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«TRAINING ACTIVITIES ON FOOD CONTAMINATION CONTROL AND MONITORING WITH SPECIAL REFERENCE TO MYCOTOXINS»

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THE INFLUENCE OF FOOD PROCESSING PRACTICES ON THE CONTENT OF MYCOTOXINS IN FOOD PRODUCTS

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I. Introduction

Contamination of vegetable raw materials such as oil bearing crops, corn, rice, wheat, and barley with mycotoxins, i.e. toxic and carcinogenic metabolites of microscopic fungi was reported from many countries. According to national legislations the content of aflatoxin B_1 must not exceed 2.5-20 µg/kg. Most countries, however, have not yet arranged a total supervision of contamination of grain and oil-bearing crops with aflatoxins and other mycotoxins. More often than not there is only a selective supervision of grain batches which have external signs of the development of microscopic fungi, or which have been stored in unfavourable conditions. In keeping with the operative standards in many countries grain batches containing excessive amount of grains spoiled by microorganisms or by spontaneous heating are barred to be processed for food needs. In a number of instances, however, aflatoxins may be present in grain which outwardly does not differ from normal grain. Intensive reproduction of insects may lead to contamination of grain with aflatoxins, through excrements which the insects carry from contaminated layers of the bulk grain. Contamination of grain with aflatoxins may occur also in a purely mechanical manner, when the spores of Aspergillus flavus which frequently incorporate high levels of aflatoxins (500 µg/kg) reach the grain, and also when grain of different batches

are mixed. Another possibility is the accumulation of toxic products as a result of disintegration of mycotoxins in the process of detoxication. All this leads to a situation when batches of grain and seeds containing considerable amount of aflatoxins and other mycotoxins go into processing.

Detoxication of products contaminated with aflatoxins is impeded by a high thermostability of toxins, considerable economic expenditures and the worsening of technological and food properties.

The processing and cooking of products contaminated with mycotoxins puts them under mechanical, physical and chemical actions which in their turn either destroy or redistribute the toxins content. The detoxification effect is attained by the following techniques:

1. Mechanical removal of toxins with the most contaminated components of the grain mass (hulled rice, spoiled and broken seeds) or with parts of kernels (husks, surface layers of seeds). This occurs during separation when grain is put to wet or dry cleaning.

2. Separation of intermediate stocks, which are the least contaminated with mycotoxins since they are distributed in the bulk grain not uniformly. Extensive use has been made of this practice in wheat and corn milling.

3. Selective extraction of mycotoxins or the product by water or organic solvents. This is carried out in case of wet milling of maize and in the production of vegetable oils.

4. Destruction of aflatoxins or turning them into less toxic substances by heating or chemical treatment (oxidation, the action of acids, alkalis).

2. Mechanical removal and redistribution of mycotoxins in the processing of food products

Prior to storing and also in the mill when grain is being prepared for milling it is subjected to cleaning. The main task of cleaning is to remove weeds and grain admixtures and to treat grain surface with the removal of the upper, the most contaminated layers. Conditioning is also applied to improve technological properties of grain when it is prepared for milling.

2.1. Separation

The kernels of maize and other crops containing aflatoxins are structurally weakened and are easily crushed owing to their affection with microscopic fungi. Toxins are generally found, in broken kernels and in grain admixtures. When 21 samples of maize were examined by Johnson et al. (1969) they found in 20 instances only traces of aflatoxins in whole kernels. As for dockage, aflatoxins were present only at 8-112 µg/kg.

As a rule, manual separation of these fractions reduces the aflatoxins level down to trace quantity. The methods of physical separation which are applied for maize in the USA are not efficient enough to reduce markedly the aflatoxins level in it. In some instances it was found that the concentration of aflatoxins even increased. The problem is rendered even more difficult since under low concentrations mycotoxins may be present in outwardly sound kernels which practically cannot be separated in grain cleaning.

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Therefore, though there is the opinion, that separation may considerably reduce the mycotoxins content in the gr in mass,

there are only a few instances of its effective use. In our experiments with rice separation wastes as few as 5–16% of aflatoxin B_1 was removed. Separation and dry cleaning of corn prior to milling reduced the content of zearalenon by 3–10% only (Shotwell O. et al., 1979; Brekke O. et al., 1975; Benneth C. et al., 1976).

Separation by size of wheat grain contaminated by ochratoxin A failed to give any positive result since the main grain and the small fractions (< 2.5 mm) consisting of broken kernels, foreign admixtures, contained similar concentrations of ochratoxin A (Chelkowski J. et al., 1981).

The practice of separation proved to be most effective for large grain crops such as peanuts and cotton seeds. Successive use of mechanical, electronic and manual separation made it possible to decrease the content of aflatoxins in a batch of peanuts from 150 µg/kg to 3 µg/kg (Table 2).

Table 2

Influence of different techniques of separation upon aflatoxin content in peanuts (Kensler, 197)

Methods of separation	Yield of fraction, %	Aflatoxin content, µg/kg
Initial sample	100	150
Mechanical sorting	0.7	2500
Separation with photoelement	14.9	30
Manual sorting	0.7	150–375
Final product	83.7	3

When peanuts imported to the USA where put to a secondary cleaning with pneumatic separation it helped to bring down the share of rejected lots from 32% in 1968 to 1% in 1971-1974.

Electronic sorting (Ashworth et al., 1968) helps to remove the cotton-seeds emitting green fluorescence and containing a greater part of aflatoxins. Successful use has been likewise made of pneumatic separators for the more damaged seeds of peanuts and cotton.

There is special interest in separation of hulled kernels from corn in cobs and paddy. Under unfavourable storage conditions fungi start to develop preferably on hulled and broken kernels and only then affect sound grain. It was established by our investigations that the probability of aflatoxins' appearance is considerably higher on shelled rice than on the remaining part of grain. The study of 10 samples of paddy contaminated with aflatoxins as a result of self-heating under experimental conditions showed that the concentration of aflatoxin B_1 in hulled grains in a number of cases was 10 times higher than the concentrations of aflatoxin observed in unhulled kernels (Table 3).

The maximum separation of hulled kernels from the mass of corn cobs, just as of shelled grains from paddy help to increase grain safety, and also the removal of those components which are most frequently damaged by fungi and may contain mycotoxins.

2.2. Dry and wet grain cleaning

The main part of the vegetative mass and organs of fungal sporification at the initial stage of development affects the surface of grain or its coating. Then the fungus penetrates the germ and only later on it attacks the endosperm. Aflatoxins

Table 3

Degree of contamination of hulled and unhulled kernels of rice with aflatoxin B_1

No.	Maximum temperature in storage, °C	Duration of self-heating, days	Content of aflatoxin B_1, µg/kg		
			Original paddy	Unhulled kernels	Hulled kernels
1	37	7	133	85	1000
2	28	3	108	80	600
3	46	2	14	12	40
4	28	4	40	40	40
5	30	9	27	25	60
6	35	12	67	63	133
7	40	19	67	61	166
8	30	15	133	77	1200

grow in grain in a similar sequence. At low concentrations (less than 10–30 µg/kg) up to 80–100% of aflatoxins are concentrated in the grain coatings. With the increase in level of contamination with toxins, considerable amount of the toxins grows in the germ and in the endosperm. The degree of the endosperm contamination decreases from outer layers to the inner ones.

Therefore, the removal of aflatoxins from grain may be carried out using technologies which clean the surface of grain and which separate the upper branny layers: washing, scouring and aspiration. Dry cleaning of grain prior to milling is the most effective technique. Scouring machines reduce bacterial and fungal contamination of grain by 50–90%, brush machines, by 20–50%. The effectiveness of cleaning is improved by repeated passing of grain through scouring and brush machines

though the effect of each subsequent pass is diminished. Removal of 3.5-5.0% of the wheat hull helpes to reduce mould fungi content down to 0.1 thousand/g and the overall number of microorganism - to 100 thousand/g.

When grain is prepared for processing 1-3,5% of its mass primarily the fruit and seed hulls is removed. The concentration of aflatoxins in the waste exceeds from 2 to 7-fold their initial content in grain. A 5% removal of hulls with scouring machines leads to elimination of 29% of aflatoxin B_1 and 26% of aflatoxin G_1 (Fig. 1). The use of Lopatinsky's worm-screw machine which removes large fragments of hulls from kernels, is most likely, more effective in terms of grain decontamination since the removal of as few as 2.8% of surface layers results in the elimination of 19-26% of aflatoxins (Fig. 1).

The application of scouring machines and of the practices which simulate grain washing makes it possible to relieve grain from 50% of aflatoxins. In this case the toxins are eliminated mechanically together with the surface layers and fungi spores, and are partially dissolved in water.

In connection with the in-depth penetration of ochratoxin A into the grain of wheat and barley, dry and wet cleaning of contaminated grain proved to give a negligible effect (Chelkowski J. et al., 1981).

To improve the health and sanitary state of grain and to remove insecticides, benzpyrene, heavy metals and mycotoxins Tschiersch R. (1978) recommends to increase the removal of hulls prior to milling up to 3 5-5.0% regardless of the type of milling.

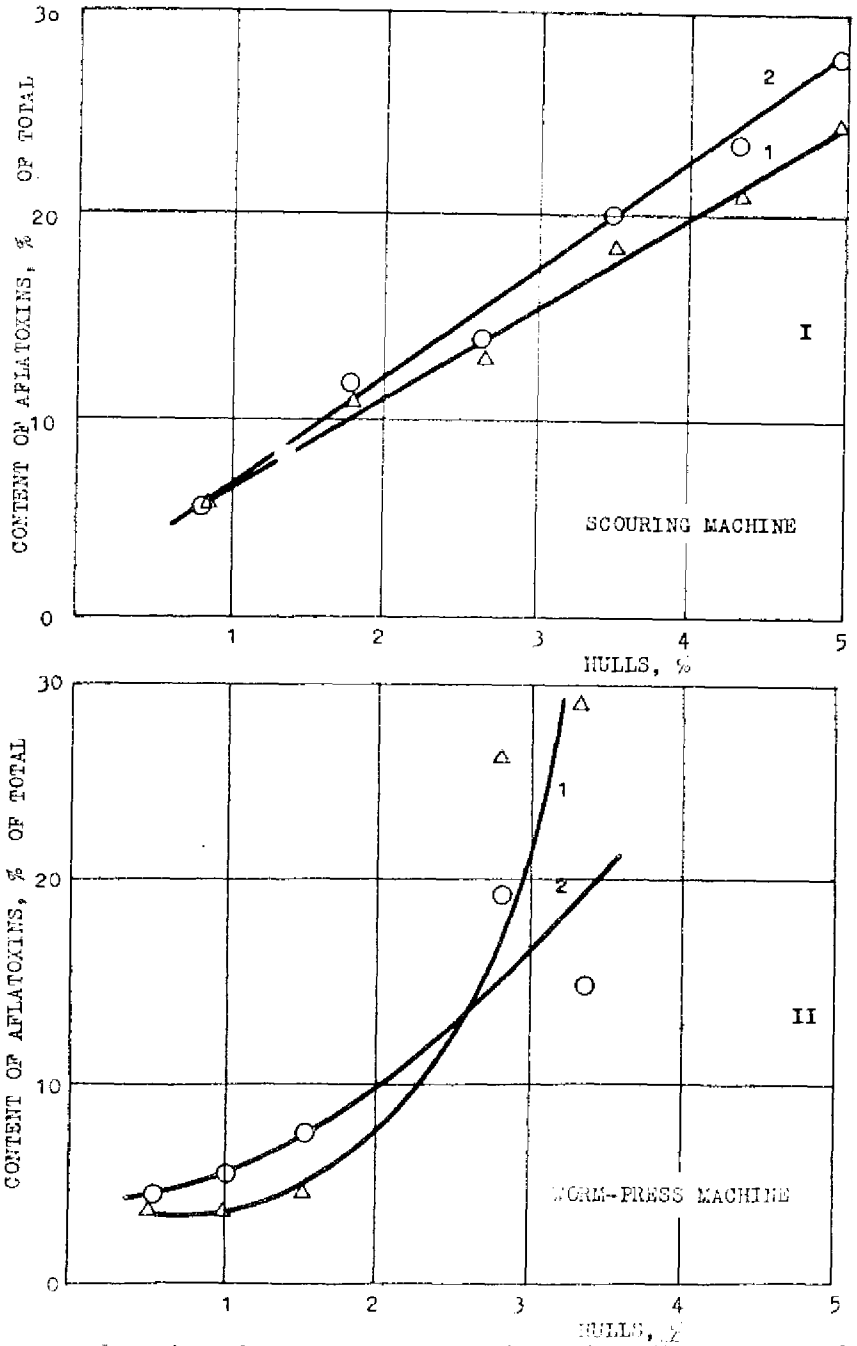

Fig.1. Lowering of aflatoxin content in grain with hull removal. 1. Aflatoxin G_1, 2. Aflatoxin B_1.

2.3. Wheat milling

Milling offers the possibility of obtaining high quality products from internal parts of the grain mass, which are to a lesser extent contaminated with aflatoxins (flour of top grade and first grade, semolino).

In scouring grinding of slightly contaminated wheat grain the largest part of toxins is eliminated with brands (Kao, Robinson, 1972). More detailed investigation of the influence of 78% milling and rapid conditioning of wheat grain on distribution of aflatoxins was carried out by Lvova L.S. et al., 1977. (Table 4).

In general for all types of milling the content of aflatoxins increases from the top grade to lower grades and attained the maximum in brands. As the distance from the centre of the endosperm to outlying parts of the kernel is increasing the content of aflatoxins is growing too. This explains why the flour of reduction systems 1,2,3 and break systems I, II and III contains the lowest aflatoxins level 4-5 times lower the initial level flour with a considerable amount of the aleuron layer and the outlaying parts of the endosperm (tail flour II) contained from 2 to 3 times more aflatoxins than it was revealed in the original grain.

When milling wheat subjected to a brief attack of mould, aflatoxins were found to be concentrated for the most part in the outlying parts of the kernel (milling 2). Therefore smaller quantities of aflatoxins passed to the top and first-grade flours as compared with the results obtained when milling wheat grain subjected to a prolonged attack of moulds (25 and 49%, respectively).

Table 4

Distribution of aflatoxin B_1 in the wheat milling stock (Lvova L. et al., 1977)

Milling stock	Milling 1		Milling 2		Milling 3	
	µg/kg	% to the original grain	µg/kg	% to the original grain	µg/kg	% to the original grain
Original grain	1.80	100	0.64	100	0.64[1)] 0.34[2)]	100 53
Top grade flour	0.89	49	0.16	25	0.08	12
1st grade flour	1.12	62	0.36	56	0.12	19
2nd grade flour	2.43	135	0.91	140	0.39	61
Brans	6.66	370	2.51	391	0.50	78

NOTE. 1) Before conditioning

2) After rapid conditioning

The application of rapid conditioning decreased the content of aflatoxins in flour, specifically in second-grade flour and brans. Most likely, the effect of moistening and high temperatures in rapid conditioning manifests itself chiefly in the surface, the most contaminated parts of the kernel.

Ochratoxin A, unlike aflatoxins, spreads in the kernel in a more uniform manner. It contaminates also the endosperm, therefore the level of ochratoxin content in flour and brans is approximately one and the same (Table 5).

Table 5

Distribution of ochratoxin A in flour and brans after milling of wheat and barley (Chelkowski J. et al., 1984)

Sample	Flour yield, %	Ochratoxin A content, mg/kg	
		in flour	in brans
1	80	2.4	2.5
2	70	1.6	3.1
3	50	0.46	0.92
4	50	2.7	1.4
5	50	1.9	1.6
6	60	1.8	1.7
7	50	1.0	0.8

2.4. Rice processing

One of the main peculiarities of rice kernels which determines its comparative resistance to aflatoxin contamination, is the presence of a flour gloom which obstructs the penetration of A. flavus within the kernel. Therefore, major part

1456

of the toxins present in the paddy (up to 70%), accumulates in broken kernels. As for the unbroken kernels, aflatoxins were distributed on the surface: up to 67% was concentrated in the flour gloom, up to 25% — in the germ, fruit and seed coatings.

These peculiarities of rice kernels have made it possible to suppose its considerable decontamination with traditional practices of treatment and processing.

It has been demonstrated that during cleaning, together with the waste, an average of 5.7% of aflatoxin B_1 was removed, however, this may be increased by a fuller separation of damaged kernels.

Owing to surface distribution of toxins, considerable effect was attained in the production of peeled rice: 55-74% of aflatoxin B_1 was removed with hulls, from 18 to 32% — with middlings, so that not more than 12% of the initial content of aflatoxin remained in peeled rice (Table 6).

The application of hydrothermal processing of rice kernels in the production of peeled rice entails the destruction of 91% of aflatoxin B_1 and 92-93% of aflatoxin G_1. In the process the larger part of toxins was destroyed in the damping of kernels at a temperature of 50 to 60°C for six hours.

Further destruction of aflatoxin occurred during cooking (6-56%).

The consideration of the entire pattern of rice treatment including its cleaning, the production of groats and cooking (Fig. 2), shows a considerable total effect of decontamination which reaches 92.4%, and when hydrothermal treatment is applied — 99.3%.

Table 6

Distribution of aflatoxin B_1 in products of rice kernels processing

Initial kernels:	Cleaning waste		Husks		Middlings 1		Middlings 2		Groats	
Aflatoxin B_1	Aflatoxin B_1 [1]		Aflatoxin B_1 [2]		Aflatoxin B_1 [2]		Aflatoxin B_1 [2]		Aflatoxin B_1 [2]	
µg/kg	µg/kg	%	µg/kg	%	µg/kg	%	µg/kg	%	µg/kg	%
130	67	4.9	440	70.6	360	20.2	100	2.9	13	6.3
160	130	8.1	440	55.5	500	23.0	400	9.3	33	12.2
140	270	16.0	330	66.8	270	14.6	270	11.0	13	7.6
190	1000	14.2	500	67.1	330	17.6	330	8.7	20	6.6
670	-	-	1000	74.4	760	13.0	430	5.2	44	7.4
M±	10.8±5.1		66.9±13.8		17.7±8.0		7.4±6.5		8.0±4.3	

1 - % of total toxins in initial grain.

2 - % of total toxins in cleaned grain.

Thus, owing to a comparatively rare distribution and low concentration of aflatoxins which are frequent in rice kernels in moderate climate (≤ 50 µg/kg) and also owing to the surface distribution of toxins in kernels, the traditional techniques of processing ensures the decontamination of a considerable part of samples of rice kernels given the present-day levels of aflatoxin contamination.

2.5. Processing of maize

Maize, more often than other grain crops, is contaminated with mycotoxins during its maturing and in storage. It is rather often that zearolenon, toxin T-2 and vomitotoxin are found in corn grain besides aflatoxins.

Dry milling is most often used for maize grain in food industry since it yields groats, flour and fodder fractions, and also the wet milling which is used to obtain starch. Technologically, dry milling of maize is close to wheat milling. During this process we observe a redistribution of aflatoxins among the products of processing. Toxins, in the main, are concentrated in the hull, germ and small-size waste. Only 9% of the total quantity of aflatoxins is found in middlings and flour. Thus, considerable detoxification of major cereal products is attained, the yield of which was 60% (Brekke O. et al., 1975). The same products contained but 10-22% of the total zearalenon (Benneth et al., 1976). The highest concentration of mycotoxin was found in the germ (from 10 to 20 times higher than in grain), 42-75% of aflatoxins and 34-53% of zearalenon fell on the germ. When the germ is not sufficiently thoroughly separated from 15 to 32% of aflatoxins remain in the principal product (Lvova L. et al., 1978).

Starch and a number of other products are produced by means of wet maize milling. Grain is first damped for several hours for better separation of the germ and coating. A considerable part of aflatoxins (up to 40%) goes into damping water; approximately 60% of toxins remain in seed fractions (the germ, fiber, protein). The principal product -- starch -- contains as few as 1% of the original aflatoxins level. When contamination with aflatoxins is not high (5-30 µg/kg, starch is practically free from aflatoxins (Yahl K. et al., 1971) (Table 7).

Benneth C. et al. (1978) have shown that the distribution of zearalenon in products of wet milling differs from the distribution of aflatoxin. Up to 72-75% of zearalenon fell on protein fractions and water soluble substances, aflatoxin, however, was found, for the most part, in damping water and water soluble substances (40%) and in fiber (coatings) (38%). This is due to the insolubility of zearalenon in water. The wet milling of corn which contains 0.9-9.4 µg/kg zearalenon ensures the obtaining of starch which is practically free from zearalenon.

3. Application of selective extraction for elimination of mycotoxins

The process of obtaining vegetable oil is a classical example of detoxication of food products during their processing with the application of solvents.

When producing vegetable oil the predominant part of toxins remains in the cake or oil-seed meal. Thus, in extracting oil from corn germs by hexane the concentration of aflatoxin B_1 in the oil-seed meal was 750 µg/kg while in oil it

Table 7

Distribution of mycotoxins in products of wet maize milling (Benneth C. et al., 1971; Yahl K. et al., 1978)

Fractions	Sample 1			Sample 2		
	Yield of fractions, %%	Aflatoxin B_1		Yield of fractions, %%	Zearalenon	
		Concentration, µg/kg	In %% of total		Concentration, µg/kg	In %% of total
Initial corn	100	120	2	100	900	2
Starch	53.7	2.2	1	67.8	0	0
Protein	10.7	140	13	9.7	6800	51.5
Germ	5.2	140	6	6.9	1700	9.1
Fiber	12.5	340	38	9.0	2700	19.0
Damping water and water soluble substances	7.2	610	39.5	6.7	3900	20.4

was only 8 µg/kg. In those cases when oil was extracted by means of chloroform a bigger quantity of aflatoxins remain in the oil. Clarification and refining makes it possible to obtain product which contains not more than 1 µg/kg of aflatoxin B_1, regardless of the method of extraction or the initial concentration of the toxin. The decomposition of aflatoxin in oil is due to the alkaline treatment during which the non-fluorescent derivatives of aflatoxins are produced. Their toxicity is weak (Parker W. et al., 1966).

A somewhat larger quantity of aflatoxins (6%) passes into oil which is produced by pressing though even in this case the refining reduces their content to traces (Natarajan et al., 1975).

4. Decomposition of mycotoxins by exposure to chemical and physical factors

When grain is prepared for milling or when it is being milled the main development is redistribution of toxins between the main and secondary products. The developments involved in the grain fermentation in the distillery industry, brewing, partially, in bread baking it is the decomposition of mycotoxins are being exposed to heat, moisture and, in a number of cases, enzymes of microorganism.

Grain processing in alcohol production develops conditions which favour the decomposition of the larger part of aflatoxins and obtaining distillate which is absolutely free from toxins, and also of secondary products which are but slightly contaminated (Damm R. et al., 1977). The larger part of toxins (65%) are decomposed during malting. This is facilitated by

high temperatures (75 and 95°C) and considerable ambient humidity (70%). The post fermentation waste retains only 35% of the initial content of aflatoxins. The waste is used to produce protein concentrates by a double alkaline extraction. The treatment with alkalines entails further decomposition of aflatoxins. Their total content in the products of alkaline extraction is 7%, and in protein concentrates -- only 2.2%. Thus, two related technologies ensure practically full decontamination of the main and secondary products.

The processing of maize contaminated with zearalenone leads to the appearance of alcohol which is free from toxins. The brewing technology ensures decomposition of 73-82% of aflatoxins, and 72-86% of ochratoxin A, which, at times, contaminates barley. The fullest decomposition of toxins is noted during the protein and carbohydrate hydrolysis which goes at a high temperature, and also in wort boiling. During this process ochratoxin A may change into ochratoxin α (Chu F. et al., 1975; Nip W. et al., 1975). It can be assumed that ochratoxin A is decomposed in the brewing process as a result of hydrolysis. Nevertheless, from 14 to 28% of mycotoxins contaminate beer.

In the process of prolonged fermentation of soya bean wort one can observe the transformation of aflatoxin B_1 to less toxic aflatoxin B_{2a} which is a result of the effect of lactic acid produced by lactobacillus delbrueckii. There is partial conversion of B_1 into B_{2a} under the effect of lactic bacteria also in ensilaging of contaminated maize.

During the isolation of protein isolates and concentrates from peanuts it was found that the larger part of aflatoxins is concentrated in the protein fraction. Nabarajan K. et al.,

1975, explain the phenomenon of concentration of aflatoxins in protein products by the ability of aflatoxins to form charge-transfer complexes with donors of π-electrons in macromolecules. Such donors are aminoacids histidine, phenylalanine, tyrosin and tryptophan.

The results are similar when isolates are taken from peanut meal: 50-60% of the overall amount of aflatoxins is isolated with the protein, 30% — remain in the sediment of protein isolation and 10-18% — in the supernatant fraction (Basappa S. et al., 1972). Preliminary washing of peanut and cotton seed flour with ethanol lowers the concentration of aflatoxins almost to one thirtieth of its initial level. The application of active carbon as an adsorbent in alkali solutions of protein entailed the lessening of aflatoxin content by 90% (Stoloff L. et al., 1976).

The techniques of cooking like frying and boiling on the whole are poorly effective in terms of their composition of mycotoxins. Let us consider a few examples. Approximately 50% of nivalenol and desoxinivalenol which were added to flour remained in the product during its baking (210°C) during frying (140°C) and boiling (Kamimura, 1978). Heating of grain to 150° did not bring about any loss of zearalenon. A considerable part of ochratoxin A (34-53%) remains in canned beans even after cooking and conserving at 121°C for 1-4 hours (Harwig J. et al., 1974). Boiling of fruit juices and sauces does not lead to a loss of patulin (Andersson A., Josefsson E., 1979). Decomposition of aflatoxin B_1 rice boiling is shown in Table 8.

Table 8

Decomposition of aflatoxin B_1 under different techniques of rice cooking

Rice and water ratio (weight)	Cooking conditions	Aflatoxin B_1 content, µg/kg		Aflatoxin decomposition, %
		initial	final	
1:2	40-45 min; atmospheric pressure	65 ± 8	61 ± 3	6
1:8	ditto	57 ± 0	36 ± 4	37
1:2	20 min; pressure 0.15	57 ± 0	40 ± 0	30
1:8	ditto	57 ± 0	25 ± 1	56

In the experiments carried out by Indian scientists (Rehana F. et al., 1979) ordinary cooking of rice decomposed 49% of aflatoxin B_1, pressure cooking of rice decomposed 73%, and boiling rice in a surplus of water — 82%.

Ordinary frying of food when oil temperature was 170-180°C did not entail considerable decomposition of aflatoxins present in conventional peanut butter. The content of aflatoxin B_1 decreased only when heating over 250°C (Peers F., Linsell C., 1975). It has been suggested in recent years to treat vegetable oils in the tropics by solar light which provokes decomposition of aflatoxins (Shanta T., Murty V., 1977; Okonkwo P., Nwokolo C., 1978).

When peanut was fried for 30 minutes at 150°C aflatoxin B_1 was decomposed to 80% and aflatoxin B_2 — 60%. When the initial content of aflatoxins was 1500 µg/kg, frying led to complete decomposition of aflatoxins (Lee W. et al., 1968). Dry frying lessened the content of aflatoxin B_1 and G_1 somewhat more radically (69 and 67%, respectively), than frying

with oil (65 and 62%) (Lee W. et al., 1969). Similar results were obtained for maize. When maize was fried, from 40 to 80% of aflatoxin B_1 was decomposed (Bagley E., 1978). Decomposition of toxins was intensified if the product was treated with ammonia prior to frying (Conway H. et al., 1978).

If the content of aflatoxin B_1 in fodder $\geqslant 50$ µg/kg, it is found in milk as aflatoxin M_1. This aflatoxin is partially retained even during pasterization and dehydrating of milk, it passes on to butter and dairy products (Kiermeier F., 1973). Aflatoxin M_1 is most frequently found in cheese in the winter period when mixed feeds contaminated with aflatoxin B_1 are widely used.

In wine-making, up to 50% of aflatoxins, which grow during the harvesting and storage of grapes are decomposed during fermentation, the remaining part passes on to wine. Aflatoxins usually contaminate, in low concentrations (2.6 µg/l) primarily red wines (Lehtonen M., 1973).

A producent of patulin, Penicillium expansum is of greatest importance among microorganisms which cause the rottening of apples and the spoiling of apple juice. It has been shown by experiments that patulin, in the production of apple juice passes from the initial material into the juice and that its amount is but slightly lowered in the technology of production. The decomposition of 50% of patulin occurs during the fermentation of apple cydre. As a real means which decreases patulin content to trace amounts is the treatment of juice with active carbon (Harwig J. et al., 1973; Andersson A., Josefsson, E., 1979).

5. Bread baking

It has been demonstrated experimentally by Reiss J. (1975, 1976 and 1977) that aflatoxin B_1 and G_1, patulin, ochratoxin A, cytrinin and sterigmatocystin may develop in bread. However, only aflatoxin, patulin and ochratoxin A have been found in spontaneously moulded bread.

Aflatoxins were also found in a mixed rye-and-wheat bread in Federal Germany. After 10% of samples of moulded bread made of coarse milled flour contained aflatoxin B_1 in an amount reaching 7000 µg/kg (Frank H., 1968). When 91 loaves of various kinds of bread with indications of moulding were examined Spicher G. (1973) found aflatoxins in 15 samples. The rye-and-wheat bread was damaged more than any other kind of bread.

The ability of aflatoxins to migrate and to get concentrated in the depth of under the focus of contamination within the loaf (Hanssen E., Yung M., 1973). Patulin, most likely, cannot grow in molded flour and bread owing to its instability when exposed to sulphydril containing aminoacids of cereals (Reiss J., 1977). Therefore there are doubts about the report of Tyllinen et al., (1977) about the finding of patulin in moulded bread. Ochratoxin A, most likely, also is not a problem for bread, since when 50 samples of spontaneously moulded bread were analyzed in England by Osborne B. (1980) only this toxin was found but in one sample (0.21 µg/kg).

5.1. Effect of bread baking on aflatoxin content

The most probable way of aflatoxin's penetration to bread is introduction with the principal component -- flour. A number

of investigations showed the role of bread baking in decreasing the content of aflatoxins in the end product — bread (Jemmali M. et al., 1972; Reiss J., 1978; Lvova L. et al., 1977). In dough mixing and leavening the content of aflatoxin B_1 was lowered to 38–86%, that of aflatoxin G_1, remained practically the same (80–103%). This confirms the supposition of Jemmali M., Lafont P. (1972) about the oxidizing changes of B_1 during dough mixing which lead to its change into aflatoxin G_1. Most likely oxidation occurs not due to air oxygen but in connection with some conjugated biological systems with participation of lypoxygenases, since an increase in the number of revolutions and the lengthening of the time of operation of dough kneader has not led to any greater decomposition of toxins.

The baking process did not notably decrease the content of aflatoxins. From 50 to 57% of aflatoxin B_1 and from 83 to 84% of aflatoxin G_1 was retained in the bread. Longer time of leavening somewhat increased the decontamination of bread and only 31% of aflatoxin B_1 and 50–62% of aflatoxin G_1 was found in it; there is also a possibility of microbiological change of aflatoxin B_1.

5.2. Formation of toxins in durable storage of bread

The temperature of storage and the acidity of grain, and also its contamination with spores of toxigenous strains of fungi are the main factors which determine the possibility of the formation of mycotoxins in bread. The best possible temperatures for the growth of aflatoxins in bread is the 20–35°C range, however, at 10°C more toxins is formed than at 40°C. A weakly acid medium with pH 5 favours the synthesis of aflatoxins. The synthesis of aflatoxin B_1 depends in a larger

measure upon the acidity of the medium, than the accumulation of aflatoxin G_1 (Roiss J., 1975; Schroider H., Hein, 1967; Spicher G., 1973). The illumination does not influence the synthesis of aflatoxins in bread.

Sliced bread which was kept in hermetically sealed,has shown that the synthesis of aflatoxins depends largely upon oxygen concentration. There was concurrent growth, sporilation of fungi and synthesis of aflatoxin up to the time the starting store of oxygen was used up. With the exhaustion of oxygen sporilation discontinued, though the growth of the fungus continued. No synthesis of aflatoxins was observed during that period (Reiss J., 1975).

The development of patulin is possible at lower temperatures since the producent of patulin Penicillium expansum has the optimum growth on bread at a temperature of 10°C. The synthesis of patulin poorly depends upon the acidity of the medium within the range of change of pH from 3.0 to 8.6. Sterigmatocystin was accumulated in bread contaminated with A. versicolor, particularly intensively at a temperature of 20°C and pH 9.

In bread storage there is the possibility of a drop in aflatoxin and patulin concentration which may be explained, for instance, by the binding of patulin by the SH-groups of bread protein.

Not only the crust but the adjacent layers of crumb are dangerous in moulded bread since aflatoxins can reach up to 7 cm into bread and even more than that either with the mycelium of fungi or owing to their migration with moisture (Frank H., 1974).

The spores of microscopic fungi introduced with the initial material during bread baking are killed. Secondary insemination of bread with spores with mould fungi occurs at bakeries and during storage in the trading network.

To prevent contamination of bread with mould fungi and the growth of mycotoxin it has been suggested to keep working premises in an air tight condition and to purify incoming air against fungi spores (Spicher G., 1976), ozonification and treatment with ultraviolet light. The addition to bread of fungistatic substances like the salts of sorbic and propionic acids does slow down the growth of fungi and the synthesis of toxins. These substances, however, have not received wide spread application in most European countries owing to the stringent limitations of concentration of the majority of conservants (Reiss G., 1976). The thermal method of decontamination of bakery products against mould fungi by exposure to high temperature (15 minutes at 90°C) is a more safe technique.

The nature of the packing material and the extent of its contamination with spores of mould fungi also has an important influence upon the intensity of bread moulding.

As a whole during bread baking there is almost a total destruction of toxigenous fungi and partial destruction of aflatoxins. This, however, does not ensure complete detoxication of the finished product which predetermines the necessity of further search of ways to lessen the contamination of bread with mycotoxins by thorough inspection of the initial material and the development of technologies which facilitate the destruction of toxins.

An instance of this, more safe technology of bread baking are the tortilas made of maize flour which are wide spread in Mexico and Latin America. Maize corn is first treated with calcium oxide, for swelling and elongation of the envelopes which leads to the destruction of 60% of aflatoxins. The final product -- tortilas -- retain 15-25% of the initial content of aflatoxins (Ulloa-Sosa M. et al., 1969).

6. Conclusion

The aforesaid leads to a conclusion that different technologies of processing agricultural materials which are contaminated with mycotoxins ensure partial (and in a number of cases complete) detoxification of staple food products.

Elaboration of specific techniques of processing which improve the effect of detoxification is a promising trend in this area. For instance, it is advisable to remove a bigger mass of the surface layers (upto 5%) in grain cleaning. It is expedient to investigate a possibility of using liquid yeast with lactic acid bacteria and extension of the fermentation period in bread making.

In a number of technologies (dry and wet milling of corn, wheat milling, vegetable oil production) the concentration of toxins is observed in by-products of feeding value (brans, germ, pulp, cake, oil seed meal etc.). These products may have high concentrations of toxins even with insignificant initial contamination of raw materials with mycotoxins, thus restricting their use as animal feed.

Since quite a number of technologies ensure a considerable decrease in the content of mycotoxins in processed products

it is feasible to establish higher maximum permissible levels of aflatoxins for raw materials to be processed with regard to the detoxification effect attained in processing.

Thus, lots of corn, containing aflatoxin B_1 in the quantity of up to 50 µg/kg, may be used for starch and molasses production, those containing up to 10 µg/kg, -- for dry milling. Similar rates may be established for wheat grain in the milling industry as well as for materials used for brewing purposes.

For the purposes of the alcohol industry, as well as for the production of vegetable oil, the original content of toxins in raw materials may be rather high since the applied technology ensures complete detoxification of the principal finished products.

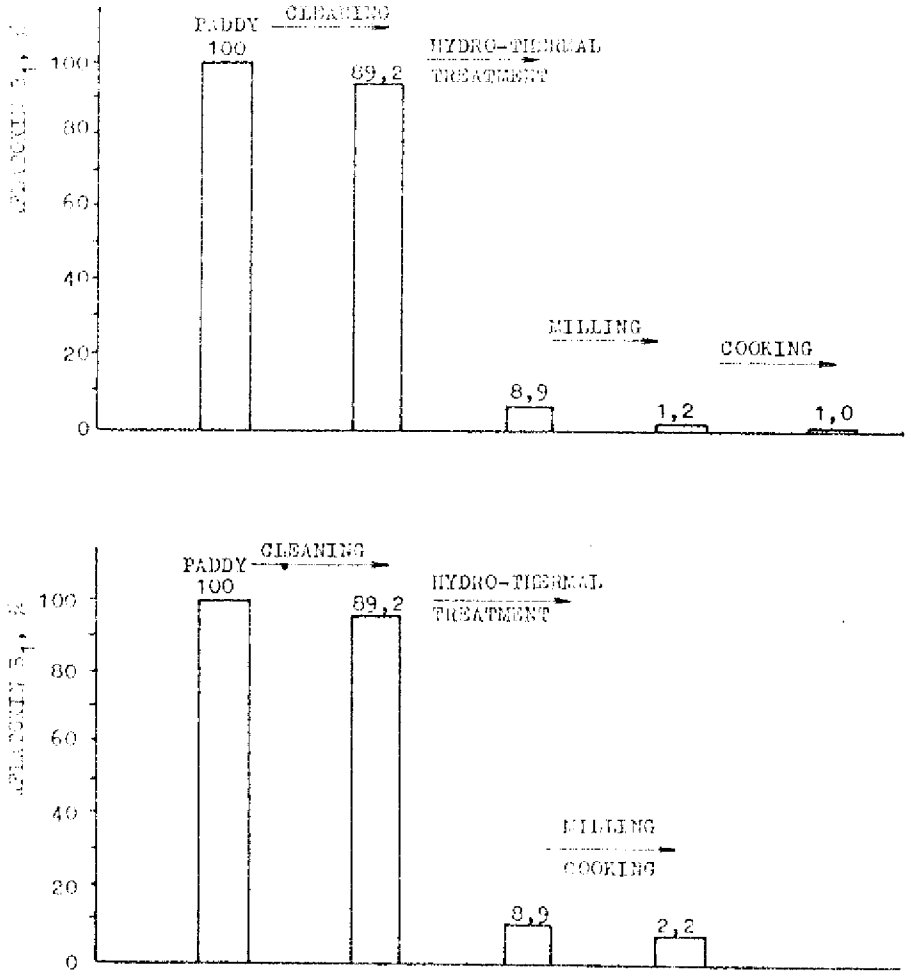

Fig.2. Reducing of aflatoxin B_1 content during various methods of rice processing.

I - with hydro-thermal treatment.
II - without hydro-thermal treatment.

Зак. 1457 [illegible]