The Occurrence of Moulds in Fermented Raw Meat Products

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Abstract

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The consumption of food contaminated with moulds (microscopic filamentous fungi) and their toxic metabolites results in the development of food-borne mycotoxicosis. The spores of moulds are ubiquitously spread in the environment and can be detected everywhere. In this study, the presence of various moulds was determined in pork and beef used as a raw material, in salami emulsions, as well as in five kinds of fermented raw meat products. *Penicillium* sp., *Acremonium* sp., *Mucor* sp., *Cladosporium* sp., and *Aspergillus* sp. were the most frequently isolated genera of moulds. Flavourings added to meat during the production of fermented raw meat products were heavily contaminated with moulds. The widest spectrum and the highest counts of microscopic filamentous fungi were observed in the following spices: milled black pepper, nutmeg, garlic powder and crushed caraway. The level of contamination depended upon the season, being higher in the summer months.

Keywords: fermented meat products; moulds; microscopic filamentous fungi; mycotoxins; spices

In recent decades, the question of mould toxicity has attracted attention, especially in the fields of agriculture and food industry. Microscopic filamentous fungi often contaminate vegetal and animal products, becoming a source of diseases in man and slaughter animals (LACIAKOVÁ & LACIAK 1994). The reason for an increasing interest is the ability of moulds to produce secondary metabolites – mycotoxins – that have unfavourable effects, such as carcinogenesis, mutagenicity, and high thermostability.

The conditions of the environment in the manufacturing rooms, stores, refrigerators and shops are very suitable for the development of moulds inside the products, but more frequently on the surface of various sorts of meat and meat products (JESENSKÁ 1987). According to HANSSEN (1995), the spoilage of food caused by bacteria, yeasts, and moulds is a complex process that is determined by different factors; among them the composition of food and the environmental conditions involved are of great importance. If these conditions are suitable for all three groups, then bacteria will often grow more quickly than yeasts and yeasts will grow more quickly than moulds (REISS 1986). Feeds and foods are often contaminated with various moulds. When the temperature and relative humidity are optimal after contamination, there is also a risk of mycotoxin production (ŠTYRIAK *et al.* 1998). Relatively low water activity ($a_w < 0.9$) and low pH-values (pH < 6.0) are particularly favourable for mould development (HANSSEN 1995).

There are a number of moulds that have been described as pathogens. These are particularly found in products that have ripened for a long time, such as sausages and hams. *Penicillium* species, *Aspergillus* species, and *Cladosporium* species have been identified in such products. LEISTNER (1986) and CASADO *et al.* (1991) supposed that fermented sausages are spontaneously colonized by domestic microflora. The composition and development of mycoflora depends on the type of products, processing time, and the conditions of ripening. The dominant mycoflora usually belongs to the *Penicillium* species (HWANG *et al.* 1993), but the *Aspergillus* and *Scopulariopsis* species are also prominent (GRAZIA *et al.* 1986).

The aim of this study was to determine the occurrence of moulds in the production of fermented raw meat products, starting with microbiological examination of raw materials and flavourings and finishing with final products in shops.

MATERIAL AND METHODS

In the course of one year, 70 samples of fermented raw meat products were collected at several monthly intervals. The samples were taken from meat (bacon without skin, prime beef rump steak, industrially prepared deboned ham, lean pork for production, pork flank without skin), from flavourings (garlic, crushed caraway, nutmeg, sweet paprika, hot paprika, cloves, ground pepper, commercially available nitrite salting mixture, and sugar), from salami emulsions as well as from final products (Sivec, Barčianska, Hornád, Púchov salami; and Gombasek sausage).

Ten grams were taken from each sample with the help of a sterile scalpel, and 90 ml of dilutant (physiological saline) were added to the sample in a sterile homogenizing vessel. The propeller homogenizer type K3K8 (MEZ Náchod, Czech Republic) with 10 000 rev. was used for homogenization; the homogenization time was 2.5 min. The samples of flavourings were prepared in the same way. They were homogenized in a sterile bag with the help of a Stomacher apparatus for 1–2 min.

0.2 ml of the basic dilution was spread on the surface of Sabouraud's agar using a sterile pipette. Inoculated samples were incubated at a temperature of 25°C for 5 days. Then the number of moulds was determined in 1 g of the sample, and the identification of genera was performed. The species of mould within a specific genus can be distinguished microscopically according to their characteristic morphological properties. However, this procedure requires a lot of experience and it was not included in this study.

RESULTS AND DISCUSSION

The results of this study are presented in Tables 1–4. As seen from the tables, various moulds were detected in raw material (pork and beef), salami emulsions, as well as in fermented raw meat products (Tables 1, 3 and 4). The most frequently isolated genera were *Penicillium* sp., *Acremonium* sp., *Mucor* sp., *Cladosporium* sp., and *Aspergillus* sp.

78 species of moulds have already been isolated from meat and various meat products. However, only 50 of them are reported to be toxicogenic (OSTRÝ 2001). Toxicogenic moulds are able to produce toxic metabolites known as mycotoxins. Some species produce one or more mycotoxins. On the other hand, the same type of mycotoxin can be produced by several different mould species. Since not all genera of moulds are toxicogenic, the presence of microscopic filamentous fungi in meat products does not confirm the presence of mycotoxins.

Once the production of a mycotoxin by a certain species of mould has been determined, all the strains belonging to the same species are considered to be potentially toxicogenic, i.e. they are able to produce mycotoxins under suitable conditions. According to OSTRÝ (2001), the production of mycotoxins in meat and meat products is enabled by the following factors:
– presence of oxygen,
– temperature between 4°C and 40°C,

Table 1. The occurrence of microscopic filamentous fungi in meat

Period	Species of isolated microscopic filamentous fungi	Moulds with highest frequency (g^{-1})
Summer months	*Acremonium* sp.	**Prime beef rump steak**
	Penicillium sp.	15 *Acremonium* sp.
	Cladosporium sp.	5 *Penicillium* sp.
	sterile mycelia	**Industrially prepared deboned ham**
		23 *Penicillium* sp.
		25 sterile mycelia
Winter months	*Acremonium* sp.	**Pork flank without skin**
	Penicillium sp.	20 *Penicillium* sp.
	Mucor sp.	15 *Mucor* sp.
	Aspergillus sp.	**Prime beef rump steak**
		50 *Penicillium* sp.
		10 *Acremonium* sp.
Spring months	*Penicillium* sp.	**Prime beef rump steak**
	Cladosporium sp.	12 *Aspergillus flavus*
	Aspergillus flavus	**Bacon without skin**
	Mucor sp.	25 *Penicillium* sp.
	sterile mycelia	5 *Mucor* sp.
		15 sterile mycelia

Table 2. The occurrence of microscopic filamentous fungi in flavourings

Period	Species of isolated microscopic filamentous fungi	Moulds with highest frequency (g^{-1})
Summer months	*Penicillium* sp.	**Crushed caraway**
	Cladosporium sp.	75 *Penicillium* sp.,
	Mucor sp.	30 *Mucor* sp.
	Aspergillus niger	**Garlic powder**
		305 *Penicillium* sp.
		5 *Cladosporium* sp.
		25 *Mucor* sp.
		Milled black pepper
		400 *Aspergillus niger*
		120 *Penicillium* sp.
		75 *Mucor* sp.
Winter months	*Penicillium* sp.	**Crushed caraway**
	Mucor sp.	60 *Penicillium* sp.
	Aspergillus flavus	15 *Mucor* sp.
	Aspergillus niger	**Nutmeg**
		15 *Aspergillus flavus*
		45 *Penicillium* sp.
		20 *Mucor* sp.
		Milled black pepper
		300 *Aspergillus niger*
		115 *Penicillium* sp.
		60 *Mucor* sp.
Spring months	*Penicillium* sp.	**Sugar**
	Mucor sp.	15 *Penicillium* sp.
	Aspergillus flavus	**Milled black pepper**
	Aspergillus niger	20 *Penicillium* sp.
		15 *Mucor* sp.
		15 *Aspergillus flavus*
		Nutmeg
		20 *Aspergillus niger*
		85 *Aspergillus flavus*
		45 *Penicillium* sp.
		35 *Mucor* sp.

– pH-value between 2.5 and 8 (with an optimum between 5 and 8),
– minimum water activity of 0.80,
– maximum salt concentration of 14%.

Currently, numerous studies aimed at the detection of microscopic filamentous fungi and their toxins in plants, slaughter animals and foods are carried out worldwide. ANDERSEN (1995) reported a 90% occurrence of *Penicillium* spp. and a 4% occurrence of *Aspergillus* spp. and *Mucor* spp. in fermented salami. ZAKY *et al.* (1995) found the aflatoxigenic moulds (*Aspergillus flavus* and *Aspergillus oryzae*) in luncheon meat. The results of aflatoxin analyses revealed that about 14% of the samples were positive for aflatoxins B_1 or both B_1 and G_1, while all the samples were negative for aflatoxins B_2, G_2, M_1 and M_2. CVETNIC and PEPELNJAK (1995) reported a 20% average occurrence of *Aspergillus flavus* and *Aspergillus parasiticus* in smoked meat products, pork salami and sausage, bacon and ham.

In this study we did not determine the production of mycotoxins. However, among the genera isolated from fermented raw meat products two (*Penicillium* sp. and *Aspergillus* sp.) are potentially toxicogenic. Members of the genus *Penicillium* are reported to produce the widest range of mycotoxins. Among them ochratoxin A; patulin; citrinin; citreoviridin; griseofulvin; rubratoxin; roque-

Table 3. The occurrence of microscopic filamentous fungi in salami emulsions

Period	Species of isolated microscopic filamentous fungi	Moulds with highest frequency (g^{-1})
Summer months	*Penicillium* sp. *Acremonium* sp. *Aspergillus niger* *Mucor* sp. *Cladosporium* sp. sterile mycelia	**Púchov salami** 15 *Penicillium* sp. 5 *Acremonium* sp. **Hornád salami** 125 *Penicillium* sp. **Gombasek sausage** 130 *Aspergillus niger* 50 *Mucor* sp. 45 sterile mycelia
Winter months	*Penicillium* sp.	**Hornád salami** 5 *Penicillium* sp. **Barčianska salami** 15 *Penicillium* sp.
Spring months	no microscopic filamentous fungi were detected	–

Table 4. The occurrence of microscopic filamentous fungi in final meat products

Period	Species of isolated microscopic filamentous fungi	Moulds with highest frequency (g^{-1})
Summer months	*Penicillium* sp. *Aspergillus flavus* *Cladosporium* sp. *Mucor* sp. *Absidium* sp.	**Hornád salami** 5 *Cladosporium* sp. 15 *Penicillium* sp. **Gombasek sausage and Púchov salami** 25 *Penicillium* sp. 15 *Absidium* sp. 20 *Mucor* sp. **Barčianska salami** 5 *Aspergillus flavus* 20 *Penicillium* sp. 5 *Cladosporium* sp.
Winter months	*Penicillium* sp. *Mucor* sp.	**Hornád salami** 25 *Penicillium* sp. 5 *Mucor* sp. **Barčianska salami** 20 *Penicillium* sp. 15 *Mucor* sp. **Gombasek sausage** 40 *Penicillium* sp. 15 *Mucor* sp.
Spring months	*Penicillium* sp. *Mucor* sp.	**Hornád salami** 35 *Penicillium* sp. 10 *Mucor* sp.

fortin C; penicillic, cyclopiazonic, secalonic, or mycophenolic acids are the most important. *Aspergillus flavus* produces aflatoxins and cyclopiazonic acid. The growth of *Aspergillus niger* can be accompanied by the production of ochratoxin A. Before now, the other mould isolates listed in Tables 1–4 (i.e. *Cladosporium* sp., *Mucor* sp., *Acremonium* sp., and *Absidium* sp.) have not been reported to be able to produce mycotoxins in meat and meat products (OSTRÝ & RUPRICH 2001). No information concerning the occurrence of mycotoxins in fermented raw meat products is available in the Slovak Republic.

Flavourings, especially spices, were heavily contaminated. The highest level of mould contamination was noticed in milled black pepper, garlic powder, nutmeg and crushed caraway (Table 2). The occurrence of microscopic filamentous fungi was influenced by the season; it was higher in summer months. As for spices commonly used in the meat industry, the presence of mould genera *Aspergillus*, *Penicillium*, and *Fusarium* has been detected by EL-KADY *et al.* (1995). Aflatoxins were determined in eight kinds of spices (aniseed, black pepper corns, caraway seeds, black cumin seeds, fennel seeds, peppermint leaves, coriander seeds and marjoram leaves). GARRIDO *et al.* (1992) examined 31 different spices and herbs in Spain. Moulds belonging to the genera *Aspergillus* and *Penicillium* (49.3% and 15.7%) were isolated in most of the spices. AKIYAMA *et al*. (1998) determined the presence of *Aspergillus ochraceus* and *Penicillium verrucosum* in red pepper. HÜBNER *et al*. (1998) isolated *Aspergillus ochraceus* from coriander, marjoram, white pepper, paprika, nutmeg and thyme. OLSEN *et al.* (1998) examined a number of samples of white pepper, chilli, nutmeg, and paprika. They confirmed the presence of *Aspergillus flavus* and *Aspergillus parasiticus*.

Conclusion

The spores of moulds are always present in the environment and they enable the moulds to survive even in extreme conditions. Therefore, it is practically impossible to eliminate them from food. In general, the conditions during the production of fermented raw meat products (ambient temperature, relative humidity, air circulation) are suitable for the development of microscopic filamentous fungi. Flavourings, especially spices, added to meat can also considerably contribute to the total mould contamination of final products. The main causes for the growth of moulds in fermented meat products include an inadequate process of drying resulting in a high water activity within the whole products or only on the surface of the products, as well as unsuitable storage conditions. In such cases, the spores can germinate, and the potentially toxicogenic microscopic filamentous fungi can produce toxic metabolites endangering the consumer's health. Therefore, the prevention of mould development in the meat of slaughter animals, in all the ingredients used, as well as in the manufacturing rooms, stores, and shops is of great importance in order to avoid the risk of mycotoxin production.

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Súhrn

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Sledovali sme výskyt rôznych druhov plesní vo východiskovej surovine (bravčovom a hovädzom mäse), v diele ako aj v samotných finálnych výrobkoch (päť druhov trvanlivých tepelne neopracovaných mäsových výrobkov). Z výsledkov vyplýva, že najčastejšie boli izolované plesne rodu *Penicillium*, *Acremonium*, *Mucor*, *Cladosporium* a *Aspergillus*. Prísady pridávané k surovinám počas výroby trvanlivých tepelne neopracovaných mäsových výrobkov boli masívne kontaminované plesňami. Najširšie spektrum a najvyššie koncentrácie mikroskopických vláknitých húb boli zistené u mletého čierneho korenia, muškátového orecha, cesnakového prášku a drvenej rasce. Úroveň kontaminácie bola rozdielna v závislosti od ročného obdobia, pričom vyššia bola v letných mesiacoch.

Kľúčové slová: trvanlivé mäsové výrobky; plesne; mikroskopické vláknité huby; mykotoxíny; korenie

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