Food Control 38 (2014) 61–66

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Co-occurrence and evaluation of mycotoxins in organic and conventional rye grain and products

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ARTICLE INFO

Article history:
Received 27 April 2013
Received in revised form
26 September 2013
Accepted 1 October 2013

Keywords:
Mycotoxins
Rye
Organic and conventional farming

ABSTRACT

A study on the co-occurrence of *Fusarium* toxins in conventional and organic grain and derived products was carried out. A total of 117 samples were collected during the period 2009–2012. Eight mycotoxins were determined using the LC-MS/MS method. Among the investigated mycotoxins, four were of major importance: DON, ZEN, T-2 and HT-2. DON was present at the highest concentration in both the agricultural systems, with its maximum level of 254 ± 23 μg kg^{-1} being present in conventional rye grain. The co-occurrence of two or more mycotoxins was observed in more than 50% of samples, with the most frequent combination being DON + ZEN. The correlation between the concentrations of T-2 and HT-2, DON and ZEN, as well as T-2 and ZEN was confirmed statistically. The concentration of DON, HT-2 and T-2 was significantly higher in conventional products. Also the higher level of ZEN in organic grain in comparison to derived products was significant. None of the samples contained DAS, while NIV, MAS and 3ADON concentrations were close to the detection limits.

1. Introduction

The major part of Polish organic cultivations is dominated by cereals, mainly winter rye (Sławiński, 2011). This is because the production of rye is adaptable on soils with low fertility (Bushuk, 2001; Verwimp, Vandeputte, Marrant, & Delcour, 2004). This cereal species grows well on acidic subsoils, which is typical for majority of Polish soils. Therefore, about 20% of the rye harvested worldwide comes from Poland. In 2010, Poland along with Germany and Russia was the major producer of rye with the total production volume accounting for 63% of the world output (FAO, 2012). The largest area for the cultivation of rye is in the temperate and cool regions, mainly in Central and Eastern Europe – in the so-called Rye Belt. In autumn 2010, 5 million hectares of agricultural land in this region was sown with rye, most of which was located in Russia and Poland (RYE BELT, 2012). Rye, in spite of many advantages, is underestimated in conventional agriculture (Mohr, 2012). Researchers have suggested that organic crops are more susceptible to mycotoxin contamination, because the use of fungicides, i.e. antifungal pesticides is forbidden in the alternative farm management (Pussemier, Larondelle, Van Peteghem, & Huyghebaert, 2006). Unfortunately, there is still not much information in the literature about the impact of the type of agricultural system on the mycotoxin content; therefore the issue remains an open question. The presence of mycotoxins in cereals and animal feeds is a significant problem worldwide, and is monitored in most countries. Risk arises from the fact that mycotoxins occur naturally in food and are difficult to eliminate (Norred, 2000). In Poland, cereals and cereal products are mainly contaminated with trichothecenes, fumonisins, and zearalenone and their metabolites. Among these groups, deoxynivalenol is a mycotoxin most frequently found in the studied samples (Stanisławczyk, Rudy, & Świątek, 2010). Rye plays an important role in whole grain food production, of which consumption can be associated with reduced incidence of chronic diseases such as diabetes, cardiovascular diseases, and some types of cancer. Furthermore, rye apart from wheat is the only cereal suitable for baking bread. Thus it is particularly important to ensure the safety of the consumer so that the positive nutritional effects of these food grains and products derived from them are not compromised by the presence of mycotoxins (Bondia-Pons et al., 2009).

Taking into account such a significant role of Poland in rye production, the aim of the present study was to determine and to compare the contents of trichothecenes and zearalenone in

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http://dx.doi.org/10.1016/j.foodcont.2013.10.003

samples of organic and conventional grain and products - including determining the coexistence of these mycotoxins in the above group of materials.

2. Material and methods

2.1. Rye samples

A total of 117 unprocessed rye grain and rye product samples, both from organic ($n = 75$) and conventional ($n = 42$) farming, were collected during a four year period (2009–2012) from farmers as well as purchased randomly from retail traders in the Kuyavian-Pomeranian province of Poland. The samples included grain (organic, $n = 52$; conventional, $n = 24$), breakfast cereals (organic, $n = 10$; conventional, $n = 2$), pasta (organic, $n = 6$), flour (organic, $n = 5$; conventional, $n = 8$) and bran (organic, $n = 2$; conventional, $n = 8$). The samples were ground (particle size of 0.75 mm) with ZM 200 ultra-centrifugal mill (Retsch, The Netherlands), mixed and aliquots were taken for determination of mycotoxins. The analyses were performed in triplicate.

2.2. Mycotoxins analysis

The standard solutions of mycotoxins: deoxynivalenol (DON), nivalenol (NIV), 3-acetyldeoxynivalenol (3ADON), T-2 toxin (T-2), HT-2 toxin (HT-2), monoacetoxyscirpenol (MAS), diacetoxyscirpenol (DAS), zearalenone (ZEN), ^{13}C deoxynivalenol and zearalanone (internal standard for ZEN) were purchased from Biopure (Tulln, Austria). Ammonium acetate, acetic acid, acetonitrile (gradient grade), and methanol (gradient grade) were supplied by Merck (Darmstadt, Germany). Deionized water was obtained using a Simplicity 185 water purification system (Millipore, Bedford, USA). The samples were prepared according to Klötzel, Lauber, and Humpf (2006) with some modifications (Grajewski, Błajet-Kosicka, Twarużek, & Kosicki, 2012). Mycotoxins were determined using HPLC with MS/MS detection. HPLC: Agilent 1200 (Agilent Technologies Inc., Santa Clara, CA, USA), mass spectrometer: 3200 QTRAP (Applied Biosystems, Foster City, CA, USA), chromatographic column: Gemini C18 (150 × 4.6 mm, 5 μm) (Phenomenex Inc., Torrance, CA, USA), mobile phase: A: H_2O + 5 mM CH_3COONH_4 + 1% CH_3COOH, B: MeOH + 5 mM CH_3COONH_4 + 1% CH_3COOH, flow rate: 0.7 mL/min, injection volume: 20 μL. Detection and quantification limits: NIV: 7.0 μg kg^{-1} and 20 μg kg^{-1}, DON: 5.0 μg kg^{-1} and 15 μg kg^{-1}, 3ADON: 5.0 μg kg^{-1} and 15 μg kg^{-1}, MAS: 1.0 μg kg^{-1} and 3.0 μg kg^{-1}, DAS: 1.0 μg kg^{-1} and 3.0 μg kg^{-1}, HT-2 toxin: 2.0 μg kg^{-1} and 6.0 μg kg^{-1}, T-2 toxin: 0.7 μg kg^{-1} and 2.0 μg kg^{-1} and ZEN: 0.2 μg kg^{-1}and 0.6 μg kg^{-1}, respectively.

2.3. Statistical analysis

Statistical processing of the data was carried out using PQstat (v. 1.4.6.320) software (PQstat Polska). The variable distribution was verified applying Kołmogorov-Smirnov and Lilliefors tests, which allowed for the rejection of the null hypothesis on the distribution normality. Subsequent statistical analyses required non-parametric tests. For samples, where concentrations were lower than the detection limit, a level of 50% of LOD was accepted for calculations (the U Mann–Whitney test). The U Mann–Whitney test was used to evaluate the differences between groups ("grain vs. product", "conventional vs. organic"), while correlation of concentrations for two mycotoxins was assessed by applying the Spearman's rank correlation test. The correlation verification was performed for samples, in which the concentration was higher than LOQ. For all calculations, a level of $p < 0.05$ was accepted as statistically significant.

3. Results and discussion

The trichothecenes and zearalenone content in rye grain and rye products from both conventional and organic farms are presented in Tables 1 and 2.

Of the eight mycotoxins identified, DON, ZEN, T-2, and HT-2 were found in predominant amounts. DON was present in the highest concentration in the analyzed samples (up to 254 ± 23 μg kg^{-1} in rye grain from conventional farming). In the group of conventional rye grain, the percentage of contaminated samples was 79%. Rye products from conventional farming were also more contaminated with DON (percentage of contaminated samples was 83%) with a maximum concentration of 120 ± 11 μg kg^{-1} in rye bran. Organic rye products contained DON in three times lesser number of samples than the conventionally farmed products. Presence of ZEN was recorded in 89% of products from conventional farming (with the highest value of 6.81 ± 0.72 μg kg^{-1}) and in 71% of grain samples from the same agricultural system (with a maximum concentration of 148 ± 17 μg kg^{-1}). DAS was not detected in any of the analyzed samples, while the concentrations of NIV, 3ADON, and MAS were close to the detection limit. The highest contents of toxins T-2 and HT-2 were recorded in rye grain: 24.8 ± 3.2 μg kg^{-1} (T-2, organic) and 44.1 ± 5.4 μg kg^{-1} (HT-2, conventional). Mean DON concentration in conventional products (29.6 μg kg^{-1}) was lower as compared to analogous value in the rye grain (45.6 μg kg^{-1}), similarly in the case of ZEN, its mean level in conventional products (1.21 μg kg^{-1}) was lower as compared to the grain (9.80 μg kg^{-1}). For grain and products from organic cultivation the mean concentration of DON was for both below 15 μg kg^{-1}. However, the mean content of ZEN was higher in grain (2.1 μg kg^{-1}) as compared to the products (<0.6 μg kg^{-1}). The fact might be explained by food processing influence on this mycotoxin level (Bullerman & Bianchini, 2007; EFSA, 2011a; Kabak, 2008).

In none of the analyzed samples, the content of mycotoxins exceeded the maximum acceptable levels (Commission Regulation (EC), 2006). Due to statistically relevant amount of data, evaluation of results was carried out for the compounds, that occurred most frequently in the material (DON, ZEN, T-2, HT-2). A higher level of ZEN in organic rye grain as compared with products turned out to be statistically significant – M_{OG} (median for organic grain) = 1.43 μg kg^{-1}, M_{OP} (median for organic products) = 0.6 μg kg^{-1}, $p = 0.0397$). When comparing the two farming systems, significantly higher levels of DON was recorded in conventional grains – M_{CG} (median for conventional grain) = 15.0 μg kg^{-1}, M_{OG} (median for organic grain) = 2.5 μg kg^{-1} ($p = 0.0007$). In the case of products, amounts of DON, T-2, HT-2 were remarkably higher also in conventional samples: DON M_{CP} (median for conventional products) = 20.1 μg kg^{-1}, M_{OP} (median

Table 1
Trichothecenes and zearalenone content in samples of rye grain from conventional and organic farms (μg kg^{-1}).

Mycotoxin	Contamination rate (%)		Mean (μg kg^{-1})		Maximum (μg kg^{-1})	
	C $n = 24$	O $n = 52$	C $n = 24$	O $n = 52$	C	O
NIV	8	0	nd	nd	<20	–
DON	79	37	45.6	<15	254	140
3ADON	8	0	nd	nd	<15	–
MAS	17	2	nd	nd	<3.0	<3.0
DAS	0	0	nd	nd	–	–
HT-2	42	21	<6.0	nd	44.1	24.3
T-2	38	27	<2.0	<2.0	7.84	24.8
ZEN	71	46	9.80	2.10	148	72.4

C – conventional grain, O – organic grain, nd – not detected.