chrom. A* **2002**, *959*, 327-330; Ringot, R.; Cango, A.; Schneider, Y. and Larondelle, Y. *Chemico-Biol. Interactions* **2006,** *156*, 18-46.
2. Almela, L.; Rabe, V.; Sanchez, B.; Torella, F. *et al*. *Food Microbiol.* **2007**, *24*, 319-327.
3. Joosten, H.; Goetz, J.; Pittet, A.; Schellenberg, M and Bucheli, P. *Int. J. Food Microbiol.* **2001***, 65*, 39-44.
4. Solfrizzo, M.; Avantaggigiato, G. and Visconti, A. *J. Chrom. A* **1998**, *815*, 67-73.
5. Ratola, N.; Barros, P.; Simoes, T.; Cerdeira, A. *et al. Talanta* **2006**, *70*, 720-731.
6. Medina, A.; Valle-Algarra, F.; Gimeno-Adelantado, J.; Mateo, R. *et al. J. Chrom. A* **2006**, *121*, 178-183.
7. Shaw, I.; Stanton, D. and Mitchell, J. *Survey of susceptible foods for ochratoxin A*. A report to the NZ Ministry of Health, June 2000.
8. Lobeau, M.; Saeger, S.; Sibanda, L.; Barna-Vetro, I. And Peteghem, C. *Food Additives Contam.* **2007**, *24*, 398-405.
9. AquaLab Operators Manual - Water Activity Meter: Decagon Devices, Inc.; see. *www.decagon.com/aw*.