

CONTAMINATION OF SPICES USED IN MEAT PROCESSING WITH TOXIGENIC MOULDS AND OCHRATOXIN A
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Spices used in meat processing may contain large numbers of fungi that can cause spoilage of final products, such as salami, sausages etc. The presence of toxigenic fungi is especially undesirable since heat process is not used in processed meat manufacture. Under such conditions moulds can grow and produce toxic metabolites (Pitt and Hocking, 1985).

Having in mind high frequency of fungal species in the environment, the aim of this study was to investigate the presence of fungi, with special attention to toxigenic ones, and ochratoxin A in mixture of spices and corn black pepper used in meat processing.

Material and Methods

Contamination of mixture of spices (8 samples) and corn black pepper (4 samples) by fungi, with special attention to toxigenic species, and ochratoxin A (OA) was investigated. The composition of mixture of spices was as follows: samples 1 and 2 - white pepper, black pepper, allspice, garlic, nutmeg; sample 3 - black pepper, red pepper, hot pepper, garlic, cumin, coriander; sample 4 - black pepper, white pepper, garlic, allspice, cumin; samples 5 and 6 - white pepper, garlic, red pepper, coriander, cumin, black pepper; sample 7 - white pepper, black pepper, garlic, cumin, allspice; sample 8 - black pepper, garlic, allspice, coriander.

Mycological analyses. The dilution plate technique was used for isolation of fungi and their total viable counts per g. The following media were used: a) Sabouraud maltose agar (SMA) - peptone 10 g, maltose 40 g, agar 15 g, distilled water to 1000 ml, b) malt extract yeast extract 50% glucose agar (MY50G) - malt extract 10 g, yeast extract 2.5 g, agar 10 g, distilled water to 500 g, glucose 500 g and c) Czapek yeast extract agar with 20% sucrose (CY20S) - K₂HPO₄ 1.0 g, Czapek concentrate (NaNO₃ 30 g, KCl 5 g, MgSO₄ x 7 H₂O 5 g, FeSO₄ x 7 H₂O 0.1 g, distilled water to 100 ml) 10 ml, yeast extract 5 g, sucrose 200 g, agar 15 g, distilled water to 1000 ml. 1 ml of 1% chloramphenicol and 1 ml of 1% oxytetracycline per 100 ml of medium was added. Incubation was done at 25 C for 7 days.

Identification of species was performed according to Raper and Thom (1949), Raper and Fenel (1965), Ellis (1971), Pidopličko and Milko (1971) and Samson and van Reenen-Hoekstra (1988).

Mycotoxycological analyses. Qualitative and quantitative determination of OA was carried out using a TLC method according to the Official Methods of Analysis of the A.O.A.C. (1990).

Results and discussion

Mycological analyses. All spices tested were contaminated with fungi at various degree. The largest number of fungi per g was determined on MY50G and the smallest on SMA (Table 1). These results could be expected since the most contaminants of spices are xerophilic.

Table 1. Total viable counts of moulds in mixture of spices and corn black pepper

Medium	Total viable counts of moulds per g											
	Sample no.											
	1	2	3	4	5	6	7	8	9	10	11	12
SMA	3.5×10^3	2.4×10^3	2.4×10^3	1.5×10^3	9.2×10^2	7.2×10^2	5.0×10^2	5.3×10^2	2.1×10^3	1.6×10^3	1.6×10^3	8.2×10^1
MY50G	8.0×10^3	5.6×10^3	3.7×10^3	5.8×10^3	2.4×10^3	2.7×10^3	1.4×10^3	3.1×10^3	2.9×10^3	5.9×10^3	4.3×10^3	7.2×10^2
CY20S	5.2×10^2	2.8×10^3	2.7×10^3	2.1×10^3	2.1×10^3	3.2×10^3	9.5×10^2	2.7×10^3	2.7×10^3	5.3×10^3	4.3×10^3	3.8×10^2

1 - 8 mixture of spices, 9 - 12 corn black pepper

Isolated fungal strains were classified into 10 genera (Absidia, Aspergillus, Emmericella, Eurotium, Mucor, Paecilomyces, Penicillium, Rhizopus, Scopulariopsis, Syncephalastrum) and 38 species (Table 2). Genera Aspergillus and Penicillium were presented with the largest number of different species. Eurotium herbariorum, a potential producer of sterigmatocystin (Frisvad, 1988), was found to be the most frequent fungal species.

About 53% of isolated fungi belonged to toxigenic species. According to literature data (Fassatiouva, 1986, Frisvad, 1988) 11% of isolated species are ochratoxigenic, 13% of them are producers of sterigmatocystin, 16% are aflatoxigenic and the rest can produce other toxins (patulin, cyclopiazonic acid, isofumiclavine etc.).

Mycotoxycological analyses. OA was found in two samples of mixture of spices (no. 6 and 7) at concentration of 32.00 µg/kg (sample no. 6) and in traces (sample no. 7).

Conclusions

All of spices were contaminated with fungi. The largest number of fungi per g was determined on MY50G. Isolated strains were classified into 10 genera and 38 species. Genera Aspergillus

Table 2. Mould species isolated from the mixture of spices and corn black pepper

Mould species	Sample no.											
	1 SMC	2 SMC	3 SMC	4 SMC	5 SMC	6 SMC	7 SMC	8 SMC	9 SMC	10 SMC	11 SMC	12 SMC
<i>Absidia corymbifera</i>	+++		+		++	+++	++	+++		+		
<i>Aspergillus caespitosus</i>												+
<i>A. candidus</i>												+
<i>A. flavus</i>	+++	+++	+++					+	++	++		
<i>A. niger</i>	+++	++	+++	+		+			++	++		
<i>A. ochraceus</i>									+++		++	
<i>A. protuberus</i>										++	+	
<i>A. repens</i>			++	+	+	++		++				+
<i>A. restrictus</i>									+			
<i>A. sydowii</i>			+	+++								
<i>A. terreus</i>	+										+	
<i>A. versicolor</i>	+	++	+	+	+			+	++	+++	++	+
<i>A. wentii</i>											+	
<i>Emmericella nidulans</i>							+					
<i>Eurotium herbariorum</i>	+++	+++	+	++	++	++	++	++	+	+++	+++	+++
<i>Mucor circinaloides</i>									+			
<i>M. hiemalis</i>					++	+						
<i>M. plumbeus</i>	+											
<i>M. racemosus</i>	+		+++			++		+				
<i>Paecilomyces variotii</i>	+++	+++		+++								
<i>Penicillium</i>												
<i>aurantio-griseum</i>			+				+		+++		+	
<i>brevi-compactum</i>		+						++	+			
<i>chrysogenum</i>									++		+++	
<i>claviforme</i>									+			
<i>decumbens</i>									+			
<i>funiculosum</i>									+			
<i>griseofulvum</i>		++		+			+				+	
<i>godlewskii</i>									+			
<i>implicatum</i>		++		+						+	+	
<i>jenssenii</i>	+	++		+								
<i>nigricans</i>	+	++		+				++		+		

Table 2. Continuation

Mould species	Sample no.											
	1 SMC	2 SMC	3 SMC	4 SMC	5 SMC	6 SMC	7 SMC	8 SMC	9 SMC	10 SMC	11 SMC	12 SMC
<i>P. notatum</i>					++							
<i>P. variabile</i>											+	
<i>Rhizopus nigricans</i>			+									+
<i>R. oryzae</i>										++		
<i>Scopulariopsis brevicaulis</i>									++			
<i>S. fusca</i>		+			+	+++	+++	++	++			
<i>Syncephalastrum racemosum</i>	++										++	

1 - 8 mixture of spices, 9 - 12 corn black pepper, S - SMA, M - MY50G, C - CY20S

and *Penicillium* were presented with the largest number of different species. The most common fungus was *Eurotium herbariorum*. About 53% of isolated species are known as mycotoxin-producing moulds. Two samples of mixture of spices were contaminated with OA (no. 6 - 32.00 µg/kg, no. 7 - in traces).

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