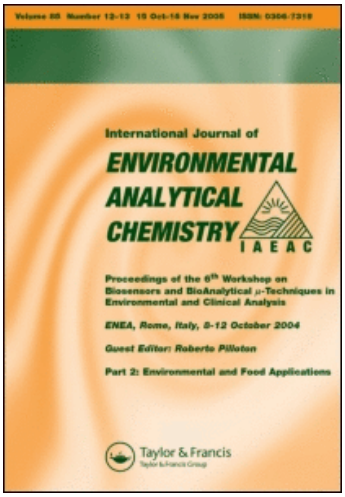

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Incidence of trichothecenes in wheat-based foods from China

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The occurrence of trichothecene toxins was determined in different kinds of wheat-based foods (convenience noodles, biscuits, bread and cakes) in Nanjing, China. A total of 74 commercially available samples were collected from the supermarkets in Nanjing from September 2006 to April 2007. Three trichothecenes named deoxynivalenol (DON), 3-acetyldeoxynivalenol (3ADON) and 15-acetyldeoxynivalenol (15ADON) were detected and quantified by gas chromatography with electron capture detection. The incidence of DON, 3ADON and 15ADON were 73.0, 8.1 and 23.0%, respectively, the content ranges were 0.07–11.38, 0.25–1.47 and 0.03–2.06 mg kg^{-1}, respectively, and the average contents were 2.47, 0.71 and 0.48 mg kg^{-1} in positive samples, respectively. The average DON contents in convenience noodles, biscuits, bread and cakes were 2.36, 2.79, 1.86 and 0.62 mg kg^{-1}, respectively. Thirty-seven of 74 samples contained more than 1 mg of DON per kilogram, which is the regulatory limit defined by the Chinese government.

Keywords: wheat-based foods; China; trichothecene; deoxynivalenol; acetyldeoxynivalenol

1. Introduction

Deoxynivalenol (DON), 3-acetyldeoxynivalenol (3ADON) and 15-acetyldeoxynivalenol (15ADON) are trichothecene toxins mainly produced by the fungal species *Fusarium graminearum* and *F. culmorum* [1]. The two pathogens cause wheat head blight in most wheat production areas worldwide, but especially in the temperate regions of America, Asia and Europe [1]. Trichothecene toxins are a group of sesquiterpenes epoxides, which are the potential inhibitors to protein biosynthesis in eukaryotes and prevent polypeptide chain initiation or elongation by binding to 60S ribosomal subunits, and are capable of producing a wide range of toxic effects in humans and animals [2,3]. The clinical symptoms to humans include necrotic changes in the mouth and gastrointestinal tract, emesis, diarrhoea, anorexia, haematological and immunological disorders [4]. Chronic consumption of low levels of these toxins may result in impaired immunity and decreased resistance to infectious diseases [4].

The risk assessment and tolerable daily intake of trichothecenes was considered by various international committees. A total of 1 μg of DON was established as a temporarily acceptable daily intake per kilogram of bodyweight (BW) [5,6]. The World Health

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Organisation (WHO) has set wheat advisory contents of 1 and 5 mg kg^{-1} of DON in food and feed for human and livestock consumption, respectively [7]. The European Commission set advisory levels of 750 μg kg^{-1} of DON content in cereal flour with the exception of 500 μg kg^{-1} of DON in bread and related products for food use [8]. Chinese government set a national advisory standard of 1000 μg kg^{-1} of DON content in wheat and wheat flour for human consumption [9].

Contaminations of DON are common in human diets, especially in wheat diets [4]. The reported levels of DON in samples of wheat, oat crackers and cookies in the USA ranged between 1600 and 5400 μg kg^{-1} [10]. DON was detected in 92.8% of wheat bread and pastries samples in Argentina, with levels ranging from 200 to 2800 μg kg^{-1} [11]. In Canada, DON was found in 43% of bakery products samples with a range from 9 to 4060 μg kg^{-1} [12]. The mean content of DON in positive samples of bakery products from German markets was 92 μg kg^{-1} [13]. An incidence of DON in bread collected from German market was 92%, the mean content was 155 μg kg^{-1} with a range of 15–690 μg kg^{-1} [14].

Although wheat is one of the most important crops in China, and usually suffers from wheat head blight [15,16], little is known about the incidence of trichothecene toxins in wheat-based foods in China. Additional data about trichothecene occurrence in foods in China is urgently needed as a basis of improved risk assessment. The main objective of the present study, therefore, was to monitor the content of the trichothecenes DON, 3ADON and 15ADON in samples of different groups of wheat-based foods sold in the supermarkets located in Nanjing.

2. Experimental

2.1 *Samples*

A total of 74 samples of wheat-based foods were collected from supermarkets located in Nanjing from September 2006 to April 2007. These samples represented the most wheat foods sold in the supermarkets in Nanjing during the survey period. The following commodities were collected: biscuits ($n = 36$), convenience noodles ($n = 23$), bread ($n = 9$) and cakes ($n = 6$). The number of each kind of commodity reflected its abundance relative to other commodities in the Nanjing markets during the survey period. Production dates of these samples ranged from July 2006 to April 2007. The places for producing the food samples included the provinces of Jiangsu ($n = 13$), Guangdong ($n = 9$), Zhejiang ($n = 9$), Fujian ($n = 7$), Anhui ($n = 6$), Hubei ($n = 2$), Shandong ($n = 1$) and municipal cities of Shanghai ($n = 15$), Beijing ($n = 7$) and Tianjin ($n = 5$). The sources of the wheats, which were used to produce these samples, were not surveyed at the food factories, but it is very probable that the raw materials were bought near the areas of the food factories. The collected samples were stored at −4°C before analysis.

2.2 *Chemicals*

Standards of DON, 3ADON and 15ADON and chromatographic grade purity isooctane were purchased from Sigma (St Luis, MO, USA). Purification column of superclean LC-18 (C-18), superclean LC-alumina (Aluminum oxide), trimethylsilylimidazole (TMSI) and trimethylchlorosilane (TMCS) were purchased from Supelco (Bellefonte, PA, USA). Solid standards of DON, 3ADON and 15ADON were dissolved in acetonitrile at 1.0 mg mL^{-1}

and stored at −20°C in a sealed vial until use. Working standards (10.0, 5.0, 2.0, 1.0, 0.5, 0.25, 0.1, 0.05 and 0.03 μg mL^{-1}) were prepared by appropriate dilution of known volumes of the stock solution with acetonitrile and used to obtain calibration curves after derivatisation and injection in the chromatographic system.

2.3 *Sample preparation for trichothecenes*

Chemical analysis was carried out as described previously by Goswami [17]. After 50 g of the food sample was milled with a grinder, 25 g of milled sample was well-mixed and then extracted with 100 mL of acetonitrile : water (84 : 16) in an Erlenmeyer flask, the mixture was shaken for 1 h. A total of 4 mL of the extract was filtered through a purification column (a 5 mL plastic syringe packed with 1 g of 1 : 3 [wt/wt] C18: Aluminum oxide mixture) and 1.0 mL of the filtrate was transferred into a labelled 4 mL screw vial and evaporated to dryness under a stream of nitrogen. After evaporation, the residue was derivatised with 100 μL of TMS reagent (TMSI : TMCS = 100 : 1). The sample was shaken for 10 min at room temperature to allow toxins to react with derivatising reagent. Afterwards, the sample was performed by adding exactly 1.0 mL of isooctane and 1.0 mL of ultrapure water to stop reaction in the hood. The sample was then mixed with a vortex mixer so that the milky isooctane layer became transparent. The upper isooctane layer was finally transferred into a 1.5 mL GC vial for gas chromatography (GC) analysis.

2.4 *GC conditions*

Analysis was carried out using a Thermo Chromatopac Gas Chromatograph with Electron Capture Detection (GC-ECD). Chromatographic separation was done with a Permabond SE-54-DF-0.25 column (30 m × 0.25 mm, i.d., Machery Nagel). An aliquot (1.0 μL) of the upper isooctane layer solution was injected in splitless mode. The carrier gas was helium with a flow rate of 1.0 mL min^{-1}. The GC conditions were as described by Goswami with modification [17]. The temperatures of the injection port and the detector were 250 and 300°C, respectively. The initial column temperature was 80°C for 1 min, it was increased to 150°C at a rate of 40°C min^{-1} and held 1 min, finally increased to 280°C at 30°C min^{-1} and kept at this temperature for 5 min.

2.5 *Analytical quality control*

The calibration curves of three trichothecenes used for quantitative determination were constructed on the basis of the area under the chromatographic peaks. The linearity of the method was assessed by using nine concentrations of each trichothecene ranged from 0.03 to 10.0 μg mL^{-1}. The correlation coefficient for all three standards was 0.999. The limits of detection for DON, 3ADON and 15ADON were 0.008, 0.012 and 0.009 mg kg^{-1} at a signal to noise ratio of 2, respectively, and the limits of quantification were 0.021, 0.034 and 0.026 mg kg^{-1}, respectively. The contents of DON, 3ADON and 15ADON were determined by external standard method.

For testing recovery, blank milled samples of convenience noodles, biscuits, bread and cakes were, respectively, spiked at three different levels (0.5, 1.0 and 1.5 mg kg^{-1}) of each mycotoxin with four replicates. Samples were cleaned up through a purification column and analysed in GC, the average recoveries and relative standard deviations (RSD) were calculated.

Table 1. Average recoveries and RSD in percentage obtained for DON, 3ADON and 15ADON from spiked convenience noodles, biscuit, bread and cake samples (spiking levels of 0.5, 1.0 and 1.5 mg kg^{-1}), after clean-up with purification columns ($n = 4$).

		Recovery (%) ± RSD (%), three levels, $n = 4$			
Toxin	Spiking levels (mg kg^{-1})	Convenience noodles	Biscuit	Bread	Cake
DON	0.5	97 ± 8.9	94 ± 5.6	84 ± 15.2	82 ± 5.1
	1.0	88 ± 8.9	83 ± 5.7	86 ± 5.6	76 ± 9.3
	1.5	92 ± 9.0	84 ± 6.1	87 ± 10.8	77 ± 12.2
3ADON	0.5	89 ± 7.4	87 ± 4.5	87 ± 7.5	80 ± 12.2
	1.0	85 ± 7.0	87 ± 6.7	83 ± 10.1	73 ± 11.4
	1.5	91 ± 9.9	87 ± 13.7	81 ± 16.9	76 ± 15.6
15ADON	0.5	93 ± 6.3	86 ± 8.1	81 ± 5.6	73 ± 5.3
	1.0	87 ± 10.7	80 ± 12.3	79 ± 7.7	71 ± 2.6
	1.5	86 ± 11.9	83 ± 7.2	84 ± 4.5	74 ± 13.8

In order to test the repeatability of the method, a naturally contaminated wheat sample with DON, 3ADON and 15ADON was analysed with 10 replicates. The RSDs of DON, 3ADON and 15ADON contents were evaluated.

2.6 *Statistical analysis*

One-way analysis of variance (ANOVA) and Tukey–Kramer's multiple comparison tests (SAS software system) were used for determination the statistical significance of the differences of mycotoxin mean contents in different food groups.

3. Results

The concentrations of DON, 3ADON and 15ADON on all collected samples were detected and corrected according to the recoveries of three trichothecenes from each series of food groups of the tested sample which are shown in Table 1. The RSD of the repeatability of the method for DON, 3ADON and 15ADON contents were 4.7, 5.7 and 5.6%, respectively.

The incidences of DON in convenience noodles, biscuits, bread and cakes samples were 82.6, 80.6, 33.3 and 50%, respectively, and the average contents were 2.36, 2.79, 1.86 and 0.62 mg kg^{-1} with ranges 0.07–7.52, 0.08–11.38, 0.75–3.45 and 0.17–1.35 mg kg^{-1}, respectively (Table 2). 3ADON was found in 8.7 and 11.1% of convenience noodles and biscuits samples, respectively, with ranges 0.51–0.93^{-1} and 0.25–1.47 mg kg^{-1}, and the average concentrations were 0.77 and 0.71 mg kg^{-1} in positive samples, respectively, whereas 3ADON was not detected in bread and cake samples (Table 2). The incidences of 15ADON in convenience noodles, biscuits, bread and cakes were 13.0, 33.3, 11.1 and 16.7%, and the average contents were 0.25, 0.57, 0.08 and 0.56 mg kg^{-1} with ranges 0.06–0.60, 0.03–2.06, 0.08–0.08 and 0.56–0.56 mg kg^{-1}, respectively (Table 2). No significant differences were found among the average contents of three toxins for all food groups.

Table 2. Concentrations and incidences of three trichothecene toxins in different groups of food samples collected in Nanjing, China.

Trichothecene	Food group	Number of positive samples (%)	Toxin in positive samples ($mg\,kg^{-1}$)		
			Range	Mean	Median
DON	Convenience noodles	19 (82.6)	0.07–7.52	2.36	1.88
	Biscuit	29 (80.6)	0.08–11.38	2.79	2.02
	Bread	3 (33.3)	0.75–3.45	1.86	1.37
	Cake	3 (50)	0.17–1.35	0.62	0.34
3ADON	Convenience noodles	2 (8.7)	0.51–0.93	0.77	0.77
	Biscuit	4 (11.1)	0.25–1.47	0.71	0.56
	Bread	0 (0)	ND	ND	ND
	Cake	0 (0)	ND	ND	ND
15ADON	Convenience noodles	3 (13.0)	0.06–0.6	0.25	0.09
	Biscuit	12 (33.3)	0.03–2.06	0.57	0.48
	Bread	1 (11.1)	0.08	0.08	0.08
	Cake	1 (16.7)	0.56	0.56	0.56

Note: ND – under detection limit.

Table 3. Concentrations and incidences of three trichothecene toxins in all wheat-based foodstuff samples collected in Nanjing, China.

Trichothecene	Incidence		Toxin in positive samples ($mg\,kg^{-1}$)		
	Positive samples	Percentage of positive samples	Range	Mean	Median concentration
DON	54	73.0	0.07–11.38	2.47	1.86
3ADON	6	8.1	0.25–1.47	0.71	0.68
15ADON	17	23.0	0.03–2.06	0.48	0.42

Overall, the incidence of DON, 3ADON and 15ADON was 73.0, 8.1 and 23.0% in all samples, respectively (Table 3). Of the three toxins, DON had the highest content, with an average content of $2.47\,mg\,kg^{-1}$ in positive samples. Whereas the average content of the 3ADON and 15ADON in positive samples was 0.71 and $0.48\,mg\,kg^{-1}$, respectively (Table 3).

An average content of DON in 74 food samples was $1.80\,mg\,kg^{-1}$, the number of samples containing 0.00, 0.01–1.00 and $>1.00\,mg\,kg^{-1}$ of DON was 20, 17 and 37, respectively. These values represented 27, 23 and 50% of all samples.

4. Discussion

4.1 *Trichothecenes were widely existed in wheat-based foodstuff in China*

The present study found that the trichothecenes DON, 3ADON and 15ADON are common in wheat-based foods produced in 10 provinces of China. In China, wheat head

blight occurs in more than 10 provinces [16] and the major pathogen of wheat head blight is *F. graminearum* [15,16]. Trichothecenes are produced in wheat grains by head blight pathogens during the ripening of the grain before harvest [4,18]. Zhang *et al.* [19] found that 52 and 30% of *F. graminearum* are 3ADON and 15ADON toxin producer chemotypes, respectively, in China. Ninety-five percent of 3ADON chemotype was originated from the warm regions where the annual average temperature was above 15°C, while 59% of 15ADON chemotype originated from cool regions where the annual average temperature was equal to or lower than 15°C. Results of the present study were consistent with Zhang *et al.* [19] in that 3ADON was detected in samples from warmer regions (Fujian, Shanghai, Guangdong, Zhejiang and Anhui) but not from cooler regions (Tianjing and Beijing), while 15ADON was detected in samples from both warmer and cooler regions. The variation on the distribution of trichothecenes in foodstuff produced in different provinces probably reflects the occurrence of wheat head blight in China during the investigation period.

4.2 *Food processing might affect toxin contamination*

The fermentation methods for wheat flour used in the production process of some biscuits can be obtained from the instruction of products. The growth temperature of *F. graminearum* in wheat flour was 4–32°C at moisture content in flour above 19% during fermentation process [6,18], and the growth of the fungus during fermentation might increase trichothecene content. Because the moisture content of stored wheat grain in China is usually less than 14% [20], production of trichothecenes during storage is unlikely [18].

The ratios of trichothecene toxin contents could indicate the source of the toxin. In our unpublished studies, the ratio of DON/3ADON in diseased wheat grains was greater than 21.8 and the ratio of DON/15ADON was greater than 8.9; in potato dextrose agar (PDA), however, a 3ADON chemotype of *F. graminearum* pathogen produced a ratio of DON/3ADON <0.1. In another study, the ratio of DON/3ADON in disease wheat kernels was greater than 26.9 and the ratio of DON/15ADON was greater than 9.7 [21]. In some fermented biscuits in the present study, the ratios of DON/3ADON and DON/15ADON were 5.1 and 0.82, respectively. Although the relationship between DON and ADON may be influenced by strain, matrix, temperature and other factors during grain ripening [18], the low ratios of DON/3ADON and DON/15ADON in fermented biscuits suggest that the toxins were probably produced by the pathogen during fermentation. However, the available data on the effects of fermentation on DON levels are conflicting. Some studies reported an increase in DON during fermentation, while other works indicated that DON was reduced by over 40% during dough fermentation [4].

Milling methods and other food processing procedures can also affect trichothecene contamination levels and could explain some of variation in trichothecenes in different foods in the present study. For example, wholemeal flour and white flour may differ in trichothecene content because of the redistribution of toxins during milling [4]. Samar *et al.* [22] also found that the processing of food may affect the content of DON. The effect of food process on the concentrations of trichothecenes might be a reason for the variation of trichothecenes on different groups of food in our study.

4.3 *The risk assessment of trichothecenes*

The toxin detected most frequently in our samples was DON, and 50% of the samples contained more than 1.00 mg of DON per kilogram, which is the regulatory limit described by Chinese government [9]. Based on these results, DON consumption from wheat-based food of Chinese consumers can be calculated at 328 μg per consumer per day; this value is based on an average DON content of 1.80 mg kg^{-1} and an average wheat grain consumption of 182 g per consumer per day in China [23]. It follows that a person weighing 60 kg will take in 5.5 μg of DON per kg of body weight. This suggests that the DON intake in China during the survey and in the survey area exceeded the acceptable daily intake of 1.0 μg kg^{-1} body weight proposed by JEFCA [6]. The consumption of trichothecene-contaminated foods with high concentration probably causes chronic health problems for humans in China. In Germany, the estimated daily DON intake per kg body weight was 0.5 μg in 1999 and 0.3 μg in 1998 [14]. In Russia, DON was detected in 69% of 2166 samples from Krasnodar region, which is considered to be the major *Fusarium* endemic region of that country, and the contamination levels ranged from 0.1 to 8.6 mg kg^{-1} [24]. In a recent study in north Asia, DON was found in 71% of 3420 wheat samples; the average DON content was 925 μg kg^{-1}, and the highest content (19 mg kg^{-1}) was detected in wheat from China [25].

5. Conclusions

The present study revealed that trichothecene toxins are frequent contaminants of wheat-based foods in China. These results also stress the need for regular screening of even greater number of wheat-based foods for trichothecene toxins in China.

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