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# Ochratoxin A Contamination in Coffees, Cereals, Red Wines and Beers in Taiwan

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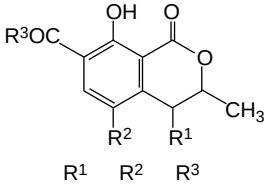
## ABSTRACT

The determination of ochratoxin A (OTA) was performed using immunoaffinity column for cleanup, HPLC for quantification and chemical derivation and LC-electrospray tandem mass spectrometry for confirmation. The detection limits for coffees, cereals and red wines and beers were 0.1, 0.3 and 0.2 ppb, respectively. The recoveries of OTA from coffees were 97.2 ± 4.9, 97.3 ± 3.1 and 95.4 ± 0.4% at 2, 4 and 8 ppb spiked levels, respectively. Spiked 0.3, 3 and 5 ppb OTA in cereals (mainly rice and wheat based food), the recoveries were 89.6 ± 5.1, 99.6 ± 1.4 and 90.2 ± 1.4%. The recoveries of red wines and beers were 91.9 ± 6.6. 99.4 ± 6.1 and 90.7 ± 0.6% at 0.2, 1 and 3 ppb spiked levels, respectively. In order to survey the OTA contents in daily diet in Taiwan, 51 samples of coffee, 114 samples of cereal (75 were rice or rice based samples and the rest were wheat flour or wheat based samples), and 10 samples of red wine and 18 samples of beer were analyzed. The results showed that OTA was detected in 13 (25%) coffee samples, 5 (50%) red wine samples and 2 (5%) wheat flour and wheat flour based samples at 0.1~0.5 ppb levels. According to the "Nutrition and Health Survey in Taiwan 1993~1996", the OTA consumption of adults were much lower than the established tolerable daily intake (TDI) of 5 ng/kg bw (European Commission) or tolerable weekly intake (TWI) of 100 ng/kg bw (JECFA).

Key words: ochratoxin A, coffee, cereal, red wine, beer, immunoaffinity column, high performance liquid chromatography (HPLC)

## INTRODUCTION

Ochratoxin A (OTA), a phenylalanyl derivative of a substituted isocumarin (shown in Figure 1), is a mycotoxin produced mainly by *Penicillium verrucosum*, by *Aspergillus ochraceus*, and by *Aspergillus carbonarius* together with a low percentage of isolates of the closely related *Aspergillus niger*[1]. These three groups of species differ in their ecological niches, in the commodities affected, and in the frequency of their occurrence in different geographical regions. *P. verrucosum* grows only at temperatures below 30˚C where water activity is above 0.80[2]. It is therefore found only in cool temperate regions and is the primary source of OTA in cereals and cereal products in Canada and Europe[3]. As cereals became widely used in animal feeds and OTA amounts remained relatively stable, this toxin is also found in some animal products, especially pig kidney and liver[3]. *A. ochraceus* grows at moderate temperatures where water activity is above 0.8[4]. It is found in stored food commodities and may also infect coffee beans during sun-drying[3]. *A. carbonarius* grows at high temperatures and was associated with maturing fruits, especially grapes[3]. Its presence had also been reported in beans, beer, dried fruit, grape juice, spices, wine, nuts, human blood plasma and mother's milk[5]. Some studies had shown that OTA contamination was mainly associated with storage; therefore, suitable post-harvest conditions such as temperature and moisture are important in preventing the growth of fungi and the production of the mycotoxin[3].




| | $R^1$ | $R^2$ | $R^3$ | |
|---|---|---|---|---|
| 1 | H | Cl | phenyl-CHCH(COOH)NH | **Ochratoxin A, OTA** |
| 2 | H | H | phenyl-$CH_2$CH(COOH)NH | **Ochratoxin B, OTB** |
| 3 | H | Cl | phenyl-$CH_2$CH($COOC_2H_5$)NH | **Ochratoxin C, OTC** |
| 4 | OH | Cl | phenyl-$CH_2$C(COOH)NH | **4-Hydroxy OTA** |
| 5 | H | Cl | OH | **Ochratoxin** α |

**Figure 1.** Chemical structures of ochratoxins.

OTA has nephrotoxic, carcinogenic, teratogenic and immunotoxic properties. Its main target is the renal proximal tubule, where it exerts cytotoxic and carcinogenic effects[3]. OTA can induce DNA damage, DNA repair and chromosomal aberrations in mammalian *in vitro* and DNA damage and chromosomal aberrations in mice treated *in vivo*; however, the mechanism of its genotoxicity is unclear. This mycotoxin can cross the placenta, and it is embryotoxic and teratogenic in rats and mice[6]. It also can inhibit the proliferation of B and T lymphocytes and affected the late stages of T-lymphocyte activation *in vitro*[3]. Moreover, it had been associated with Balkan endemic human nephropathy. Based on sufficient evidence in animals for kidney carcinogenicity of OTA but inadequate evidence in humans, the International Agency of Research on Cancer (IARC) classified OTA as a possible human carcinogen[7].

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Many countries have set regulatory limits of OTA for different commodities (Table 1), and the tolerable intakes have also been estimated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Canada and European Commission at 100 ng/kg bw/week, 1.5~5.7 ng/kg bw/day and not more than 5 ng/kg bw/day, respectively[8]. Up to now, Taiwan has not determined any regulatory limits for any commodities.

Taking the diet of people in Taiwan and the dietary exposure of OTA into consideration, the major dietary sources of OTA are cereals, but significant levels of contamination might be found in red wines and coffees[6]. Based on the "Nutrition and Health Survey in Taiwan 1993~1996"[8] conducted by the Department of Health (DOH), this survey was designed to develop methods to determine the OTA contamination status in retail coffees, cereals (including rice, rice based products, wheat flour and wheat flour based products), red wines and beers to understand the quantitative exposure of OTA in food in Taiwan.

## MATERIALS AND METHODS

### I. *Materials*

#### (I) *Sample collection*

The samples were collected from supermarkets, Taiwan Agricultural Chemicals and Toxic Substances Research Institute (TACTRI) and the Council of Agriculture (COA). TACTRI was holding a "Taiwan Total Diet Survey" from 2002~2004 and the samples they provided were collected from 8 counties of Taiwan in the spring and fall of 2003. Fifty-one samples were coffees: 27 were soluble coffees (of which 16 are instant coffees with sugar and creamer), 19 were ready-to-drink coffees, 3 were roasted ground beans and 2 were roasted beans. Seventy-five samples included 27 rice (1 rice, 17 brown rice and 9 rice mixed with several kinds of cereals) and 48 cooked rice based products (32 cooked rice and 16 traditional Chinese food: Wan-Quei and Rou-Zong). Ten brown rice samples and all the cooked rice based products were kindly provided by COA and TACTRI, respectively. Thirty-nine samples were 7 wheat flour (of which 4 were whole wheat flour) and 32 wheat flour based products (including 8 toasts and 8 cooked noodles). All the wheat based products were provided by TACTRI. Twenty-eight samples were 10 red wines (of which 8 were imported) and 18 beers (of which 9 were imported). The minimum sample size are 250 g or mL.

#### (II) *Reagents*

The OTA standard was purchased from Supelco (Bellefonte, PA, USA). The immunoaffinity columns OchraTest™ were purchased from Vicam (Watertown, MA, USA). Sodium hydroxide, sodium bicarbonate, sodium chloride, potassium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate, acetic acid and polyethylene glycol (PEG) were reagent grade. Acetonitrile and methanol used for the liquid chromatographic mobile phases were of LC grade. Distilled, deionized water was used throughout the procedure.

### II. *Methods*

#### (I) *Sample preparation*

##### (i) *Coffee samples*

The procedures were modified from Entwisle *et al.*[9]. Coffee beans were milled before extraction. Five grams test portion were weighted into 50-mL centrifuge tube, and added 25 mL of extraction solution (3% $NaHCO_3$/methanol = 1/1). The mixture was shaken gently for 30 min and centrifuged for 10 min at 4˚C at 2,500×g. The extract was filtered through filter paper. Ten milliliter of filtrate was pipetted and mixed with 10 mL phosphated-buffered saline (PBS, 8 g NaCl, 1.16 g $Na_2HPO_4$, 0.2 g $KH_2PO_4$ and 0.2 g KCl in 1 L water), followed by filtration through glass microfibre filter.

##### (ii) *Cereal samples*

The procedures were modified from Entwisle *et al.*[10]. Rice samples were milled before extraction. Twenty five

**Table 1.** Regulatory limits for OTA of different commodities in various countries[22]

| Country | Commodity | Limit (ppb) | Country | Commodity | Limit (ppb) |
|---|---|---|---|---|---|
| Austria | Wheat, rye, durum wheat | 5[a] | Romania | All foods, all feedstuffs | 5 |
| Brazil | Rice, barley, beans, maize | 50 | Sweden | Cereals | 5 |
| Czech Republic | All foods, children's foods, infant's foods | 20 | Switzerland | Cereals | 2 |
| Denmark | Cereals, pig kidney | 25 | The Netherland | All foods | 10 |
| France | Cereals | 5[a] | Uruguay | Rice, barley, beans, coffee, maize | 50 |
| Greece | Raw coffee beans | 20 | European Union | Cereals (raw) | 5 |
| Israel | Cereal (product) pulse (product), grain for feed | 50[b] 50 | | Cereals (products) Dried vine fruit | 3 10 |

[a]Guideline level.
[b]Proposal.

grams of test portion were weighted into a 500-mL blender jar and added 100 mL of extraction solution (acetonitrile/methanol = 6/4). The mixture was blended for 3 min and centrifuged for 10 min at 4˚C at 2,500×g. The extract was filtered through filter paper. Four milliliter of filtrate was pipetted and mixed with 44 mL of phosphate-buffered saline (PBS), followed by filtration through glass microfibre filter.

(iii) *Red wine and beer samples*

The procedures were modified from Visconti *et al.*[11]. Beers were degassed by sonicating for 1 hr. Ten milliliter of test portion were poured into a 50-mL centrifuge tube and added 25 mL of extraction solution (10 g PEG and 50 g $NaHCO_3$ in 1 L water). The mixture was shaken vigorously and centrifuged for 10 min at 4˚C at 2,500×g, followed by filtration through glass microfibre filter.

(II) *Immunoaffinity clean up*

All the filtrates of cereal samples and 10 mL of filtrates of coffee and red wine and beer samples were passed at about 1-2 mL/min through an OchraTest™ immunoaffinity column. Twenty milliliter of water was used to wash the loaded immunoaffinity column at a steady flow rate. The column was blown to dryness with a stream of nitrogen, and OTA was eluted with 2 mL of methanol. The methanol eluate was evaporated to dryness under a gentle stream of nitrogen, and the residue was redissloved by vortexing with 1 mL of acetonitrile, which was then filtered through a 0.45 µm microfilter for HPLC analysis.

(III) *HPLC analysis*

The LC system consisted of a Shimadzu (Kyoto, Japan) LC-10$AT_{VP}$ pump, a RF-10$A_{XL}$ fluorescence detector, and a Waters $717_{plus}$ autosampler, all under the control of a Shimadzu SCL-10$A_{VP}$ system. Data acquisition was performed on a SISC program. The column (250 × 4.6 mm, Cosmosil 5C18-AR-II, 5 µm, Nacalai, Japan) was maintained at 30˚C. Injection volume was 100 µL and the detector wavelength settings were 333 nm (excitation) and 460 nm (emission). The mobile phase, water/acetonitrile/acetic acid (99/99/2, v/v), was pumped at a constant flow rate of 1.0 mL/min.

(IV) *Calibration*

The OTA standard was diluted with mobile phase to prepare a series of working solutions containing 0.02–10 ng OTA / 200 µL. A calibration curve was constructed by plotting the peak area for each standard against the mass of OTA injected. Slope and intercept data of the calibration curve were used to compute the quantity of the analyte in the sample extracts.

(V) *Validation*

Samples, spiked with various concentrations of standard solutions, were analyzed. Limits of detection were based on a signal to noise (S/N) ratio with 3:1 as the minimum. Recovery tests were performed in triplicate by spiking standards at 3 different levels into OTA-free samples: 2, 4, and 8 ppb in coffee, 0.3, 3, and 5 ppb in cereal (rice powder), 0.2, 1, and 3 ppb in red wine. The spiked samples and blank samples without standard were analyzed by HPLC. Recovery was determined by the comparison of the amount of OTA added with the amount of OTA found.

(VI) *Confirmation*

The identity of OTA was confirmed by methyl ester formation according to Zimmerli and Dick[12] and LC-electrospray tandem mass spectrometry. Five hundred microliter of the LC sample injection was evaporated to dryness at 40˚C under nitrogen and redissolved in 500 µL of methanol. One hundred microliter of concentrated HCl was added and the mixture was vortexed and allowed to sit overnight. The mixture was again evaporated to dryness, reconstituted in LC mobile phase and reinjected. The disappearance of the OTA peak and the appearance of a new peak corresponding to the methyl ester of OTA confirmed the original presence of OTA.

Mass spectra were acquired using a Quattro Ultima MS/MS (Micromass, MA, USA). The electrospray capillary voltage was set at 112 kV and collision energy was 21 V. Under negative electrospray ionization (ESI) and using a 4.6 × 250 mm Cosmosil 5C18-AR-II (5 µm) analytical column with water/acetonitrile/acetic acid (33/35/32, v/v), OTA was monitored by multiple reaction monitoring. In the ESI full spectrum, the most intense ion was the $M\text{-}H^-$ ion at *m/z* 402.0. By $MS^2$, the most ion important fragment was the $[M\text{-}H\text{-}CO_2]$ ion at *m/z* 357.9.

(VII) *Quantification*

OTA mass concentration of the test sample was calculated using the following equation:

$$W_m = W_a \times (V_f/V_i) \times (1/V_s)$$

Where $W_m$ = the numerical value of OTA mass concentration in the test sample in ppb (ng/g or ng/mL); $W_a$ = the numerical value of the amount of OTA corresponding to the area of OTA peak of the sample extract (ng); $V_f$ = the numerical value of the final volume of redissolved eluate (µL); $V_i$ = the numerical value of the final volume of injected eluate (µL); $V_s$ = the numerical value of volume or mass of prepared test portion passing through the column (mL or g).

Express the results to 3 significant figures.

## RESULTS

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I. *Analytical Method Performance*

(I) *Calibration*

Within the calibration range of 0.02-10 µg/200 µL of OTA, the HPLC responses (peak area) were positively regressed with injected OTA mass and gave a correlation coefficient of $R^2 = 0.9989$, as shown in Figure 2.

(II) *Validation*

The method detection limits of coffee, cereals and red wines and beers were 0.1, 0.3 and 0.2 ppb, respectively. Specificity of the OTA peaks were clearly shown in HPLC chromatograms of Figure 3, which showed the absence of interfering signals at the OTA retention time for coffee, cereals and red wines and beers. The recoveries of OTA in coffees, cereals and red wines were 95.4-97.3, 89.6-99.6, and 90.7-99.4%, respectively, as shown in Table 2.

(III) *Confirmation*

The presence of OTA was confirmed in the extracts of 20 samples (13 samples of coffee, 2 samples of cereal and 5 samples of red wine). In each case, the original OTA peak was substantially diminished and a new peak corresponding to the methyl ester of OTA appeared, confirming the presence of OTA in the samples (Figure 4). LC-electrospray tandem mass spectrometry was applied to two samples and

**Table 2.** Recoveries of OTA in different samples

| Samples | Spiked levels (ppb) | Recovery[a] (%) | CV[b] (%) |
|---|---|---|---|
| Coffee | 2 | 97.2 ± 4.9 | 5.0 |
| | 4 | 97.3 ± 3.1 | 3.0 |
| | 8 | 95.4 ± 0.4 | 0.4 |
| Rice powder | 0.3 | 89.6 ± 5.1 | 5.7 |
| | 3 | 99.6 ± 1.4 | 1.4 |
| | 5 | 90.2 ± 1.4 | 1.5 |
| Red wine | 0.2 | 91.9 ± 6.6 | 7.1 |
| | 1 | 99.4 ± 6.1 | 6.1 |
| | 3 | 90.7 ± 0.6 | 0.6 |

[a]Average of triplicate analysis ± standard deviation.
[b]CV: coefficient of variation.

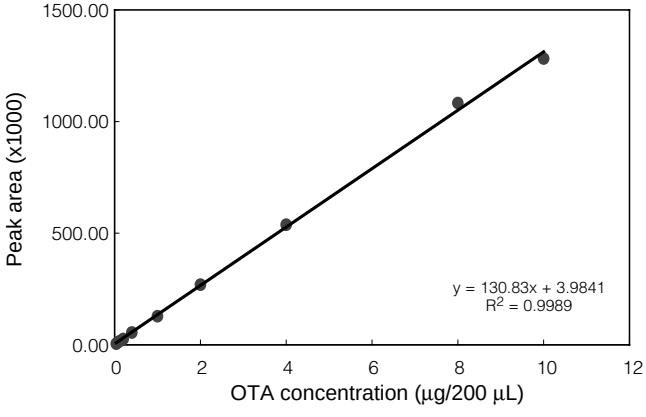


**Figure 2.** Calibration curve of OTA analyzed by HPLC.

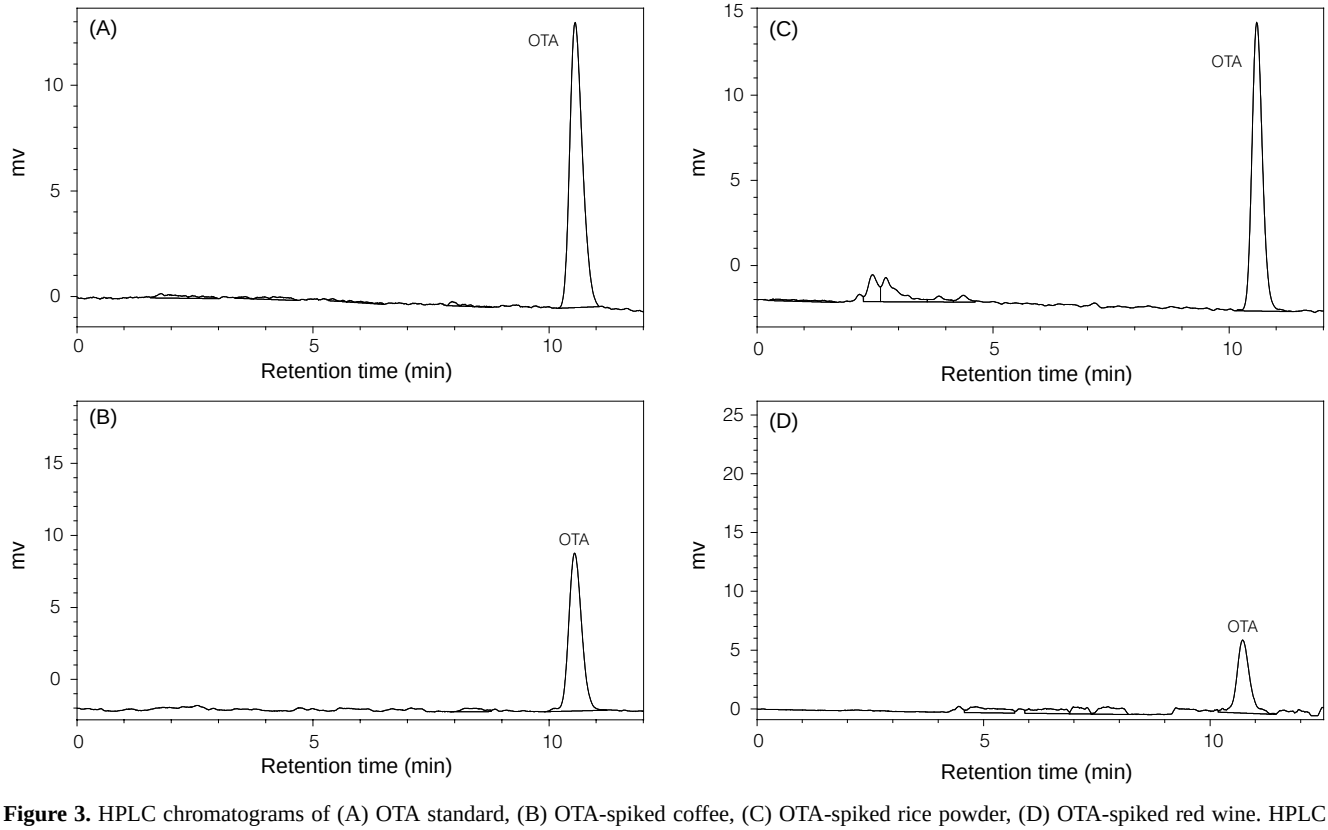


**Figure 3.** HPLC chromatograms of (A) OTA standard, (B) OTA-spiked coffee, (C) OTA-spiked rice powder, (D) OTA-spiked red wine. HPLC conditions: Cosmosil 5C18-AR-II; mobile phase, acetonitrile/water/acetic acid = 68/24/8; flow rate, 1.0 mL/min.

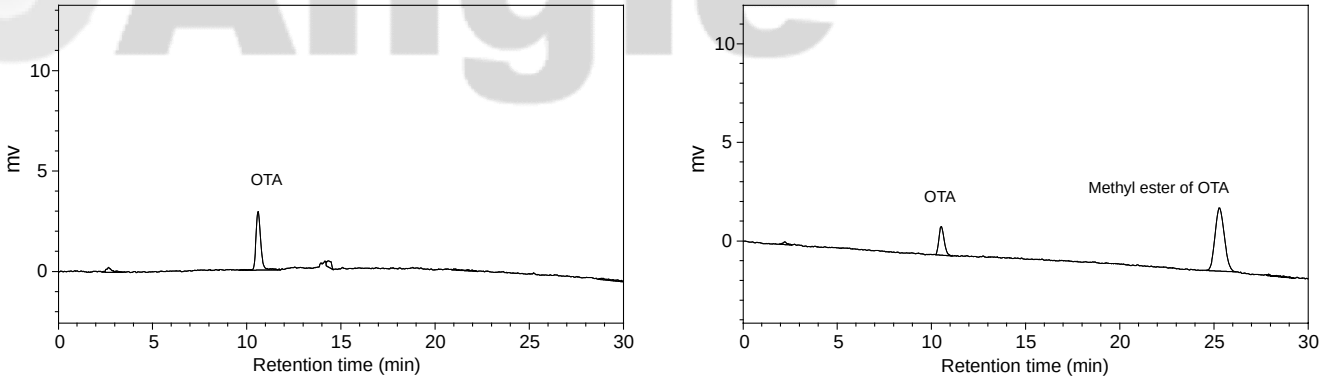


**Figure 4.** HPLC chromatograms of instant coffee extract (0.5 ppb sample) before (left) and after (right) mehyl esterification. For conditions, see text.

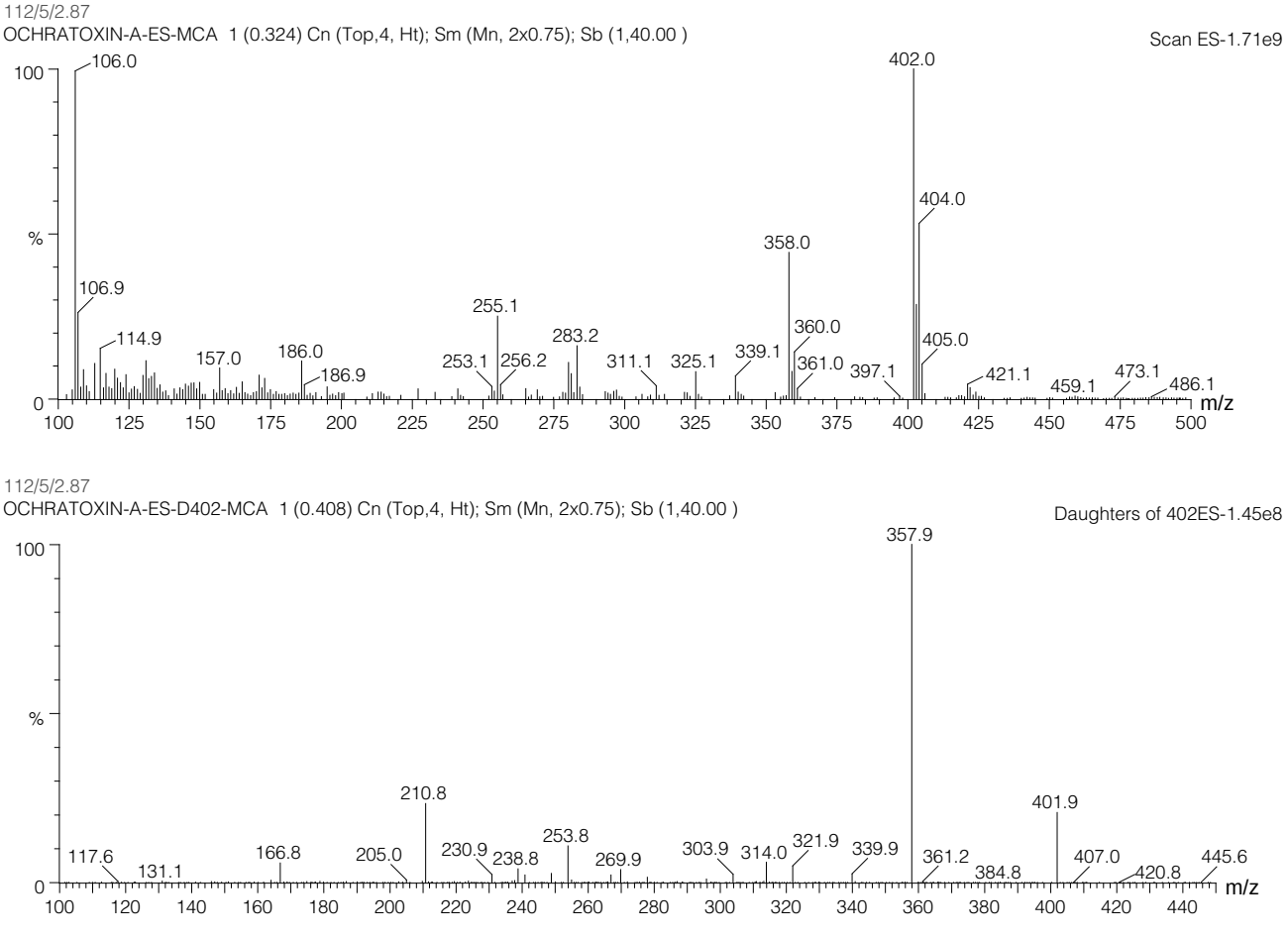


**Figure 5.** LC-electrospray tandem mass spectra of OTA: in the ESI full spectrum, the most intense ion was the M-H$^-$ ion at *m/z* 402.0 (above). By MS$^2$, the most ion important fragment was the [M-H-$CO_2$] ion at *m/z* 357.9 (below). For conditions, see the text.

the OTA standard. The identity of OTA in samples was further confirmed by the fragmentation patterns obtained in MS$^2$ spectra of the key ion [M-H-$CO_2$] at *m/z* 357.9 (Figure 5). These patterns were similar to those found in the MS$^2$ spectra of the standard of OTA (Figure 6).

II. *Survey Results (Table 3)*

(I) *Coffee Samples*

OTA was present in 13 (25%) of 51 coffee samples, including five instant coffee samples (with sugar and creamer) and eight ready-to-drink coffee samples. The OTA levels ranged from 0.1~0.5 ppb and the mean OTA level in the positive samples was 0.3 ppb. It was also the

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**Table 3.** OTA contents of different samples

| Samples | No. of samples | No. of positive samples (%) | OTA contents (ppb) | |
|---|---|---|---|---|
| | | | 0.1~0.3 | 0.3~0.5 |
| Coffee | 51 | 13 (25%) | | |
| Soluble coffee | 11 | 0 | 0 | 0 |
| Instant coffee | 16 | 5 | 2 | 3 |
| Ready-to-drink coffee | 19 | 8 | 5 | 3 |
| Roasted ground coffee been | 3 | 0 | 0 | 0 |
| Roasted coffee bean | 2 | 0 | 0 | 0 |
| Red wine and beer | 28 | 5 (18%) | | |
| Red wine (domestic) | 2 | 1 | 1 | 0 |
| Red wine (imported) | 8 | 4 | 2 | 2 |
| Beer (domestic) | 9 | 0 | 0 | 0 |
| Beer (imported) | 9 | 0 | 0 | 0 |
| Rice and rice based products | 75 | 0 (0%) | | |
| Rice | 1 | 0 | 0 | 0 |
| Brown rice | 17 | 0 | 0 | 0 |
| Rice mixed with cereals | 9 | 0 | 0 | 0 |
| Cooked rice | 32 | 0 | 0 | 0 |
| Wan-Quei | 8 | 0 | 0 | 0 |
| Rou-Zong | 8 | 0 | 0 | 0 |
| Wheat flour and wheat flour based products | 39 | 2 (5%) | | |
| Wheat flour | 3 | 0 | 0 | 0 |
| Whole wheat flour | 4 | 1 | 0 | 1 |
| Toast | 16 | 0 | 0 | 0 |
| Noodle | 16 | 1 | 1 | 0 |
| Total | 193 | 20 (10%) | 11 | 9 |

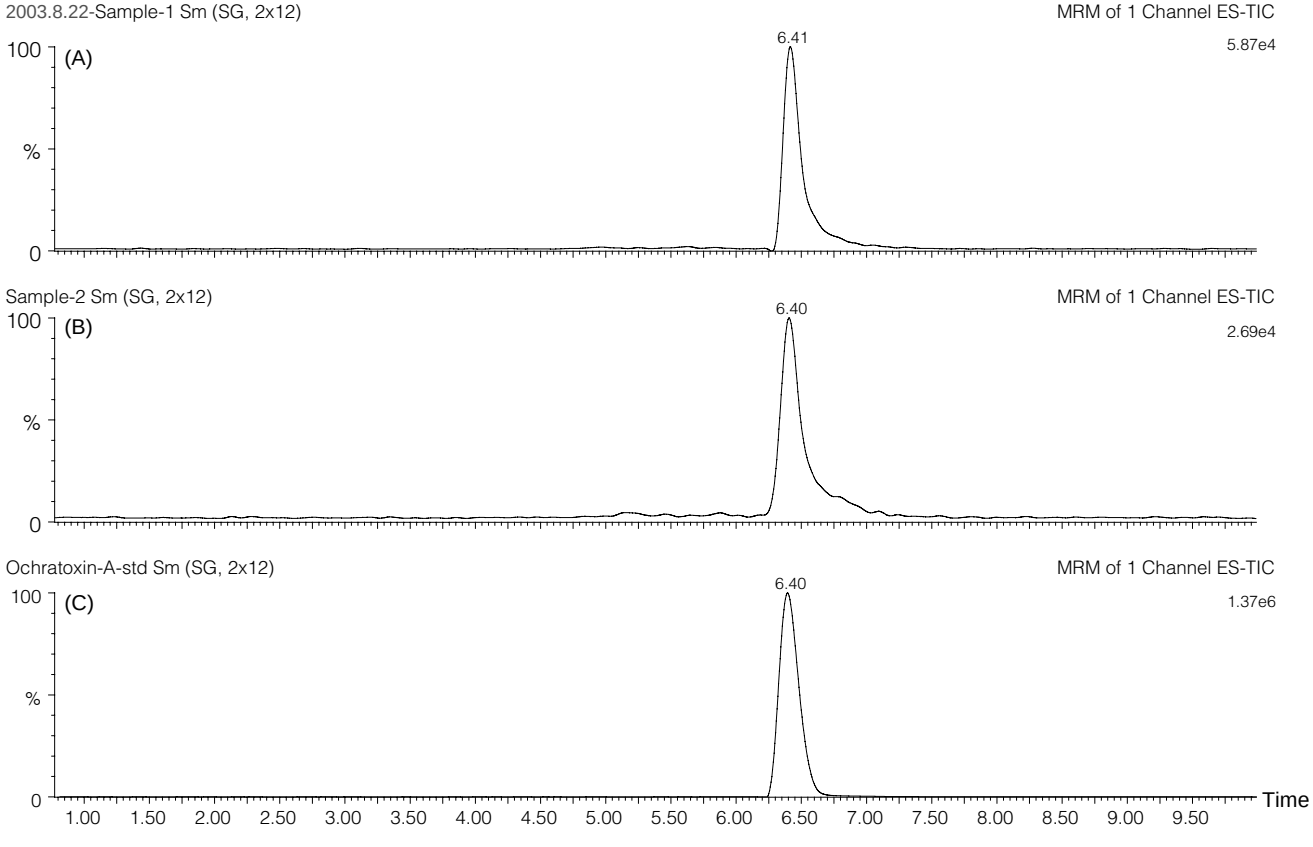


**Figure 6.** $MS^2$ spectra of the red wine sample (A), coffee sample (B) and OTA standard (C). For conditions, see text.

first time OTA contents were determined in ready-to-drink coffee samples in Taiwan and the incidence of OTA in the tested samples was quite high (42%).

(II) *Cereal samples*

There was no OTA present in 27 rice samples and 48

rice based products based on the limit of detection of 0.3 ppb. OTA was present in 1 of 7 (14%) wheat flour samples, and the positive sample was whole wheat flour at the level of 0.5 ppb. Thirty-two wheat based products were 16 toasts and 16 cooked noodle samples, and only 1 (0.3%) cooked noodle sample was contaminated with OTA at the level of 0.4 ppb.

(III) *Red wine and beer samples*

Quantifiable levels of OTA were found in 5 (50%) red wines, two were domestic products and three were imported from Italy and France. The OTA levels ranged from 0.2~0.5 ppb and the mean OTA level in the positive samples was 0.3 ppb. There is no OTA present in 9 domestic beer and 9 imported (from America, China, Japan, Mexico and the Netherlands) beer samples.

## DISCUSSION

The detection limits of OTA for retail coffees, cereals, red wines and beers were 0.1, 0.3 and 0.2 ppb, respectively. These were not significantly different compared to previous studies[9,10,11]. Confirmation of the identity and quantity of OTA was achieved by HPLC analysis of the methyl ester of OTA and by LC-electrospray tandem mass spectrometry.

For convenience, instant coffees including ready-to-drink coffee are becoming more popular in Taiwan. This survey is the first to determine OTA contamination in instant coffee (with sugar and creamer) and ready-to-drink coffee samples. The result showed that 5 out of 16 (31%) instant coffee and 8 of 19 (42%) ready-to-drink coffee samples were contaminated with OTA. Three roasted ground beans and two roasted beans were free of OTA. To date, the European Commission have discussed the proposed regulatory limit for OTA, with suggestion that 8 and 4 ppb OTA in roasted and instant coffees, respectively, might be appropriate[9]. According to the reference, more than 90% of the total OTA load was located in the husks of sun dried processed coffee[13]. An 85% reduction in OTA load of green coffee occurred during the processing of green coffee to roasted and soluble coffees[14]. Soluble coffee adulterated with husks might contain relatively large amounts of OTA[15]. A previous survey conducted by our lab in 2000 showed that 19 of 44 (43%) coffee samples were contaminated with OTA, and 15 of these 19 (79%) positive samples were soluble coffee[16]. Compared to the survey mentioned above, 11 soluble coffees were free of OTA in this study, but the high incidence (31%) of OTA in instant coffee (with sugar and creamer) was really a warning sign.

Rice, rice based foods, wheat flour, and wheat flour based foods are the main stables in Taiwan. This study collected 75 rice samples and products based on rice, and 39 wheat flour samples and products based on wheat flour. The result showed that only 1 of 4 (25%) whole wheat flour and 1 of 16 (6%) cooked noodles were contaminated with OTA at the levels of 0.5 and 0.4 ppb, respectively. Milling had been reported to reduce substantially the concentration of OTA in white flour[3], hence the higher incidence of OTA in whole wheat flour was reasonable. Though the incidence and quantity of OTA in cooked noodles was low, the quality of noodle manufacturing should be monitored. In this survey, COA collected and provided 10 brown rice samples from 5 rice warehouses located in 5 counties in Taiwan. Each rice warehouse donated two kinds of brown rice harvested in 2001 and 2002. This meant that when we analyzed these samples, they had been stored for over a year. All of these samples were OTA free. Seven fresh brown rice samples purchased from supermarkets were also free of OTA. This revealed that the quality of brown rice in Taiwan was good and the storage conditions were well controlled.

Beers are the major liquor sold in Taiwan, and red wines are becoming more popular due to the positive health effects. Owing to the relative stability of OTA during heating and fermentation[17,18], this 'ubiquitous' and 'unavoidable' contaminant can be 'carried over' during food processing. Thus, OTA occasionally can be found in barley and grape can partially persist during fermentation and finally they could be found in beers and red wines[19]. According to the Codex Alimentarius Commission's limited data, 23% of the total intake of OTA was due to beers and red wines[20]. In this survey, 18 beers (of which 9 were imported) were OTA free. Tangni *et al.* surveyed the OTA contents in 62 Belgium beers and 20 European brand beers, and the result showed that 60 of the 62 Belgium beers and all the European brand beers were contaminated with OTA[21]. None of these beers exceeded the previous suggested EU limit of 0.2 ppb. In this survey, the limit of detection in beers was 0.2 ppb and none of the 18 bees were positive. It confirmed the result of the previous survey.

Two domestic red wines and three of eight imported red wines were contaminated with OTA in this survey. The available evidence indicated that *A. carbonarius* and *A. niger* were not pathogens on fruit such as grapes and hence could not gain entry to undamaged fruit. However, mechanical or chemical damage to fruit or damage caused by insects or microorganisms might permit fungal invasion of fruit tissue[3]. No tolerance in wines had been established by EU, although the topic was under discussion by European authorities[11].

According to the "Nutrition and Health Survey in Taiwan 1993~1996"[8], the daily average intake of adult male and female were 39.1 g and 38.3 g for wheat and wheat flour, 19.3 g and 10.2 g for coffee, and 1.43g and 0.5 g for red wines and beers. The mean body weight of adult male and female were 64 and 56 kg, respectively. Based on the highest OTA level of 0.5 ppb in this survey, the daily possible OTA intake was estimated as follows:

(The intake sum of wheat and wheat flour, coffee and red wine and beer) × OTA level/mean body weight.

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The results were 0.47 and 0.44 (ng/kg bw/day) for adult male and female, respectively. Consequently the intakes were much lower than the tolerable intakes estimated by JECFA (100 ng/kg bw/week), Canada (1.5 to 5.7 ng/kg bw/day) and European Commission (not more than 5 ng/kg bw/day).

## CONCLUSIONS

By the combination of immunoaffinity column for cleanup and LC with fluorescence for detection, the OTA contents in coffees, cereals and red wines and beers could be measured with good recoveries and reasonable LOD. Through chemical derivation and LC-electrospray tandem mass spectrometry, confirmation of the identity and quantity of OTA could be achieved.

In 51 samples of coffees, 114 samples of cereals, and 28 samples of red wines and beers, OTA was detected in 13 (25%) coffee samples, 2 (5%) wheat flour and wheat flour and 5 (50%) red wine samples at 0.1~0.5 ppb levels. None of these positive samples exceeded the regulation set or suggested by EU.

The daily OTA consumption of adult male and female (0.47 and 0.44 ppb, respectively) were much lower than the established tolerable daily intake (TDI) of 5 ng/kg bw (European Commission) or tolerable weekly intake (TWI) of 100 ng/kg bw (JECFA).

## ACKNOWLEDGEMENTS

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