Ochratoxin A in Cereals and Cereal Products

J. WOLFF

Bundesanstalt für Getreide-, Kartoffel- und Fettforschung, Detmold, Germany

Introduction

In recent years, a considerable number of publications has appeared reporting Ochratoxin A (OTA) contents in cereals and cereal products (2,3,6). The British Food and Science Group (MAFF) reported OTA contents in 3 out of 67 samples (flour and breakfast cereals) in the range of 4.1 to 7.0 µg/kg. A survey of 153 cereal samples in 1997 showed OTA contents in the range of 0.3 to 8.4 µg/kg (8). Unfortunately, no information was provided about whether the cereal grains analysed were cleaned or in the state after threshing.

The limited information available so far does not constitute a sufficient data base to calculate the overall OTA contamination, considering the large variety of foods retailed and consumed. It was thus not possible to estimate, with adequate precision, the total dietary OTA intake from the consumption of cereal products. Therefore, a joint research project, which involved seven Federeal Research Institutes in Germany, was sponsored by the German Ministry of Health, with the aim to establish the analytical data base necessary to calculate human exposure to OTA. The objective of the study presented here was twofold: to provide (i) a representative record of the OTA contents in cereal products retailed in Germany and (ii) to determine, based on the analytical data, the OTA-intake of the German consumer through cereal products.

Materials and Methods

Samples

The samples were purchased four times a year from retail stores situated in the areas of the participating research institutes. These locations are spread over Germany (Berlin, Detmold, Jena, Karlsruhe, Kiel, Kulmbach, and Trier). Within a few hours after purchase, breads, rolls, and other cereal products were cut into cubes and dried at ambient temperature. Fresh and dry weight was determined. The dried samples were wrapped in paper bags and mailed for analysis. After receipt, the samples were ground (ZM 100, 1 mm sieve; Retsch GmbH & Co. KG, Germany) and thoroughly mixed (Inversina mini-manuel, Bioengineering AG, Germany). Aliquots of 150 g were further ground (ZM 100, 0.5 mm sieve; Retsch) and mixed again. Aliquots of 40 g were used for analysis.

Determination of Ochratoxin A

OTA was determined as described in CEN/ISO (4). This HPLC method is similar to that described in the official collection of analytical methods according to Article 35 of the German food law (Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG [5]). The reliability and precision of the method was checked by intercomparison studies performed by the participating laboratories (7).

HPLC conditions:
Pump: 480 micro; flowrate: 0,22 ml/min (Gynkotek, Germany);
Mobile Phase: acetonitrile/water (41) + acetic acid (2); 580/420 ml v/v;
Column oven (Gynkotek) set at 30 °C;
Columns: 125 mm x 2.1 mm; Kromasil 100, C18, 5µm particle size (MZ-Technik, Germany);
Fluorescence detector: RF 1002 (Gynkotek), set at an excitation wavelenght: of 330 nm and an emission wavelenght of 460 nm.
Two narrow-bore columns were set in line (11). Peak height was used to calculate OTA concentration.If the OTA concentration exceeded 0.3 µg/kg, two identifcation procedures employing methylation of OTA (5) and enzymatic degradation (9) were used for confirmation of the results.

Results and Discussion

After evaluation of the analytical data collected over a period of three years (1996–1998), no evidence for a relationship between seasonal or regional characteristics and the OTA content in cereals, flour, breads, and other cereal products could be found. It is interesting to note that the data do also not provide any evidence for a correlation between OTA content and the price level of the products.

Cereals and buckwheat

OTA was detected in nearly two thirds of the 204 samples (Table 1). However, the calculation of median values revealed that the contamination levels were low. OTA contents usually ranged form 0.01 to 0.1 µg/kg. It should be noted that further identification of OTA by methylation or enzymatic treatment was possible only in a few samples because of the low overall toxin concentrations.

Table 1: **Ochratoxin A contents in cereals and cereal products**

Commodity	Number of samples	Number of samples above d.l.	% Samples below d.l.	Minimum (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	Mean µg/kg	90th percentile (µg/kg)
Rye	37	14	62.2	< 0.010	0.800	0.100	0.113	0.142
Wheat	35	14	60.0	< 0.010	0.650	0.100	0.109	0.264
Barley	22	19	13.6	< 0.010	0.495	0.049	0.070	0.100
Oats	30	24	20.0	< 0.010	0.140	0.058	0.064	0.100
Maize	31	19	38.7	< 0.010	3.347	0.046	0.172	0.258
Buckwheat	23	10	56.5	< 0.010	0.594	0.011	0.074	0.239
Millet	26	23	11.5	< 0.010	0.831	0.086	0.111	0.161

d.l.: detection limit (0.01 µg/kg)

Cereal samples for bread-baking (wheat and rye) were of 'ready-to-eat' quality, which means that they had been thoroughly cleaned in the mill. No OTA was detected in 61 % of these samples. The highest OTA concentration detected in bread cereals was 0.8 µg/kg (Figure 1). A 90th percentile of 0.26 µg/kg for wheat suggests that considerable OTA levels can occur in semolinas, bran, and germs. The maximum OTA content found in maize was four times higher than that found in wheat or rye. Low levels of contamination were observed in oats.

Flour

OTA was detected in 320 (85.6 %) of the 374 cereal and buckwheat flour samples (Table 2). The median values ranged from 0.09 to 0.11 µg/kg. In rye flour, OTA contents were generally higher than in wheat flour, probably because rye flour contains a higher portion from the outer kernel. A positive correlation between OTA and ash content was observed for wheat and rye flour (Figure 2). The OTA levels determined for buckwheat and spelt flour were significantly higher than that found in bread cereal flour (wheat and rye).

In earlier studies, the effect of milling on the distribution of OTA has been determined for the different cereals under investigation. Typically, the OTA content in flour was only half of that found for the respective grain (10, 11). In the present study, such differences could not be seen because the cereal samples analysed were thoroughly cleaned for direct consumption and not for milling.

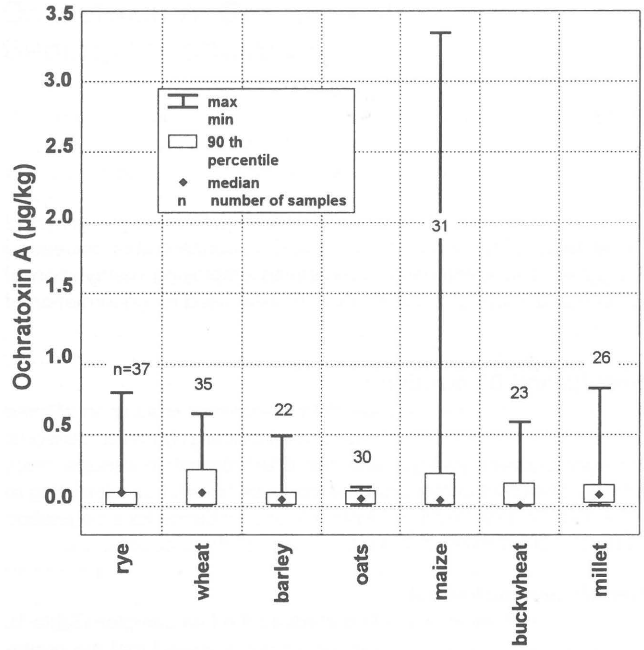

Fig. 1: Comparison of the ochratoxin A content in different cereals and in buckwheat

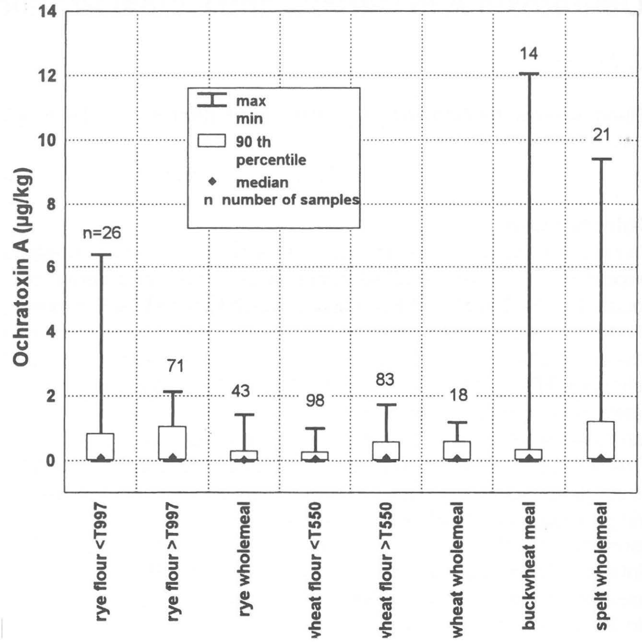

Fig. 2: Comparison of the ochratoxin A content in different flours

Table 2: **Ochratoxin A contents in flour**

Commodity	Number of samples	Number of samples above d.l.	% Samples below d.l.	Minimum (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	Mean µg/kg	90th percentile (µg/kg)
Rye flour <T997	26	20	23.1	< 0.010	6.400	0.100	0.422	0.873
Rye flour >T997	71	68	4.2	< 0.010	2.145	0.108	0.316	1.211
Rye whole meal	43	32	25.6	< 0.010	1.431	0.033	0.115	0.358
Wheat flour <T550	98	82	16.3	< 0.010	1.000	0.059	0.105	0.263
Wheat flourl >T550	83	77	7.2	< 0.010	1.732	0.099	0.203	0.595
Wheat whole meal	18	18	0	0.018	1.197	0.082	0.201	0.660
Buckwheat meal	14	5	64.3	< 0.010	12.07	0.100	0.965	1.205
Spelt whole meal	21	18	14.3	< 0.010	9.429	0.090	0.660	1.242

d.l.: detection limit (0.01 µg/kg)

Table 3: **Ochratoxin A contents in breads and rolls**

Commodity	Number of samples	Number of samples above d.l.	% Samples below d.l.	Minimum (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	Mean µg/kg	90th percentile (µg/kg)
Mixed wheat bread	125	110	12.0	< 0.010	2.089	0.100	0.188	0.440
Mixed rye bread	128	121	5.5	< 0.010	2.244	0.120	0.245	0.550
White bread	57	48	15.8	< 0.010	1.905	0.067	0.111	0.203
Toast bread	59	52	11.9	< 0.010	0.584	0.046	0.081	0.197
Rye wholemeal bread	96	89	7.3	< 0.010	5.488	0.095	0.219	0.317
Milk & water roll	89	79	11.2	< 0.010	0.524	0.063	0.090	0.214
Multigrain bread	49	48	2.0	< 0.010	1.757	0.126	0.245	0.730
Multigrain bread w. oilseeds	101	98	3.0	< 0.010	2.440	0.100	0.173	0.335
Crispbread	87	62	28.7	< 0.010	0.437	0.039	0.076	0.202
Wheat wholemeal bread	13	13	0	0.026	0.401	0.122	0.134	0.339
Pumpernickel	64	55	14.1	< 0.010	2.228	0.088	0.145	0.245
Wholemeal rolls	31	31	0	0.013	0.773	0.135	0.169	0.277
Multigrain rolls	49	48	2.0	< 0.010	5.542	0.104	0.357	0.306
Rye rolls	38	37	2.6	< 0.010	0.441	0.101	0.160	0.386

d.l.: detection limit

In 1998, the average consumption of wheat and rye flour was about 65 kg per capita, which was the highest consumption in Germany since 1990 (1). About 5 % of the German milled products (mainly rye) was consumed as whole meal products.

Breads and Breadrolls

As shown in Table 3, OTA was found in 891 samples (90.4 %) out of a total of 986 analysed samples. Median values ranged from 0.04 to 0.14 µg/kg in the different products. The percentage of samples with detectable OTA was higher for rye than for wheat products. A few samples of rye, wholemeal bread (Figure 3), and speciality rolls (Figure 4) with very high OTA concentrations were considered as extreme single values, because both the median values and the 90th percentile were much lower. The proportion of samples with OTA contents above the detection limit and the median values were only slightly higher for breads and rolls than for flour. In conclusion, the data shows that OTA occurs at very low levels in breads and rolls.

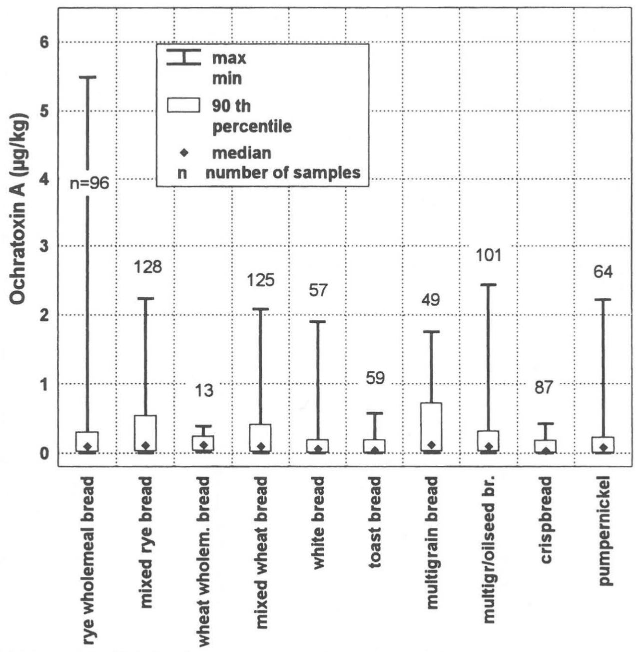

Fig. 3: Comparison of the ochratoxin A content in different breads

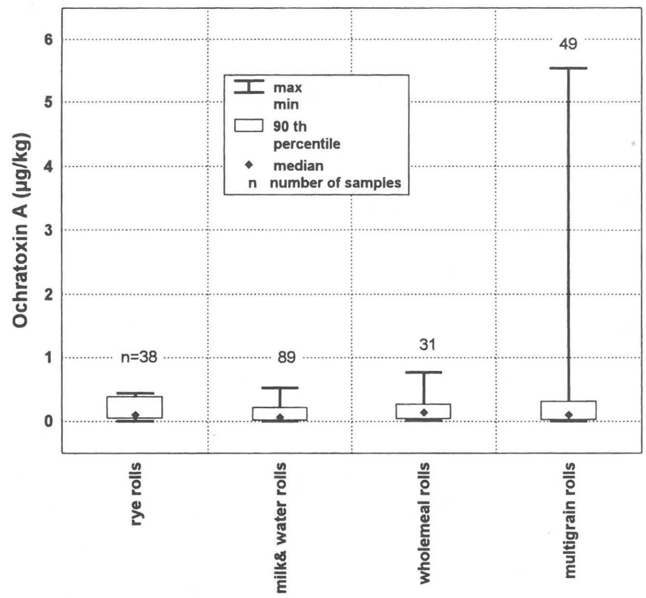

Fig. 4: Comparison of the ochratoxin A content in rolls

Other cereal based foods

In cereal based compound foods, including infant foods, OTA was detected in 270 samples (36 %) out of a total of 476 analysed samples. The median values ranged from 0.02 to 0.10 µg/kg in the different products (Table 4). These results show that both contamination frequency (68 %, 97 samples) and levels of OTA in infant cereal food samples (max. 2.13 µg/kg) were high. The OTA content was highest in baby foods containing soy products (Figure 5).

As for rice-based products, four samples (18.2 %) out of a total of 22 samples of brown rice were found to contain

Table 4: **Ochratoxin A contents in other cereal based foods and pulses**

Commodity	Number of samples	Number of samples above d.l.	% Samples below d.l.	Minimum (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	Mean (µg/kg)	90^{th} percentile (µg/kg)
Brown rice *	22	4	81.8	<0.100	0.280	0.100	0.109	0.117
Parboiled rice*	21	0	100.0	<0.100				
Long-grain rice *	24	0	100.0	<0.100				
Round grain rice *	15	0	100.0	<0.100				
Pasta without egg*	50	29	42.0	<0.100	1.750	0.165	0.282	0.606
Pasta with egg *	84	27	67.9	<0.100	0.950	0.100	0.199	0.590
Whole meal pasta *	27	10	63.0	<0.100	29.770	0.100	2.002	2.319
Mixed products (Tortellini)*	30	12	60.0	< 0.100	0.860	0.100	0.205	0.597
Oat flakes	66	26	60.6	< 0.010	0.251	0.100	0.069	0.100
Oat bran	26	14	46.2	<0.010	0.330	0.100	0.089	0.109
Semolina*	25	10	60.0	< 0.100	2.580	0.100	0.410	1.494
Wheat bran *	25	3	88.0	< 0.100	1.590	0.100	0.218	0.716
Wheat germs	19	13	31.6	< 0.010	0.448	0.100	0.112	0.270
Barley groats	31	21	32.3	< 0.010	0.950	0.057	0.094	0.100
Polenta	29	6	78.6	< 0.010	1.530	0.100	0.204	0.366
Unripe spelt wheat	17	2	88.2	< 0.010	0.100	0.100	0.069	0.100
Infant cereal foods	97	66	32.0	< 0.010	2.130	0.020	0.118	0.142
Peas, lentils, beans	103	1	99.0		0.840			
Soy beans	31	26	83.9	< 0.010	0.100	0.080	0.059	0.100

d.l.: detection limit (0.01 µg/kg), *detection limit: 0.10 µg/kg

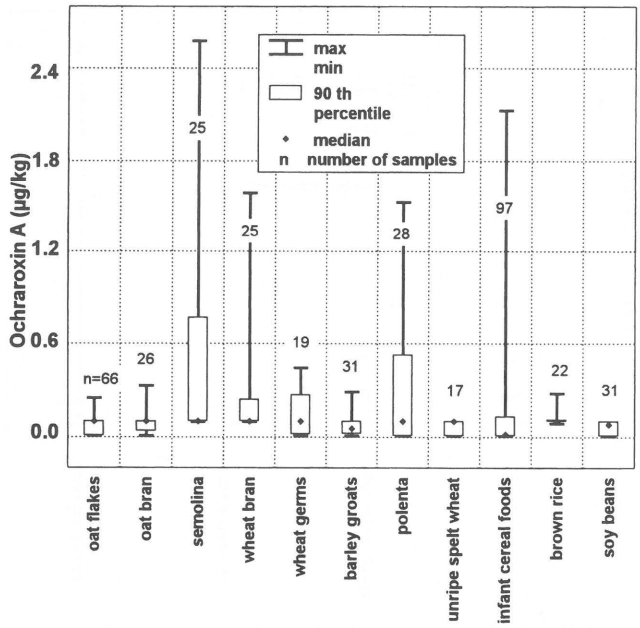

Fig. 5 Comparison of the ochratoxin A content in different cereals-based foods

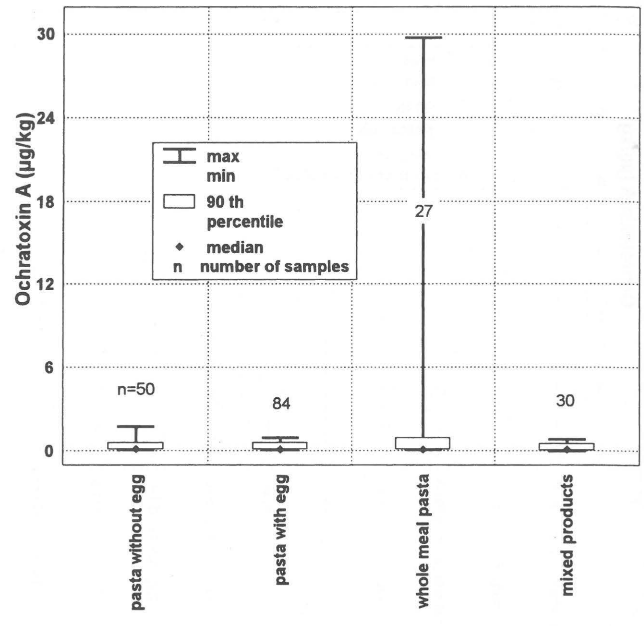

Fig. 6: Comparison of the ochratoxin A content in different pasta

OTA, at a detection limit of 0.1 µg/kg. None of the other rice samples (parboiled, long- and round-grain rice) contained detectable levels of OTA.

In pasta type samples produced without chicken egg, OTA occurred more frequently (58%) than in pasta produced with chicken egg (34 %), and frequently OTA was present at higher concentrations (median values: 0.165 and 0.100 µg/kg, respectively). For whole meal pasta, OTA was detected in 37 % of the samples. Here the median value was low (0.10 µg/kg) compared with the 90th percentile (2.3 µg/kg), as shown in Figure 6.

The summarized data for rice, wheat bran, peas, lentils and beans as listed in Table 4 show that only a few of these samples of contained OTA at concentrations above the detection limit of 0.1 µg/kg.

Summary

Ochratoxin A (OTA) was determined in cereals and in a large variety of cereal products (flour, bread, breadroll, pasta, other cereal-based foods) and in a variety of pulses. The detection limit for OTA was 0.01 µg/kg, except for rice and pasta (0.1 µg/kg). A total of 2374 samples were analysed, 68.6 % were found to contain OTA. However, only 1.4 % of the samples contained OTA at levels exceeding 3 µg/kg. The median values for the different groups of foods were between 0.011 µg/kg and 0.165 µg/kg. The mean OTA values for each type of cereal and cereal products were calculated, which constitute a part of a data base to determine the total OTA intake by the German consumer through all types of food.

Zusammenfassung

Ochratoxin A (OTA) wurde in Getreide, Mehl, Brot, Brötchen, Teigwaren und anderen Lebensmitteln auf Getreidebasis (Nährmitteln) sowie in Leguminosen bestimmt. In 68,6 % der insgesamt 2374 Muster wurde OTA nachgewiesen. Die Nachweisgrenze betrug 0,01 µg/kg (bei Reis und Teigwaren 0,1 µg/kg). Gehalte über 3 µg/kg wurden bei Getreide und Getreideprodukten in nur 1,4 % der Muster bestimmt. Die Mediane der OTA-Gehalte lagen in den einzelnen Produktgruppen zwischen 0,011 µg/kg und 0,165 µg/kg. Mittelwerte wurden für die Ermittlung der Belastung des deutschen Verbrauchers mit OTA berechnet.

References

(1) AGRA-EUROPE (AgE) (1999): 18. Januar 1999, Kurzm. 4: Mehlverbrauch in Deutschland auf Rekordniveau gestiegen (Pressemitteilung).

(2) ATKINS, D. (1997): Survey of aflatoxins and ochratoxin A in cereals and retail products. MAFF, Food Contaminats Division, London, Information Sheet Nr. 130, November 1997.

(3) BAUMANN, U., ZIMMERLI, B. (1988): Einfache Ochratoxin-A-Bestimmung in Lebensmitteln. Mitt. Gebiete Lebensm. Hyg. **79,** 151–158.

(4) DIN 10900-32: Bestimmung von Ochratoxin A – Teil 1: Hochleistungsflüssigkeitschromatographisches Verfahren für Weizenmehl (ISO/DIS 15141-1: 1996), E DIN 10900-32 : 1996-05.

(5) LMBG § 35, L 15.00-1 1992-12: Bestimmung von Ochratoxin A. Verfahren zur Probenahme und Untersuchungen von Lebensmitteln, Tabakerzeugnissen, kosmetischen Mitteln und Bedarfsgegenständen, Bundesgesundheitsamt, Loseblattausgabe Bd. 1, Beuth Verlag Gmbh: Berlin, Köln.

(6) MAJERUS, P., CUTCA, I., DREYER, A., EL-DESSOUKI, S., EYRICH, W., REUSCH, H., SCHURER, B., WAIBLINGER, H. U. (1993): Zur Belastungssituation von Ochratoxin A in Lebensmitteln pflanzlichen Ursprungs. Dtsch. Lebensm.-Rundsch. **89,** 112–114.

(7) MAJERUS, P., WEBER, R., WOLFF, J. (1994) Nachweis und Bestimmung von Ochratoxin A in Getreide und Getreideprodukten. Bundesgesundheitsbl. **37,** 454–458.

(8) MOORE, A. (1996): Ochratoxin A in cereals and flour, and carry-over into retail processed foods, MAFF, Food Safety and Science Group, London, Information Sheet Nr. 95, October 1996.

(9) STUDER-ROHR, I. (1996): Ochratoxin A in humans: exposure, kinetics and risk assessment. Dissertationsschrift, Bundesinstitut für Technologie, Zürich, Schweiz.

(10) WOLFF, J. (1990): Verteilung von Ochratoxin A beim Vermahlen von belastetem Getreide. Die Mühle + Mischfuttertechnik **127,** 706–709.

(11) WOLFF, J., RICHTER, W. I. S. (1992): Ochratoxin A in Getreide. Getreide Mehl und Brot **46,** 355–360.

Address of author:

J. Wolff, Bundesanstalt für Getreide-, Kartoffel- und Fettforschung, Schützenberg 12, 32756 Detmold, Germany.