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Application of isotope dilution mass spectrometry: determination of ochratoxin A in the Canadian Total Diet Study

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Analytical methods are generally developed and optimized for specific commodities. Total Diet Studies, representing typical food products 'as consumed', pose an analytical challenge since every food product is different. In order to address this technical challenge, a selective and sensitive analytical method was developed suitable for the quantitation of ochratoxin A (OTA) in Canadian Total Diet Study composites. The method uses an acidified solvent extraction, an immunoaffinity column (IAC) for clean-up, liquid chromatography-tandem mass spectrometry (LC-MS/MS) for identification and quantification, and a uniformly stable isotope-labelled OTA (U-[$^{13}C_{20}$]-OTA) as an internal recovery standard. Results are corrected for this standard. The method is accurate (101% average recovery) and precise (5.5% relative standard deviation (RSD)) based on 17 duplicate analysis of various food products over 2 years. A total of 140 diet composites were analysed for OTA as part of the Canadian Total Diet Study. Samples were collected at retail level from two Canadian cities, Quebec City and Calgary, in 2008 and 2009, respectively. The results indicate that 73% (102/140) of the samples had detectable levels of OTA, with some of the highest levels of OTA contamination found in the Canadian bread supply.

Keywords: total diet; market basket survey; liquid chromatography-mass spectrometry (LC/MS); mycotoxins; ochratoxin A; processed foods

Introduction

Ochratoxin A (OTA) is a toxic secondary metabolite of certain *Aspergillus* spp. (e.g. *A. ochraceus* and *A. carbonarius*) and of *Penicillium verrucosum* (Abarca et al. 2001; Varga et al. 2002). The chemical structure of OTA is 7-L-β-phenylalanylcarbonyl-5-chloro-8-hydroxy-3,4-dihydro-3-*R*-methylisocoumarin. OTA is carcinogenic to rodents, possesses nephrotoxic, immunotoxic, teratogenic and genotoxic properties, and has been associated with human and animal kidney disease (Petzinger and Ziegler 2000; Clark and Snedeker 2006; Pfohl-Leszkowicz and Manderville 2007; Pfohl-Leszkowicz et al. 2007). The International Agency for Research on Cancer (IARC) has given a Group 2B classification to OTA, i.e. possibly carcinogenic to humans (Clark and Snedeker 2006). There is considerable information on the natural occurrence of OTA in human foods and foodstuffs, including cereals and cereal-derived foods, beer, coffee, beans, cocoa, dried vine fruit and other dried fruits such as figs, wine, grape juice, olives, nuts, spices, liquorice, botanicals, milk, and pork meat (particularly liver and kidney), as well as in human blood and mother's milk (Clark and Snedeker 2006; Duarte et al. 2009; Kuiper-Goodman et al. 2010). A wide variety of analytical methods has been used for surveillance of OTA in all kinds of foods (Monaci and Palmisano 2004; Visconti and De Girolamo 2005). They include immunoaffinity column (IAC) clean-up and liquid chromatography-tandem mass spectrometry (LC-MS/MS) determination. In addition, the commercial availability of stable isotope-labelled OTA allowed the development of isotope dilution mass spectrometry for the analysis of OTA (Noba et al. 2009), so extension to Total Diet Study (TDS) composites was a natural progression. To our knowledge, this is the first instance of this combination of technologies being successfully applied for a mycotoxin in a TDS.

Materials and methods

Sampling

The design of the Canadian TDS was described in detail by Conacher et al. (1989). Briefly, in each city, foods from four different supermarkets and a variety of fast-food chains were collected over a 5-week period

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during September and October by the Canadian Food Inspection Agency. The samples were shipped to the Department of Food Science, University of Guelph, Kemptville, ON, where they were prepared as they would be in an average Canadian household using standard recipes. The prepared foods were homogenized and combined, as per Canadian TDS protocol, into 149 different food composites. For the OTA determinations, 100 g of each composite were stored in glass jars with Teflon-lined caps at −20°C until analysis.

Only those foods deemed likely to contain OTA were selected for analysis.

Chemicals and materials

The OTA standard in crystal form was purchased from Sigma-Aldrich (St. Louis, MO, USA). The U-[$^{13}C_{20}$]-OTA was purchased from Biopure (Tulin, Austria). Toluene and chloroform were obtained from Caledon Laboratories Ltd (Georgetown, ON, Canada). Acetonitrile (ACN) and high-performance liquid chromatography (HPLC)-grade methanol (MeOH) were obtained from Fisher Scientific (Fair Lawn, NJ, USA). HPLC-grade acetic acid, American Chemical Society (ACS)-grade disodium hydrogen orthophosphate, potassium dihydrogen phosphate, and potassium chloride were obtained from EMD Science (Gibbstown, NJ, USA). ACS-grade sodium chloride was obtained from J. T. Baker (Philipsburg, NJ, USA). ACS-grade potassium dichromate (Baker Analyzed) was used to calibrate the ultraviolet (UV) light spectrophotometer. Water used throughout was generated with the Purelab Ultra Water Purification System from ELGA LabWater (VWS (UK) Ltd., Marlow, Bucks, UK). OchraTest WB columns were obtained from Vicam (Watertown, MA, USA).

Preparation and calibration of OTA

An OTA stock solution was prepared to give a concentration of about 25 μg ml^{-1} in toluene–acetic acid (99 : 1, v/v) and was calibrated using a UV spectrophotometer according to Association of Analytical Communities (AOAC) International Official Methods of Analysis 970.44, 971.22 and 973.37 (Horwitz and Latimer 2005). The calibrated OTA stock solution was diluted with additional toluene–acetic acid (99 : 1, v/v) to give a working standard solution of 2 μg ml^{-1}. An aliquot of 62.5 μl was pipetted into a 50 ml volumetric flask and the solvent was evaporated to dryness under a gentle stream of nitrogen. The residue was redissolved and diluted to volume with acetonitrile to prepare the standard calibrants. The OTA solutions were kept at –20°C until use.

Sample extraction and clean-up (IAC)

Approximately 10 g or 10 ml of the sample, spiked with a known quantity of U-[$^{13}C_{20}$]-OTA, were extracted with 30 ml chloroform and 30 ml phosphoric acid–saline solution (33.7 ml of phosphoric acid and 18 g of NaCl in 1 L of water). The mixture was homogenized and centrifuged. The bottom chloroform layer was then transferred to a 50 ml centrifuge tube and extracted three times with 15 ml phosphate-buffered solution (PBS) at pH 7.4. The resultant three PBS solutions were combined and passed through an IAC. Toxins bound to the antibody were eluted four times, with 1 ml methanol each time, into a silanized tube. The eluate was evaporated under a gentle stream of nitrogen. The residue was reconstituted in 200 μl of acetonitrile–water (1 : 9, v/v). For samples such as wine, beer and juice, approximately 10 ml of sample were diluted with 50 ml of PBS and spiked with a known quantity of U-[$^{13}C_{20}$]-OTA. The mixture was homogenized, centrifuged and passed through an IAC as previously described.

Liquid chromatography-tandem mass spectrometry analysis (LC-MS/MS)

A Waters Acquity ultra-performance liquid chromatograph (uPLC) coupled to a Waters Quattro Premier Mass Spectrometer (Waters, Milford, MA, USA) was used. The uPLC was equipped with a BEH C18 column (50 × 2.1 mm, 1.7 μm; Waters). The mobile phase consisted of a variable mixture of solutions A (water–formic acid, 99 : 1 v/v) and B (acetonitrile–formic acid, 99 : 1 v/v) at a flow rate of 0.3 ml min^{-1}. The linear gradient program was set at: 90% A at 0 min, 10% A at 7 min, 90% A at 8 min and 90% A at 10 min. The column temperature was maintained above the ambient temperature at 30°C. Injection volume was 20 μl.

The mass spectrometer was operated in the positive electrospray ionization mode with argon as the collision gas. Multiple reaction monitoring (MRM) mode was configured to monitor the following mass-to-charge ratio (m/z) transitions: both 404 to 239 and 404 to 358 for OTA, as well as 424 to 250 for U-[$^{13}C_{20}$]-OTA. The most abundant product ion (m/z 239) was used for quantitation while the second product ion (m/z 358) was for confirmation.

Calibration and data analysis

A five-point OTA standard curve ranging from 0.25 to 25 ng ml^{-1}, with a known quantity of U-[$^{13}C_{20}$]-OTA as internal standard, was prepared for sample analysis. Data collection and reduction was achieved using Micromass Masslynx software release number 4.1.

Quality assurance (QA) and quality control (QC) measures

The following measures were taken to ensure the validity of results: before sample analysis, a low concentration OTA standard ($0.25\,ng\,ml^{-1}$) was run in order to verify adequate system performance, defined as a total ion chromatograph (TIC) signal-to-noise (S/N) ratio greater than 10 : 1. In addition, the correlation coefficient (r^2) for the calibration range was required to be greater than 0.995. As previously mentioned, OTA stock solution was calibrated by standard AOAC International methods in order to ensure accuracy and multi-year consistency of results.

During sample analysis, each batch was prepared to include a reagent blank to control for background contamination and duplicate spiked samples ($0.25\,ng\,g^{-1}$) to confirm OTA recovery of $100\% \pm 33\%$. An intermediate-level calibration standard followed each sample batch; when compared with the leading calibration standard, a retention time within $\pm 3\%$ and response $\pm 20\%$ was considered to confirm system stability. An ion ratio of $1.9 \pm 20\%$ for m/z 239/358 represented the minimum requirement for the identification and confirmation of OTA. In addition, the per cent recovery for U-[$^{13}C_{20}$]-OTA for each sample needed to be greater than 15%.

Results and discussion

Method development

Background

The goal was to develop a single method that would work for the wide variety of food products present in a TDS, and would also be highly sensitive in order to account for the dilution effect from mixtures of contaminated and uncontaminated raw ingredients.

Several analytical approaches were considered. The direct analysis of crude extracts by LC-MS/MS with isotope-labelled OTA internal standard was considered but this would not achieve the sensitivity required for a TDS. A purification and concentration step such as IAC clean-up is needed to improve sensitivity.

IAC clean-up with HPLC and fluorescence detection was also considered. However, it is known that solvent extraction efficiencies, and therefore recoveries for mycotoxins can vary depending on the solvent and food product combination (Bradburn et al. 1995; Meister 1999; Ribeiro and Alves 2008; Malone 2010). Indeed, even liquid food products with no extraction may require method optimization of IAC conditions to improve recoveries (Noba et al. 2009). The different recoveries expected within a TDS would result in compromised quantitations. In order to ensure accurate quantitations, isotope dilution mass spectrometry with IAC clean-up to improve sensitivity was deemed the best option for a single TDS method. Isotope-labelled OTA (U-[$^{13}C_{20}$]-OTA) was added to the food products at the beginning of the process and carried through to the final quantitation, thereby correcting for the expected recovery differences.

Chloroform extraction

There are many solvent-extraction combinations used in OTA analysis. Chloroform was selected since it had been successfully used in a previous TDS (Sizoo and van Egmond 2005), in a duplicate diet study (Gilbert et al. 2001), in official methods of analysis for grains such as those of AOAC International (Horwitz and Latimer 2005, Method 991.44), and in a variety of non-grain matrices such as serum/blood, milk and meat (Zimmerli and Dick 1995; De Saeger et al. 2004; Moreno et al. 2005; Boudra and Morgavi 2006; Lino et al. 2008). The wide breadth of use suggested it as a good generic extraction solvent for the many types of matrices expected in the present TDS.

IAC purification and fortification

IAC was selected since it is an established technology and can be used both to purify and concentrate a sample in order to maximize sensitivity. An issue was identified during the developmental stage: the IACs contained varying levels of residual OTA incurred during the manufacturing process. The levels ranged from non-detected to above the limit of detection (LOD). In order to avoid potential false-positives, it was decided to precondition all IACs as follows: the PBS was drained and 10 ml of water were passed through the column, which was then washed with two 1-ml portions of methanol; next, 20 ml of the PBS were added to the column and allowed to drain to the top of the supporting material. This procedure removed the residual OTA from the IACs, which helped eliminate potential false-positives and maximized method sensitivity. Other IAC brands were not evaluated for residual OTA contamination.

Method validation and performance characteristics

Accuracy and precision estimation by spikes

To determine precision and recovery, duplicate samples of various commodities were spiked with 0.25 or $0.25\,ng\,ml^{-1}$ OTA. Based on a total of 17 duplicates (34 data points) spread over 2 years of samples, the total method precision was estimated to have a relative standard deviation (%RSD) of 5.5% with 101% recovery (Table 1). A one-way analysis of variance (ANOVA) was applied to the results in order to separate the error components. The %RSD of within- and

Table 1. Method accuracy and precision.

	Accuracy, duplicate recoveries (%)[a]		Precision, analysis of variance[b]	
Food product	1	2	Type of precision	Results (%RSD)
Year 1				
Tea	98.5	95.8	Within-run	4.4
Alcoholic drinks, wine	103.1	101.0		
Desserts	102.7	95.1	Between-run	3.3
Formulae, milk base	102.5	98.1		
Vegetables, peas, fresh	105.3	99.0	Total	5.5
Dinners, cereals plus vegetables plus meat	103.4	94.9		
Cereals, mixed	103.6	90.2		
Bread, white	104.9	111.6		
Pork, fresh	103.7	114.0		
Year 2				
Milk, 2%	102.6	99.8		
Lamb, fresh	95.3	102.4		
Cereals, corn	93.0	97.3		
Rice	106.4	108.3		
Peas, processed	96.0	90.2		
Tea	105.6	104.9		
Desserts	99.2	97.0		
Dinners, cereals plus vegetables plus meat	94.6	99.5		
	Average	101		

Notes: [a]Percentage recovery was calculated as $[(C_{obs} - C_{native})/C_{spike}] \times 100$, where C_{obs} is the observed concentration of the spiked composite; C_{native} is the concentration of the unspiked composite; and C_{spike} is the spiking level. C_{obs} and C_{native} were recovery-corrected using ^{13}C-labelled ochratoxin A (OTA). C_{spike} is 0.25 ng g^{-1} or 0.25 ng ml^{-1}for dry or wet composites, respectively.
[b]Analysis of variance (ANOVA) is expressed as percentage relative standard deviation (RSD).

between-run batch variability were 4.4% and 3.3%, respectively.

Accuracy evaluation by external reference materials

Two wine samples (T1755, T1785) were purchased from the Food Analysis Performance Assessment Scheme (FAPAS). The OTA concentrations in these samples were sufficiently well characterized from the results of laboratories participating in a proficiency test that they may be used as quality-control materials. Analysis of duplicate samples gave 1.63, 1.51 and 0.74, 0.76 µg l^{-1} for T1755 and T1785, respectively. The acceptance criteria were 0.91–2.35 µg l^{-1} (assigned value of 1.63 µg l^{-1}) and 0.50–1.27 µg l^{-1} (assigned value of 0.88 µg l^{-1}) for T1755 and T1785, respectively, indicating that the method was accurate.

Accuracy evaluation by method comparison

An oat sample was analysed by both this new method and an International Organization for Standardization (ISO)-accredited (standard) method. The accredited method was based on IAC clean-up with ultra-HPLC and fluorescence detection. Both results were recovery corrected. There was close agreement between the two methods, with 0.87 and 0.80 ng g^{-1} found with the new and standard methods, respectively.

Table 2. Ochratoxin A homogeneity and stability in select food products.

	Analysed in Year 1 (ng g^{-1})	Reanalysed in Year 2 (ng g^{-1})	
Food product		Duplicate 1	Duplicate 2
Bread, rye	1.00	1.08	1.06
Bread, white	0.77	0.84	0.85
Bread, whole wheat	1.27	1.23	1.25
Buns and rolls	1.02	1.00	1.01
Flour, white (wheat)	0.39	0.42	0.44

Limit of detection (LOD) and limit of quantitation (LOQ)

Due to the unique nature of each food product, the LOD and LOQ were estimated for each food product (Table 3). The LOD estimates were low and ranged from 0.001 (rice) to 0.008 ng g^{-1} (cheese). The LOD and LOQ were estimated as 3 : 1 and 10 : 1 *S*/*N* respectively.

Table 3. Results from two Canadian cities: Quebec City and Calgary.

Code	Food product	Results[a,b] Quebec City, 2008	Results[a,b] Calgary, 2009	Limit of detection (LOD), $S/N = 3:1$	Limit of quantification (LOQ), $S/N = 10:1$	Units
KK01	Alcoholic drinks, beer	0.04	0.01	0.002	0.005	$ng\,ml^{-1}$
KK02	Alcoholic drinks, wine	0.02	n.d.[c]	0.002	0.005	$ng\,ml^{-1}$
GG01	Baked beans, canned	0.01	n.d.	0.003	0.009	$ng\,g^{-1}$
GG02	Beans, string	0.53	n.d.	0.002	0.006	$ng\,g^{-1}$
HH05	Blueberries	n.d.	n.d.	0.002	0.005	$ng\,g^{-1}$
FF21	Bread, other	0.45	0.42	0.002	0.006	$ng\,g^{-1}$
FF03	Bread, rye	1.0	0.57	0.003	0.009	$ng\,g^{-1}$
FF01	Bread, white	0.77	0.67	0.003	0.010	$ng\,g^{-1}$
FF02	Bread, whole wheat	1.3	0.83	0.004	0.012	$ng\,g^{-1}$
FF20	Buns and rolls	1.0	1.2	0.003	0.011	$ng\,g^{-1}$
FF04	Cake	0.07	0.04	0.002	0.005	$ng\,g^{-1}$
JJ02	Candy	0.01	n.d.	0.003	0.010	$ng\,g^{-1}$
FF05	Cereals, cooked wheat	0.04	0.01	0.002	0.009	$ng\,g^{-1}$
FF06	Cereals, corn	0.01	0.01	0.003	0.010	$ng\,g^{-1}$
LL01	Cereals, mixed	0.43	0.01	0.003	0.010	$ng\,g^{-1}$
FF07	Cereals, oatmeal	0.11	0.11	0.003	0.009	$ng\,g^{-1}$
FF08	Cereals, rice and bran	0.27	0.26	0.004	0.011	$ng\,g^{-1}$
AA09	Cheese	n.d.	n.d.	0.008	0.025	$ng\,g^{-1}$
AA11	Cheese, processed	n.d.	n.d.	0.006	0.019	$ng\,g^{-1}$
NN04	Chicken burger	0.32	0.23	0.003	0.010	$ng\,g^{-1}$
NN06	Chicken nuggets	0.09	0.04	0.003	0.010	$ng\,g^{-1}$
JJ01	Chocolate bars	0.30	0.13	0.003	0.009	$ng\,g^{-1}$
KK04	Coffee	0.02	0.01	0.002	0.008	$ng\,ml^{-1}$
FF09	Cookies	0.21	0.20	0.003	0.008	$ng\,g^{-1}$
GG27	Corn chips	0.03	n.d.	0.003	0.008	$ng\,g^{-1}$
FF10	Crackers	0.44	0.03	0.003	0.010	$ng\,g^{-1}$
FF11	Danish, donuts and croissants	0.24	0.35	0.003	0.008	$ng\,g^{-1}$
LL02	Desserts	n.d.	n.d.	0.006	0.021	$ng\,g^{-1}$
LL03	Dinners, cereal plus vegetable plus meat	n.d.	0.01	0.002	0.008	$ng\,g^{-1}$
CC01	Eggs	n.d.	n.d.	0.005	0.017	$ng\,g^{-1}$
FF12	Flour, white (wheat)	0.39	1.7	0.004	0.014	$ng\,g^{-1}$
LL05	Formulae, milk base	n.d.	n.d.	0.005	0.017	$ng\,ml^{-1}$
LL06	Formulae, soya base	0.01	0.02	0.002	0.006	$ng\,ml^{-1}$
MM02	Frozen entrees	0.06	0.05	0.002	0.006	$ng\,g^{-1}$
HH10	Grape juice, bottled	n.d.	n.d.	0.002	0.006	$ng\,ml^{-1}$
HH11	Grapes	n.d.	n.d.	0.002	0.007	$ng\,g^{-1}$
NN03	Hamburger	0.44	0.22	0.001	0.003	$ng\,g^{-1}$
PP06	Herbs and spices	0.08	0.07	0.006	0.021	$ng\,g^{-1}$
NN05	Hot dogs	0.38	0.41	0.004	0.012	$ng\,g^{-1}$
AA07	Ice cream	0.02	0.03	0.002	0.007	$ng\,ml^{-1}$
BB07	Lamb	n.d.	n.d.	0.004	0.012	$ng\,g^{-1}$
BB09	Luncheon meats, canned	0.01	0.01	0.002	0.008	$ng\,g^{-1}$
LL08	Meat, poultry or eggs	n.d.	n.d.	0.002	0.007	$ng\,g^{-1}$
AA02	Milk, 2%	n.d.	n.d.	0.002	0.007	$ng\,ml^{-1}$
AA13	Milk, chocolate, 1%	0.02	0.01	0.002	0.006	$ng\,ml^{-1}$
FF13	Muffins	0.20	0.19	0.002	0.007	$ng\,g^{-1}$
JJ12	Nuts	0.02	0.03	0.002	0.007	$ng\,g^{-1}$
BB10	Organ meats	n.d.	0.01	0.004	0.015	$ng\,g^{-1}$
FF14	Pancakes and waffles	0.10	0.16	0.003	0.009	$ng\,g^{-1}$
FF15	Pasta, mixed dishes	0.13	0.07	0.002	0.005	$ng\,g^{-1}$
FF16	Pasta, plain	0.34	0.07	0.002	0.005	$ng\,g^{-1}$
JJ07	Peanut butter	0.04	n.d.	0.002	0.007	$ng\,g^{-1}$
GG14	Peas	n.d.	n.d.	0.003	0.010	$ng\,g^{-1}$
FF17	Pie, apple	0.11	0.13	0.002	0.006	$ng\,g^{-1}$
FF18	Pie, other	0.11	0.09	0.001	0.005	$ng\,g^{-1}$
NN01	Pizza	0.21	0.22	0.002	0.007	$ng\,g^{-1}$
MM01	Popcorn, microwave	n.d.	n.d.	0.002	0.007	$ng\,g^{-1}$

(*Continued*)

Table 3. Continued.

Code	Food product	Results[a,b] Quebec City, 2008	Results[a,b] Calgary, 2009	Limit of detection (LOD), $S/N = 3:1$	Limit of quantification (LOQ), $S/N = 10:1$	Units
BB05	Pork, cured	0.06	0.20	0.003	0.012	$ng\,g^{-1}$
BB04	Pork, fresh	0.03	0.23	0.004	0.011	$ng\,g^{-1}$
JJ08	Puddings	0.03	0.02	0.002	0.005	$ng\,g^{-1}$
HH17	Raisins	2.3	0.17	0.004	0.013	$ng\,g^{-1}$
FF19	Rice	n.d.[c]	n.d.	0.001	0.004	$ng\,g^{-1}$
JJ11	Seeds, shelled	0.07	0.35	0.003	0.008	$ng\,g^{-1}$
KK07	Soy beverage, fortified	0.05	0.02	0.002	0.006	$ng\,ml^{-1}$
PP07	Soya sauce	n.d.	n.d.	0.002	0.007	$ng\,ml^{-1}$
KK06	Tea	n.d.	n.d.	0.002	0.007	$ng\,ml^{-1}$
PP05	Vanilla extract	n.d.	0.02	0.002	0.006	$ng\,ml^{-1}$
BB06	Veal, cutlets	n.d.	n.d.	0.004	0.012	$ng\,g^{-1}$
LL09	Vegetables, peas	n.d.	n.d.	0.003	0.008	$ng\,g^{-1}$
BB11	Wieners and sausages	0.12	0.06	0.002	0.007	$ng\,g^{-1}$

Notes: [a]Results were corrected for recovery.
[b]Values below the estimated LOD are provided for potential statistical analysis. It has been reported that the relative uncertainty at the LOD is 100% and at the LOQ is 30%, both with a 95% confidence level (Taylor 1987).
[c]n.d., Not detected.

Matrix effects study

The type of food analysed may impact on the accuracy of the results. The *F*-test was used to evaluate whether there was any significant difference between commodities when results were corrected for recovery with isotope-labelled OTA (U-[$^{13}C_{20}$]-OTA). Statistical analysis of the 17 duplicate recoveries from various food products over 2 years (Table 1) indicated no significant difference in food products at the 95% confidence level when results were recovery corrected.

Results

Study considerations

The TDS is an important part of the Canadian government's surveillance programme. Among other purposes, the data can be used to estimate dietary intakes of nutrients and exposures to contaminants, to monitor trends in levels thereof, and to inform planning of targeted surveys. A consideration for interpreting the results of this study is that while high values demonstrate the presence of contaminants, low values cannot be taken as definitive proof of their absence. This is due to the high variability in mycotoxin levels observed in targeted surveys; the %RSD between samples can range from around 40% to 260% (Counil et al. 2005), while the variability between different lots of product can reach several orders of magnitude (Kuiper-Goodman et al. 2010). A good example from the present study to highlight this variability would be the raisin values for the 2 years: 0.17 and $2.3\,ng\,g^{-1}$. Another consideration is that the mixed nature of the foods analysed in the TDS makes it difficult to determine the main source of contamination.

Occurrence, levels and trends of OTA contamination

All the results are presented in alphabetical order by food product (Table 3). The highest value in 2008 was for raisins at $2.3\,ng\,g^{-1}$. The highest value in 2009 was for wheat flour at $1.7\,ng\,g^{-1}$. In both years, 73% (51/70) of the samples had detectable levels of OTA (greater than the LOD). In 2008, 67% (47/70) were above the LOQ, while in 2009, 61% (43/70) were above the LOQ. To help identify trends, Table 4 presents the top 20 OTA-contaminated samples sorted by the 2-year average result. Bread is a high-consumption staple food for both adults and children. Some of the highest levels of OTA contamination were found in the domestic bread supply for both sampling years (Table 4). Indeed, cereal-containing food products represent the majority (16/20) of the top 20 OTA contaminated samples (Table 4). This is not surprising, as cereals and cereal-derived products have been previously shown to be the main contributors to human exposure in both Europe (Miraglia and Brera 2002) and Canada (Kuiper-Goodman et al. 2010).

OTA homogeneity and stability in selected food products

In order to test for homogeneity and stability, five samples from 2008 were reanalysed in duplicate after 1 year in frozen storage (Table 2). The data indicate that

Table 4. Top 20 food products with the highest average level of OTA contamination.

Code	Food product	Description	Results (ng g^{-1})[a]			
			Quebec City, 2008	Calgary, 2009	Average	Cereal[c]
HH17	Raisins	Straight	2.3	0.17	1.3	No
FF20	Buns and rolls	Hotdog/hamburger buns : kaiser rolls (1 : 1) made into crumbs	1.0	1.2	1.1	Yes
FF02	Bread, whole wheat	Bread : toast (1 : 1) made into crumbs	1.3	0.83	1.1	Yes
FF12	Flour, white wheat	All-purpose	0.39	1.7	1.0	Yes
FF03	Bread, rye	Bread made into crumbs	1.0	0.57	0.78	Yes
FF01	Bread, white	Bread : toast (1 : 1) made into crumbs	0.77	0.67	0.72	Yes
FF21	Breads, other	Plain bagels : pita bread : English muffins (2 : 2 : 1)	0.45	0.42	0.44	Yes
NN05	Hot dogs	With : without condiments (1 : 1) from fast food outlets	0.38	0.41	0.40	Yes
NN03	Hamburger	Hamburger : cheeseburger (1 : 1) from fast food outlets, with condiments	0.44	0.22	0.33	Yes
FF11	Danish, donuts and croissants	Danish pastry-or-cinnamon buns : cake donuts : yeast donuts-or-dutchies : croissants (1 : 1 : 1 : 1)	0.24	0.35	0.30	Yes
NN04	Chicken burger	Plain, no condiments from fast food outlets	0.32	0.23	0.28	Yes
FF08	Cereals, rice and bran	Rice cereal: bran cereal : bran and raisin cereal (1 : 1 : 1)	0.27	0.26	0.26	Yes
GG02	Beans, string	String beans (raw or frozen) : string beans (canned) (1 : 1)	0.53	n.d.[b]	0.26	No
FF10	Crackers	Saltines or soda biscuits	0.44	0.03	0.24	Yes
NN01	Pizza	One medium combination pizza (pepperoni, mushroom, green pepper) from fast food outlets	0.21	0.22	0.22	Yes
LL01	Cereals, mixed	One box from each store, mixed together (types not specified), prepared with whole milk (AA01)	0.43	0.01	0.22	Yes
JJ01	Chocolate bars	Plain milk chocolate bars	0.30	0.13	0.22	No
JJ11	Seeds, shelled	Sunflower	0.07	0.35	0.21	No
FF09	Cookies	Oreo-type : chocolate chip : oatmeal (1 : 1 : 1)	0.21	0.20	0.21	Yes
FF16	Pasta, plain	Spaghetti (enriched):macaroni (enriched) (1 : 1)	0.34	0.07	0.21	Yes

Notes: [a]Results were corrected for recovery.
[b]n.d., Not detected.
[c]Cereal: contains a cereal such as wheat, oat, rye, etc. as a raw ingredient.

in these food products the samples remain homogeneous and OTA is stable after 1 year in frozen storage. Year-to-year consistency of results also minimizes between-year bias and suggests that differences in contaminant levels between 2008 and 2009 samples of the same product are true differences.

Bread is a high-consumption staple for both adults and children. The results indicate that bread and, more generally, cereal-containing food products, appear to be a primary source of OTA exposure for the 2008 and 2009 sampling years. Further research is needed to characterize and perhaps mitigate risk more fully.

Summary and conclusions

Previously, TDS composites were analysed for OTA using different methods for different food products. This approach was labour intensive and often lacked sufficient sensitivity. This paper describes a single, cost-effective, practical, in-house-validated analytical method that is both accurate and precise. It is well suited for the technical challenge imposed by the different food products in TDS.

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