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Occurrence of aflatoxin B1, citrinin and ochratoxin A in rice in five provinces of the central region of Vietnam

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Abstract

The possible coexistence of three mycotoxins in rice, including aflatoxin B1 (AFB1), citrinin (CIT) and ochratoxin A (OTA), was investigated. The samples of rice were collected in large markets in five provinces of the central region of Vietnam. These toxins were extracted, purified and finally quantified by HPLC with fluorimetry detection. Contamination of AFB1 was found to be the most, followed by OTA, while contamination of CIT was insignificant. The coexistence of CIT with AFB1/OTA in rice was found in high percentage. Some samples overpassed the authorized limit by Europe in OTA and/or AFB1.

Keywords: Aflatoxin B1; Ochratoxin A; Citrinin; Mycotoxin analysis; Rice

1. Introduction

One of the most serious problems to confront the quality of rice is the presence of mycotoxins which are produced by different species of the genus *Aspergillus* or *Penicillium*. Aflatoxins are of greatest concern as they are highly toxic, mutagenic, teratogenic and carcinogenic compounds that have been implicated as causative agents in human hepatic and extrahepatic carcinogenesis (Castegnaro & Pfohl-Leszkowicz, 1999; Hussein & Brasel, 2001; IARC, 1993; INSPQ, 2002; Massey, Stewart, Daniels, & Ling, 1995).

Ochratoxin A and citrinin are produced by some Aspergilli (*Aspergillus ochraceus*, *Aspergillus carbonarius*, *Aspergillus niger*) or Penicillia (*Penicillium viridicatum*, *Penicillium verrucosum*, and *Penicillium cyclopium*). It has been reported that ochratoxin A is a teratogenic, potent renal carcinogen (for review see IARC, 1993; Manderville & Pfohl-Leszkowicz, 2006; Pfohl-Leszkowicz & Castegnaro, 1999). Some studies have found the implication of ochratoxin A in certain epidemic nephropathies in animals and humans (Bennett & Klich, 2003; Castegnaro et al., 2006; Pfohl-Leszkowicz, Petkova-Bocharova, Chernozemsky, & Castegnaro, 2002).

Similarly, some scientific reports show a link between citrinin and nephrotoxic (kidney damaging) effects and possibly a carcinogenic effect for humans (Arai & Hibino, 1983; NTP, 1989). Citrinin enhances carcinogenicity induced by OTA (Kanizawa, 1984).

The central region of Vietnam is narrow and long (Fig. 1). So there are various types of climate there. In the region Quang Nam–Quang Ngai, with high level of rain and humidity, the average relative humidity is high, up to 90% and higher than the region Binh Dinh-Phu Yen-Nha Trang (average 80%) (Dan & Dac, 1993). In Vietnam, the conditions of storage of rice vary but local commercial rice may be badly stored, and sold without packing; it is directly exposed to moist warm air. Mould and mycotoxin contamination is highly risky. It can seriously affect consumer health.

Some studies have shown that the contamination of rice samples, collected in the south of Vietnam, by aflatoxin B1

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Fig. 1. Map of Vietnam. Rice has been collected in five areas X1 – Quang Nam; X2 – Quang Ngai; X3 – Binh Dinh; X4 – Phu Yen; X5 – Nha Trang.

and OTA were high, reaching levels up to 27 ng/g and 26 ng/g, respectively. (Son et al., 1998; Trung, Bailly, Querin, Le Bars, & Guerre, 2001).

The objective of our study is to investigate the presence of three mycotoxins (OTA, CIT and AFB1) in rice samples collected from five provinces (Quang Nam, Quang Ngai, Binh Dinh, Phu Yen and Nha Trang) of central regions in Vietnam (Fig. 1). To quantify the amounts of these toxins in rice, a simultaneous analytical method was developed, using an HPLC system with fluorimetry detection.

2. Materials and methods

2.1. Samples

One hundred samples of rice were randomly collected from five provinces (Quang Nam, Quang Ngai, Binh Dinh, Phu Yen, and Nha Trang) of the central region of Vietnam. They were collected in November (rainy season) and in April (dry season). A minimum sample size of 500 g was applied and samples were kept at −20 °C in PE bags while awaiting analysis.

2.2. Reagents

All reagents (potassium chloride, phosphoric acid, hydrochloric acid) were of PA grade. All solvents (methanol, acetonitrile, propanol-2-ol, *n*-hexane, chloroform) were of HPLC grade. Deionised water was used for the preparation of all aqueous solutions and for HPLC. Standard toxins, AFB1, CIT, OTA, were supplied by Sigma Chemicals.

2.3. Preparation of standard solutions

Standard solutions AFB1, CIT and OTA were prepared by dissolving 10 mg of AFB1, CIT or OTA in 1 ml of methanol. The concentration of the AFB1 stock solution was determined by measuring the UV absorbance at 360 nm and calculating by using the molar extinction coefficient ε of 21 800 mol^{-1} cm^{-1} (IARC, 1982). The concentration of the CIT stock solution was determined by measuring the UV absorbance at 321 nm and calculating by using the molar extinction coefficient ε of 5490 mol^{-1} cm^{-1} (Molinié, Faucet, Castegnaro, & Pfohl-Leszkowicz, 2005). The concentration of the OTA stock solution was determined by measuring the UV absorbance at 333 nm and calculating by using the molar extinction coefficient ε of 5440 mol^{-1} cm^{-1} (IARC, 1993).

A series of working standards ranging from 3.13 to 100 ng of each mycotoxin per ml of methanol was prepared by dilution (equivalent to a concentration of mycotoxin in rice from 0.078 to 2.5 ng/g). They were used to calibrate the LC detector responses.

2.4. Confirmation of the presence of OTA

The confirmation of the presence of OTA in some rice samples was achieved by the following technique: an aliquot, taken from the purified extract of a sample where OTA was detected by the HPLC analysis, was dried. The pellet was dissolved in 975 µl of a buffer solution of 0.04 M Tris–HCl, 1 M NaCl, pH 7.5. Then, 25 µl of carboxypeptidase (100 U/ml H_2O) was added and the mixture was incubated at room temperature overnight. The sample was analysed under the same HPLC chromatographic conditions as used above. The OTA peak disappeared whereas the peak of α-OT appeared.

2.5. Confirmation of the presence of AFB_1

Underivatised AFB_1 was separated by HPLC and detected by spectrofluorimetry after post-column derivatisation in the Kobra cell® where it was converted into the 9,10-dibromo derivative.

2.6. Procedure

2.6.1. Extraction of toxins

Ground uncoated rice (20 g) were extracted with 100 ml of acetonitrile–4% aqueous solution of potassium chloride (9:1). This mixture was adjusted to pH 1.5 with undiluted hydrochloric acid, and then shaken on an orbital shaker for 20 min and filtered through a Whatman No. 4 paper under vacuum.

2.6.2. Purification

One hundred ml of *n*-hexane were added to the filtrate and shaken 10 min. After separating, the upper phase (*n*-hexane) was discarded. This step was repeated with 50 ml of *n*-hexane. To the lower phase, 50 ml deionised water and 50 ml chloroform were added. The solution was shaken for 10 min. After separation, the lower phase (chloroform) was collected. The upper phase was re-extracted, twice, with 25 ml of chloroform, using the above conditions. The chloroform extracts were pooled. Then the chloroform was evaporated to near dryness under vacuum by using a rotary evaporator in a 40 °C water bath at low speed. Two milliliter of methanol were added and the solution was sonicated and filtered through a 0.45 μm filter and finally evaporated to dryness under nitrogen. For analysis in the HPLC system, 500 μl of methanol were added.

2.6.3. HPLC conditions

A Shimadzu (Kyoto, Japan) LC-10, equipped with an injector 20 μl loop, a C18 spherisorb column (3 μm C18, 0.46 × 25 cm) and a fluorescence detector (Spectra physic 2000), was used. Different excitation and emission fluorescence parameters (AFB1 365 and 440 nm; OTA 335 and 465 nm; CIT 331 and 500 nm) were used to achieve the optimal conditions of detection for each toxin. The system was run isocratically, with mobile phase (0.33 M) H_3PO_4/acetonitrile/propan-2-o1 (650/400/50); flow rate was 0.5 ml/min; elution times of AFB1, CIT and OTA were about 14, 28 and 56 min, respectively.

The chromatograms were analyzed by a Class-LC10 software version 1.6 Shimadzu (Fig. 2).

3. Results and discussion

3.1. Simultaneous analytical method for AFB1, CIT and OTA in rice

3.1.1. Recoveries

Five different uncontaminated rice samples were spiked with each toxin at 5 ng/g. The average recoveries and the relative standard derivations (RSD) for AFB1, CIT and OTA are presented in Table 1.

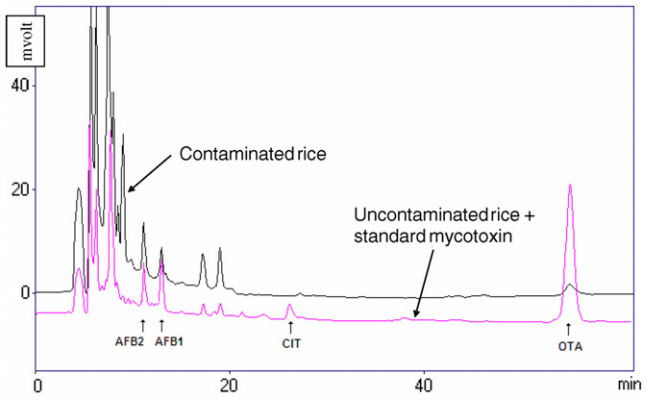

Fig. 2. Example of separation of mycotoxins extracted from rice.

The results demonstrate the high reproducibility of the method. They are satisfactory in regard to the EU legislation (Nos. 472/2002 and 26/2002). It appears that, between 1 and 10 ng/g, the recoveries are acceptable in the range 70–110% and the RSD should be <20%.

3.1.2. The limit of detection (LOD) and the limit of quantification (LOQ)

LOD and LOQ were determined by taking 3.3 and 10 times the standard deviation, (respectively), using the slope of calibration of each toxin. Results are presented in Table 2.

3.2. Sample analysis

As can be seen from Tables 3 and 4, AFB1 was found in just over one half of the total number of samples (51%). It should be noted, however, that AFB1 was present in all five provinces of the central region of Vietnam. CIT, on the other hand, was only quantified in two samples out of 13 contaminated samples. OTA was not quantified in rice in the province of Quang Nam; however, other provinces had rice contaminated in approximately one third of the total number of samples.

It is clear from Table 4 and Figs. 3 and 4 that the season had an impact on the contamination by these mycotoxins. In the rainy season, the ratio of detectable samples and average of quantifiable samples of AFB1 in rice were higher than in the dry season ($p < 0.05$).

Except for the province of Binh Dinh, the occurrence of AFB1 in other provinces in the rainy season was much higher than those in the dry season. For example, the high-

Table 1
The average recoveries and the relative standard deviations of each mycotoxin

Mycotoxin	Average recoveries (%)	RSD (%)
AFB1	90.1	6
OTA	84.1	6.7
CIT	103	5.4

Table 2
LOD and LOQ of AFB1, CIT and OTA

	AFB1	CIT	OTA
LOD (ng/g)	0.07	0.11	0.08
LOQ (ng/g)	0.22	0.35	0.25

Table 3
Analytical results of 100 samples from 5 provinces of central region of Vietnam

Mycotoxin	Detectable samples	Quantifiable samples		
	Number	Number	Average (ng/g)	Maximum (ng/g)
AFB1	51	35	3.31	29.8
CIT	13	2	0.38	0.42
OTA	35	20	0.75	2.78

est amount of AFB1 found in rice in the province of Quang Nam was seven-folds higher in the rainy season than in the dry season.

Conforming with the WHO regulations for AFB1 (30 ng/g), there are no samples that exceed this limit. However, the European Community agreed, on 16 July 1998, a limit of 2 ng/g of AFB1 in a range of foods for human consumption. There were 10 samples that exceeded this limit (Adams & Moss, 2002).

The major staple food in many Asian countries, including Vietnam, is rice. The daily intake of rice by the average adult Vietnamese is estimated to be 500 g. For the highest AFB1-contaminated sample (29.8 ng/g), the level of AFB1 contamination was up to 15 μg/person/day and daily intake of AFB1 for a 60 kg person would be 296 ng/kg b.w./day. According to Wild et al. (1992), this is a risky factor for cancer.

The maximum amounts of CIT detected in rice in the provinces of Binh Dinh and Phu Yen in the rainy season were 0.42 ng/g and 0.38 ng/g, respectively, whereas, no CIT was found in rice in the dry season.

In the dry season, OTA can be found in rice in the provinces of Binh Dinh and Phu Yen up to 1.63 ng/g and 1.87 ng/g, respectively, compared to 0.35 and 0.29 ng/g in the rainy season.

In the same way, the maximum amounts of OTA found in the provinces of Quang Ngai and Nha Trang in the dry

Table 4
Analytical results of samples from each of five provinces of central region in Vietnam

Province	Season sampling	Number of samples analysed	Type of mycotoxin	Detectable samples > LOD	Quantifiable samples > LOQ		
				Number	Number	Average (ng/g)[a]	Highest value (ng/g)
Quang Nam	Rainy season	10	AFB1	7/10	7/7	10.08	29.82
			CIT	1/10	0	–	–
			OTA	4/10	0	–	–
	Dry season	10	AFB1	5/10	5/5	1.77	4.44
			CIT	0/10	0	–	–
			OTA	4/10	0/4	–	–
Quang Ngai	Rainy season	10	AFB1	8/10	5/8	4.5	12.43
			CIT	0/10	0	–	–
			OTA	0/10	0	–	–
	Dry season	10	AFB1	4/10	2/4	0.55	0.65
			CIT	1/10	0	–	–
			OTA	8/10	7/8	0.58	0.87
Binh Dinh	Rainy season	10	AFB1	7/10	5/7	0.58	0.75
			CIT	4/10	1/4	0.42	0.42
			OTA	2/10	1/2	0.35	0.35
	Dry season	10	AFB1	1/10	1/1	1.34	1.34
			CIT	0	0	–	–
			OTA	4/10	2/4	1.03	1.63
Phu Yen	Rainy season	10	AFB1	7/10	5/7	0.95	2.33
			CIT	4/10	3/4	0.34	0.38
			OTA	2/10	1/2	0.29	0.29
	Dry season	10	AFB1	1/10	1/1	0.89	0.89
			CIT	0/10	0	–	–
			OTA	4/10	4/4	0.85	1.87
Nha Trang	Rainy season	10	AFB1	8/10	5/8	2.21	7.2
			CIT	0/10	0	–	–
			OTA	0/10	0	–	–
	Dry season	10	AFB1	2/10	2/2	0.96	1.41
			CIT	0	0	–	–
			OTA	5/10	4/5	0.99	2.78

[a] Average take into account only the samples above LOQ.

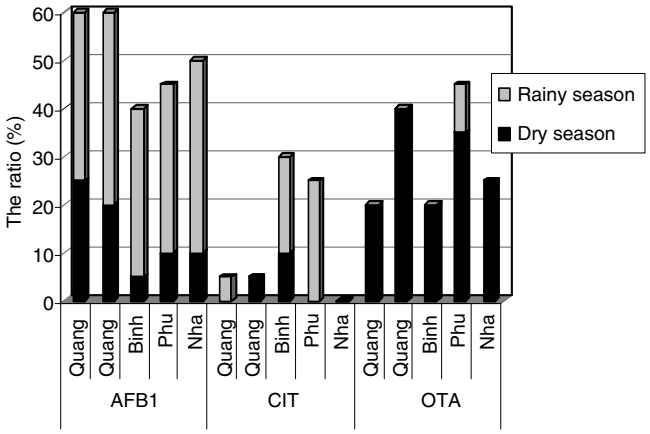

Fig. 3. The proportion (ratio) of AFB1-, CIT- and OTA-contaminated samples in rice from five provinces.

season were 0.87 ng/g and 2.78 ng/g, respectively and, in Quang Nam, OTA was not quantified, whereas, no OTA was detected in rice in the rainy season of these provinces.

The European Community has put limits of 5 ng/g in raw cereal and 3 ng/g in processed food. According to the joint expert committee on food and additives (JECFA), the provisional tolerable weekly intake (TWI) of OTA (based on nephrotoxicity in pig) is 100 ng/kg b.w./week, equivalent to 6 μg/week for a person weighing 60 kg. If we take into account the EU legislation based on carcinogenicity of OTA, the daily intake (TDI) is much lower and corresponds to 5 ng/kg b.w./day. This means a consumption of 300 ng/day for a person weighing 60 kg. Consumption of the most contaminated rice corresponds to 1390 ng/day. This is five times higher than the EU recommendation and 1.6 times higher than JECFA recommendation. Our result suggests that human exposure to OTA via contaminated rice contributes significantly to public health risk.

As can be seen from Table 5, among AFB1 and OTA co-contaminated rice samples, 69.23% and 61.54% are contaminated by CIT, respectively.

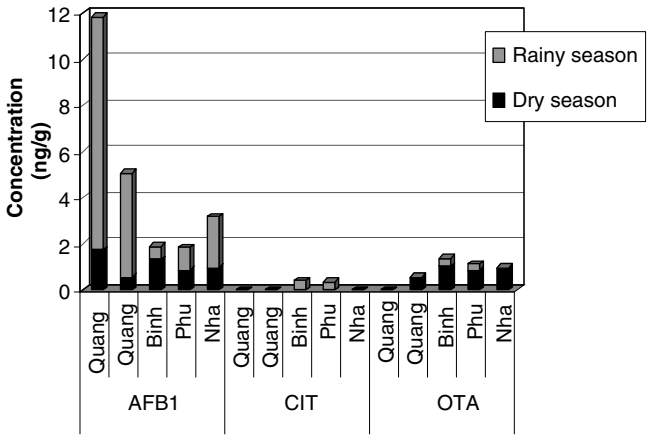

Fig. 4. The quantifiable averages of AFB1-, CIT- and OTA- contaminated samples in rice from five provinces.

Table 5
The simultaneous contamination percentage of each mycotoxin (A) to total number of other mycotoxin (B) contaminated samples

B	A		
	AFB1	CIT	OTA
AFB1	–	17.65%	21.57%
CIT	69.23%	–	61.54%
OTA	31.43%	22.86%	–

This is particularly important in regard to possible synergism and additive effects of these mycotoxins. Such co-contamination has been previously observed with other food samples, such as wheat (Vrabcheva, Usleber, Dietrich, & Martlbauer, 2000) breakfast cereal (Molinié et al., 2005; Pfohl-Leszkowicz, Molinié, & Castegnaro, 2004) or olives (El Adlouni, Tozlovanu, Natman, Faid, & Pfohl-Leszkowicz, 2006; Heperkan, Meric, Sismanoglu, Dalkiliç, & Güler, 2006).

4. Conclusion

The contamination of these mycotoxins in rice in five provinces of the central region of Vietnam was alarmingly high, especially AFB1, predominantly, but also OTA for some samples.

The level of contamination by these toxins in rice was greatly affected by the season of the year, particularly the rainy season which proved to be the major risk factor for the presence of AFB1 and CIT. So it is necessary to have an appropriate method for rice preservation during the distribution to consumers, particularly in the markets.

The coexistence of CIT with AFB1/OTA in rice was found in a high percentage of samples and thus should be taken into consideration as claimed by the European community (Scientific committee on Food opinion on Ochratoxin A. CS/CNTM/MTC/14 Final annex II to document XXI/2210/98, Brussels: CEC, 1998).

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