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Mycotoxin content of organic and conventional oats from southeastern Poland

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ABSTRACT

In this study, we investigated the influence of organic and conventional farming systems on mycotoxin levels in oats from 2006 to 2008. The relationship between mycotoxin contents was also calculated. Data were analysed separately for each factor (year – three values and production systems – two values) and their combinations, e.g. analysis of samples harvested in entire three-year period of this study from organic farming. Only one significant difference occurred between organic and conventional farming systems when samples from entire three-year period of this study were included – the concentration of diacetoscirpenol (DAS) was higher in samples from conventional farms. Statistical analysis did not reveal any influence of the cropping system on the concentrations of remaining mycotoxins. However, the number of mycotoxin-positive samples was higher in organic farming in comparison to conventional production. Furthermore, considering only the mycotoxin-positive samples, the concentrations of deoxynivalenol (DON), T-2, HT-2, DAS, nivalenol (NIV), and aflatoxins (B1, B2, G1 and G2) were slightly higher, but not statistically significant, in samples from conventional farming. Most correlations of the mycotoxin content occurred in oat grain samples from organic rather than conventional farms. Numerous positive correlations were found between NIV and DAS and between NIV, DAS and other mycotoxins. Only one low negative correlation was detected for the concentration of DON and NIV when all samples from organic production system were considered. Low and moderate positive correlations were found between aflatoxins, ochratoxins and other mycotoxins. The level of aflatoxins and ochratoxins was correlated only in samples from organic farming. This is one of the few reports presenting correlations between mycotoxins on the background of organic and conventional farming systems.

1. Introduction

Fungi can develop in many types of food, leading to changes in its texture, taste and smell, mainly through the production of enzymes and other chemicals. Currently, over 31,000 known metabolites produced by fungi occur in cereals. Some of these chemicals, called mycotoxins, are harmful to both humans and animals. The production of mycotoxins by these microorganisms is one of the most important points of food contamination (AntiBase, 2005; Sulyok, Krska, & Schuhmacher, 2010).

Mycotoxins are secondary metabolites produced by fungi, mainly from the genera *Fusarium*, *Aspergillus* and *Penicillium* (Sherif, Salama, & Abdel-Wahhab, 2009). Currently, there are more than 400 known mycotoxins. The most studied mycotoxins are aflatoxins, trichothecenes, zearalenone (ZEN), and ochratoxins (Binder, Tan, Chin, Handl, & Richard, 2007; Filtenborg, Frisvad, & Samson, 2000).

Biological and chemical properties of mycotoxins vary, as well as their toxicity (Salem & Ahmad, 2010). Many of these compounds have oestrogenic, teratogenic, mutagenic and carcinogenic effects (Binder et al., 2007; D'Mello, Placinta, & Macdonald, 1999; Prelusky, Rotter, & Rotter, 1993). Mycotoxins can enter the human body in different ways, mainly through the consumption of contaminated food (Berthiller, Sulyok, Krska, & Schuhmacher, 2007; Salem & Ahmad, 2010).

In the case of crop production, economic losses caused by toxigenic fungi can be substantial. In the United States, losses resulting from the occurrence of Fusarium head blight (FHB) of wheat and barley between 1998 and 2000 were valued at $ 2.7 billion (Waalwijk et al., 2004; Windels, 2000).

The FAO report (2000) concerning the content of mycotoxins in agricultural crops showed no clear differences between organic and conventional farming systems. It was stated however that in certain circumstances, such differences might occur. Most studies

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published after the FAO report (2000) also failed to identify significant differences between organic and conventional cropping systems (Champeil, Fourbet, Dore, & Rossignol, 2004; Cirillo, Ritieni, Visone, & Cocchieri, 2003; Jestoi et al., 2004). Nevertheless, in some cases differences in mycotoxin levels between organic and conventional production systems were reported (Knudsen, Elmholt, Hockenhull, & Jensen, 1995; Skaug, 1999; Woese, Lange, Boess, & Bogl, 1997).

There are a several possible ways to carry out the studies that deal with mycotoxin content in the products from organic and conventional production systems, namely comparison of the products from the market, surveys of samples from organic and conventional farms, and direct cultivation trials in experimental fields (Malmauret, Parent-Massin, Hardy, & Verger, 2002). In this study, a survey was conducted.

The aims of this survey were (1) to investigate the distribution of mycotoxins in oat grains from southeastern Poland, (2) to calculate the relationships between mycotoxin content in analysed samples (3) and to analyse the data using each of the factors and their values (year: 2006, 2007 and 2008; production system: organic and conventional).

2. Material and methods

2.1. Sampling

The study was conducted in 2006, 2007 and 2008. A total of 58 samples were collected (Table 1). The survey was undertaken in two Polish provinces, i.e. Lublin and Swietokrzyskie province. Oat grain samples (2 kg per farm) were acquired from conventional and certified organic farms. To obtain representative samples, primary large samples were prepared by combining several smaller ones, collected from different parts of storage sacks or containers. The primary samples were homogenised and quartered to obtain a 250 g sample for laboratory analysis.

Samples were collected up to a week after harvest and stored at −20 °C until analysed.

2.2. Enzyme-linked immunosorbent assay (ELISA) for mycotoxin determination

Assays for the detection of mycotoxins were performed according to the protocols described in the AgraQuant® Total Aflatoxin (COKAQ1100), AgraQuant® Ochratoxin (COKAQ2000), AgraQuant® DON (COKAQ4000) and AgraQuant® T-2 Toxin (COKAQ6000) ELISA Test Kit manuals (Romer Labs, Tulln, Austria) with slight modifications. The overview of kits characteristics is presented in Table 2.

A finely ground 20 g sample of oat grain was homogenised in 100 ml of distilled water (DON) or 100 ml of methanol:water (7:3, v/v) (aflatoxins, ochratoxins, ZEN, T-2 toxin) by blending for 3 min and filtering through Whatman No. 1 paper. One ml of such prepared extract for aflatoxins and ochratoxins, 0.2 ml for DON, 0.25 ml for ZEN and 0.1 ml for T-2 analysis were aliquoted to the 1.5 ml Eppendorf tubes, filled to 1 ml of total volume with distilled water for DON and 70% methanol (v/v) for other mycotoxin determinations. One hundred microlitres of diluted extract was used further in the procedure. Plates were read using a Tecan Sunrise microwell reader with an absorbance filter of 450 nm and a differential filter of 630 nm. The optical densities of the samples were compared to standards.

The recovery of mycotoxins was not studied, however calibration curves had a high correlation in the range of 0.952–0.997.

Table 1
Number of oat samples analysed for the presence of mycotoxins in this study.

Harvest year	Farming system		Total
	Organic	Conventional	
2006	12	12	24
2007	13	4	17
2008	11	6	17

Table 2
Characteristic of AgraQuant® ELISA test kits used in this study.

ELISA kit	Item no.	Quantitation range (μg kg^{-1})	Limit of detection (μg kg^{-1})
AgraQuant® Total Aflatoxin	COKAQ1100	1–20	1
AgraQuant® Ochratoxin	COKAQ2000	2–40	2
AgraQuant® DON	COKAQ4000	250–5000	200
AgraQuant® T-2 Toxin	COKAQ6000	75–500	35

2.3. GC–ECD and GC–MS

Gas chromatography was used to determine NIV, HT-2 and DAS toxins presence. Samples from 2006 to 2007 were analysed using gas chromatography with an electron capture detector (GC–ECD) and samples from 2008 were analysed using gas chromatography with mass spectrometry (GC–MS) due to the failure of GC–ECD.

A sample extraction was performed according to Valle-Algarra et al. (2005) with modifications. One hundred ml of extraction buffer (acetonitrile:H_2O, 84:16 v/v) was added to 25 g of ground kernels, then shaken for 30 min. After filtering through Whatman No. 4 filter paper, 5 ml of sample extract was cleaned up using MycoSep 225 column (Romer Labs). A 2 ml volume of the purified extract was transferred to a vial and concentrated to dryness using a vacuum pump (Barnant).

Derivatisation was performed with 0.1 ml 4-dimethylaminopyridine (DMAP) solution (2 mg/l) in toluene:acetonitrile (80:20, v/v) with an addition of 0.05 ml of pentafluoropropionic anhydride (PFPA) in a screw cap vial with dry extract. After tight capping, the reaction mixture was heated at 60 °C for 60 min in a heater block and cooled. One millilitre of a 3% (w/v) aqueous solution of $NaHCO_3$ was added to the mixture, and the vial was vortexed for 15 s. The two layers were allowed to separate. The top layer (organic phase) was transferred to a glass insert and put into a GC autoinjector vial and analysed by GC–ECD and GC–MS.

The results for the recovery, detection and quantification limits of NIV, HT-2 and DAS are presented in Table 3. On average, the recovery for NIV, HT-2 and DAS was 74.1%, 89.3%, and 104.6%, respectively. Detection and quantification limits were determined according to DIN 32645.

GC–ECD analyses were performed on the Varian GC 3380 with column factor-FOUR VF-1ms, 30 m × 0.32 mm, id = 0.25 μm film thickness. In splitless mode 1.0 μl of solution was injected. The temperatures of the injection port and the detector were 250 and 320 °C, respectively. The oven temperature programme was: 100 °C held for 1 min, 10 °C/min to 170 °C, 3 °C/min to 200 °C, 5 °C/min to

Table 3
The recovery, detection and quantification limits with the GC–ECD and GC–MS methods used for NIV, HT-2 and DAS determination.

Mycotoxin	Recovery for MycoSep 225 and PFPA mean ± S.D. (%)	Detection limit (μg kg^{-1})		Quantification limit (μg kg^{-1})	
		GC–MS	GC–ECD	GC–MS	GC–ECD
NIV	74.1 ± 16.7	11	4	32	13
HT-2	89.3 ± 18.6	7	3	21	10
DAS	104.6 ± 15.8	9	5	30	16