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ORIGINAL PAPER

Mycotoxins in foods in Lower Saxony (Germany): results of official control analyses performed in 2009

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Abstract In this presentation, the mycotoxin levels—as analysed by the analytical centre for mycotoxin surveillance of the state food laboratory (LAVES Braunschweig)—for approximately 500 food samples are reported. The samples were collected in the year 2009 at retail in the German federal state of Lower Saxony. Aflatoxin and ochratoxin A were analysed in dried fruits, spices, cereals and tree nuts. Ochratoxin A was detected in all samples of dried vine fruits, at levels up to 8.1 μg/kg. Aflatoxins and ochratoxin A were also found in nutmeg and curry powder: the maximum regulatory levels for aflatoxins were exceeded in 25% of the nutmeg samples. Nearly all samples of basmati rice contained aflatoxins, although at levels below the maximum regulatory level in all but one sample. Aflatoxins were also detected in about 50% of hazelnut samples, in 20% of the samples the maximum levels was exceeded (maximum 23.2 μg/kg). In contrast, aflatoxin contents in pistachios were surprisingly low. Fusarium toxins were analysed in cereals and cereal products such as flour, bread, and pasta. Deoxynivalenol (DON) was the predominant toxin found in these samples: DON was found in about 40% of the samples, although the maximum levels were not exceeded (max. 418 μg/kg). Fumonisins (FBs) and zearalenone (ZEA) were specifically analysed in maize products (snacks, flour and oil). Most of these samples (80%) were positive, but at levels not exceeding the maximum levels. Maximum levels were 98 μg/kg (ZEA) and 577 μg/kg (sum of FB_1 and FB_2). Ergot alkaloids (six major alkaloids) were analysed in rye flour, and approximately 50% were positive. The highest concentration of ergot alkaloids was 1,063 μg/kg; the predominant alkaloids were ergotamine and ergocristine. In conclusion, the results indicate that continuous and efficient control measures for mycotoxins in a wide range of critical foods are necessary to ensure compliance with maximum levels. Although the mycotoxin levels in the vast majority of samples were below maximum levels, year-to-year variation and changes in the production of relevant commodities may result in a different picture in the future.

Keywords Mycotoxins · Aflatoxins · Ochratoxin A · Deoxynivalenol · Fumonisins · Zearalenone · Ergot alkaloids · Food commodities

Introduction

Scientific mycotoxin research in food started in 1960, when toxic metabolites of mould fungi were found to cause death of thousands of turkeys ("turkey-x-disease", Mücke and Lemmen 2004). The first official regulation concerning mycotoxins in Germany was implemented in 1976 (Aflatoxin-Verordnung 1976). Since that time, a lot of new regulations have come into force. The basic principles of EU legislation on contaminants in food are laid down in Council Regulation (EEC) No 315/1993 and maximum levels for certain contaminants in food, including mycotoxins, were set in Commission Regulation (EC) No 1881/2006. The latest amendments of maximum levels, listed in Commission Regulation (EC) No 1881/2006, concern aflatoxins (AFs) and ochratoxin A (OTA) and were implemented at the beginning of 2010 with Commission Regulations (EU) No

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165/2010 and 105/2010, respectively (see Table 1b). In addition to these European Union regulations, in Germany, the Kontaminanten-Verordnung (2010) defines rules concerning mycotoxins and other contaminants. Until 19th March 2010, similar regulations were laid down in the Mykotoxin-Höchstmengenverordnung (1999), which have now been repealed. All maximum levels applied in this work are based on these European and German regulations. A brief overview is given in Tables 1 and 2.

To ensure consumer protection, a large number of food samples has to be analysed. In Lower Saxony, one of the 16 federal states of Germany, the analytical centre for mycotoxin surveillance of official food samples is located in the Food institute of the Lower Saxony Office of Consumer Protection and Food Safety (LAVES), in Braunschweig. In this article, the mycotoxin contents of about 500 samples are presented, which were mainly taken at retail in 2009.

Materials and methods

Sample origin and sample collection

In Germany, the responsibility for the enforcement of official food control lies with the federal states ("Länder"). Based on the general food control legislation and general principles, each of the 16 federal states has a slightly different system concerning official food control. In Lower Saxony, food samples for official control are collected by trained and experienced staff of the local veterinary authorities. These samples are transported to, and analysed by, the institutes of the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES). The analytical centre for mycotoxin investigation of LAVES is located in the food institute at Braunschweig. Based on the results of the analyses, food law enforcement is achieved by action of the responsible local authorities.

Table 1 Maximum levels of some foodstuffs according to Commission Regulations (*CR*) 1881/2006 (valid in 2009), 165/2010 and 105/2010

Mycotoxin	Foodstuffs	Maximum levels (μg/kg)	
AFs (CR 1881/2006)		AFB_1	Total AFs
	Groundnuts and nuts and processed products thereof, intended for direct human consumption or use as an ingredient in foodstuffs	2.0	4.0
	Dried fruit and processed products thereof, intended for direct human consumption or use as an ingredient in foodstuffs	2.0	4.0
	All cereals and all products derived from cereals, including processed cereal products, with some exceptions	2.0	4.0
	Certain species of spices (nutmeg, ginger, pepper, chilli, paprika, turmeric)	5.0	10.0
AFs (CR 165/2010)	Groundnuts and other oilseeds and processed products thereof, intended for direct human consumption or use as an ingredient in foodstuffs with some exceptions	2.0	4.0
	Almonds, pistachios and apricot kernels, intended for direct human consumption or use as an ingredient in foodstuffs	8.0	10.0
	Hazelnuts and Brazil nuts, intended for direct human consumption or use as an ingredient in foodstuffs	5.0	10.0
	other tree nuts	2.0	4.0
OTA (CR 1881/2006)	All products derived from unprocessed cereals, including processed cereal products and cereals intended for direct human consumption with some exceptions	3.0	
	Dried vine fruit (currants, raisins and sultanas)	10.0	
OTA (CR 105/2010)	Certain species of spices (nutmeg, ginger, pepper, chilli, paprika, turmeric)	30 μg/kg from 1.7.2010 to 30.6.2012, 15 μg/kg from 1.7.2012	
Deoxynivalenol (CR 1881/2006)	Cereals intended for direct human consumption, cereal flour, bran and germ as end product marketed for direct human consumption, with some exceptions	750	
	Pasta (dry)	750	
	Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals	500	
Zearalenone (CR 1881/2006)	Cereals intended for direct human consumption, cereal flour, bran and germ as end product marketed for direct human consumption with some exceptions	75	
	Refined maize oil	400	
	Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals, excluding maize snacks and maize-based breakfast cereals	50	
	Maize intended for direct human consumption, maize-based snacks and maize-based breakfast cereals	100	
Fumonisins (sum of B_1 and B_2) (CR 1881/2006)	Maize intended for direct human consumption, maize-based foods for direct human consumption, with some exceptions	1000	
	Maize-based breakfast cereals and maize-based snacks	800	

Table 2 Maximum levels of some foodstuffs based on the German Kontaminanten-Verordnung

Mycotoxin	Foodstuffs	Maximum levels (μg/kg)	
AFs		(AFB_1	Total AFs
	Foodstuffs not regulated in the Commission Regulation 1881/2006	2.0	4.0
OTA	Dried fruits, with the exception of dried fruits regulated in the Commission Regulation 1881/2006 and dried figs	2.0	
	Dried figs	8.0	

The vast majority of the samples, including those for which results are presented in this report, originated from retail shops in Lower Saxony. Some samples were collected directly from producers located in Lower Saxony, including food processing companies. Other samples, in particular those analysed for AF content, were collected within the regime of official import controls.

Analytical methods

AFs

The homogenized samples were extracted with methanol/water. Deviating from this, cinnamon was extracted with acetonitrile/water. The extracts were cleaned up with immunoaffinity columns. Quantification was made by HPLC with post-column derivatization (pyridinium bromide perbromide) and fluorescence detection (ASU 2004, L23.05-2).

OTA

Cinnamon samples were extracted with chloroform after acidification. This chloroform solution was extracted again with sodium hydrogencarbonate solution. Other spices and dried fruits were extracted directly with sodium hydrogencarbonate solution after homogenisation. Then the extracts were cleaned up with immunoaffinity columns and measured by HPLC with fluorescence detection (Koch et al. 1996; Scheuer et al. 1997).

Fusarium toxins

In cereals other than maize *Fusarium* toxins (deoxynivalenol, T-2 and HT-2 toxin, zearalenone) were measured after extraction with acetonitrile/water and clean-up with solid-phase extraction by HPLC/tandem mass spectrometry (Klötzel et al. 2006).

Zearalenone

In maize products (except maize oil), zearalenone was extracted with methanol/water, cleaned up with immunoaffinity columns and determined by HPLC/fluorescence detection. Determination of zearalenone in maize oil was carried out by extraction and clean-up with gel permeation chromatography, followed by HPLC/tandem mass spectrometry (Biopharm 2009; Kappenstein et al. 2005).

Fumonisins

Maize products were extracted with methanol/acetonitrile/water. After clean-up with immunoaffinity columns, the fumonisins were quantified by HPLC with pre-column derivatization (ortho-phthaldialdehyde and 2-mercaptoethanol) and fluorescence detection (ASU 2006, L15.05-3).

Ergot alkaloids

Samples were extracted with methanol/phosphoric acid, then cleaned up by cation exchange solid-phase extraction and measured by HPLC/tandem mass spectrometry (Ware et al. 2000).

Results and discussion

AFs in cereals and tree nuts

The analysis of cereals (basmati rice, millet seed, unripe spelt grain) for AF yielded positive results for basmati rice only. More than 90% of the tested basmati rice samples, all of which were collected from retail shops, contained total AF contents (sum of AFB_1, B_2, G_1 and G_2) up to 5.1 μg/kg, the mean concentration was 1.1 μg/kg. In one sample, the content of AFB_1 and total AFs exceeded the maximum level as set by Commission Regulation 1881/2006. In basmati rice, and for samples collected in 2010 (n=22), AFs were detectable in about 70% of the samples, with a maximum value (total AFs) of 1.53 μg/kg. Similar reports from Sweden (Fredlund et al. 2009) and Iran (Mazaheri 2009) confirm that AFs are common contaminants in rice.

In contrast to the years 2007 and 2008 (Hülsdau and Reinhold 2009), in 2009 almost all tested pistachios were free of AFs (see Table 3). In 2007 and 2008, five pistachio samples exceeded the maximum level for AFB_1 (2 μg/kg) and total AFs (4 μg/kg), but in about 70% of the samples

Table 3 AFs in cereals and tree nuts

	n^a	Content<LOD[b]	AFB_1 (μg/kg)			Total AFs (μg/kg)		
			Mean[c]	Median	Max	Mean[c]	Median	Max
Basmati rice	17	1	0.96	0.65	4.61	1.11	0.78	5.09
Unripe spelt grain	5	5	-	-	-	-	-	-
Millet seed	14	14	-	-	-	-	-	-
Hazelnuts	42	18	1.37	0.26	4.99	4.11	0.61	23.16
Pistachios	32	30	0.3	<0.03	0.37	0.3	<0.03	0.37

LOD limit of detection: 0.01 μg/kg, *LOQ* limit of quantification: 0.03 μg/kg

[a] Total number of samples

[b] Number of samples with contents<LOD

[c] Mean of contents>LOQ

AFs were not detectable. Compared with 2007 and 2008, the contamination of hazelnut samples with AFs increased in 2009. Total AF contents up to 23.2 μg/kg were found in more than 50% of the hazelnut samples (Table 3). In almost 20% of the hazelnut samples, the maximum levels for AFB_1 (2 μg/kg) and/or the sum of AFs (4 μg/kg) were exceeded. Six of these eight samples were sampled in retail shops and two were taken for import control into the European Union (origin: Turkey). Because of exceedance of maximum levels, the two concerned consignments were rejected. In 2010, maximum levels for AFs, e.g. in almonds, pistachios and hazelnuts, were amended in order to consider maximum levels set by Codex Alimentarius and new scientific results (Commission Regulation 165/2010, see Table 1b). The maximum levels for AFB_1 and total AFs in hazelnuts for direct human consumption have been increased from 2 μg/kg to 5 μg/kg and 4 μg/kg to 10 μg/kg, respectively. The Scientific Panel on Contaminants in the Food Chain (Contam Panel) of the European Food Safety Authority (EFSA) concluded that increasing the levels for total AFs in almonds, pistachios and hazelnuts would slightly affect the dietary exposure and cancer risk (EFSA 2007). If the hazelnut samples of 2009 are judged by the new AF maximum levels, only 2% of the samples instead of 20% would have exceeded those new levels.

AFs and OTA in dried fruits and spices

None of the analysed dried fruits, which included products such as grapes (24 samples), banana slices (26 samples), figs (4 samples) and dates (13 samples) contained detectable levels of AFs. OTA was found only in dried grapes, and indeed in all samples (maximum 8.1 μg/kg, mean 3.0 μg/kg), however without any exceedance of the maximum level of 10 μg/kg. Similar results were obtained by MacDonald et al. (1999): most of the dried vine fruits (53 out of 60 samples) contained OTA, but AFs could not be detected.

Among the analysed spices (Table 4), all samples of curry were contaminated with AFs and OTA. In ginger, small amounts of these mycotoxins were found, and in cinnamon only OTA was detected in about half of the samples. In nutmeg powder, comparatively high contents of AFs and OTA were found and all samples were contaminated. In 25% of the nutmeg samples, the maximum levels for AFB_1 (5 μg/kg) and/or the sum of AFs (10 μg/kg) were exceeded. These samples originated from food companies which process (grind) raw materials of spices. Effecitve from 1st July 2010, the maximum levels for OTA have been set for some spices such as nutmeg (30 μg/kg, Commission Regulation 105/2010). With a maximum OTA

Table 4 AFs and OTA in spices

		AFs					OTA (μg/kg)		
			AFB_1 (μg/kg)		Total AFs (μg/kg)				
	n^a	Content<LOD[b]	Mean[c]	Max	Mean[c]	Max	Content<LOD[b]	Mean[c]	Max
Curry	6	0	0.41	1.03	0.45	1.17	0	1.44	2.74
Ginger	7	5	0.16	0.2	0.35	0.39	7		
Cinnamon	13	13	-	-	-	-	6	0.60	2.21
Nutmeg	20	0	4.11	19.69	5.24	23.74	0	2.56	10.3

LOD (AFs): 0.03-0.2 μg/kg; LOD (OTA): 0.02-0.06 μg/kg

[a] Total number of samples

[b] Number of samples with contents<LOD

[c] Mean of contents>LOQ

Table 5 Deoxynivalenol in cereal products

	n^a	Content<LOD[b]	Content<LOQ[b]	DON (μg/kg)		
				Mean[c]	Median	Max
Flour	18	14	2	156	156	177
Bulgur	10	10	-	-	-	-
Bread	13	6	5	53	<25	54
Pre-baked rolls	33	24	4	78	<25	168
Ciabatta	24	9	0	115	80	338
Pasta	28	15	3	87	<25	126
Cereal burger	16	10	2	189	<50	418

LOD: 25 μg/kg, LOQ: 50 μg/kg, cereal burger LOD: 50 μg/kg, LOQ: 100 μg/kg

[a] Total number of samples

[b] Number of samples with contents<LOD and<LOQ, respectively

[c] Mean of contents>LOQ

content of 10.3 μg/kg, all samples collected in 2009 contained OTA at levels below the new maximum level. Results reported for nutmeg samples collected in 2007 and 2008 also showed that most nutmeg samples were contaminated with AFs and OTA, but the highest AF and OTA contents were far below the new maximum levels. Compared with the results for spices analysed within the food monitoring programme in 2007 (BVL 2008), the contents of AFs and OTA in the nutmeg samples investigated in 2009 are in the same range, in some cases even slightly higher.

Fusarium toxins in cereals and cereal products

Fusarium toxins occur more often and with higher contents in cereals in years with adverse climatic condition (Mücke and Lemmen 2004), therefore periodic investigations are necessary. DON is the most frequently *Fusarium* toxin in cereals (Mücke and Lemmen 2004). In our study, DON was the predominant mycotoxin present also and was found in 38% of the analysed samples (Table 5). The highest amount, 418 μg/kg, was found in burger rolls made of cereals—a dry mixture for making "organic spelt burger". Most (62%) of the "ciabatta" type bread samples contained DON, with a maximum content of 338 μg/kg. In pasta samples, DON contents ranged up to 126 μg/kg. Most of these samples were collected as offered to the consumer in retail shops.

In all samples of cereal products (flour, bread, pasta) analysed in 2009 (Table 5), the DON content did not exceed the permitted maximum level of 500 μg/kg (bread) and 750 μg/kg (cereals intended for direct human consumption and pasta). In 2008, about 45% of the investigated cereals and cereal products contained DON with a maximum value of 746 μg/kg in wheat flour (Verbraucherschutzbericht Niedersachsen 2008). About 200 cereal products (flour, bread, pasta) were analysed for ZEA, T-2 toxin and HT-2 toxin but all were negative.

Zearalenone (ZEA) and fumonisins in maize products

For maize and maize products, maximum levels for ZEA and fumonisins have been set by Commission Regulation 181/2006. According to Mücke and Lemmen (2004), maize is frequently contaminated with fumonisins. In 2009, ZEA and fumonisins (B_1 and B_2) were analysed in about 100 samples of maize products, and about 80% were found to be contaminated (Tables 6 and 7). The highest ZEA

Table 6 Zearalenone in maize products

	n^a	Content<LOD[b]	Content<LOQ[b]	ZEA (μg/kg)		
				Mean[c]	Median	Max
Popcorn maize	12	6	4	16.2	2.5	22.0
Maize snacks	18	2	8	8.0	<4	19.8
Maize flour	8	2	1	31.7	12.0	71.8
Maize germ oil	20	0	0	63.9	63.7	97.7

LOD: 1 μg/kg, LOQ: 4 μg/kg; maize oil: LOD: 5 μg/kg, LOQ: 10 μg/kg

[a] Total number of samples

[b] Number of samples with contents<LOD and<LOQ, respectively

[c] Mean of contents>LOQ

Table 7 Sum of fumonisin B_1 and B_2 in maize products

	n[a]	Content<LOD[b]	Content<LOQ[b]	Sum of fumonisin B_1 and B_2 (μg/kg)		
				Mean[c]	Median	Max
Popcorn maize	12	4	4	176	<25	577
Maize snacks	30	8	16	48	<25	80
Maize flour and polenta	23	0	11	117	26	340
Tortilla chips	10	0	5	163	30	260

LOD: 7 μg/kg, LOQ: 25 μg/kg

[a] Total number of samples

[b] Number of samples with contents<LOD and<LOQ, respectively

[c] Mean of contents>LOQ

concentrations were found in popcorn maize, maize snacks and maize flour (22 μg/kg, 19.8 μg/kg and 71.8 μg/kg, respectively). ZEA was detected in all maize oil samples (sampled in retail shops). The content ranged from 16 μg/kg to 98 μg/kg. The maximum levels for ZEA (maize oil: 400 μg/kg; other investigated foodstuffs: 100 μg/kg) were not exceeded in any sample.

The maximum value for the sum of fumonisin B_1 and B_2 was found in popcorn maize (577 μg/kg). The maximum contents of fumonisins in maize snacks, maize flour, and tortilla chips were 62 μg/kg, 340 μg/kg, and 260 μg/kg, respectively. The fumonisin levels in all analysed samples were far below the maximum levels for fumonisins (Table 1). These results confirm other findings which showed that contamination of maize products with fumonisins is very common, although toxin levels are usually relatively low (Reinhold 2009, 2010).

Ergot alkaloids in rye flour

Six main alkaloids and their corresponding epimers were determined in 31 rye flour and wholemeal rye flour samples, mostly taken at retail. In Table 8, each ergot alkaloid and its respective epimer is combined (alkaloid+epimer).

About 50% of the samples contained ergot alkaloids, with a maximum value for the sum of 1,063 μg/kg. However, only 10% of the samples exceeded a level of 500 μg/kg. For ergot alkaloids, there are still no maximum levels set within the European Union. A value of 1,000 μg/kg for the sum of ergot alkaloids is generally regarded as a guidance value (Taschan 2009). The predominant alkaloid isomeric pair, which was detected in all positive samples, was ergotamine/ergotaminine, followed by ergocristine/ergocristinine and ergosine/ergosinine. The maximum content of the individual alkaloids ranged from 86 μg/kg for ergocornine to 365 μg/kg for ergotamine. Appelt and Ellner (2009) also found that ergotamine and ergocristine seem to be the two main alkaloids, although the alkaloid composition varies.

Conclusions

Although the maximum levels for AFs were exceeded in some samples, the majority of samples contained the various mycotoxins at relatively low levels. As growth of mould fungi is dependent on weather, harvest, production and storage, the conditions for the formation of mycotoxins can strongly vary from year to year. Therefore, it is necessary to continue and strengthen official controls by state authorities, in order to encourage the responsible parties in their efforts to minimize conditions for mycotoxin formation and content as far as possible.

Table 8 Ergot alkaloids in rye flour and wholemeal rye flour (n=31)

	Content<LOD[a]	Mean[b] (μg/kg)	Median (μg/kg)	Max (μg/kg)
Ergocornine+-inine	22	58.9	<10	86
α-Ergocryptine+-inine	18	53.1	<10	143
Ergotamine+-inine	18	94.5	<10	365
Ergocristine+-inine	20	83.3	<10	320
Ergosine+-inine	17	62.4	<10	183
Ergometrine+-inine	24	42.4	<10	96
Sum of alkaloids	15	213	<20	1,063

LOD: 10 μg/kg, LOQ: 20 μg/kg

[a] Number of samples with contents<LOD

[b] Mean of contents>LOQ

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