THE OCCURRENCE OF MYCOTOXINS IN ORGANIC SPELT PRODUCTS

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Abstract: Mycotoxins have a proven toxic effect on the health of humans and animals. Nowadays, there is a focus on having a healthy lifestyle and consuming organic foods. High quality grain products, especially spelt products, which are an important element of a well-balanced diet have become more popular. The aim of this study was to determine the mycotoxin content in spelt products available on the Polish market. Spelt products were collected in 2009 and 2010 in eastern Poland. The Enzyme-Linked ImmunoSorbent Assay (ELISA) method was used to identify aflatoxins, ochratoxin A, T-2 toxin, deoxynivalenol, and zearalenone. The study confirmed that all investigated mycotoxins were present in spelt products.

Key words: spelt, *Fusarium* toxigenic fungi, mycotoxins

INTRODUCTION

Spelt (*Triticum spelta* L.) is one of the oldest subspecies of common wheat along with *Triticum monococcum* L. or *Triticum dicoccum* L. This ancient wheat is known from the Neolithic period, in North and Central Europe (Achremowicz *et al*. 1999; Krawczyk *et al*. 2008; Zarzycki 2009). In XIX century spelt was replaced by common wheat which is easier to cultivate and process. Nowadays spelt is enjoying a comeback due to its pro-health properties (Ziobrowski 2006; Radomski *et al*. 2007). Spelt helps decrease the cholesterol level in blood, fosters digestion, and helps the circulatory system. It has been associated with prevention of liver and kidneys diseases. Spelt slows down ageing, reduces sugar levels in blood, and enhances concentration and brain activity (Tyburski and Babalski 2006; Ziobrowski 2006; Radomski *et al*. 2007; Czerwińska 2009). The current focus on health food and a healthy lifestyle have helped increase consumption of spelt products. Nowadays, this cereal is cultivated mostly by organic methods. Thanks to a higher stalk and hard adherent husks, spelt has poor fungal infestation, less pest damages as well as less contamination by heavy metals and pesticide residues (Wiwart and Perkowski 2005; Tyburski and Babalski 2006; Ziobrowski 2006; Radomski *et al*. 2007; Krawczyk *et al*. 2008).

Cereal products are major ingredients of the human diet but they are also an important source of mycotoxins. Fungi from *Aspergillus, Penicillium* and *Fusarium* genera are main producers of the mycotoxins (Binder *et al*. 2007). Mycotoxins are secondary metabolites, which are low-molecular, thermostable toxic substances. The division of mycotoxins is as follows: aflatoxins B_1, B_2, G_1, G_2 and their metabolites M_1 and M_2, ochratoxin A, B and C, trichotecenes A: T-2 toxin, HT-2 toxin, diacethoxyscirpenol/DAS, neosolaniol/NEO and B: deoxynivalenol/DON, nivalenol/NIV, fuzarenon-X/FUS-X, zearalenon/ZEN and fumonisines B_1, B_2, B_3. In cereals, mostly trichotecenes and ochratoxins are found (Hussein and Brasel 2001; Pokrzywa *et al*. 2007).

Fungi toxins are dangerous for both humans and animals. They can cause acute food poisoning or even death. They have mutagenic, carcinogenic, immunosuppressive, teratogenic, neurotoxic or estrogenic properties. In 1993, the International Agency for Research on Cancer added ochratoxin A, aflatoxin M_1, fumonisin B_1 and B_2 and fusarin C as possible carcinogenic substances for humans and zearalenon, deoxynivalenol, nivalenol, fusarenon X, and T-2 toxin as substances in which there is little evidence for being classified as carcinogenic for humans. In a 2002 supplemented list, aflatoxins were listed as a natural mixture which has a proven carcinogenic potential for humans (IARC monographs on the evaluation of carcinogenic risks to humans 2002; http://monographs.iarc.fr/ENG/Classification/ClassificationsGroupOrder.pdf 2010).

The aim of this study was to determine the mycotoxin content in spelt products available on the Polish market.

MATERIALS AND METHODS

Samples

23 spelt products were collected from local market in eastern Poland in 2009 and 2010. There were 14 spelt flour samples in 2009 and 3 flour samples and 6 other products in 2010.

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Method

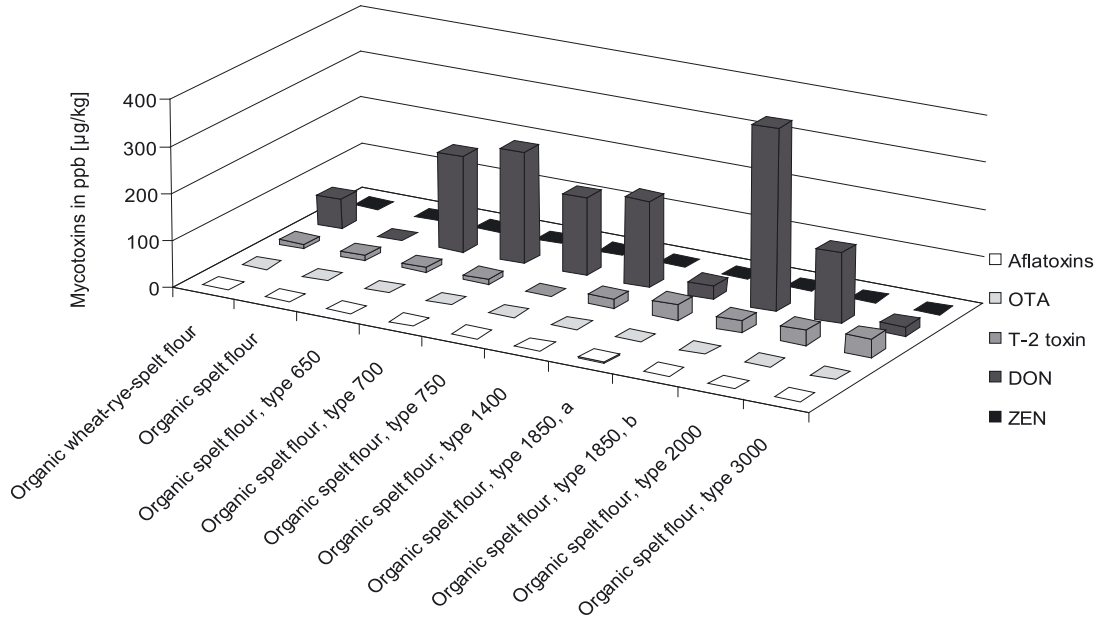
All samples were examined for aflatoxins, ochratoxin A, deoxynivalenol, zearalenon, and T-2 toxin content by enzyme-linked immunosorbent assay.

Commercial The Enzyme-Linked ImmunoSorbent Assay (ELISA) kits: Ridascreen Aflatoxin Total Assay, Ridascreen T-2 Toxin Assay, Ridascreen Ochratoxin A 30/15 Assay, Ridascreen DON Assay and Ridascreen Zearalenon Assay were used in the study. The kits are direct competitive immunoassays with horseradish peroxidase conjugate. ELISA test was performed according to the procedure described in the Ridascreen Assay kit manual. Ground samples (20 g) were extracted and filtered through Whatman No. 1 paper. Samples were diluted with distilled water. Aliquots of 100 ml of all extracts were further used in the procedure. Absorption in microwells was measured with a Tecan Sunrise microwell reader using a 450 nm absorbance filter.

RESULTS

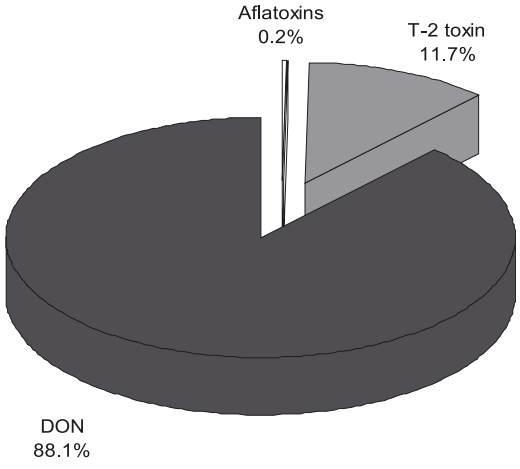
In all samples from 2009 and 2010, T-2 toxin was found in an amount below 75 μg/kg. In samples collected in 2009, ochratoxin A and zearalenon were not found. T-2 toxin was found in 93% of samples. There was one sample with aflatoxins and eleven with deoxynivalenol. Deoxynivalenol (DON) was indentified in a maximum amount of 386.37 μg/kg. In all examined samples, the tolerated level of mycotoxins was not exceeded (Fig. 1).

In samples from 2009, mostly DON was found. This toxin was in 90% of the samples in an average amount of 144.40 μg/kg, but in one sample up to 386.37 μg/kg. Aflatoxins showed the lowest frequency in products amounting to 0,2% of the total sample number (Fig. 2.).



OTA – ochratoxin A; DON – deoxynivalenol; ZEN – zearalenone

Fig. 1. Content of mycotoxins in spelt flours in 2009 in ppb [μg/kg]



DON – deoxynivalenol

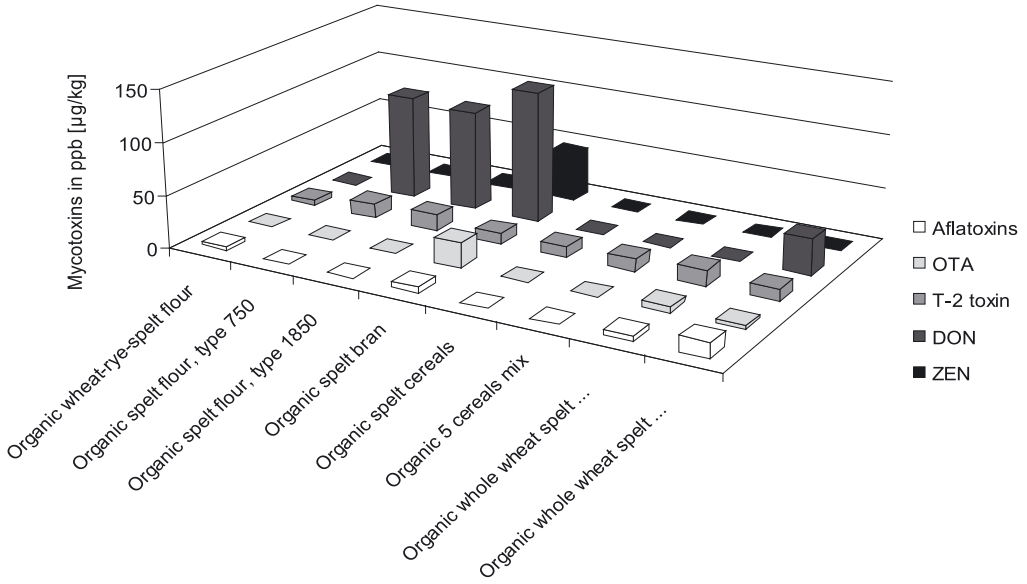
Fig. 2. Frequency of mycotoxins occurence in 2009

In 2010, aflatoxins were found in four samples. In two of them, the tolerated level of mycotoxins was exceeded. In one sample, zearalenon was detected in the amount of 45.2 μg/kg, two samples contained deoxynivalenol (34.3 μg/kg and 125.0 μg/kg) and three ochratoxin A (OTA). In two samples, the evaluated amount of ochratoxin A exceeded the tolerated level. The highest level of OTA was in spelt bran – 23.99 μg/kg. Also, in whole wheat spelt pasta (5.77 μg/kg), the tolerated level of that mycotoxin was exceed (Fig. 3.).

In samples from 2010, DON was also mostly found. In all the examined samples, T-2 toxin was detected in amounts below 75 μg/kg. Zearalenone (ZEN) and OTA occurred in 8% and 6% of the samples, respectively. Figure 4 shows that, of all sampled spelt products, 5% had the least degree of detected contamination by aflatoxins.

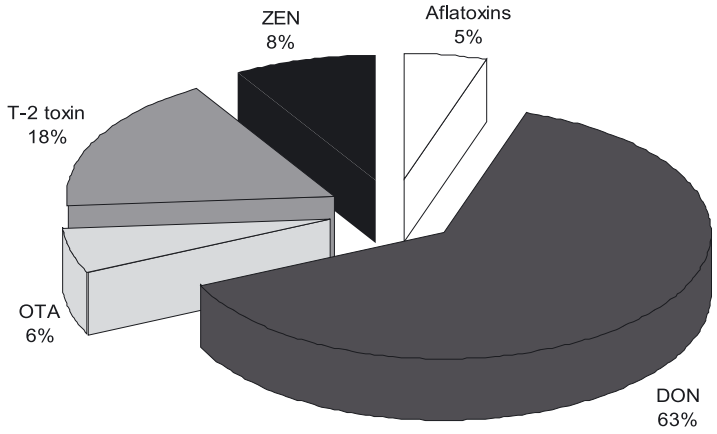
The most contaminated sample was spelt bran in which all the evaluated mycotoxins were detected. Spelt flour has a total concentration of mycotoxins above 100 μg/kg. The least contaminated samples was wheat-rye-spelt flour. Only aflatoxins and T-2 toxin were found in minor amounts in wheat-rye-spelt flour (Fig. 5.).

In 2010, contamination of mycotoxins was less than in 2009 in the same type of organic spelt products. In 2009, there were higher amounts of DON and T-2 toxin (Fig. 6).



OTA – ochratoxin A; DON – deoxynivalenol; ZEN – zearalenone

Fig. 3. Content of mycotoxins in spelt products in 2010



OTA – ochratoxin A; DON – deoxynivalenol; ZEN – zearalenone

Fig. 4. Frequency of mycotoxins occurence in 2010

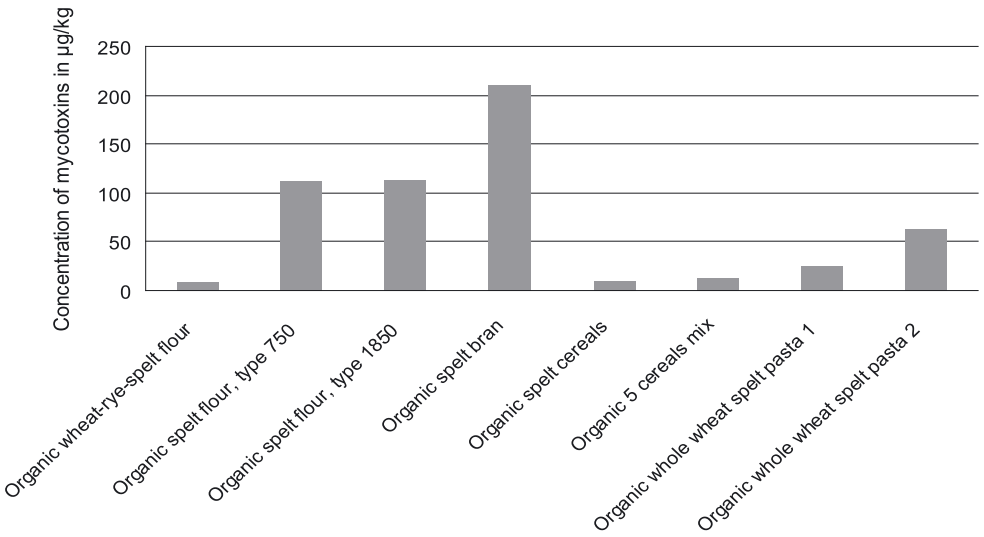
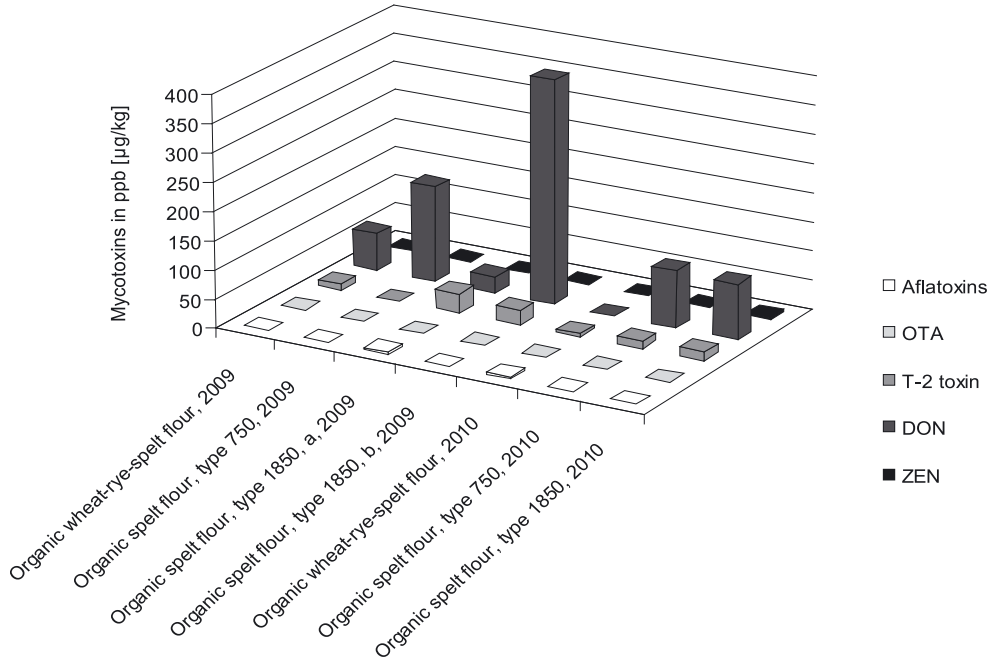


Fig. 5. Mycotoxin contamination in spelt products in 2010



OTA – ochratoxin A; DON – deoxynivalenol; ZEN – zearalenone

Fig. 6. Comparison of mycotoxin contamination in some organic spelt products from 2009 and 2010

DISCUSSION

Fusarium species are main producers of mycotoxins in cereals. Errors in crop rotation, like cearls having too much of a share, are a major cause of high mycotoxin grain contamination (FAO 2003). Information from literature about mycotoxin contamination in spelt is scarce. Slight amounts of deoxynivalenol, T-2 toxin and ochratoxin A were found on spelt. Because of its tough husks it is one of the cereals which is least infected by fungi (Wiwart and Perkowski 2005; Tyburski and Babalski 2006; Ziobrowski 2006; Radomski *et al*. 2007; Krawczyk *et al*. 2008). The results of the study indicated T-2 toxin as the most often occurring mycotoxin in spelt products. T-2 toxin is one of the most toxic mycotoxin belonging to the trichotecenes group. *Fusarium sporotrichioides* and *Fusarium poae* are the main producers of T-2 toxin. This toxin has been shown to induce haematological and immunological toxicity and to impair synthesis of DNA and cellular proteins [8]. To date, in the European Union there is no maximum tolerated level for that mycotoxin in food [Rozporządzenie Komisji (WE) nr 466/2001 z dnia 8 marca 2001; Rozporządzenie Komisji (WE) nr 1126/2007 z dnia 28 września 2007]. Tolerated daily intake (TDI) for the mixture of T-2 and HT-2 toxins is 0.06 µg per 1 kg of body weight per day (Hussein and Brasel 2001; Pokrzy-

wa *et al*. 2008; Rozporządzenie Komisji (WE) nr 856/2005 z dnia 6 czerwca 2005; Rozporządzenie Komisji (WE) nr 1126/2007 z dnia 28 września 2007). Grain products are a base element of the daily human diet. Adults need from 245 to 455 g and more grain products per day, children and teenagers from 175 to 455 g. T-2 toxin is the most dangerous for children because of their high daily intake of grain products and lower body weight (http://web.mit.edu/athletics/sportsmedicine/wcrfoodpyr.html; http://www.choosemyplate.gov/foodgroups/grains_amount_table.html). Solarska *et al*. (2009) found in one sample of ancient wheats cultivated in Poland, a high T-2 toxin content in the amount of 179.6 μg/kg. In assaying product content for the T-2, this mycotoxin was found to be below 75 μg/kg. In whole-grain products, the concentation of T-2 toxin is higher than in white flours (Schollenberger *et al*. 2002). The second frequently occurring mycotoxin was deoxynivalenol. The tolerated DON daily intake is 1 μg per 1 kg of body weight per day according to EU determinations (Hussein and Brasel 2001; Rozporządzenie Komisji (WE) nr 856/2005 z dnia 6 czerwca 2005; Pokrzywa *et al*. 2008). Wiwart *et al*. (2009) indicated that the average concentration of DON is up to 486.50 μg/kg in spelt kernels and up to 1,508.55 μg/kg on husks. Other authors consistently found lower contamination levels of DON in spelt (Castoria *et al*. 2005; Wiwart *et al*. 2011). Solarska *et al*. (2009) found 169.37 μg/kg as the average concentration of deoxynivalenol in ancient wheats. In spelt flours, the maximum tolerated contamination is 750 μg/kg. In our study, there are no significant difference in the DON contamination level in both flour types (Schollenberger *et al*. 2002; González-Osnayaa *et al*. 2011; Wiwart *et al*. 2011). The most contaminated products are whole-grains, like bran. Often, all investigated mycotoxins are present. The reason is that there is a high mycotoxin content on the kernel cover (Schollenberger *et al*. 2002; Wiwart *et al*. 2011). Aflatoxins and ochtatoxin A are frequently detected mycotoxins in spelt products. This cereal without its husks, is difficult to store. Bad storage conditions, especially in high moisture conditions and when there is not enough airing, may cause an increase in contamination by aflatoxins and OTA. Common spelt kernel contamination of ochratoxin A was indicated by Elmholt and Rasmussen (2005), on average, to amount to 0.1 μg/kg. A higher OTA concentration in spelt was indicated by Castoria *et al*. (2005) in their study. Zearalenon and aflatoxins were particularly detected in some products from 2010. In the literature, any information about spelt contamination by these mycotoxins or lack of aflatoxins, were noticed in spelt kernels (Solarska *et al*. 2009). There is a definite need to monitor the occurrence of mycotoxins in grain products before market introduction.

CONCLUSION

All the investigated mycotoxins were indentified in the spelt products. Occurrence of dangerous substances especially in health foods, indicates the need for constant monitoring of these products.

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