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FUNGAL CONTAMINATION OF CEREALS AND FLOUR-BASED PRODUCTS, INCLUDING BREADS AND DETERMINATION OF OCHRATOXIN A WITH ELISA TECHNIQUE

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Abstract

Fungi are able to grow on all kinds of food: cereals, meat, milk, fruit, vegetables, nuts, fats and their products. Their growth on foods may results in several kinds of food-spoilage: off-flavours, toxins, discolouration, rotting and formation of pathogenic or allergenic propagules. The most important aspect of fungal spoilage of foods is, however, the formation of mycotoxins. More than 400 mycotoxins are known today, and aflatoxins being the best known in our country, but there is an increasing knowledge and understanding of the role also of other mycotoxins such as: ochratoxins, trichothecenes, zearalenone, fumonisins etc. Ochratoxin A is a toxic secondary metabolite produced by species belonging essentially to the *Aspergillus* and *Penicillium* genera under diverse conditions. Its presence in the human body is due to the ingestion of small quantities present in a wide range of food commodities. The favoured substrate for fungal growth and nephrotoxicity and probable human carcinogen ochratoxin A production appears to be cereals and flour-based products, including bread.

For that reason, in different cereals, flours, pastas and breads (from commercial markets, private firms or manufacturers) were determinate total number of fungae with standard microbiological examination and ochratoxin A concentration with ELISA technique.

Results shown that species belonging to *Aspergillus* and *Penicillum* genera are dominants in all samples and although ochratoxin A content was below the maximum limit established by European legislation for raw cereals (5 µg/kg) and for bread or processed cereals (3 µg/kg), in few of the analysed samples, it was above these value.

Maybe this will be motive more frequent checks of fungal food contamination to conducted, especially in terms of the presence of mycotoxins and in order to provide healthy and safety food for overall population in Republic of Macedonia.

***Key words**: Cereals, Flour-based products, Bread, Fungi, Ochratoxin A.*

1. Introduction

The occurrence of fungal growth in food and food products causes many changes related mostly to their physico-chemical characteristics which negatively affect nutritional and sensory properties of food (spoiled food), but also they can cause serious diseases and infections in human beings. There are more than 100,000 species of fungi but only some of them are pathogenic [1].

Aspergillus and *Penicillium* belong to the group of pathogenic fungi. They are able to produce many mycotoxins, among which is ochratoxin A. Ochratoxin A is detected in foods of plant origin (wheat, corn, rice, rye, flour, buckwheat, various types of cereals, muesli, etc.) mainly in Eastern Europe [2], [3], and [4].

Ochratoxin A presents a potential serious risk for the health of humans and animals. Its presence is demonstrated in almost all animal species and their viscera (the kidneys, liver and circulatory system). Ochratoxin A is nephrotoxic metabolite which exhibits immunotoxic, genotoxic, teratogenic, mutagenic and cancerous influence [3]. International Agency for Research on Cancer (IARC) includes the ochratoxin A in the group 2B of mycotoxins as a potential carcinogenic substance to humans [5].

2. Materials and Methods

This study is about the fungal contamination and the presence of ochratoxin A in wheat and flour-based commodities is given in Table 1. Analyzed products were taken from commercial markets, private companies or manufacturers located in different areas in Republic of Macedonia.

Table 1. Analyzed products types

Cereals	Flour	Noodles	Bread
Wheat	Flour T-400	Vermicelli with eggs	Bread T-500
Corn	Flour T-400 for meat pie and bakery products	Noodles from integral flour and oat flour	Bread "elite"
Barley	Flour T-500	Noodles from parsley and integral flour	Grill-baked bread
Mercantile wheat	Flour for baked goods	Noodles from buckwheat and integral flour	Integral bread
Mercantile barley	Livestock flour	Noodles from barley and integral flour	Bread with olives, Chiabata

Identification of the fungi was performed using the Standard Koch method. The samples were inoculated in Sabouraud-maltose agar plates and then were incubated at T = 30 ^{0}C from 24 to 72 hours. After the incubation period enumeration of the grown colonies was performed [6]. The identification of the grown colonies was done by micro- and macroscopical observation.

Using the ELISA method, enzymatic immunologic quantitative analysis, ochratoxin A was detected. RIDASCREEN OCHRATOXIN A 30/15 (R-Biopharm) test was used according to the manufacture's guide. The test is based on antigen-antibody reaction. The tests were photometrically measured with ELISA Human Reader Single Plus at 450 nm [7].

3. Results and Discussion

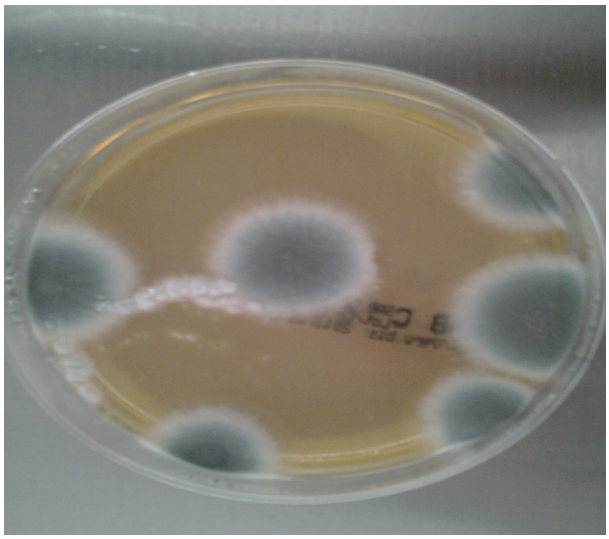
The results defining the total number of isolated fungi in the analyzed samples from wheat, corn, barley, flour, flour-based products, noodles and bread are shown in Table 2. The values of total number of detected fungi are in the limits of 7 cfu/g isolated from vermicelli with eggs to 509 cfu/g in livestock flour. According to the Regulation for special demands regarding microbiological criteria for food (Official Gazette of Republic of Macedonia no.100/2013) [8], all results are in the range of maximal allowed (permitted) concentrations (cereals and flour 10^4 - 10^5 cfu/g, noodles and bread 10^3 - 10^4 cfu/g).

Table 2. Total number of isolated fungi in the analyzed samples

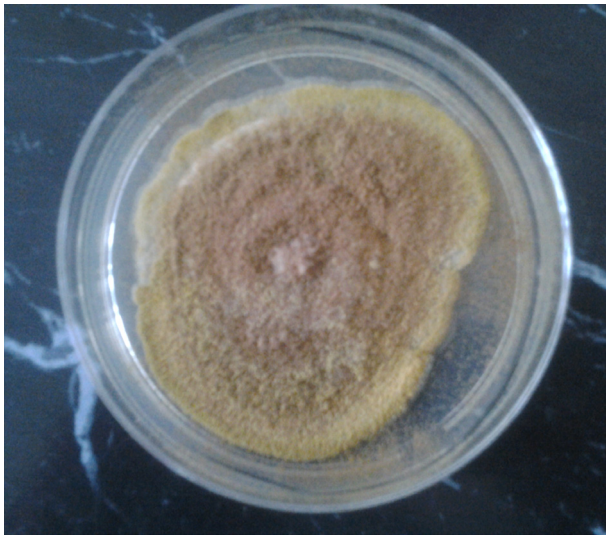
Analyzed sample	Isolated fungi number cfu/g
Wheat	368
Corn	280
Barley	102
Mercantile corn	336
Mercantile barley	111
Flour T-400	250
Flour T-400 for meat pie and bakery products	120
Flour T-500	405
Flour for baked goods	120
Livestock flour	509
Vermicelli with eggs	7
Noodles from integral flour and oat flour	29
Noodles from parsley and integral flour	35
Noodles from buckwheat and integral flour	47
Noodles from barley and integral flour	31
Bread T-500	50
Grill-baked bread	24
Bread "Elite"	46
Integral bread	35
Bread "Chiabata"	11

Nevertheless, this minimal contamination should not be overlooked because the number of fungi varied depending from the type of the food commodities. Boukuline and Dotourion [9] examined the fungal contamination in food product which could not be sterilized at high temperatures. They isolated fungal cultures at Sabouraud-plus-chloramphenicol (SC) broth and malt-plus-chloramphenicol (MC) agar plates (Bio-Rad, Marnes la Coquette, France) at 37 ^{0}C for 24 hours and showed that the degree and the type of fungal contamination varied a lot according to the type of food. In our experiment the detected and identified

fungi belongs to the species *Aspergillus* and *Pencilium* which are potential pathogenic fungi and are the major producers of Ochratoxin A.



a) *Penicillium*



b) *Aspergillus*

Figure 1. *Penicillium* (a) and *Aspergillus* (b) colonies detected in food samples inoculated in Sabouraud-maltose agar plates using standard Koch method

The results from the mycotoxicological examination and detection of ochratoxin A with the ELISA method are shown in Table 3. They show that in 10% of the total number of the samples an increased concentration of ochratoxin A is reported, compared to the maximal permitted concentrations regarding this mycotoxin (5µg/kg for cereals and 3 µg/kg for bread, flour and noodles) according to the Regulation for general requirements regarding maximum levels of certain contaminants [10]. An increased concentration of ochratoxin A with 0.65 µg/kg above the permitted i.e. 3.65 µg/kg was detected in the bakery product flour, while in the samples from livestock flour these concentrations were 0.24 µg/kg above the MPC.

In all the other samples (wheat, corn, noodles, bread) the concentrations of ocratoxin A is in normal range between 1,18 µg/kg to 3.10 µg/kg.

Table 3. The concentration of ochratoxin A in the analyzed samples

Analyzed sample	**Measured Concentration of Ochratoxin A mg/kg**
Wheat	2,91
Corn	2,68
Barley	1,98
Mercantile corn	3,10
Mercantile barley	1,24
Flour T-400	2,69
Flour T-400 for meat pie and bakery products	1,73
Flour T-500	2,26
Flour for baked goods	3,65
Livestock flour	3,24
Vermicelli with eggs	1,18
Noodles from integral flour and oat flour	1,97
Noodles from parsley and integral flour	1,42
Noodles from buckwheat and integral flour	2,19
Noodles from barley and integral flour	1,71
Bread T-500	2,81
Grill-baked bread	1,49
Bread "Elite"	2,68
Integral bread	1,92
Bread "Chiabata"	1,68

4. Conclusions

The conducted fungal contamination and mycotoxicology analyses of samples in different types of food products indicate that:

- The total number of fungi (cfu/g) in all analyzed samples is in the range of maximal allowed (permitted) concentrations according to the Regulation for speacial requirements about microbiological criteria for food [8].

- The detected and indentified fungi belong to *Aspergillus* and *Penicillium* species.

- An increased ochratoxin A concentration is reported in the samples from bakery products flour and livestock flour.

- It should be noted that although the total number of fungi in all analyzed samples are in the eligible concentrations according with the Regulation for speacial requirements about microbiological criteria for food [8], increased and not permitted concentrations of ochratoxin A are detected in some of them (bakery product flour, livestock flour).

5. References

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