Mycotoxin Res (2017) 33:219–227
DOI 10.1007/s12550-017-0280-3

ORIGINAL ARTICLE

Mycotoxins in organic and conventional cereals and cereal products grown and marketed in Croatia

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Received: 22 March 2017 / Revised: 28 April 2017 / Accepted: 1 May 2017 / Published online: 9 May 2017

Abstract In this study, the levels of aflatoxin B_1 (AFB_1), ochratoxin A (OTA), zearalenone (ZEN), deoxynivalenol (DON) and fumonisins (FUM) in unprocessed cereals ($n = 189$) and cereal-based products ($n = 61$) were determined using validated ELISA methods. All samples originated from either conventional or organic production corresponded to the 2015 harvest in Croatia. Based on the mean mycotoxin concentrations, the risk for the consumer to exceed the tolerable daily intake (TDI) for these toxins by the consumption of both types of cereals and cereal-based products was assessed. Mycotoxin contamination of organic cereals and organic cereal-based products was not significantly different ($p > 0.05$). Given that the exposure assessment resulted in a small fraction of the TDI (maximum: DON, 12% of TDI), the levels of the investigated mycotoxins in both types of cereals and cereal-based products from the 2015 harvest did not pose a human health hazard.

Keywords Mycotoxins · Organic · Conventional · Cereals · Cereal products · Exposure assessment

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Introduction

Mycotoxins are secondary fungal metabolites mainly produced by species of the *Aspergillus*, *Penicillium* and *Fusarium* genera. These toxins are found all over the world as natural contaminants of numerous commodities of plant origin, especially in cereal grains, but also in nuts, oilseeds, fruits, dried fruits, vegetables, cocoa and coffee beans, wine, beer, herbs and spices. Mycotoxins can also be found in food made from animals fed on contaminated feed, that is to say, in meat, eggs, milk and milk products. It is also known that fungal infection of plants and the production of mycotoxins within them depend on temperature, humidity, host species and cultivars, agronomy and other environmental conditions (Brodal et al. 2016).

The interest in organic food has increased worldwide in response to concerns about conventional agricultural practices, food safety, human health, animal welfare and the environment. In the EU, organic farming is an agricultural practice and a food production modality that combines favourable environmental and animal welfare standards and is supported by the EU law (834/2007/EC; 889/2008/EC). Nowadays, many consumers prefer organic rather than non-organic food, given that organic production makes no use of synthetic fungicides and mineral fertilisers. In general, organic practices are thought to decrease the risk of plantal infection (Juan et al. 2008), but, at the same time, there exists the awareness that poorer use of fungicides may go in favour of mycotoxin presence in 'natural' or 'homemade' chemical-free products (Pussemier et al. 2006a). Furthermore, environmental conditions such as improper storage, including high temperature,

poor drying conditions and elevated moisture, which are often linked to organic production, may represent the causal background of fungal presence and promote fungal development and contamination of cereals and cereal-based products with mycotoxins as secondary fungal metabolites.

A number of scientific surveys from Europe and only a few from other temperate countries have compared mycotoxin content in organic and conventional food, especially in cereals and cereal products (Brodal et al. 2016). Mycotoxin concentrations have been shown to greatly vary across various cereals; dependent on the investigated mycotoxin, some organic food has been shown to be less or equally contaminated as its conventional counterpart (Juan et al. 2008). On top of that, contradictory results have been reported regarding the safety of these two types of products (Magkos et al. 2006). Therefore, the question about potentially higher contamination of organic food has been raised, justifying scientific assessments of the quality of products coming from different production systems. However, the information on the impact of agriculture practices on cereal mycotoxin contents is still rather poor; therefore, the issue remains open for discussion and investigation (Błajet-Kosicka et al. 2014).

The aim of this study was to investigate into the occurrence of the most representative mycotoxins such as aflatoxin B_1 (AFB_1), ochratoxin A (OTA), zearalenone (ZEN), deoxynivalenol (DON) and fumonisins (FUM) in different unprocessed organic and conventional cereals and cereal-based products produced from both types of cereals that are available on the Croatian market and to assess the risk for consumers coming from the consumption of both types of food and food products. Whereas many European countries have already reported on mycotoxin levels in organic and conventional cereals, Croatia insofar failed to do so, so that no such data have been published. To the best of our knowledge, this study represents the first comprehensive research on the incidence of mycotoxins in organically and conventionally cultivated cereals and cereal products sampled from Croatian fields and markets.

Materials and methods

Sampling and sample preparation

During the 2015–2016 timeframe, a total of 250 samples were sampled, out of which 189 of unprocessed cereals (maize, wheat, oat, barley and rye) and 61 of cereal-based products (maize meal, maize flour, oat mash and wheat flour). Among them, 115 samples were declared as organic (89 cereals and 26 cereal-based products), while 135 samples were produced in a conventional manner (100 cereals and 35 cereal products). Unprocessed crop season 2015 cereals were sampled from agricultural fields situated in the northeastern, central and eastern Croatia during the harvesting period (July to October 2015). Cereal products were sampled from January 2015 to June 2016 in form of packaged commercial products produced by different domestic manufacturers present on the Croatian market.

The sampled organically produced products were labelled according to the Regulation 834/2007/EC on organic production and labelling of organic products that repeals the Regulation (EEC) No 2092/91. These products also bear national or private company logos of organic food producers, the code of the competent body and the indication of the cultivation site.

Sampling and sample preparation were performed in full line with the Commission Regulation 401/ 2006, laying down the methods of sampling for the purpose of official control of the levels of mycotoxins in foodstuffs. Samples were stored in a cool and dry place and transported to the laboratory within 48 h. The prepared test portions were ground into a fine powder having a particle size of 1.0 mm using an analytical mill (Cylotec 1093, Tecator, Sweden) and then stored at 4 °C pending analyses.

Chemicals and reagents

The ELISA kits used for mycotoxin analyses were produced by R-Biopharm (Darmstadt, Germany). Each kit contains a micro-titre plate with 96 wells coated with antibodies, standard solutions containing different concentrations of mycotoxins, an enzyme conjugate, an anti-antibody, a substrate, a chromogen solution (urea peroxide/tetramethylbenzidine), a stop solution and washing and dilution buffers. Solid mycotoxin standards employed with the quality control of each analytical method were provided by Sigma-Aldrich Chemie GmbH (Steinheim, Germany). All other chemicals used for analyses were of an analytical grade.

Determination of mycotoxins

For the purpose of AFB_1, ZEN and FUM determination, the extraction was performed with 25 mL of 70% methanol. For the purpose of OTA analysis, 100 mL of 0.13 M sodium hydrogen carbonate buffer (pH 8.1) was used, whereas with DON analyses, 25 mL of distilled water was used. The extraction was carried out by virtue of agitation on a shaker for 30 min at the speed of 300 r/min (HS 260 Control, Ika, Germany). The whole contents were then filtrated in 50-mL Erlenmeyer flasks, the filtrates subsequently being used for the ELISA tests.

The ELISA tests were performed according to the ELISA kits' manufacturer using a ChemWell auto-analyzer (Awareness Technology Inc. 2910, USA), the absorbance thereby being measured at 450 nm. In order to determine mycotoxin concentration in the sampled material, a standard

curve was plotted for each analysed mycotoxin. When establishing the final mycotoxin concentrations in a given sample, the dilution factor and the mean recovery rate determined for each mycotoxin were taken into account.

Quality control of the ELISA methods

The limit of detection (LOD) was calculated from the average of ten toxin-negative cereal mixture samples (containing maize, wheat and barley in equal proportions; earlier analysed for the presence of mycotoxins and used for validation as a blank material) plus tripled standard deviation (LOD = mean ± 3SD). To determine the limit of quantification (LOQ), the mean concentration determined with ten toxin-negative cereal mixture samples was summed up with the sixfold standard deviation (LOQ = mean ± 6SD).

The trueness was established using the maize-appropriate certified reference material (CRM) (n = 6) manufactured by Fapas (T04209QC, York, England), to which mean values and acceptable ranges were assigned, as follows: 8.01 μg/kg and 4.49–11.5 μg/kg for AFB_1; 5.57 μg/kg and 3.12–8.03 μg/kg for OTA; 1780 μg/kg and 1260–2300 μg/kg for DON; and 344 μg/kg and 214–473 μg/kg for ZEN. For each mycotoxin, the recovery rate was determined at three different 'spiking' levels by virtue of fortifying a toxin-negative cereal mixture standard working solution of the given mycotoxin (100 μg/L for AFB_1 and OTA; 300 μg/L for DON, ZEN and FUM) followed by the analysis of three replicates at each level.

Statistical analysis

Statistical analysis was performed using the Statistica Ver. 10.0 Software (StatSoft Inc. 1984–2011, USA), with a statistical significance set at 95% (p = 0.05). In order to determine the differences in concentrations of the studied mycotoxins found in various cereals during various sampling years, parametric tests like t test and one-way and two-way ANOVA were used, with the statistical significance set at $p < 0.05$.

Results and discussion

Data published insofar have shown that the presence of mycotoxins as frequent contaminants of different foodstuffs and feedstuffs greatly varies dependent on many parameters of influence, such as the type of cereal, weather conditions and else. However, contradictory results have been reported regarding mycotoxins and safety of organic and non-organic food (Magkos et al. 2006). In this study, the occurrence of the most representative mycotoxins in different unprocessed cereals cultivated on Croatian fields in an organic and conventional manner, and in cereal-based products available on the Croatian market, was investigated. Based on the determined mean mycotoxin concentrations, human health risk associated with the consumption of both types of cereals and cereal-based products was assessed.

Due to the large number of samples analysed within the frame of this study, which were analysed for the presence of five mycotoxins in a short period of time, validated ELISA methods implemented in earlier studies (Pleadin et al. 2012; Pleadin et al. 2013; Pleadin et al. 2015) were used. Although the ELISA method has been perceived as an easy-to-use technique with a lesser need for an extensive cleanup, when it comes to the determination of some mycotoxins, this method lacks accuracy at very low mycotoxin concentrations and may be prone to cross-reactivity resulting in overestimation of concentrations of certain mycotoxins (Krska et al. 2007; EFSA 2011). Prior to the determination of mycotoxin levels, the ELISA methods were validated and then applied for the analyses of the sampled materials. Quality control results pertaining to the ELISA methods implemented within this study frame for each mycotoxin analysed are shown in Table 1. The obtained trueness values were compared to the assigned CRM values, whereas the recovery values were checked for their compliance with the performance criteria (recovery 60–130%) given under the Commission Regulation 401/ 2006. Based on the obtained quality control results, the ELISA methods were perceived as suitable tools capable of reliable and efficient determination of the studied mycotoxins in different cereals and cereal-based products.

Mycotoxin concentrations determined in this study in organic and conventional unprocessed cereals are shown in Table 2. Among cereals, the highest contamination was that of maize, both when it comes to organic and conventional maize samples. The second most contaminated cereal was wheat, followed by oat and barley, while the lowest level of contamination with nearly all studied mycotoxins was observed for rye, both organic and conventional. The highest contamination was that with *Fusarium* mycotoxins (DON, ZEN and FUM), as already established in earlier cereals-oriented studies performed in Croatia (Pleadin et al. 2012; Pleadin et al. 2013).

Mycotoxin concentrations determined in this study in organic and conventional cereal products available on the market are shown in Table 3. In accordance to the results obtained for the presence of *Fusarium* mycotoxins in cereals, the highest percentage of positives and the highest level of contamination were determined among maize by-products, such as maize meal and maize flour, followed by oat mash and wheat flour; the above applies both to organic- and conventional cereal-based products. The percentage of samples not complying to the maximum levels (MLs) defined by the legislation (EC 1881/ 2006) for each mycotoxin is given in Table 4. However, samples incompliant to the above criteria and therefore inappropriate for use as foodstuffs can be used as feedstuffs, as foreseen by the legislation governing the

Table 1 Quality control results pertaining to the ELISA methods used for the determination of mycotoxins within this study frame

Mycotoxin	LOD (μg/kg) (n = 10)	LOQ (μg/kg) (n = 10)	Trueness ± SD[a] (μg/kg) (n = 6)	Spiked level (μg/kg)	Recovery (%) (n = 3)	CV (%)	Cross-reactivity[b] (%)
AFB_1	1.10	1.50	7.75 ± 0.321	5	96.8	5.12	AFB_1 100; AFB_2 13; AFG_1 29; AFG_2 3
				10	98.1	6.71	
				20	97.5	6.43	
OTA	1.30	1.80	6.14 ± 0.210	5	87.8	6.61	OTA 100; OTC 44; OTB 14; OTα < 0.1
				10	90.1	7.30	
				20	92.6	5.90	
DON	19.1	25.5	1630 ± 147	50	95.4	5.42	DON 100; 3-acetyl DON >100; 15-acetyl DON 19; nivalenol 4
				100	96.1	6.60	
				200	102	7.21	
ZEN	1.90	2.50	334 ± 27.0	50	88.5	5.83	ZEN 100; α-zearalenol 42; zeranol 28; β-zearalenol 14
				100	97.2	6.34	
				200	101	8.12	
FUM	27.2	34.3	–	50	76.7	6.64	FUM B_1 100; FUM B_2 40; FUM B_3 100
				100	78.5	8.70	
				200	81.9	9.21	

A mixture of cereals was used in the validation process; a mixture containing maize, wheat and barley in equal proportions was analysed for mycotoxins under study and represented a blank (toxin-negative) material

AF (B_1, B_2, G_1, G_2) aflatoxin (B_1, B_2, G_1, G_2); *OT* (*A*, *B*, *C*, α) ochratoxin (A, B, C, α); *DON* deoxynivalenol; *ZEN* zearalenone; *FUM* fumonisins B_1, B_2, B_3; *LOD* the limit of detection; *LOQ* the limit of quantification; *SD* standard deviation; *CV* coefficient of variation

[a] CRM-assigned value (FAPAS T04209QC): AFB_1 8.01 μg/kg (4.49–11.5 μg/kg); OTA 5.57 μg/kg (3.12–8.03 μg/kg); DON 1780 μg/kg (1260–2300 μg/kg); and ZEN 344 μg/kg (214–473 μg/kg)

[b] Approximate percentages of cross-reactivity given by the ELISA kit manufacturer

presence of undesirable substances in animal feed (2003/100/EC).

Percentage of the tolerable daily intake (TDI) or the tolerable weekly intake (TWI) based on the data on average dietary intakes of these foodstuffs is shown in Table 5. Since in Croatia, human average daily intakes of some grains and grain milling products have not been established yet, the exposure assessment made within the frame of this survey made use of the data on mean daily intakes (g/day) provided by the European Food Safety Authority (EFSA) for our neighbouring countries (Italy, Austria and Hungary) (EFSA 2017). Of further note, no separate data on the consumption of conventional and organic grains and grain milling products are available. It should also be kept in mind that in this study not only final products but also unprocessed cereals were analysed for the mycotoxin presence; it is namely common knowledge that unprocessed cereals are not consumed directly (in their original form) but rather following some sort of treatment (post-harvest treatment, sieving or any other procedure that involves water, temperature, humidity and time), which can significantly reduce the amount of mycotoxins in them (Ariño et al. 2007).

In this study, significant differences in AFB_1 concentrations found in organic cereals and organic cereal-based products vs their conventional counterparts were not observed ($p > 0.05$) with slightly higher concentrations of this mycotoxin in a few samples in comparison with the MLs. Because AFB_1 was evidenced to be a genotoxin and carcinogen, it is not possible to determine the threshold levels below which this mycotoxin would have no effects on human health; therefore, no TDI or TWI levels have been recommended and percentage of these values were not calculated within this study frame.

Mean OTA values determined in cereals under this study ranged from the minimum of 1.91 μg/kg both in organically and conventionally produced oat, to the maximum of 3.50 μg/kg in organically cultivated rye and 2.74 μg/kg in conventionally produced maize and wheat (Table 2). In cereal products of both types, OTA mostly failed to be found (<LOQ), except for organic and conventional maize meal and wheat flour, in which only low OTA concentrations were determined (Table 3) making none of the samples incompliant to the pertaining regulations (Table 4). Significant differences in OTA concentrations established in organic cereals and cereal-based products as compared to their conventional counterparts were not observed ($p > 0.05$). The maximal % of the TWI was observed in organically produced wheat flour (7.66%) and conventionally produced barley (7.00%) (Table 5); in both cases, these levels do not pose as a human health risk. Earlier studies also reported no differences in OTA

Table 2 Mycotoxin concentrations observed in organically and conventionally cultivated unprocessed cereals (grains)

		Maize		Wheat		Oats		Barley		Rye	
		O (n = 33)	C (n = 37)	O (n = 25)	C (n = 27)	O (n = 13)	C (n = 14)	O (n = 11)	C (n = 13)	O (n = 7)	C (n = 9)
AFB_1	Positives (%)	6.06	10.8	4.00	3.70	7.71	7.11	–	7.69	–	11.1
	Mean ± SD (μg/kg)	3.70 ± 0.71	3.31 ± 1.49	2.10	2.40	1.63	1.70	<LOQ	2.22	<LOQ	2.10
	Range (μg/kg)	3.20–4.20	2.00–5.75	–	–	–	–	<LOQ	–	<LOQ	–
OTA	Positives (%)	9.13	16.2	16.0	3.70	15.4	14.3	–	23.1	28.6	33.3
	Mean ± SD (μg/kg)	3.20 ± 1.31	2.74 ± 1.22	2.42 ± 0.610	2.66	1.91 ± 0.101	1.91 ± 0.010	<LOQ	2.40 ± 0.703	3.50 ± 0.902	2.42 ± 0.203
	Range (μg/kg)	1.93–4.52	1.81–4.34	1.90–3.20	–	1.80–2.02	1.81–1.92	<LOQ	1.81–3.20	2.91–4.12	2.14–2.61
DON	Positives (%)	93.9	81.1	56.0	59.3	61.5	57.1	54.5	61.5	57.1	22.2
	Mean ± SD (μg/kg)	564 ± 605	350 ± 419	256 ± 204	252 ± 351	119 ± 122	207 ± 207	71.8 ± 44.3	140 ± 119	68.6 ± 33.4	35.4 ± 6.81
	Range (μg/kg)	34.7–2260	27.8–1430	62.4–678	27.1–1220	32.2–377	33.4–546	32.3–157	42.6–389	33.8–113	30.6–40.2
ZEN	Positives (%)	100	97.3	60.0	55.6	46.2	35.7	36.4	46.2	28.6	22.2
	Mean ± SD (μg/kg)	106 ± 102	87.8 ± 142	32.6 ± 29.6	41.5 ± 26.1	17.6 ± 16.7	25.0 ± 23.0	6.42 ± 3.29	14.0 ± 12.6	5.69 ± 3.02	5.83 ± 0.801
	Range (μg/kg)	4.71–396	5.60–778	4.72–115	10.6–94.5	3.20–45.3	6.40–63.1	3.31–10.3	3.53–36.8	3.60–7.81	5.22–6.31
FUM	Positives (%)	93.9	89.2	40.0	25.9	38.5	21.4	27.3	53.8	28.6	33.3
	Mean ± SD (μg/kg)	528 ± 637	350 ± 353	99.7 ± 46.5	59.1 ± 26.4	44.2 ± 7.39	38.3 ± 3.21	50.3 ± 7.13	74.6 ± 29.9	68.5 ± 7.61	38.2 ± 2.80
	Range (μg/kg)	56.2–1980	28.1–1380	41.2–215	38.9–115	34.6–52.3	34.9–41.2	42.6–56.7	43.3–126	63.1–73.8	35.6–41.1

AFB_1 aflatoxin B_1, *OTA* ochratoxin A, *DON* deoxynivalenol, *ZEN* zearalenone, *FUM* fumonisins, *SD* standard deviation, *LOQ* limit of quantification, *O* organic cultivation, *C* conventional cultivation

Table 3 Mycotoxin concentrations observed in organically and conventionally cultivated cereal products

		Maize meal		Maize flour		Oat mash		Wheat flour	
		O (n = 9)	C (n = 10)	O (n = 5)	C (n = 9)	O (n = 6)	C (n = 7)	O (n = 6)	C (n = 9)
AFB_1	Positives (%)	11.1	10.0	–	11.1	–	–	–	11.1
	Mean ± SD (μg/kg)	1.70	1.60	<LOQ	1.61	<LOQ	<LOQ	<LOQ	1.63
	Range (μg/kg)	–	–	<LOQ	–	<LOQ	<LOQ	<LOQ	–
OTA	Positives (%)	11.1	–	–	–	–	–	33.3	22.2
	Mean ± SD (μg/kg)	2.02	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.30 ± 0.203	1.92 ± 0.104
	Range (μg/kg)	–	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.23 ± 2.51	1.84–2.05
DON	Positives (%)	77.8	80.0	60.0	55.6	50.0	42.9	50.0	66.7
	Mean ± SD (μg/kg)	173 ± 146	80.3 ± 83.9	101 ± 101	53.8 ± 29.4	39.5 ± 6.69	40.4 ± 15.0	68.9 ± 46.4	56.3 ± 41.4
	Range (μg/kg)	38.3–456	26.6–272	31.2–217	26.7–102	35.3–47.2	26.3–56.2	32.8–121	27.1–126
ZEN	Positives (%)	55.6	80.0	60.0	44.4	50.0	42.9	33.3	33.3
	Mean ± SD (μg/kg)	30.5 ± 22.1	20.4 ± 21.7	16.1 ± 14.3	14.3 ± 20.7	7.90 ± 3.55	5.75 ± 1.70	4.85 ± 1.06	7.81 ± 2.23
	Range (μg/kg)	4.91–56.3	4.10–62.1	5.60–32.3	3.30–45.3	3.80–10.1	4.51–7.82	4.12–5.60	5.70–10.1
FUM	Positives (%)	77.8	70.0	80.0	77.8	33.3	28.6	33.3	44.4
	Mean ± SD (μg/kg)	150 ± 93.3	168 ± 114	60.5 ± 31.2	148 ± 155	50.2 ± 3.70	36.0 ± 0.501	45.3 ± 11.0	44.9 ± 12.0
	Range (μg/kg)	48.3–322	52.1–312	37.5–106	40.2–456	47.5–52.8	35.6–36.3	37.5–53.1	35.2–62.3

AFB_1 aflatoxin B_1, *OTA* ochratoxin A, *DON* deoxynivalenol, *ZEN* zearalenone, *FUM* fumonisins, *SD* standard deviation, *LOQ* limit of quantification, *O* organic cultivation, *C* conventional cultivation

levels found in organic vs conventional cereals and cereal products or considerably lower OTA levels in organically in comparison to conventional produced (Biffi et al. 2004; Pussemier et al. 2006b; Czerwiecki et al. 2002). Significant differences in AFB_1 and OTA concentrations between organically and conventionally cultivated cereals and organic vs conventional cereal-based products observed in certain studies were related to the drying and storage conditions rather than to agricultural practices (Brodal et al. 2016).

In this study, the maximal mean concentrations were determined for DON both in organically (564 ± 605 μg/kg) and conventionally (350 ± 419 μg/kg) cultivated maize (Table 2). As for the finished products, the highest DON concentrations were determined in organically (173 ± 146 μg/kg) and conventionally produced maize meal (80.3 ± 83.9 μg/kg) (Table 3). Significant differences in DON levels found in these two types of cereals and cereal products were not observed ($p > 0.05$), and the maximal % of the TDI were observed in organically produced maize (12.0%) and conventionally produced wheat (10.7%) (Table 5). Although the majority of studies suggesting that it is unlikely that organic cultivation increases the risk of a more substantial DON occurrence (Brodal et al. 2016), some studies showed lower DON contents in organically than in conventionally produced cereals (Smith-Spangler et al. 2012). De Galarreta et al. (2015) pointed that weather conditions, the cultivation year and the cultivation location, crop rotation and tillage may be more important for its production than the type of cultivation.

Table 4 Samples incompliant to the maximum levels (MLs) of mycotoxins in unprocessed cereals and cereal products

	% of non-compliant samples[a]								
	Maize O/C	Wheat O/C	Oats O/C	Barley O/C	Rye O/C	Maize meal O/C	Maize flour O/C	Oat mash O/C	Wheat flour O/C
AFB_1	0/3	0/0	0/0	0/8	0/11	0/0	0/0	0/0	0/0
OTA	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
DON	6/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
ZEN	18/19	4/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
FUM	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

AFB_1 aflatoxin B_1, *OTA* ochratoxin A, *DON* deoxynivalenol, *ZEN* zearalenone, *FUM* fumonisins, *O* organic cultivation, *C* conventional cultivation

[a] The percentage of samples in which mycotoxin concentration was higher than the ML defined for foodstuffs (Commission Regulation 1881/ 2006)

Table 5 Estimation of mycotoxin dietary intakes coming from the consumption of organically and conventionally cultivated cereals and cereal products

Cereal/ cereal product	Mean daily intake[a] (g/day)	Cultivation	% TDI[b]/% TWI[c]			
			OTA	DON	ZEN	FUM
Maize	16.0	O	4.02	12.0	9.07	5.63
		C	3.39	7.47	7.49	3.73
Wheat	32.0	O	6.02	10.9	5.56	2.13
		C	6.77	10.7	7.08	1.26
Oats	12.5	O	1.86	1.98	1.17	0.37
		C	1.86	3.44	1.67	0.320
Barley	37.2	O	0.00	3.56	1.27	1.25
		C	7.00	6.93	2.78	1.85
Rye	20.0	O	5.49	1.83	0.611	0.914
		C	3.76	0.94	0.620	0.512
Maize meal	19.9	O	3.12	4.60	3.24	1.99
		C	0.0	2.13	2.17	2.23
Maize flour	19.9	O	0.0	2.67	1.71	0.801
		C	0.0	2.86	4.54	3.94
Oat mash	33.3	O	0.0	1.76	1.40	1.12
		C	0.0	1.80	7.18	0.802
Wheat flour	42.5	O	7.66	3.90	1.11	1.28
		C	6.33	3.19	1.77	1.27

OTA ochratoxin A, *DON* deoxynivalenol, *ZEN* zearalenone, *FUM* fumonisins

[a] Mean daily intake (g/day) of cereal grains and cereal milling products established for consuming adults (average weight, 75 kg) residing in our neighbouring countries (Italy, Austria and Hungary) (EFSA 2017)

[b] Tolerable daily intake (*TDI*)—DON 1 μg/kg b.w./day (EC 2002); FUM 2 μg/kg b.w./day (EC 2003); ZEN 0.25 μg/kg b.w./day (EFSA 2011)

[c] Tolerable weekly intake (*TWI*)—OTA 120 ng/kg b.w./day (EFSA 2006)

ZEN concentrations determined in maize and maize-based products within this study were significantly higher in comparison to other cereals and cereal products, with the highest percentage of incompliant samples in comparison to samples contaminated with other mycotoxins under study (Table 4). The mean ZEN concentration determined in organically cultivated maize was 106 ± 102 μg/kg, while that in conventionally cultivated maize amounted to 87.8 ± 142 μg/kg, indicating huge variations across the analysed samples (Table 2). Among the analysed cereal-based products, the highest mean ZEN concentration was determined in organically produced maize meal (30.5 ± 22.1 μg/kg) (Table 3) In general, no proof that agricultural practices play a role in ZEN content found in cereals and cereal products was found (Lacko-Bartosova and Kobida 2011; Ibáñez-Vea et al. 2012; Twaruzek et al. 2013). The maximal % of the TDI was observed in organically (9.07% of the TDI) and conventionally (7.49% of the TDI) cultivated maize grains (Table 5). Huge variations in ZEN concentrations observed in this study for some organic and conventional cereals and their products could also reflect the impact of different factors of influence mentioned above when discussing DON presence, rather than the impact of the cultivation practice.

Alike in the earlier study on *Fusarium* mycotoxins by Pleadin et al. (2013), the highest FUM concentrations determined in this study were those found in maize which are 528 ± 637 μg/kg in organically and 350 ± 419 μg/kg in conventionally cultivated maize (Table 2). The same applies to maize by-products such as conventionally produced maize flour (148 ± 155 μg/kg) and maize meal (168 ± 114 μg/kg) (Table 3). However, unlike other mycotoxins investigated under this study, statistically significant differences between mycotoxin content found in organic as compared to conventional cereals were observed only for FUM in oat mash ($p = 0.00330$) and rye ($p = 0.00660$), but not in other unprocessed cereals and cereal products. Taking into account the TDI and the mean determined FUM concentrations, the highest human exposure to FUM comes from organically cultivated maize (5.63% of the TDI) and conventionally produced maize flour (3.94% of the TDI) (Table 5).

The two-way ANOVA, employed within this research in order to investigate into the statistical significance of differences in the amounts of mycotoxins found in unprocessed cereals as compared to final products coming from the same type of cereal as well as in order to investigate into the relevance of agricultural practices for the amount of mycotoxins

present in cereals and cereal-based products, revealed a significant difference in DON and ZEN presence in unprocessed cereals as compared to final products coming from these cereals. The above applies to unprocessed maize, maize meal and maize flour as well as to wheat, in which a significantly higher DON content was found as compared to that in wheat flour. As concerns the agricultural practices, no statistically significant differences in mycotoxin contents found in unprocessed cereals or cereal-based products of both types were established ($p > 0.05$). Significantly higher DON and ZEN concentrations found in unprocessed cereals as compared to the final products are all in line with literature data on the impact of mitigation techniques on the mean mycotoxin content. Namely, physical removal techniques such as manual sorting of grains done by farmers or automated sorting carried out in industrial settings can significantly lower the mean mycotoxin content. Further processing of crops, such as milling, steeping and extrusion, can also reduce the mycotoxin content (Karlovsky et al. 2016).

In general, significant differences in contents of all mycotoxins investigated under this study between organically and conventionally cultivated cereals and cereal products were not observed. Mycotoxin levels potentially hazardous for human health were not evidenced either among organic or conventional cereal and cereal product samples. However, levels higher than defined by the legislation were determined in a few cereal samples; should they be consumed in an insufficiently processed form and in a continuous manner, they might pose as a human health hazard. Results of the research conducted in Croatia are in line with the conclusions of Brodal et al. (2016), who claimed no significant differences in mycotoxin contents found in organic as compared to conventional cereals and cereal products, and who were of the opinion that other factors, such as weather conditions, cropping year, cropping location, crop rotation, tillage and the choice of cultivars may have a more profound influence on mycotoxin levels than the type of cultivation. The authors pointed out that despite of fungicide non-use, organic cultivation practice generally appears to be able to maintain mycotoxin contamination at low levels and that the contamination with mycotoxins is similarly widespread across organically and conventionally cultivated cereals.

Conclusions

On the overall, mycotoxin contamination seen in organic cereals and cereal products does not significantly differ from that witnessed in their conventional counterparts. It is likely that the preventive measures used in organic cultivation are able to keep the mycotoxin contamination of organic cereals and organic cereal by-products at levels similar to those found in conventional cereals and cereal products, despite of the fungicide non-use. Nevertheless, in certain samples analysed under this study, mycotoxin levels higher than the ML for foodstuffs were observed; in such a case, these unprocessed grain samples should be used in the production of feedstuffs. Based on the mean mycotoxin concentrations established in all unprocessed cereals and cereal by-products within this study frame, as well as given that the exposure assessment resulted in low percentage of the TDI and/or the TWI, the investigated mycotoxins found in cereals and cereal products of both types do not pose as a human health hazard.

Acknowledgements The work is supported by the Croatian Veterinary Institute, Zagreb, Croatia, without any external financial support.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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