Contamination of Pasta and the Raw Materials for its Production with Moulds of the Genera *Aspergillus*

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Abstract

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The degree of contamination of pasta (132 samples), flour (86 samples), and egg powder (44 samples) with moulds, with emphasis on the species of genus *Aspergillus* was investigated . The most frequently isolated genera in the mould samples analysed were those of *Aspergillus* (70.88%) and *Penicillium* (21.22%), followed by *Mucor*, *Cladosporium*, *Rhizopus*, *Fusarium*, *Alternaria*, *Absidia* and others. The following species of the *Aspergillus* genera were prevalent: *A. albus* (65.66%) and *A. flavus* (22.01%), followed by *A. clavatus*, *A. amstelodami*, *A. versicolor*, *A. ochraceus*, *A. niger*, and others.

Keywords: pasta; flour; egg powder; moulds; mycotoxins

Raw materials can be potential sources of pasta contamination with microorganisms (primary contamination), whereas a certain share of microorganisms come during the production process itself (secondary contamination) (Todorović *et al*. 1990).

Microorganisms which develop during the production process, as well as some of their metabolites, can be found in the packaged pasta since the thermal treatment in the drying process does not destruct them, i.e. does not decompose them completely.

Pathogen bacteria with their toxins can cause contamination of raw materials, semiproducts and final products which can eventually lead to various disorders in human organism.

Similarly, contamination of raw materials, semiproducts, and final products with moulds that are producers of mycotoxins, and with mycotoxins as such, is also considered to be of significant importance.

Moulds of the genera *Aspergillus*, *Penicillium* and *Fusarium* are known to produce mycotoxins (Duraković & Duraković 2000). Among the mycotoxins known, the most toxic are aflatoxins which are synthesised by the species *Aspergillus flavus* Link ex Fries, *Aspergillus parasiticus* Speare, and also by some other moulds (Allcroft *et al*. 1961; Purchase 1974). Flour as the raw material for the pasta production can be contaminated with aflatoxin-producing fungi and aflatoxin itself (Halt 1984a, 1989). Moreover, some other raw materials used for the pasta production can be potential sources of contamination with moulds and mycotoxins as well.

Since pasta is very commonly used in the human diet, mycotoxins are of concern due to their potentially harmful effects on both humans and animals. Therefore, we considered it to be of great importance to investigate the degree of contamination of pasta and the materials for its production, especially with the mould species of the genus *Aspergillus*.

MATERIALS AND METHODS

The samples of packaged pasta were taken from the storage space in a factory for the pasta production, and flour samples and egg powder were taken from the storage space for raw materials of the same factory, prior to the production process.

Total number of 262 samples were analysed, out of which:

1. Dried, originally packaged pasta – 132 samples;
2. Flour for the pasta production – 86 samples;
3. Egg powder – 44 samples.

The presence and the number of moulds in pasta and raw materials were determined by Koch's agar plate method and Czapek's agar based media (Službeni list RH 1992).

The determination of the isolated mould genera was performed on the basis of morphological mould properties, their reproduction, and the developmental cycle, etc., according to Gilman (1959), Raper and Fenell (1965), Pitt and Hocking (1997), Duraković and Duraković (2000) and Fassatiova (1986), as well as all other available literature on this subject.

RESULTS AND DISCUSSION

The results of the determination of the degree of contamination of pasta, flour, and egg powder samples are shown in Table 1–3. In the pasta samples analysed, the total number of mould colonies varied from 0–3.1 × 10^4 in 1 g. Next, there were 0–1.28 × 10^4 mould colonies detected in the flour samples which is in accordance with the Book of regulations on microbiological standards for food which allows 10^4 in 1 g of flour (Službeni list RH 1994). Egg powder was less contaminated, with the number of the mould colonies varying from 0–650 in 1 g (Table 1).

Table 1. Contamination of pasta, flour, and powdered eggs by moulds

No.	Sample	Total number of analysed samples	Total number of mould colonies in 1 g of the sample	Samples contaminated with *Aspergillus flavus*	
				number	(%)
1.	Pasta	132	0–3.1 × 10^4 (510)	52	39.40
2.	Flour	86	0–1.28 × 10^4 (470)	10	11.63
3.	Powdered eggs	44	0–6.5 × 10^2 (80)	0	0
Total		262	–	62	23.66

Table 2. Specific mould genera present in pasta, flour, and powdered eggs samples

No.	Mould genera	Pasta		Flour		Powdered eggs		Total	
		number	%	number	%	number	%	number	%
1.	*Aspergillus*	1697	81.51	1458	69.07	89	23.18	3244	70.88
2.	*Penicillium*	140	6.72	565	26.56	266	69.27	971	21.22
3.	Mucor	68	3.27	9	0.43	1	0.26	78	1.70
4.	*Rhizopus*	15	0.72	3	0.14	0	0	18	0.39
5.	*Absidia*	1	0.05	3	0.14	0	0	4	0.09
6.	*Fusarium*	4	0.19	4	0.19	2	0.52	10	0.22
7.	*Alternaria*	0	0	2	0.09	3	0.78	5	0.11
8.	*Cladosporium*	6	0.29	35	1.66	11	2.87	52	1.14
9.	Unidentified	151	7.25	32	1.52	12	3.13	195	4.26
Total		2082	100	2111	100	384	100	4577	100

A great variety of moulds and their species were detected by the analysis of pasta, flour, and egg powder as can be seen in Table 2.

From the products analysed, 4577 mould isolates were separated, predominately the species of the genera *Aspergillus* (70.88%) and *Penicillium* (21.22%), followed by *Mucor*, *Cladosporium*, *Rhizopus*, *Fusarium*, *Alternaria*, *Absidia* (listed in order of their frequency) (Table 2).

The results on pasta and raw materials contamination correspond to the results of our previous research (Todorović & Halt 1975; Halt 1979, 1984b, 1994a; Halt *et al*. 1985).

Some of the mould species, if developed under favorable conditions, can even synthesise mycotoxins (Allcroft *et al*. 1961; Purchase 1974; Šutić 1976; Duraković 1991; Duraković & Duraković 2003, etc.).

Moulds of the genera *Aspergillus*, *Penicillium* and *Fusarium* are known to produce mycotoxins (Duraković & Duraković 2000).

Special attention was paid to the *Aspergillus* species, out of which 50 were mycotoxic (Duraković & Duraković 2000).

Among the mycotoxins produced by the *Aspergillus* species, the following were identified: aflatoxins, ochratoxin A, sterigmatocystin, citrinin, penicillium acid, etc. (Duraković & Duraković 2000).

The following *Aspergillus* species were most commonly detected in pasta and the raw materials for its production: *A. albus* (65.66%), *A. flavus* (22.01%), *A. clavatus* (1.30%), *A. amstelodami* (1.02%), *A. versicolor* (0.83%), *A. ochraceus* (0.12%), *A. niger* (0.12%), etc. (Table 3).

The special attention was paid to the detection of the pasta and its raw materials contamination with *A. flavus*, the producer of the most toxic mycotoxin-aflatoxin.

A higher occurrence of *A. flavus* was observed in pasta than in flour and egg powder. Namely, out of 132 pasta samples, 52 (39.40%) revealed the presence of *A. flavus*. At the same time, the presence of *A. flavus* was detected in 10 out of 86 samples (11.63%), whereas egg powder did not contain this mould species at all.

Contamination with this mould type occurred most likely during the drying of pasta and the storage process, and, indirectly, from flour itself. This can be proven by the previous researche conducted by Halt (1994b), the author of this paper, when the results of the analysis of the swab and moist pasta revealed a significant degree of contamination with moulds which were present at the working surfaces of the pasta factory.

Apart from *A. flavus*, some other *Aspergillus* species, which were isolated from pasta and raw materials, are capable of producing aflatoxins and other mycotoxins.

Pasta contamination, and especially flour contamination with *A. albus*, the potential producer of citrinin, is very significant (Duraković & Duraković 2000) (Table 3). In comparison wih other *Aspergillus* species, this one was detected in 65.66% of pasta and raw materials samples, and even in 88.41% flour samples. These results correspond to the previously conducted research by Halt (1979, 1984a) in which a considerable flour contamination with this mould species was observed. According to the

Table 3. Contamination of pasta, flour, and powdered eggs by moulds of *Aspergillus* genera

No.	Species of *Aspergillus* genera	Pasta		Flour		Powdered eggs		Total	
		number	%	number	%	number	%	number	%
1.	*flavus*	687	40.48	27	1.85	0	0	714	22.01
2.	*albus*	827	48.73	1289	88.41	14	15.73	2130	65.66
3.	*amstelodami*	16	0.94	14	0.96	3	3.37	33	1.02
4.	*clavatus*	4	0.24	38	2.61	0	0	42	1.30
5.	*ochraceus*	3	0.18	1	0.07	0	0	4	0.12
6.	*versicolor*	0	0	2	0.14	25	28.09	27	0.83
7.	*niger*	4	0.24	0	0	0	0	4	0.12
8.	sp.	156	9.19	87	5.97	47	52.81	290	8.94
Total		1697	100	1458	100	89	100	3244	100

results obtained, the following mould species were detected in flour: *A. clavatus* (2.61%), *A. amstelodami* (0.96%), *A. versicolor* (0.14%), *A. ochraceus* (0.07%) (Table 3), the potential producers of patulin, sterigmatocistin, ochratoxin, penicillium acid, and aflatoxin (Duraković & Duraković 2000).

Furthermore, a significant degree of contamination with *Penicillium* of pasta and raw materials for its production was observed, out of which 32 species are capable of producing 27 different mycotoxin types (Pitt & Hocking 1997; Duraković & Duraković 2000).

Moulds of *Fusarium* species which are the potential producers of zearalenon, fumonisin, trihotecen etc. (Duraković & Duraković 2000) were present in 0.22% samples.

Furthermore, moulds of the genera *Mucor*, *Rhizopus* and *Alternaria* were also isolated. Some of the species of these genera are known to be mycotoxic (Duraković & Duraković 2000).

In other words, the results obtained by this research, as well as those obtained by the previous research in the Slavonia and Baranya regions (Todorović & Halt 1975; Halt 1981, 1984a, b, 1989, 1994a, b, etc.) show contamination of wheat, flour, pasta, and egg powder with mould species that are potential mycotoxin producers.

The presence of moulds, however, does not necessarily mean that the stored material is contaminated with mycotoxins. Certain mould species which are mycotoxin producers can be identified without the presence of mycotoxin. Namely, it is well known that mycotoxin synthesis occurs only when the moulds are found under favourable conditions. The influence of these factors in the toxin production has been investigated in a great number of papers. For example, Thung and Ling (1968) claimed that, although aflatoxins can be synthesised even at 83% relative humidity optimum for their production is considered to be 99%.

Moreover, Rabie and Smalley (1965) stated that the optimal temperature for the development of *A. flavus* and the toxin biosynthesis ranges from 18 to 24°C. Diener and Davies (1970) obtained maximal toxin quantity after 15 and 11 days of growing at the temperatures of 20°C and 30°C, respectively. They stated that for aflatoxin production in peanut humidity above 10% is needed.

Jarvis (1971) demonstrated that aflatoxins will not be biosynthesised at temperatures below 13°C or above 42°C. According to Muntanola-Cvetković (1987), in order to produce aflatoxins, it is necessary to fulfill the following criteria: moulds should be genetically capable of synthesis, relative humidity should be 85%, water content 30%, air temperature of 25°C, and an adequate medium is necessary.

The research by Reiss (1986) showed that aflatoxin production in poultry food contaminated with *A. flavus* and *A. parasiticus* occurred at the relative humidity above 14%, air temperature between 25–45°C, with the corresponding content of Zn ions.

Hence, the appropriate storage, low air temperatures, a low relative air humidity, and an insignificant degree of humidity of the stored materials can, to a certain extent, prevent the synthesis of aflatoxin and other mycotoxins regardless of the presence of the mould producers.

In the process of the pasta production, besides special attention paid to the presence and number of saprophyte and pathogen bacteria in the raw materials, the mould species and their number in semiproducts and final products should also be taken into special consideration.

The moulds of genus *Aspergillus* whose numerous identified species, especially *A. flavus*, as has already been mentioned, can synthesise mycotoxins – the potential aflatoxin producer.

The presence of *A. flavus* and aflatoxin B_1 in pasta and flour was also proven in the previous research conducted by the same authors (Halt 1994b).

Out of 406 flour samples analysed, 43 samples were contaminated with *A. flavus*. Also, out of 59 pasta samples analysed, the presence of *A. flavus* was detected in 15 samples. In 27 flour samples contaminated with *A. flavus*, the level of aflatoxin B_1 varied from 2 to 10 ppb. Aflatoxin B_1 was found in only 2 pasta samples contaminated with *A. flavus*, with the level of 2 ppb that most likely originated from flour.

These aflatoxin quantities were in the acceptable limits according to the standards proposed by FAO/WHO/UNICEF (FAO/WHO/UNICEF 1968) in which aflatoxin contents in food products should not exceed 30 ppb.

Similar results with regard to the aflatoxin content in flour samples contaminated with *A. flavus* were obtained in the previous research as well (Halt 1981, 1984a, 1989, 1994a, b; Halt *et al*. 1985).

Products in the form of bread, rolls, pasta, cakes, cookies, or flour are very common in everyday nutrition, hence, the likelihood of ingesting aflatoxin in human organism is of great significance.

Aflatoxin is extremely thermostable. It decomposes partially or completely only at temperatures

above the boiling point, which was also mentioned by a great number of other authors (Feuell 1966; Fishbach & Campbell 1965; Haberle *et al*. 1978; Šutić & Pantović 1976, etc.).

Our research (Halt 1984b) revealed that during the baking of bread which was previously artificially contaminated with aflatoxin B_1 at the temperature between 260–300°C, up to 60% of aflatoxin decomposed in 32 min. In other words, a part of aflatoxin which is transmitted by flour in the process of production of bread, rolls, pasta, can also be expected in the final product since the temperature treatment throughout the production process is issuficient to decompose it.

Confectionary and bread analysed by Šutić *et al*. (1989) revealed a certain degree of contamination of these products with aflatoxin, ochratoxin, and zearalenon.

All these data speak in favour of the idea of taking special care as concerns the mycological and mycotoxicological acceptability of flour, as well as of the products that are made of flour, especially those that are often used in human diet.

Although very small amounts of aflatoxin are ingested in human organism as a results of products, it is known that it accumulates in liver the uptake of these causing serious damage and liver cancer (Duraković *et al*. 1989; Uragucki & Yamazaki 1978, etc.). Therefore, it is necessary to avoid or at least reduce its ingestion in human and animal organisms.

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Souhrn

Halt M., Kovačević D., Pavlović H., Jukić J. (2004): **Kontaminace těstovin a surovin pro jejich výrobu plísněmi rodu** *Aspergillus*. Czech J. Food Sci., **22**: 67–72.

Sledovali jsme kontaminaci těstovin (132 vzorků), mouky (86 vzorků) a vaječného prášku (44 vzorků) plísněmi. Pozornost byla věnována hlavně druhům rodu *Aspergillus*. V analyzovaných vzorcích mouky byly nejčastěji nalezeny rody *Aspergillus* (70,88 %) a *Penicillium* (21,22 %), následovány rody *Mucor*, *Cladosporium*, *Rhizopus*, *Fusarium*, *Alternaria*, *Absidia* a dalšími. Z druhů rodu *Aspergillus* byly nejvíce zastoupeny *A. albus* (65,66 %) a *A. flavus* (22,01 %), následovány *A. clavatus*, *A. amstelodami*, *A. versicolor*, *A. ochraceus*, *A. niger* a dalšími.

Klíčová slova: těstoviny; mouka; vaječný prášek; plísně; mykotoxiny

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