



## THE ABILITY OF MOLDIES EXTRACTED FROM CONDIMORIES TO PRODUCE AFLATOXINS

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**Abstract.** This paper concerns the production of aflatoxins caused by moldies extracted from condimories.

Aflatoxins are cancerogenous, cause diseases of various organs and death. The presence of aflatoxins in condimories hasn't been deeply investigated so far, so qualitative and quantitative analysis of aflatoxins in condimories is the main goal in this paper.

According to analyses the concentration of aflatoxins in some condimories are extremely high, and above the normativised level of tolerance. Further research in this area should be done due to everyday use of condimories as food of men, especially children.

### 1. Introduction

Despite the extraordinary pace of technology, nowadays the mankind is faced with a big problem of producing the healthy food in quantities that are needed. One of most frequent cause of perishables are moldies, neglected for a long time. It was assumed that moldies are not dangerous for health, causing only changes of organoleptic characteristics of product they grown upon. Not earlier than discovery that a vast of moldies during it's metabolism synthesize toxic metabolites - mycotoxins, causing sickness of men and animals, and even impacting the negative influence on microorganisms, this phenomenon received much greater significance and systematic research begun.

The level of sensitiveness to mycotoxins varies, depending on many factors, like the sort, age and state of health of the intoxicated organism, the quantity and the length of the period mycotoxins are brought in.

Aflatoxins are the group of secondary metabolites of difurano-cumarian type created after logarithmic phases of growth of some strains of moldies belonging to genus of *Aspergillus flavus*. It was experimentally proved that aflatoxins in lethal doses, received by food in the animal body cause death after 72 hours, while its presence in smaller quantities cause cancerogenous changes of

liver. Toxicity of aflatoxins is in disturbing the nucleic acids synthesis, therefore protein biosynthesis.

In lack of our own legislation in this area, the judgment of food usefulness contaminated by aflatoxins in food is 30  $\mu\text{g}/\text{kg}$ .

## 2. Experimental

### *Material and methods*

To prove and measure the presence of *Aspergillus flavus* moldies and aflatoxins in conditories and the most important row materials, microbiological analyses of 150 samples was held. Samples are taken directly from food industry departments, and the relationship between aflatoxin concentration at the beginning (in a row material) and the end of technological process (in a final product) is measured.

To prove the ability of *Aspergillus flavus* to produce aflatoxins, sugar bouillon with 20% of sucrose and 2% yeast extract is used. The bouillon is taken in quantities of 50 ml and sterilized.

Isolated moldies strains as pure culture are transferred by bacteriological essays from the solid basis from Petry cup to liquid sterile sugar bouillon. These cultures are incubated at 27°C. After the seventh, tenth and fourteenth day isolated strains of *Aspergillus flavus* are examined in order to prove the production of aflatoxin.

In the sugar bouillon chloroform in quantity of 75 ml is added and mixed in magnetic mixer for 15 minutes. The contents is filtrated for 2-3 times and pour to separation funnel. Two layers are product: the lower is the chloroform with dissolved aflatoxin, and the upper is the liquid basis with remaining impurities.

Chloroform extract is filtrated again and evaporated in vacuum evaporator at 40°C until dry residue is obtain. The dry residue is washed out with 5 ml of chloroform.

Using thin layer chromatography (TLC) method, the qualitative and quantitative measure of aflatoxin in the concentrated chloroform dissolve. Aflatoxin is identified visually under the ultraviolet long wave light according to specific fluoroscentia and RF values of the specimen compared to aflatoxin B1 standard.

## 3. Results and discussion

According to the research so far specific conclusions about the degree of contamination of conditories by moldies and mycotoxins cannot be given. In this paper from 29 conditories 150 samples of row material and final conditory product are analyzed.

*Aspergillus flavus* moldy is isolated from 10 out of 29 conditories and their row materials: coconut, grinding cookies, cocoa, fig, jam, Lambada - domestic cookies, jam and poppy pie, Čokolita and pretzel. Pure *Aspergillus flavus* culture is isolated from 21 samples, i.e. 14% of the samples being tested. Four out of 21 *Aspergillus flavus* (or 19,05%) do produce aflatoxin. Results are given in Table I.

Table I. The impact of the period of *Aspergillus flavus* mycellium cultivation to the aflatoxin production

Product	Aflatoxin concentration in µg/l		
	after 7 days	after 10 days	after 14 days
grinding cookies	0	17	60
cocoa	0	30	50
Čokolita	0	80	200
Lambada-dom cookie	0	47	180

The impact of the period of *Aspergillus flavus* mycelium cultivation to the aflatoxin production is examined. It is concluded that the extension of this period give rise to the aflatoxin production, with the maximum after 14 days.

It is proved that *Aspergillus fumigatus* also produces aflatoxin. This moldy is isolated from grinding cookies and the special mixture Granada. From 150 samples, *Aspergillus fumigatus* is isolated from 3 samples (2%). One of those three (33.33%) can produce aflatoxin. Results are shown in Table II.

Table II. The impact of the period of *Aspergillus fumigatus* mycellium cultivation to the aflatoxin production

Product	Aflatoxin concentration in µg/l		
	after 7 days	after 10 days	after 14 days
grinding cookies	0	7	15

Moldies, producers of aflatoxin, need specific conditions for producing biologically sufficient quantities of aflatoxin. On the other side, once aflatoxins are produced, moldies can vanish, but toxins remain in food. Therefore the research in this paper is concerned with qualitative and quantitative measure of aflatoxin in conditories and their row materials.

For this purpose 150 samples are also analyzed. Aflatoxin is found in 7 of them (4.67%). Results are shown in Table III.

Table III shows that aflatoxin concentrations found in conditories are extremely high. It is especially important for the cocoa having over 50  $\mu\text{g}/\text{kg}$ , because it is often used as children food. As

Table III. The contents of aflatoxin in conditories and their raw materials

Product	N° of anal. prod.	samples with aflatoxines		aflatoxins concentration in $\mu\text{g}/\text{kg}$				
		N°	%	to 30	30-50	over 50	B1	G1
grinding cookies	10	2	20	2	0	0	2	0
cocoa	9	1	11.11	0	0	1	1	0
fig	9	1	11.11	1	0	0	0	1
Lambada	10	1	10	0	1	0	1	0
jam pie	5	1	20	1	0	0	1	0
jam	7	1	14.29	1	0	0	1	0
$\Sigma$	150	7	4.67	5	1	1	6	1

aflatoxins influence stronger younger than older organism, this reflects serious problem which deserves greater attention, because aflatoxin is extremely cancerogenous, especially B1 fraction.

#### 4. Conclusion

Results given in this paper show that conditories are more or less contaminated by moldies which produce aflatoxin.

From 150 analyzed samples of conditories 76% are contaminated by moldies. Most of the isolated moldies, 45.61% belongs to the genus *Aspergillus*. Twenty one samples showed contamination by strains of *Aspergillus flavus*, presenting 14% of total analyzed samples. Three samples (2%) showed contamination by strains *Aspergillus fumigatus*. Four strains of *Aspergillus flavus* out of 21 (19.05%) can produce aflatoxins. Only one of 3 strains of *Aspergillus fumigatus* was able to produce aflatoxins.

To prove the presence of aflatoxins in conditories and their raw materials, 150 samples have been also analyzed and seven samples (4.67%) showed the presence of aflatoxins. The concentrations of aflatoxins are extremely high, especially in the cocoa, so this problem needs greater attention.

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## SPOSOBNOST PLESNI IZDOJENIH IZ KONDITORSKIH PROIZVODA NA PRODUKCIJU AFLATOKSINA

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Rad se bavi proučavanjem plesni izdvojenih iz konditorskih proizvoda, a koje proizvode aflatoksine. Aflatoksini su kancerogeni, izazivaju oboljenja različitih organa i smrt organizma. Prisustvo aflatoksina u konditorskim proizvodima nije do sada dublje izučavano, i zato je osnovni cilj ovog rada kvalitativno i kvantitativno određivanje aflatoksina u konditorskim proizvodima.

Analize govore da su koncentracije aflatoksina u nekim konditorskim proizvodima izuzetno visoke i premašuju tolerantni nivo koji je normama predviđen. Konditorski proizvodi se svakodnevno upotrebljavaju u ishrani ljudi, a naročito dece, pa zbog toga treba da se nastave istraživanja u ovom pravcu.