Research article

Contamination of breakfast cereal products by fungi and mycotoxins – a potential risk for consumer's health

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Abstract: *The aim of research was assessment of breakfast cereal snacks available in trade for their contamination with fungi and selected mycotoxins in related to potential risk of consumers' health. The contamination with fungi ranged from 1.3×10^1 cfu g^{-1} to 9.0×10^2 cfu g^{-1}. The most contaminated was muesli, that apart from cereal components comprised also dried fruit, nuts and coconut flakes. Species belonging to Aspergillus, Penicillium, Cladosporium, Rhizopus, Mucor, Chaetomium, Trichoderma, Eurotium and Fusarium genera were isolated as dominant. The isolated species included Aspergillus ochraceus, A. flavus, A. versicolor, A. sydowii, Penicillium verrucosum and Fusarium graminearum, which are well known as mycotoxin producers. None of the products was contaminated with aflatoxin B_1. The presence of ochratoxin A exceeding of 3 ng g^{-1} was discovered in 30% of samples. The contamination with deoxynivalenol equalled 587 ng g^{-1} on average. This result indicates that the cereals products may form a serious source of exposure to mycotoxins, particularly for most vulnerable group, i.e. children.*

Keywords: *cereal products, fungi, xerophils, ochratoxin A, aflatoxin B_1, deoxynivalenol.*

Introduction

Crops and cereal-based products, due to their chemical composition, are particularly susceptible to moulds. Fungi contaminating grains have been conventionally divided into two groups – field fungi and storage fungi. Field fungi are those that infect the grain throughout the vegetation phase of plants and they include *Alternaria, Fusarium, Cladosporium* and *Botrytis* species. Storage fungi infect the grain during storage in improper conditions of excessive humidity. This group comprises moulds within *Aspergillus*, *Penicillium*, *Rhizopus* and *Mucor* [1, 2]. Food spoilage by fungi has been characterized in some review articles [3-5]. Fungi contaminating crops include toxigenic species,

both belonging to the storage fungi and the field fungi, e.g. *Aspergillus flavus, A.ochraceus, Penicillium verrucosum, Fusarium graminearum, F. culmorum* and *F. sporotrichoides.* Their development leads to contamination with mycotoxins, such as aflatoxin B_1, ochratoxin A, citrinin, fumonisins, zearalenone, and deoxynivalenol. These toxins diffuse into the grain and can be found in all ground fractions and, due to their thermo-resistance properties, also in products subject to thermal processing [6]. Such products make a serious threat to the consumers' health and some of the toxins are enumerated among carcinogenic compounds for humans and animals. Aflatoxin that belongs to human carcinogens induces liver tumors, and ochratoxin A has nephrotoxic effects. Trichothecenes, and deoxynivalenol (DON) among them present a wide range of toxic influence on human beings and animals resulting in their lack of appetite, nausea, diarrhea, hemorrhages and anemia. The occurrence of mycotoxins in various raw materials and cereal products, as well as health threats related to this, have been discussed in numerous review articles [7-9].

In recent years the rules of healthy nutrition indicate the necessity to increase the amounts of unprocessed cereal products in one's diet. Therefore, the market of cereal products for direct consumption has been subject to a great development. The aim of presented research was evaluation of breakfast products available on the market as for their contamination with fungi and selected mycotoxins in related to potential risk of consumers' health.

Experimental

Materials

The following type of cereal breakfast flakes and snacks marked with the number from 1 to15, from retail trade were randomly collected: cereal snacks with cinnamon (No 1, 2), cornflakes (No 3, 4), cornflakes with nuts and honey (No 5, 6), multi-cereal products (No 7), cereal products with chocolate (11, 12, 13, 14, 15), and muesli containing dried fruit, nuts as well as cereal and coconut flakes (No 8, 9, 10). For each type of assortment three independent laboratory samples were analysed.

Methods

The concentration of fungi was determined by the dilution plating method according to PN-EN ISO 21527, 2008 [10]. Initial suspension and decimal dilutions were prepared following the PN-EN ISO 6887, 2000 [11]. Dichloran-Rose Bengal Chloramphenicol Agar (DRBC, 1.00467. Merck, Darmstadt, Germany) for estimation of total number of fungal colony forming unit and Dichloran Chloramphenicol Agar with Glycerol (DG18, 1.00465. Merck) for xerophilic fungi were used. The samples were incubated at 25^{o}C for 7 days. The results were quoted as colony forming units per 1 g of a sample (cfu g^{-1}). The identification of mould was conducted following the isolation of pure cultures

and inoculation on Czapek-Dox Agar according to identification keys [12-14]. The frequency of several species in total species expressed in % was determined.

Mycotoxins were determined by the ELISA method with the use commercial test kits: AgraQuant®Aflatoxin B_1 and AgraQuant®Ochratoxin A (Romer Labs. Diagnostic GmbH, Tulln, Austria) for aflatoxin B_1 and ochratoxin A estimation and MycoChek™ Vomitoxin (DON) Test Kit (Strategic Diagnostics Inc. Newark, the USA) for deoxynivalenol (DON). The extraction of toxins was conducted according to the guidelines of the producers. The limit of detection for cereal matrix was established according to tests producers on the following levels: 2 ng g^{-1} for aflatoxin B_1 and ochratoxin A, and 250 ng g^{-1} for DON.

All analysis was conducted in three independent samples. The results were subject to statistical analysis using Microcal ORIGIN ver. 6.0 software (Northampton, USA).

Results and Discussion

The presented study showed that almost all analysed products are contaminated with fungi on the level dependent on the kind of products in the range from 1.3×10^1 to 9.0×10^2 cfu g^{-1} (Table 1).

Table 1. Contamination of breakfast cereal products by fungi

Sample No	Total fungi [cfu g^{-1}]		Xerophilic fungi [cfu g^{-1}]	
	Mean ± SD	Median	Mean ± SD	Median
1	$2.0\times10^1\pm1.0\times10^1$	1.5×10^1	nd	
2	$3.3\times10^2\pm6.7\times10^1$	3.1×10^2	$1.3\times10^1\pm0.6\times10^1$	1.0×10^1
3	$5.0\times10^2\pm3.6\times10^1$	4.9×10^2	$1.7\times10^1\pm1.1\times10^1$	1.0×10^1
4	$3.1\times10^2\pm1.0\times10^1$	3.1×10^2	$5.3\times10^1\pm0.6\times10^1$	5.0×10^1
5	$1.3\times10^1\pm0.6\times10^1$	1.0×10^1	nd	
6	$8.0\times10^2\pm2.6\times10^1$	7.9×10^2	$1.7\times10^1\pm0.6\times10^1$	1.5×10^1
7	$6.0\times10^1\pm1.0\times10^1$	5.5×10^1	$2.3\times10^1\pm0.7\times10^1$	2.0×10^1
8	$9.0\times10^2\pm2.6\times10^1$	8.9×10^2	$8.1\times10^2\pm2.0\times10^1$	8.0×10^2
9	$2.2\times10^2\pm5.3\times10^1$	1.9×10^2	$1.1\times10^2\pm1.0\times10^1$	1.1×10^2
10	$5.0\times10^1\pm1.0\times10^1$	4.5×10^1	nd	
11	$1.7\times10^1\pm0.6\times10^1$	1.5×10^1	$4.3\times10^1\pm0.6\times10^1$	4.0×10^1
12	nd		$8.3\times10^1\pm0.5\times10^1$	8.0×10^1
13	$5.0\times10^2\pm1.0\times10^1$	4.9×10^2	$5.3\times10^1\pm0.7\times10^1$	5.0×10^1
14	$3.0\times10^2\pm1.7\times10^1$	2.9×10^2	nd	
15	$1.0\times10^2\pm2.0\times10^1$	9.0×10^1	nd	

nd – no detected, under detection limit 10 cfu g^{-1}

The most contaminated was muesli, that apart from cereal components comprised also dried fruit, nuts and coconut flakes, and were obtained from a network of discount stores and produced by a small company. In two products with chocolate (No 11 and 12), the domination of xerophilic fungi was

discovered. Currently valid microbiological criteria lack norms concerning the maximum level of moulds in cereal products. Prior to the entry of Poland to the EU there had been requirements in force, which stated that the number of fungi in breakfast products couldn't exceed the value of 10^3 cfu g^{-1}. The number of fungi in analysed products can be considered a permissible value. The research conducted in the years 2006-2007 in Poland showed a similar level of mould contamination of cereal raw material ranging from 1.0×10^1 to 1.0×10^3 cfu g^{-1} [15]. The primary source of mould contamination can be related to the raw substance used for production, though equally important is the source of the secondary contamination, e.g. from air or from human beings during the later phases, including distribution.

Table 2. Fungi identified in breakfast cereal products

Sample No	DRBC medium		DG18 medium	
	Species	F [%]	Species	F [%]
1	*Aspergillus versicolor*	100	nd	
2	*Penicillium chrysogenum* *Aspergillus versicolor*	63 37	*Aspergillus niger*	100
3	*Penicillium chrysogenum*	100	*Penicillium glabrum*	100
4	*Fusarium graminearum* *Aspergillus ochraceus*	66 34	*Cladosporium cladosporioides*	100
5	*Aspergillus sydowii* *Rhizopus nigricans*	75 25	nd	
6	*Aspergillus sydowii* *Aspergillus versicolor*	76 24	*Penicillium glabrum*	100
7	*Penicillium chrysogenum* *Penicillium verrucosum* *Aspergillus versicolor*	65 20 15	*Eurotium repens*	100
8	*Aspergillus niger* *Penicillium chrysogenum*	90 10	*Aspergillus niger*	100
9	*Aspergillus niger* *Aspergillus flavus* *Fusarium graminearum* *Penicillium chrysogenum*	85 5 5 5	*Eurotium repens* *Penicillium* sp.	63 37
10	*Penicillium verrucosum* *Aspergillus versicolor*	80 20	nd	
11	*Trichoderma viride*	100	*Cladosporium herbarum* *Penicillium glabrum*	75 25
12	nd		*Rhizopus nigricans* *Penicillium verrucosum* *Mucor racemosus*	63 24 13
13	*Aspergillus versicolor* *Penicillium chrysogenum*	50 50	*Cladosporium herbarum*	100
14	*Aspergillus flavus* *Chaetomium globosum* *Rhizopus nigricans* *Aspergillus versicolor*	30 30 30 10	nd	
15	*Aspergillus niger*	100	nd	

F – frequency, nd – not detected

The dominant species were those within *Aspergillus* and *Penicillium* (Table 2). They made from 15 to 100% of the general count of fungi in the individual samples. Apart from that, there were isolated *Cladosporium*, *Rhizopus*, *Mucor*, *Fusarium*, *Chaetomium*, *Trichoderma* and *Eurotium* species. The fungi on DG 18 medium included not only typical xerophilic fungi such as *Eurotium repens*, but also species that tolerate conditions of low water activity and that were also discovered on DRBC medium. The fungal species isolated from the analysed products frequently contaminate cereal products and their source is soil and air.

The isolated species included such fungi as *Aspergillus ochraceus*, *A. flavus*, *A. versicolor*, *A.sydowii*, *Penicillium verrucosum* and *Fusarium graminearum*, which are well known as mycotoxin producers. These strains are able to produce sterigmatocistin, aflatoxin B_1, ochratoxin A and DON, respectively [16-19]. The results obtained by [20] showed that 93.2% of fungal species isolated from wheat flour belonging to the group of toxigenic fungi.

Table 3. Contamination of analysed products by mycotoxins

Sample No	Mycotoxin [ng g^{-1}]		
	Ochratoxin A	Aflatoxin B_1	Deoxynivalenol
1	4.2±0.361	nd	526±5.6
2	nd*	nd	nd
3	nd	nd	880±9.4
4	4.0±0.458	nd	1366±11.4
5	4.8±0.265	nd	485±4.4
6	nd	nd	nd
7	nd	nd	nd
8	3.9±0.400	nd	317±3.6
9	3.6±0,200	nd	283±2.1
10	nd	nd	335±2.4
11	nd	nd	nd
12	nd	nd	820±8.9
13	nd	nd	465±3.5
14	2.4±0.361	nd	569±8.2
15	nd	nd	408±4.9

Mean ± SD, *nd below the limit of detection (2 ng g^{-1} for aflatoxin B_1 and ochratoxin A; 250 ng g^{-1} for deoxynivalenol)

According to the Regulation of the Commission of the EU No 105/2010 and 165/2010 the permissible contamination of cereal products with ochratoxin A equals 3 ng g^{-1}, and with aflatoxin B_1 2 ng/g. None of the samples was contaminated with aflatoxin B_1 (Table 4), with the detection limit on the level of 2 ng/g. Cereal products were examined and only 18% of samples are contaminated within the range from 0.04 to 1.35 ng g^{-1}. In 5 of examined

products the permissible limit level of ochratoxin A was exceeded. The most OTA contaminated products contained cornflakes with dried fruit and nuts. Similarly it was found out that 69% of breakfast products coming from French stores were contaminated with OTA within the range from 0.2 to 8.8 ng g^{-1} [21]. The highest concentrations were also revealed in the samples containing dried fruit, bran and chocolate. The other research also showed a slight share of breakfast products contaminated with ochratoxin A; the toxin was present in 2 samples per 17 in maximum concentration equalling 29.4 ng g^{-1} [22]. Maize is rarely mentioned as a source of this toxin. The results obtained by [23] showed OTA in maize on a low level equalling 2.6 ng g^{-1}. From the cornflakes where the presence of OTA was found in the concentration of 4 ng/g, a toxinogenic species was isolated, i.e. *Aspergillus ochraceus*. In case of two products containing raisins, the dominant species was *Aspergillus carbonarius*, which according to certain authors may produce this metabolite [24].

Deoxynivalenol was present in 70% samples. The average content of this toxin in positive samples equalled 587 ng g^{-1}, and the maximum reached the level of 1366 ng g^{-1} (Table 4). The EU Regulation No 1126/2007 established the highest permissible contamination of cereal snacks and breakfast flakes with deoxynivalenol on the level of 500 ng g^{-1}. In case of 5 products this value was exceeded, four times in cornflakes sample No 4. It was showed that 73% of breakfast products available in Portugal contained DON within the range from 103 ng g^{-1} to 6040 ng g^{-1} [25]. No correlation was revealed between the presence of toxigenic fungi and the production of toxins. In two samples *Penicillium verrucosum* was found, however there were no traces of OTA. It confirms literature data regarding strain specificity [24]. Therefore, the occurrence or the lack of toxinogenic species is not a sufficient indicator of mycotoxin contamination. Nevertheless, the samples examined may contain other mycotoxins not comprised by the hereby analysis. Ochratoxin A – in case of cereal samples and particularly those containing oat, wheat, maize and rice – is always accompanied by citrinin, which forms an additional risk factor [21].

Conclusions

Following the rules of rational nutrition, cereal products from full grain make an increasing share in human diet. As shown by this study, cereal products may be a potential source of toxic, and many a time carcinogenic mycotoxins, metabolic products of moulds. The presence of fungi and their metabolites in food makes a serious problem to consumers' health and especially in relation to their most vulnerable group, i.e. children. Economic factors and concern regarding health safety of a consumer force food producers to ensure proper microbiological quality of cereal products manufactured by them. It is related to the need to define and eliminate the sources of microbiological dangers at every phase of the production process, starting from the harvest, through production, storing, transport and finally to distribution.

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