Presence of Ochratoxin A (OTA), Citrinin and Fumonisin B_1 in breakfast cereals collected in french markets. Comparison of OTA analysis using or not immunoaffinity clean-up before HPLC.

Annie Pfohl-Leszkowicz
Anne Molinié
Marcel Castegnaro

Lab. Toxicology and Food Safety, Av. de L'Agrobiopole , F- 31326 Auzeville-Tolosane France

REVISTA MEXICANA DE MICOLOGÍA 19: 7-16, 2004

ORIGINAL

Presencia de Ocratoxina A (OTA), de Citrinina y de Fumonisina B1 en cereales para desayuno colectados en Francia. Comparación del análisis de OTA con utilización o no de una purificación con columna de immunoafinidad antes de la cromatografía de líquidos

Resumen. Los cereales y otros vegetales pueden ser atacados por hongos tanto en campo como en almacén. Las micotoxinas producidas por estos hongos son muy estables y pueden encontrarse en productos industrializados como son los cereales para desayuno. El objetivo de este estudio fue evaluar la presencia de Fumonisina B_1 (FB_1), de Ocratoxina A (OTA) y de Citrinina (CIT) en cereales para desayuno colectados en supermercados franceses. La FB1 purificada en columna de inmunoafinidad fue detectada en 25 de las 27 muestras, con tasas de 1 a 1110 μg/kg. La FB_1 se detecta en muestras conteniendo no sólo maíz, sino también avena y arroz. La recuperación de OTA y de CIT fue superior al 80% por el método convencional de extracción por partición, mientras tanto la recuperación de OTA sólo alcanzó los 60% con una purificación efectuada por columnas de inmunoafinidad con un amortiguador de sales de fosfatos . OTA se detectó en 72.5% de las muestras, con tasas que llegaron al límite de detección (0.1 μg a 8.8 μg/kg). La tasa más elevada se encontró en una muestra con frutas secas y fibras. El 17% de las muestras presentaron un contenido de OTA superior al límite autorizado por la Unión Europea (3 μg/kg). El 20% de las muestras presentaron contaminación por CIT.
Palabras clave: Ocratoxina A, Fumonisina, Citrinina, micotoxinas, cereales de desayuno.

Abstract. Cereals and other crops are susceptible to fungal attack either in the field or during storage. The mycotoxins produced by these fungi are very stable and could be found in final products such as breakfast cereal. The aim of this study was to evaluate the presence of Fumonisin B_1 (FB_1), Ochratoxin A (OTA) and Citrinin (CIT) in some breakfast cereals collected in French supermarkets. FB_1 was purified by immunoaffinity columns and has been detected, after derivatization, in 25/27 samples, ranging from 1 to 1110 μg/kg. FB_1 has been detected in samples containing corn, but also oat or rice. OTA and CIT recoveries were over 80 % by the conventional method of partition extraction, whereas the OTA recovery was about 60% using immunoaffinity clean-up in phosphate saline buffer. OTA has been detected in 72.5% of the samples ranging from the limit of detection (0.1μg/kg) to 8.8 μg/kg. This highest level was detected in a sample containing dry fruit and fibres. Seventeen percent of the samples contained OTA above the EU limit of 3μg/kg. Twenty percent of them were also contaminated by CIT.
Keywords: Ochratoxin A, Fumonisin, Citrinin, mycotoxins, Breakfast cereal.

Recibido 27 de enero 2004; aceptado 26 de agosto 2004.
Received 27 January 2004; accepted 26 August 2004.

Autor para correspondencia: Annie Pfohl-Leszkowicz
leszkowicz@ensat.fr

Introduction

Cereals and other farm products are susceptible to fungal attack which may produce mycotoxins [30, 32, 33, 35, 36, 38, 40]. They develop either in the field or during storage. Fumonisin B_1 is produced in the field by some *Fusarium* species growing on corn, oat or rice but also on other cereals like wheat, millet and sorghum [3, 30, 43, 48]. During storage, wheat, oat or barley contaminated by Penicillia (*Penicillium verrucosum*, *P. aurantiogriseum*, *P. citrinum* et *expansum*) can contain Ochratoxin A (OTA) and Citrinin; [25, 31]. Those contaminated by Aspergilli (*Aspergillus ochraceus*, *A. carbonarius*, *A. niger*) may contain OTA [1, 50]. These mycotoxins can be transferred during the food process [8, 9, 19, 28, 29] to the final products such as breakfast cereals: i.e; fumonisin has already been detected in numerous samples [15, 20, 24, 41, 44, 46]. OTA has been recently reported [22, 42, 50]. Citrinin have so far never been reported in breakfast cereals in the literature. Until now, no methods have been validated for the analysis of mycotoxins in breakfast cereals. There is, therefore, a tendency to extrapolate the use of AOAC international or EU validated methods to the analysis of mycotoxins in these commodities.

The aim of this study was to evaluate the presence of Fumonisin B_1 (FB_1), Ochratoxin A (OTA) and Citrinin (CIT) in some breakfast cereals collected from retails in France. For the simultaneous analysis of OTA and CIT in some breakfast cereals we developed a conventional method [27] and compared it to the published conventional methods and other methods using immunoaffinity clean-up such as the AFNOR method [4, 5]. Fumonisin was analyzed as described by Visconti *et al*. [47] but we investigated its applicability to substrates like mixed breakfast cereals.

Material and methods

Sample collection

Forty samples purchased by the "Institute National pour la consummation" from retail outlets in France were analyzed [14]. These samples included the following major ingredients: corn, bran with fruits, cereals and chocolate, rice and oat (see Table 1). OTA and CIT were analyzed in all samples whereas FBs were analysed in the 27 samples containing corn, oat, and rice.

Reagents

All reagents (potassium chloride, sodium hydrogen carbonate, sulphuric acid, phosphoric acid, hydrochloric acid, acetic acid, sodium dehydrogenate phosphate) were of analytical grade. All solvents (methanol, chloroform, acetonitrile, propanol-2, n-hexane) were HPLC grade from ICS (France). Demineralised water was used for the preparation of all aqueous solutions and for HPLC studies. OTA free from benzene, CIT, carboxypeptidase and ortho-phtalaldehyde (OPA) for the derivatization of FBs were from Sigma Chemicals (France). The immunoaffinity columns (Fumoniprep and Ochraprep) were from Rhône Diagnostic technologies (RDT, France). Fumonisin B_1 was a gift from Dr D. Miller (Carleton University, Ottawa, ON, Canada). A qualitative FB_2 solution was obtained from the International Agency for Research on Cancer (IARC)Lyon, France.

Method of analysis

FBs were analyzed by the method of Sydenham *et al.* [45] as validated by the AOAC international [47]. OTA and CIT were analysed by the method described by Molinié *et al*. [27]. Some samples were also analyzed by the method of Entwisle *et al*.[10] and according to the notice "application note" provided by Rhône Diagnostic Technologies [37].

Table 1. Summary of the results for OTA, CIT and FB_1 analysis.

Sample Number	cocoa	Fruits[1]	rice	corn	oat	Wheat flour	Wheat bran	Barley	CIT ug/kg	OTA ug/kg	FB_1 ug/kg
1				x				x	N.D.	<LOQ	21
2	x	x	x				x		N.D.	N.D.	36
3				x				x	N.D.	N.D.	8
4				x				x	N.D.	<LOQ	45
5				x					N.D.	N.D.	7
6				x				x	N.D.	N.D.	21
7				x				x	N.D.	N.D.	11
8		x					x	x	<LOQ	0.9	NA
9	x	x	x	x	x		x		19	2.5	10
10	x	x	x						N.D.	2.8	25
11*			x				x		N.D.	0.9	N.D.
12	x	x	x			x	x		42	4.1	32
13	x	x	x			x	x		N.D.	0.8	48
14	x	x	x			x			N.D.	4.6	trace
15	x					x			N.D.	4.4	NA
16*	x			x					<LOQ	3.4	1113
17	x		x					x	N.D.	2.5	trace
18		x					x	x	N.D.	1.3	NA
19			x			x	x		N.D.	N.D.	240
20*		x	x				x		N.D.	1.3	N.D.
21		x					x	x	N.D.	0.4	NA
22							x		12	1.7	NA
23	x						x		N.D.	N.D.	NA
24							x	x	N.D.	<LOQ	NA
25	x	x	x	x	x			x	12	<LOQ	30
26*	x		x	x		x			N.D.	<LOQ	50
27	x	x		x					N.D.	1.4	65
28		x				x	x	x	N.D.	8.8	NA
29	x	x	x			x	x		N.D.	1.9	120
30*	x					x			N.D.	N.D.	NA
31		x					x	x	N.D.	3.1	NA
32		x					x		N.D.	N.D.	NA
33	x	x	x			x			N.D.	N.D.	40
34	x		x						N.D.	N.D.	25
35	x	x	x			x	x		5	<LOQ	28
36	x	x			x				7	3.1	45
37*	x		x						N.D.	<LOQ	50
38		x					x	x	N.D.	<LOQ	NA
39		x					x		N.D.	2	NA
40	x	x	x			x	x		N.D.	<LOQ	15

NA: not analyzed, N.D.: below the LOD, LOQ: mycotoxin is detectable but not quantifiable.* presence of corrector of acidity (Sodium bicarbonate, calcium carbonate) ° dried raisins, nuts, hazelnuts.

HPLC system for the analysis of OTA and CIT

A C18 Spherisorb column (3 µm C18, 0.46 × 25 cm) preceeded by a C18 precolumn from ICS was used for analysis of OTA and CIT. In order to reduce the risk of false positives, 2 elution solvents were used for OTA analysis: Phase 1. methanol / acetonitrile / sodium acetate (5mM) / acetic acid (300/300/400/28); Flow rate 0.7 mL /min. Phase 2. H_3PO_4 (0.33M) / acetonitrile / propanol-2 (600/400/50); flow rate 0.7 mL /min. A third phase was used for CIT analysis : H_3PO_4 (0.33M) / acetonitrile / propanol-2 (700/300/50); flow

rate 0.7mL/min. Gilson 811B dynamic chromatographic pump coupled to a Spectra Physic 2000 fluorescence spectrophotometer and an ICS auto sampler were used.

For the analysis of OTA and CIT, the excitation and emission wavelengths were respectively (OTA) 335 and 465 nm; (CIT) 331 and 500 nm.

HPLC system for the analysis of FBs

FBs were eluted on a C18 nucleosil column, 150 x 4.6 mm, with an Ultrasep C18 10µm pre-column, 1 cm long from ICS. The mobile phase for FBs was methanol / Na H_2PO_4 0.1M (80/20) adjusted to pH 3.35 with H_3PO_4; flow rate 0.9 mL /min. HPLC is equipped with pumps provided by ICS coupled to a fluorescence spectrophotometer (Lachrom) and a manual sampler. The excitation and emission wavelengths were respectively 335 nm and 440 nm. The chromatograms were analyzed by a Normasoft software provided by ICS (France).

Confirmation of OTApresence

The confirmation of OTA presence in samples detected at 2 µg /kg was performed by the carboxypeptidase technique, in producing ochratoxin alpha (Ot alpha). Briefly, an aliquot taken from the purified extract was dried and dissolved in 0.9 mL of a buffer solution of TRIS HCl 0.04M, NaCl 1M, pH 7.5. Hundred µL of Carboxypeptidase (100 U/mL were added and the solution was incubated at 37°C for 2h. After room cooling, in sample(s) containing OTA, when using the same HPLC chromatographic conditions as for the analysis of OTA, we found the absence of the OTA peak together with the appearance of the Ot alpha peak.

Statistical analysis

The data were analyzed for the comparison of the medians by the method of Mc Gill *et al*. [26].

Results

Comparison of methods for OTAanalysis

First, the analytical method by Molinié *et al*. [27] was compared to that of Entwisle *et al*. [10] and the proposed one in the application note from Rhône Diagnostic technologies [37]. The results are presented in Table 2. Except for sample B, lower levels of OTAwere found by the method of Entwisle *et al*. [10] as compared to that of Molinié *et al*. [27]. This trend is confirmed by generally lower recoveries on samples spiked with 3µg/kg. In addition, all analysis performed by the RDT application note [38] presented much lower OTAlevels, with non detected OTA in samples when contamination were below the EU regulatory limits[12, 13].

Study of the applicability of the fumonisin AOAC International method to breakfast cereals

Four samples were spiked with 200 µg/kg FB_1 and analyzed using the AOAC International method [47]. Recoveries ranged from 40 to 74.9%. In order to elucidate the source of these low recoveries, the equivalent of 300 µg/kg of FB_1 were added to the extracts of nine different samples just before the application of the immunoaffinity column. Recoveries varied from 54.4 to 79.1 % (Table 3).

Table 2: Comparison of OTA level in function of three methods of OTA purification.

Samples / Method	A*	C	D	E*	F*	G	H	I
Molinié *et al*. (2003) [27]	3.4	<LOQ	8.8	ND	ND	1	ND	<LOQ
Entwisle *et al*. (2000) [10]	1.6	ND	7.2	ND	ND	0.45	ND	ND
RDT (application note N° A9-P14.V1 [37]	<LOQ	ND	5.6	ND	ND	ND	ND	ND
Recovery Molinié *et al*. (2003) [27]	85 %	83 %	83 %	NA	83 %	NA	87 %	85 %
Recovery Entwisle *et al*. (2000) [10]	63 %	75 %	66 %	61 %	75 %	78 %	90 %	80 %

* sample contain corrector of acidity ; NA not analysed; ND: below LOD.

Results of the sample analysis

OTAand CIT contamination

All samples were analyzed for OTA and CIT. The plain results from recoveries are presented in Table 1. OTA was detected in 72.5 % of the samples, ranging from the limit of detection (0.1 µg/kg) to 8.8 µg/kg. Fifty percent of them were above the limit of quantification. The highest level was detected in a sample containing dried fruit and bran. Eighteen percent of the samples contained OTA amounts above the EU limit of 3µg/kg, 32 % contained OTA between 0.2 and 2.99 µg/kg and 50 % had non detectable levels. Twenty percent of the samples are contaminated by CIT in the range of 1 to 42 µg/kg. Samples containing dried fruits or wheat bran were statistically more frequently (64% versus 25% and 64% versus 32%, respectively) and more contaminated (median values of 0.95 versus 0.01 and 0.95 versus 0.05 respectively) by OTA than the samples which did not contain these ingredients (Tables 4 and 5). On the contrary, samples containing corn were significantly less contaminated than sample without corn (23% versus 56%; median value: 0.1 versus 2.5).

Table 3. Recovery tests of the immunoaffinity column only for FB_1 in various samples.

Sample	Recovery in %
A	74.87
B	69.45
C	74.75
D	54.4
E	78.4
F	61.7
G	79.1
H	59
I	67

FB_1 contamination

FB_1 were detected in 93% of the samples containing maize, oat or rice. The levels ranged from the limit of detection (1 µg/kg) to 1110 µg/kg. Seven percent were below the limit of detection, 7.4 % were between 100 and 250 µg/kg and 3.7 % were above 1 mg/kg. Two samples contained also FB_2 and the most contaminated one, FB_3. These later two toxins were not quantified.

Multitoxin contamination

From the 40 samples analyzed for OTA and CIT, 8 (20%) contained both toxins. Among the 27 samples analyzed for the three toxins, 6 (22.2 %) contained the three toxins, and 17 (63 %) had OTAand FB_1.

Table 4. Statistical analysis of OTA between class of ingredient.

Ingredients	Average µg/kg	Median ug/kg	Inferior Quartile	Superior Quartile	Confidence interval	% of samples above LOQ	Significant*
Cocoa	1.45	0.5	0.4	2.8	0.8	50% (11/22)	
Without cocoa	0.95	0.1	0	1	0.33	44% (10/23)	NS
Fruits	1.62	0.95	0	2	0.63	64% (16/25)	
Without fruits	0.65	0.01	0	0.9	0.3	25 % (5/20)	S
Wheat (bran)	1.41	0.95	0.1	2	0.63	64% (14/22)	
Without wheat	0.85	0.05	0	1.4	0.55	32 % (5/16)	S
Corn	0.59	0.1	0	0.1	0	23 % (3/13)	
Without Corn	1.45	2.5	0.05	2.5	0.68	56 % (18/32)	S
rice	1.13	1.3	0	1.3	0.82	47% (9/19)	
Without rice	1.22	1.7	0	1.7	0.47	46% (12/26)	NS

* S: statistically different, NS: not statistically different.

Table 5. Comparison of samples containing cocoa and/or fruits versus sample containing neither cocoa, nor fruits.

Ingredients	Average µg/kg	Median µg/kg	Inferior quartile	Superior quartile	Confidence interval	% of sample above LOQ
Cocoa alone	1.04	0.1	0	2.5	1.31	33% (3/9)
Cocoa + fruits	3.64	1.77	0.1	3.1	1.34	66% (8/12)
Fruits alone	1	0.9	0.1	1.3	0.6	70% (7/10)
No cocoa, no fruits	0.26	0	0	0.1	0.05	20% (2/10)

Discussion

The aim of this work was to analyze some breakfast cereals for their content of OTA, CIT and FBs. No method had been validated for the analysis of these toxins in such complex mixtures. Our preliminary work has demonstrated that methods which had already been validated for one or two matrices such as that of Entwisle *et al*. [10], for the determination of OTA or that of Visconti *et al.* [47] for FB_1 cannot be satisfactorily extrapolated to the analysis of the same toxins in very complex matrices. For both methods, lower recoveries were observed with some of these complex mixtures. These may be attributed to several factors.

In the case of OTA, the trends in the extraction changed, going from extraction from an acidified medium to a non acidified [10] or even alkaline one [21, 38]. In alkaline medium, OTA is transformed to open-ring OTA (OP-OTA), but this reaction is reversible when the medium is acidified [7]. When using immunoaffinity columns for the clean-up, this molecule is no longer recognised by the antibodies, and thus not retained on the column. This results in losses of OTA and an underestimation of the true contamination. This fact has two implications: 1) when extracting OTA from a matrix in which a corrector of acidity such as sodium carbonate or sodium bicarbonate has been added (which is the case of samples 11, 16, 20, 26, 30, 37 in Table 1) and A,B, E, F in Table 2), depending of the amount added and the final pH of the matrix, OTA is not correctly quantified. This explains the generally lower levels found by the method of Entwisle *et al*. [10] and the generally lower OTA recoveries of the spiked samples. Moreover, spectral characteristic of OTA is dependent of pH, and induced of shift in the chromatogram [17]. Thus, even by the conventional method in some samples containing a corrector of acidity, the final pH could be not enough acid, and thus a part of OTA was OP-OTA. Indeed, in some chromatograms, a second peak having the same chromatographic properties than OP-OTA was detected (data not shown). 2) when extracting OTA with an alkaline reagent [37] and purifying the extract by immunoaffinity columns, the effect was even more drastic and greater losses were observed (Table 2). To confirm this result, we passed a solution containing 60 ng OTA in water and in 1M sodium bicarbonate through an immunoaffinity column, and we found that after 1 hour of contact only 40 % of the OTA was recovered through the column. These losses were even more important when we lengthen the contact time with sodium bicarbonate. These analytical problems will have serious impact on the amount of OTA detected, specially at the concentrations close to those from the EU legislation (N°472/2002) [12, 13]. In addition, we must keep in mind that in stomach, the acidic pH induce the reformation of OTA. Our method (27) avoids these drawbacks and allows simultaneous extraction of the CIT.

For the analysis of FBs, we applied the method validated by Visconti *et al.* [47] which proved to be acceptable with corn and corn flakes. When analyzing corn flakes alone, recoveries were in the range of that of the EU study: 70 to 75% (data not shown). With other types of samples (i.e.: containing, fruits, oat, rice, sugar chocolate, etc.) recoveries varied and could be very low (down to some 40%). To test the potential cause of these losses, we enriched extracts with the equivalent of 300 ng/kg FB_1, just before the immunoaffinity columns purification. Twenty five to 45% losses are observed at this step (Table 3). This can be explained by the presence of some compound(s) which were recognized by the antibodies, thus blocking the sites and reducing the trapping efficiency for FBs. When looking at the composition of the samples, we suggest that this may be due to the presence of higher levels of unsaturated fatty acids.

All samples were analyzed for OTA and CIT. Seven of the 40 samples had uncorrected OTA concentrations already above the EU regulatory level of 3 µg/kg. If the data were corrected for recoveries, three additional samples would have exceeded this limit. These results were in line with those of Lombaert *et al.* [22] who reported OTA contamination in 10/47 samples of baby cereals at concentrations up to 6.9 µg/kg.

Although the average and the median OTA amounts were higher for sample containing cocoa, comparison of the medians with confidence intervals at 95% were not statistically different. In contrast, this analysis demonstrates the potential implication of two other factors in the OTA contamination: fruits and wheat bran. On the contrary, there is no implication of the presence of corn. The data in Table 5 strengthen the implication of the presence of fruits. Indeed, the EU legislation on raisins allows up to10 µg/kg [12, 13]. A sample contaminated in the upper range, added in significant quantities to the breakfast cereals will bring the concentration of contamination of these breakfast cereals above the EU regulatory concentration of 3 µg/kg established for cereals ready for consumption.

From these data, we calculated [27] that child of 20 kg who consumes one of the 4 highest contaminated samples according to the manufacturers' suggestion will exceed the OTA tolerable daily intake of 5.8 ng/kg body weight.

When CIT was found in a sample, it was always associated to OTA. These samples contained oat (3/8), wheat (5/8), corn (2/8) or rice (4/8). The presence of OTA in oat, wheat and corn is well documented [30]. Recently, Beretta *et al*. [6] reported it in rice. Since both toxins can be produced by similar Penicillia, this is probably the source of CIT.

The samples containing wheat alone were not analyzed for FBs contamination. Some samples contained only one type of cereal but most of them were combinations of 2 or more cereals. Average FB values are higher in sample containing corn (146.33 versus 42), nevertheless, comparison of the median values with confidence intervals at 95% did not show any significant differences between corn-based sample, rice-based samples or oat-based samples (see Table 6). Two samples containing wheat and some rice did not contain any FBs. Seventeen other samples containing rice were contaminated, mostly at low concentrations (10-50 µg/kg) except 2 which reached levels of 120 and 240 µg/kg. Among the rice contaminated samples, one contained oat in addition, one corn and two oat and corn. Three samples contained only oat and were contaminated respectively with 18, 35 and 45 µg/kg. One oat sample contained corn and had 46 µg/kg FB_1. All the other samples contained corn alone as cereal. The range of contamination was 1-1113 µg/kg FB_1. The most contaminated was exclusively from corn flour. It was also contaminated by FB_2 and FB_3 (not quantified). Thus the data in Table 1 represent an underestimation of the actual contamination. In this work, we demonstrated that contaminations by FBs were not only associated to corn-based cereals but also to those containing oat or rice as was already published for these ingredients [3, 48]. Our results are in line with those from the literature. Indeed, 19 µg/kg FB_1 and 10 µg/kg FB_2 were found in "baby menu" with corn [23]. Several baby food samples were positive for FB (41) reaching 440 µg/kg FB_1 [24] and 158 µg/kg Fb_2 [15]. Two studies described presence of FB_1 in 17 out of 18 corn base breakfast cereals at concentrations up to 1 mg/kg [44]; and in 22 out 25

Table 6. Statistical analysis of FB between class of ingredient.

Ingredients	Average µg/kg	Median µg/kg	Inferior quartile	Superior quartile	Confidence interval	% of sample above LOQ
Corn alone	146.33	21	11	45	17.8	100% (9/9)
Corn *	109	21	10	45	15	100% (13/13)
Rice alone	47.93	28	15	48	14.4	84 % (11/13)
Without corn	42	25	15	45	11	89% (17/19)
Without rice	112	32	18	45	14.4	100% (13/13)

* all sample containing corn alone or in presence of rice and/or oat .

samples [20]. These authors demonstrated also that the amounts reported did not take into account the FB_1 bound to proteins which can be as important as 2.6 fold that of the unbound toxin. Our results on FBs in corn base breakfast cereals confirmed those reported by these other teams. FB_1 has been recently classified as "possible human carcinogen by IARC" (Group 2B). While no EU legislation exists at present, it is being investigated and we can calculate from the results of the carcinogenicity studies that a maximum level of 300 ng/kg b.w. may be reasonably proposed. In this case, we calculated that a person consuming the highest contaminated sample may exceed this level by 5 times. Finally, we want to point out that during the process, CIT and FBs can be degraded into very toxic compounds [16, 39]. They were not analyzed in this study.

In conclusion, our results demonstrated: 1) that a method of analysis which has been validated for one substrate, needs to be re-evaluated before analysing the same contaminant in another substrate, 2) that in the case of OTA the contamination can exceed the EU regulatory levels, 3) that some breakfast cereals can be contaminated by OTA simultaneously with CIT and/or FB_1. This is particularly important as a number of studies indicate additive or synergic toxic effects.

Acknowledgements

The authors thanks the French "Institut National de la Consommation" for supporting this work, R. Victoria and R. Reuss for helpful discussions and the Region Midi-Pyrénées.

Literature cited

1. Abarca, M.L., M.R. Bragulat, G. Castellà, F.J. Cabanes, 1994. Ochratoxin A production by strains of *Aspergillus niger* var. *niger*. Applied Environmental Microbiology 60: 2650-2652.
2. Abarca, M.L., M.R. Bragulat, G. Castellà, F. Accensi, F.J. Cabanes,1997. New ochratoxigenic species in the genus *Aspergillus*. Journal of Food Protection 60: 1580-1582.
3. Abbas, H.K., R.D. Cartwright, W.T. Shier, M.M.Abouzied, C.B. Bird, L.G. Rice, P.F. Ross, G.L. Sciumbato, F.I. Meredith, 1998. Natural occurrence of fumonisins in rice with *Fusarium sheat* rot disease. Plant Disease 88: 22-25.
4. AFNOR, 1998a. Dosage de l'ochratoxine A dans les céréales et produits derivés. Partie 1 : Méthode par chromatographie liquide haute performance comprenant une étape d'extraction par chromatographie sur gel de silice. NF EN ISO 15141-1, pp 17.
5. AFNOR, 1998b. Dosage de l'ochratoxine A dans les céréales et produits dérivés. Partie 2 : Méthode par chromatographie liquide haute performance comprenant une étape d'extraction par une solution de bicarbonate. NF EN ISO 15141-2, pp 15.
6. Beretta, B., R. De Dominico, A. Gaiaschi, C. Ballabio, C.L. Galli, C. Gigliotti, P. Restani, 2002. Ochratoxin A in cereal-based baby foods/ Occurrence and safety evaluation. Food Additives and Contaminants 19: 70-75.
7. Castegnaro, M., J. Barek, J. Jacob, U. Kirso, M. Lafontaine, E.B. Sansone, G.M. Telling, M. Vu Duc (eds), 1991. Laboratory decontamination and destruction of carcinogens in laboratory wastes : Some mycotoxins, International Agency for Research on Cancer (IARC) publication 113: 5.
8. Castellà, M.M., S.S. Sumner, L.B. Bullerman, 1998a. Stability of fumonisins in thermally processed corn products. Journal of Food Protection 61: 1030-1033.
9. Castellà, M.M., S.K. Katta, S.S. Sumner, M.A. Hanna, L.B. Bullerman, 1998b. Extrusion cooking reduces recoverability of fumonisin B1 from extruded corn grits. Journal of Food Science 63: 696-698.
10. Entwisle, A.C., A.C. Williams, P.J. Man, P.T. Slack, J. Gilbert, 2000. Liquid chromatographic method with immunoaffinity column clean up for determination of ochratoxin A in barley: collaborative study, Journal of the Association of Official Analytical Chemists International 83: 1377-1383.
11. Entwisle, A.C., A.C. Williams, P.J. Man, J. Russell, P.T. Slack, J. Gilbert, 2001. Combined phenyl silane and immunoaffinity column clean up with liquid chromatography for determination of ochratoxin A in roasted coffee: collaborative study. Journal of the Association of Official Analytical Chemists International 84: 444-450.
12. European Union, 2002a, Règlement C.E. N°472/2002 du 12 Mars 2002 portant fixation de teneurs maximales pour certains contaminants dans les denrées alimentaires.
13. European Union, 2002b, Règlement C.E. N°26/2002 du 13 Mars 2002 portant fixation des modes de prélèvement d'échantillons et des méthodes d'analyse pour le contrôle officiel des teneurs en ochratoxine A des denrées alimentaires.
14. Guibert F., R. Victoria, 2003. Petit-déjeuner, 40 céréales analysées. 60 millions de consommateurs 368: 41-50.
15. Henningen, M.R., S. Sánchez , N.M. Di Benedetto, A. Longhi J.E. Torroba, L.M. Valente- Soares, 2000. Fumonisin levels in commercial corn products in Buenos Aires, Argentina. Food Additives and Contaminants 17: 55-8.
16. Hirota, M., A.B. Menta, K. Yoneyama, N. Kitabatake, 2002. A major decomposition product, citrinin H_2, from citrinin on heating with moisture. Bioscience Biotechnology and Biochemistry 66: 206-210.
17. Ill'chev, Y.V., J.L. Perry, R.A. Manderville, C.F. Chignell, J.D. Simon, 2001. The pH-dependent primary photoreactions of ochratoxin A. Journal of Physical and Chemical Reference Data. 105: 11369-11376.
18. International Agency for Research on Cancer (IARC), 2002. IARC monographs on the evaluation of carcinogenic risks to human. Vol: 82. Some traditional herbal medicine, some mycotoxins, naphthalene and styrene. Lyon, France, pp 590.
19. Katta, S.K., L.S. Jackson, S.S. Sumner, M.A. Hanna, L.B. Bullerman, 1999. Effect of temperature and screw speed on stability of fumonisin B_1 in extrusion cooked corn grits. Cereal Chemistry 76: 16-20.
20. Kim, E.K., P.M. Scott, B.P. Law, 2003. Hidden fumonisins in corn flakes. Food Additives and Contaminants 20:161-9 (*erratum in Food Addi. Contam.* 20: 417).
21. Larsen, T.O., A. Svendsen, J. Smedsgaard, 2001. Biochemical characterization of ochratoxin A producing strains of the genus *Penicillium*. Applied and Environmental Microbiology 67: 3630-3635.
22. Lombaert, G.A., P. Pellaers, V. Roscoe, M. Mankotia, R. Nile, P.M. Scott, 2003. Mycotoxins in infant cereal foods from the Canadian retail market. Food Additives and Contaminants 20: 494-504.
23. Lukacs, Z., S. Schaper, M. Herderich, P. Schreier, H.U. Humpf, 1996. Identification and determination of fumonisin B_1 and B_2 in corn and corn products by high performance liquid chromatography electrospray ionisation tandem mass spectrometry (HPLC-ESI-MSMS). Chromatographia 43: 124-128.
24. Machinski M, L.M. Valente-Soares, 2000.Fumonisin B_1 and B_2 in Brazilian corn based food products. Food Additives and Contaminants 17: 875-879.
25. Mantle, P.J., K.M. McHugh, 1993. Nephrotoxic fungi in foods from nephropathy households in Bulgaria. Mycological Research 97: 205-212.
26. Mc Gill R., J.W. Tuckey, W.A. Larsen, 1978. Variations of box plots. The American Statistician32: 12-16.
27. Molinié A., V. Faucet, M. Castegnaro, A. Pfohl-Leszkowicz, 2004. Analysis of some breakfast cereals collected on the French market for their content in ochratoxin A, citrinin and fumonisin B_1. Development of a new method for simultaneous extraction of ochratoxin A and citrinin. Food Chemistry (in press).
28. Osborne, B.G. 1979. Reverse phase high performance liquid chromatography determination of ochratoxin A in flour and bakery products. Journal of Food Science and Agriculture 30: 1065-1070.
29. Osborne BG, F. Ibe, G.L. Brown, F. Petagine, K.A. Scudamore, J.N. Banks, N.T. Hetmanski, C.T. Leonard, 1996. The effects of milling and processing on wheat contaminated with ochratoxin A. Food Additives and Contaminants. 13: 141-153.
30. Pfohl-Leszkowicz, A., (ed), 1999. Les mycotoxines dans l'alimentation, évaluation et gestion du risque, TEC et DOC, Lavoisier, Paris..
31. Pitt, J.I., 1987. Reclassification of *Penicillium viridicatum*, *Penicilium verrucosum* and production of Ochratoxin A. Applied And Environmental Microbioliology 53: 266-269.
32. Pitt J.I., A.D. Hocking (eds), 1997. Fungi and food spoilage. Chapman and Hall, London.
33. Prelusky, D.B., B.A. Rotter, R.G Rotter, 1994. Toxicology of mycotoxins. In: J.D. Miller, and H.L., Trenholm (eds) Mycotoxins in grain, Egan Press, Saint Paul Minnesota, pp 359-403.
34. Reinhard, H., B. Zimmerli, 1999. Reversed-phase liquid chromatographic behabior of the mycotoxins citrinin and ochratoxin A. Journal of Chromatography A. 862: 147-159.
35. Resnik, S., M.L. Costarrica, A. Pacin 1995. Mycotoxins in Latin America and the Caribbean. Food Control 6: 19-28.
36. Resnik, S., A. Neira, A. Pacin, E. Martinez, N. Apro, S. Latreite, 1996. Survey of the natural occurrence of aflatoxin and zearalenone in Argentine field maize: 1984-1994. Food Additives and Contaminants 13: 115-120.
37. Rhône Diagnostics technologies, 1999. Cereal ochratoxin A extraction method, application note for analysis of Ochratoxin A in cereal using sodium bicarbonate extraction in conjunction with Ochraprep ®. Application note Ref N° A9-P14.V1
38. Ross, P.S., P.E. Nelson, J.L. Richard, G.D. Osweiller, L.G. Rice, R.D. Plattner, T.M. Wilson, 1990. Production of fumonisins by *Fusarium moniliforme* and *Fusarium proliferatum* isolates, associated with equine leucoencephalomalacia and pulmonary edema syndrome in swine. Applied And Environmental Microbiology 56: 3225-3226.
39. Saunders DS, F.I. Meredith, K.A. Voss, 2001. Control of fumonisin: effect of processing. Environmental Health Perspectives 109: 333-336.
40. Scott, P.M., 1990, Trichothecenes in grains. Cereals Food World 35: 661-666.
41. Scott, P.M., GA. Lawrence, G.A. Lombaert, 1999. Studies on extractions of fumonisins from rice, corn based foods and beans. Mycotoxin Research 15: 50-60.
42. Sewram, V., G.S. Shephard, W.F.O. Marasas, M.F. Penteoda M. de Castro, 2003. Improving extraction of fumonisin mycotoxins from Brazilian corn-based infant foods. Journal of Food Protection 66: 854-859.
43. Shephard, G.S., P.G. Thiel, S. Stockenstrom, E.W. Sydenham, 1996. Worldwide survey of fumonisin contamination of corn and corn based products. Journal of the Association of Official Analytical Chemists International 79: 671-687.
44. Solfrizzo, M., A. De Girolamo, A.Visconti, 2001. Determination of Fumonisin B_1 and B_2 in cornflakes by high performance liquid chromatography and immunoaffinity clean-up. Food Additives and Contaminants 18: 227-235.
45. Sydenham, E.W., G.S. Shephard, P.G. Thiel, 1992. Liquid chromatographic determination of fumonisins B_1, B_2 and B_3 in food and feeds. Journal of the Association of Official Analytical Chemists International 75: 313-318.
46. Usleber, E., and E. Märtlbauer, 1998. Occurence of fumonisins in foods in Germany. *In*: M. Miraglia, H. Van Egmond, C. Brera and J. Gilbert (eds) Mycotoxins and phycotoxins developments in chemistry toxicology and food safety, Allaken, Fort Collins pp 81-86.
47. Visconti A., M. Solfrizzo, A. De Girolamo, 2001. Determination of fumonisins B_1 and B_2 in corn and corn flakes by liquid chromatography with immunoaffinity column cleanup: collaborative study. Journal of the Association of Official Analytical Chemists International 84: 1828-1837.
48. Wilson, B.J., R.R. Maronpot, 1971. Causative fungus agent of leucoencephalomalacia in equine animals. Veterinary Records 88: 484-486.

49. WHO, 2002. Evaluation of certain mycotoxins in food. Report of the fiftysixth meeting of the joint FAO/WHO expert committee on food additives. WHO technical report series 906. (WHO, Geneva).
50. Wolff, J., 2000. Ochratoxin A in cereal and cereal products. Archiv für Lebensmittelhygiene 51: 81-88.