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# QUALITATIVE AND QUANTITATIVE ANALYSIS OF FILAMENTOUS FUNGI IN AIR, FOOD AND OCHRATOXIN A IN HUMAN MILK

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This work was based on a research of filamentous fungi in the air of kitchens of domestic households, the contamination of cereal products by fungi and the presence of ochratoxin A (OA) in human milk. The highest contamination by spores of filamentous fungi in 23 domestic households was recorded in bread bins. In 104 tested samples of cereal products, the most commonly isolated fungi were those from *Penicillium* and *Aspergillus* genera. The presence of ochratoxin A was recorded using ELISA method in 51% of tested human milk samples. The concentration of OA was higher in colostrums samples than in term and mature samples from the same women, which can result from the accumulation of mycotoxins in tissue and their gradual release through the breast gland.

## INTRODUCTION

The illnesses caused by fungi and their metabolites became the fundamental issue of the pathophysilogy of plants, animals and humans at the threshold of the 21st century. Spores of many types of filamentous fungi from Ascomycetes and Deuteromycetes classes, present in the environment, find ideal conditions for development in food products [Pacin *et al.*, 2003].

Fungi toxins present in food, which enter the body and cumulate in tissue causing many disorders including cancerous changes, are the subject of detailed analyses. The problem of mycotoxin migration through the placenta and breast gland during lactation is crucial due to its harmful effect [Hellen *et al.*, 1989]. On the basis of experimental tests and clinical observations, it is clear that mycotoxins react with the highest intensity during organogenesis of the embryo, between the 20th and 40th days of pregnancy.

Similarly, a newborn and a baby in the breast–feeding period is susceptible to toxic effects of the elements absorbed in the mother's body. The processes of detoxification of the baby during the intrauterine life and neonatal period are impaired because of the immature state of the microsomal liver enzyme system, restricted ability to remove the toxic substances through the kidneys and inefficient immunological system.

In the UE Member States, including Poland, a special attention is paid to the contamination of cereal and meat produced by ochratoxin A, which has a wide spectrum of toxin actions, especially nephropathic ("mycotic nephropathy") and exerts a damaging effect on the immunological system [Scudamore & Patel, 2000].

The research aimed at determining the influence of airborne mycoflora and cereal products in household kitchens with reference to the presence of ochratoxin A in human milk.

## MATERIAL AND METHODS

The material for mycological research consisted of 69 air samples from 23 household kitchens, 164 cereal product samples stored in the same households and 87 human milk samples taken at different stages of lactation.

The air tests were conducted using the "crash method" with an AIR Ideal apparatus, taking samples from 3 different checkpoints: bread bin, fridge and the space above the kitchen table.

The capacity of sucked in air going trough the sterile mesh into the culture medium ranged from 10 to 50 L. About 50 g of products, from bread, cereals, flour and groats which constituted the diet of the tested women, were put in a sterile container. Two 10 g samples from the received material were taken in sterile conditions, and then 10 times diluted using a buffer solution of 0.85% NaCl. After thorough mixing, 2 mL of the suspension of each sample were placed on the surface of culture medium for fungi in Petri dishes.

In order to isolate and identify filamentous fungi in the air and food products, the following culture media were used: Czapek-Dox (BBL) Sabouraude medium (Biomerieux), potato agar (Mar-FOUR), YGC-glucose chloramphenicol agar (Biomerieux).

The analysed material was inoculated on Petri discs and stored at 20–25°C for 7 to 14 days. Microscope specimens were prepared on microscopic glass from all cultured colonies. Their colour, the structure of their surfaces, the pres-

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ence and colour of the pigments produced (colony reverse) as well as characteristic arrangement of mycelium, type, size and arrangement of spores in microscopic specimens enabled systematic determination in accordance with an appropriate code.

The studies of ochratoxin A concentration were conducted with the use of enzyme micro immunoassay ELISA. The analysis was carried out on 78 samples of human milk obtained from breast-feeding women living in Warsaw. The samples were collected from June 2002 to May 2003. Ochratoxin test Ridascreen OA manufactured by NOACK was used to determine OA content. The samples of standards, milk samples as well as peroxidase-labelled conjugate were placed in the basins of discs coated in antibodies. Next, all the ingredients were incubated (12 h) and flushed with PBS-Tween 20 solution. Then, the substrate was added and the incubation was continued with enzymatic reaction with TMB (tetramethylbenzidine). The reaction was stopped with 1 mol/L $H_2SO_4$. The result of colour reaction given in terms of absorbance coefficient was read with the unit manufactured by AsysHitech. The content of OA in the analysed sample was read from the standardised curve.

## RESULTS

The results of mycological air control conducted with the crash method using 4 different base types enabled the estimation of the number and types of colonies grown in 1 $m^3$ read from the tables enclosed in the air IDEAL apparatus manuals (Table 1).

The bread bins were definitely the most contaminated area by fungi spores. Mycological contamination of the fridges was slightly less, but it did not show any statistically significant differences in comparison with the bread bins ($p>0.05$).

The lowest number of fungi colonies was grown on the medium, after the exposure of the open plates on the kitchen tables. The air from all checkpoints was predominated by the spores of filamentous fungi from *Aspergillus* and *Penicillium* genera. The presence of other potentially toxic fungi from *Trichoderma*, *Alternaria*, *Mucor* and *Gliocladium* genera was noted. The research results are shown in Table 2.

Filamentous fungi were also cultured from 76% of the cereal products stored in domestic households. The analyses showed that the highest number of toxigenic fungi species from *Penicillium* and *Aspergillus* genera was isolated from bread and other cereals products (Figure 1).

In 78 analysed human milk samples, the concentration of OA higher than 5 ng/L was noted in 51% of the tested samples. The highest values of OA level (18.7±7.9 ng/L) were noted in colostrum samples. The tested samples of mature milk obtained from the same women contained lower levels of OA. Regarding other women, in the samples of mature milk obtained from the first to 8th month of lac-

TABLE 2. Filamentous fungi isolates from air samples of checkpoints in kitchen.

| Species | Number of grown colonies | | |
|---|---|---|---|
| | Bread bin, n=23 | Area above kitchen table, n=23 | The inside of the fridge (second shelf), n=23 |
| *Absidia corymbifera* | 4 | 0 | 0 |
| *Absidia glauca* | 2 | 0 | 0 |
| *Aspergillus candidus* | 12 | 4 | 4 |
| *Aspergillus flavus* | 17 | 11 | 4 |
| *Aspergillus fumigatus* | 10 | 17 | 11 |
| *Aspergillus oryzae* | 6 | 5 | 7 |
| *Aspergillus parasiticus* | 24 | 9 | 4 |
| *Aspergillus restrictus* | 9 | 0 | 4 |
| *Aspergillus versicolor* | 12 | 19 | 8 |
| *Eurotium herbariorum* | 6 | 2 | 0 |
| *Aspergillus restrictus* | 9 | 18 | 6 |
| *Aspergillus versicolor* | 12 | 19 | 8 |
| *Cladosporium sp.* | 4 | 16 | 2 |
| *Eurotium herbariorum* | 6 | 2 | 0 |
| *Fusarium graminearum* | 11 | 7 | 5 |
| *Fusarium moniliforme* | 6 | 5 | 4 |
| *Fusarium sporotrichoides* | 4 | 2 | 4 |
| *Fusarium solani* | 2 | 6 | 3 |
| *Geotrichum candidum* | 4 | 5 | 18 |
| *Gliocladium ssp.* | 8 | 4 | 0 |
| *Trichoderma viride* | 4 | 7 | 0 |
| *Helminthosporum ssp.* | 2 | 5 | 0 |
| *Mucor ssp.* | 6 | 8 | 4 |
| *Mycelia sterilia* | 2 | 6 | 4 |
| *Penicillium aurantiogriseum* | 0 | 2 | 6 |
| *Penicillium notatum* | 3 | 7 | 4 |
| *Penicillium brevicompactum* | 12 | 6 | 4 |
| *Penicillium chrysogenum* | 7 | 4 | 4 |
| *Penicillium digitarum* | 4 | 6 | 0 |
| *Penicillium expansum* | 6 | 2 | 2 |
| *Penicillium notatum* | 4 | 6 | 2 |
| *Penicillium roqueforti* | 2 | 3 | 2 |
| *Phoma ssp.* | 0 | 8 | 0 |
| *Rhizopus rhizopodiformis* | 13 | 6 | 2 |
| *Trichoderma viride* | 4 | 7 | 0 |
| *Verticillium* ssp. | 6 | 4 | 4 |

TABLE 1. Number of filamentous fungi contamination in the household kitchen air.

| Checkpoint | Mean value of filamentous fungi contamination CFUs/$m^3$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Sabouraud medium | | Potato medium | | Czapek Dox medium | | YGC medium | |
| Bread bin, n=23 | total | 334.7±120.7 | total | 124.8±64.2 | total | 223.9±167.4 | total | 104.6±74.8 |
| | toxicogenic* | 106.4±59.6 | toxicogenic* | 43.7±29.0 | toxicogenic* | 120.6±48.9 | toxicogenic* | 67.4±40.2 |
| Area above kitchen table, | total | 43.3±30.1 | total | 24.7±15.7 | total | 40.7±23.6 | total | 16.4±8.6 |
| n=23 | toxicogenic * | 31.8±20.7 | toxicogenic * | 18.4±10.1 | toxicogenic* | 25.3±14.2 | toxicogenic * | 12.3±7.2 |
| The inside of the fridge | total | 65.1±45.2 | total | 28.6±11.8 | total | 54.9±23.7 | total | 32.7±20.8 |
| (second shelf), n=23 | toxicogenic * | 33.6±23.9 | toxicogenic * | 18.4±10.7 | toxicogenic * | 20.6±19.7 | toxicogenic * | 14.1±8.6 |

* from *Aspergillus*, *Penicillium* and *Fusarium* species

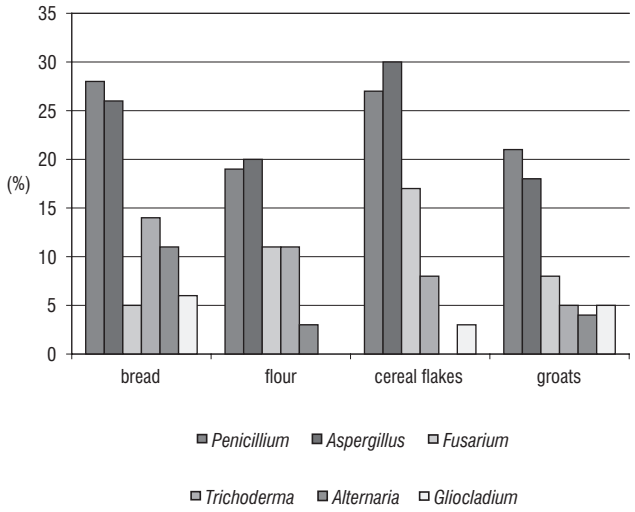


FIGURE 1. Percentage of isolated filamentous fungi of selected food products stored in domestic conditions.

tation, the OA level was between 2.4 to 16.8 ng/L. After taking into account the division into different types of human milk, taken at various time intervals during lactation, it was observed that the differences in each of the samples were statistically negligible (Table 3).

TABLE 3. Concentration of ochratoxin A in human milk samples at various times of women's lactation.

| Milk type | Average values of OA level [ng/L] |
|---|---|
| Colostrum | 18.7±7.9 |
| Premature milk | 8.9±5.8 |
| Mature milk | 6.8±4.9 |

## DISCUSSION

The spores of filamentous fungi carried by air in kitchens can easily cause the contamination of food products. With regard to the number of colonies grown from air samples greater than 300sCFU/m$^3$, the mass growth of fungi from bread stored in closed bins was noted in this personal research [Tangni *et al.*, 2002].

The results obtained can be compared only with few articles in Poland and in the world. The average number of fungi colonies in kitchen air from 23 domestic households tested amounted to 940 CFU m$^3$ and was three times higher than the indicator assumed by Żuława [1985] for kitchen.

The average values of fungi contamination in different food storage places in domestic households are hard to compare with previous research because of their general character, regarding the degree of microbiological contamination and not detailed qualification of fungi flora. Only a few studies regarding air mycoflora in gastronomic environments were found [Saxena *et al.*, 2001]. The number of fungi colonies grown in air using the crash method varied from 400 to 2880 colonies.

The taxonomic research of filamentous fungi in air cultures in the experiments carried out showed the presence of 14 different types of fungi. The most common isolated fungi were those from *Penicillium* and *Aspergillus* genera, including many toxigenic species: *Penicillium viridicatum*, *Penicillium chrysogenum*, *Aspergillus flavus*, *Aspergillus fumigatus*, and *Fusarium moniliforme*.

A comparison with research carried out by Żuława [1985] points to differences in species composition as the most common isolated fungi acknowledged by this author was *Cladosporium herbarum*.

The common occurrence of many types of filamentous fungi from *Aspergillus* and *Penicillium* genera caused the contamination of bread, flour, groats and cereals. Colonies of these fungi were grown from 76% of food samples obtained from domestic households. The results obtained are comparable with those of a research by Kononenko *et al.* [2002], who most frequently stated the presence of *Penicillium* genus in cereal products.

Numerous studies devoted to food contamination by toxigenic filamentous fungi have been published in the last 20 years. They aimed at proving the relationship between their presence and the creation of mycotoxins [Jorgensen & Jacobsen, 2002, Larsen *et al.*, 2001].

Ochratoxin A, which is present in cereal products was recognised as a danger to human health. The first ochratoxinopathy symptom is usually a kidney disorder [Walker, 2002]. At first, the damage affects interstitial fibrosis, which later leads to the degeneration of renal glomerules [Abouzied *et al.*, 2002].

In many experimental tests conducted on animals it was shown that ochratoxin is toxic for the kidneys at the level of 200 ng/L of fodder. However, even a small amount of this highly toxic mycotoxin observed in tested human milk can cause dangerous changes regarding the metabolism of proteins, fats or carbohydrates in the body of a human baby [Jorgansen & Jacobsen, 2002].

The amounts of OA recorded in this personal research varying from 2.2 to 26.4 ng/L, from 51% of human milk samples obtained at the different stages of lactation, are lower than concentration of this mycotoxin in milk obtained from women tested by Skaug *et al.* [1998] who recorded a concentration of between 10 to 182 ng/L in 21% women in Norway.

Lower amounts of ochratoxin in human milk in Italy, ranging from 0.1 to 12 ng/kg in 20% of tested samples were recorded by Micco *et al.* [1995]. Differences in the results given in many studies by many authors seem to be due to the intake of many types of food contaminated by mycotoxin and the choice of different analytical methods used in the analyses.

## CONCLUSIONS

1. Mycological control, conducted with the crash method using the air IDEAL apparatus, enabled the precise measurement of contamination levels in kitchens, especially the places used for storage of cereal products.
2. Targeted mycological research of cereal products led to the isolation of toxigenic filamentous fungi from *Aspergillus* and *Penicillium* genera.
3. Received contamination values of ochratoxin A in human milk appear to indicate an urgent need to take preventative action regarding mycological food contamination.

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