Fungal Contamination of Cookies and the Raw Materials for their Production in Croatia

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Abstract

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The study examined fungal loads in different varieties of cookies, as well as in the raw materials used for their production in Croatia. The mean presence of various fungi in the final products and most of the raw materials was within acceptable levels. A higher contribution of major mycotoxigenic molds was detected in flour which makes cookies more susceptible to the accumulation of mycotoxins.

Keywords: cookies; molds; mycotoxins

Cookies are among the most important snack foods in the developed countries (Subar *et al.* 1998a,b; Alberti-Fidanza *et al.* 1999). Due to the peculiarities of the production of some brands of cookies (e.g. short baking time, no thermal processing of the filling in gourmet cookies, etc.), they could present a particularly favourable medium for the growth of a wide range of microorganisms. This study focused on the level of contamination of cookies and raw materials with molds, with an accent on *Aspergillus flavus* as the most important aflatoxin producer.

MATERIALS AND METHODS

Samples of different cookie varieties were taken directly from the processing plant of a local manufacturer, prior to packaging and storage. Raw materials were sampled at the storage facility. A total of 153 samples were analysed:

1. Cookies (75 samples): A – Gourmet cookies (filled products and piscottes) (56 samples); B – Commodity cookies, party cookies, cut-outs, and decorated cookies (19 samples)

2. Flour (58 samples) – wheat flours with three different ash contents (0.3–0.4%, 0.45–0.55%, and 0.8–0.9% ash)

3. Other raw materials (20 samples): cocoa powder, powdered chocolate, soya flour, roasted coffee beans, hazelnuts, skimmed milk powder, roasted peanuts, poppy seeds, corn meal, sugar, walnuts.

Microbiological investigations were performed using standard laboratory methods consistent with the respective Croatian regulations on microbiological analyses of food (Službeni list RH 1980). The determination of the total microbial contamination of the samples was performed using the Koch method of agar plates. From each sample, 20 g was weighed into an Erlenmeyer flask and 180 cm^3 of sterile physiologic solution was added (dilution 10^{-1}). According to the expected contamination, a suitable dilution was further applied. After incubation (7–14 days at 28°C), the grown colonies were counted and expressed per 1 g of sample. The determination of the mold genera was carried out according to the procedures based on the morphological characteristics, reproduction

type, development cycle, etc., as described in Gilman (1959), Raper and Fenell (1965), Fassatiova (1986), Pitt and Hocking (1997) and Duraković and Duraković (2000). All the analyses of the samples were performed at least in duplicates.

RESULTS AND DISCUSSION

Considering the established range of the mold colonies in the samples of cookies (Table 1), the conclusion of a relatively low level of the mold contamination in the varieties examined can be drawn. These mold loads are well below the Croatian regulated margin of 10^2 mold colonies per gram (Službeni list RH, 1994). The expected tendency towards a greater mold presence was found with the filled products. The mold contamination is also important in view of the possible mycotoxin production by a great number of mold species (Hussein & Brasel 2001). Hepatotoxic, immunotoxic, and carcinogenic aflatoxins are the most important mycotoxins found in wheat flour and they are synthesised mainly, by a limited number of *Aspergillus flavus* and *Aspergillus parasiticus* strains, but also by some other fungi (Hussein & Brasel 2001).

The fungal genera that contributed most to the total mold counts of cookies were *Penicillium* (18%), *Mucor* (16%), *Aspergillus* (13%), *Cladosporium* (2%), and *Alternaria* (2%) (Table 2). The species *Aspergillus flavus* was not detected in any of the samples of cookies analysed (Table 1). The absence of this or any other mycotoxin-producing species does not preclude certain levels of aflatoxins/mycotoxins in the samples as these could have accumulated in the raw materials before the destruction of molds during the production process. Out of the six samples positive in *Aspergillus*, three contained *Aspergillus versicolor* which is a potential sterigmatocystin producer (Pitt & Hocking 1997; Duraković & Duraković 2000). The genus *Penicillium* is another group of mycotoxigenic fungi (Pitt & Hocking 1997; Hussein & Brasel 2001), and the species of this genus were the most common in the analysed samples of cookies (Table 2). It must also be emphasised that almost half of the mold

Table 1. Contamination of cookies and raw materials with molds

	n	Number of colonies per g	*Aspergillus flavus* positive (%)
Cookies	75	0–50	0
Flour	58	0–12.3 × 10^3	38
Other materials	20	0–300	5

Table 2. Contribution of different genera to total mold contamination of cookies and raw materials

Mold genus	Number of mold isolates		
	cookies	flour	other materials
Mucor	7	23	1
Rhizopus	0	4	0
Absidia	0	5	2
Penicillium	8	1205	66
Aspergillus	6	812	12
Cladosporium	1	20	0
Fusarium	0	6	0
Alternaria	1	21	3
Trichoderma	0	2	4
Nonidentified	22	48	30
Total	45	2146	118

isolates (49%) could not be placed into any of the main mold genera (Table 2), with an unknown mycotoxigenic impact on the final product.

On average, the flour samples had higher mold counts as compared to cookies and other raw materials (Table 1). The established levels of contamination with molds are appropriate in view of the valid Croatian regulations (Službeni list RH, 1994), with the exception of one semi-white wheat flour sample with the mold count just above the allowed upper margin of 10^4 colonies per gram of flour. The predominant mold genera in flour were *Penicillium* (56% of all isolated mold colonies), *Aspergillus* (38%), *Mucor* (1%), *Alternaria* (1%), while *Cladosporium*, *Fusarium*, *Absidia*, *Rhizopus*, and *Trichoderma* contributed less than 1% to the total mold count (Table 2).

Aspergillus flavus was determined in 38% of all flour samples analysed (Table 1). Additionally, minor presence of some other *Aspergillus* species was detected, e.g. *Aspergillus versicolor*, which is a potential sterigmatocystin producer (Duraković & Duraković 2000). Ochratoxin-producing *Aspergillus ochraceus* was determined in one flour sample. Ochratoxins are also secondary metabolites of some *Penicillium* species in temperate climates, and they were found to be hepatotoxic, nephrotoxic, teratogenic, immunotoxic, and carcinogenic for both domestic and laboratory animals (Hussein & Brasel 2001). The species *Aspergillus niger* was determined in two flour samples. According to some authors (Škrinjar 1990), this species is also capable of aflatoxin biosynthesis. *Aspergillus clavatus* was found in five flour samples; this mold produces the teratogenic and carcinogenic patulin. The samples were significantly contaminated (41 flour samples) with *Aspergillus albus*, a producer of the nephrotoxic citrinin. The isolated *Rhizopus* and *Trichoderma* species are also mycotoxigenic, as well as the *Fusarium* species which are potential producers of trichothecenes, zearalenone, and fumonisins (Duraković & Duraković 2000; Hussein & Brasel 2001).

Other raw materials, potential introducers of mold and mycotoxin contamination into cookies, were also examined here, and these were, in general, less contaminated with molds than the flour samples (Table 1). They also met the requirements of the current Croatian regulations (Službeni list RH 1994). The most frequent mold genera in these products were *Penicillium* (56%), *Aspergillus* (10%), *Trichoderma* (3%), and *Alternaria* (3%), with minor presence of *Absidia* and *Mucor* (Table 2). *Aspergillus flavus* was isolated only from the samples of corn meal. *Aspergillus albus* was another dominant species in the samples of the raw materials analysed. The prevalent presence of mycotoxigenic *Aspergillus* and *Penicillium* species could indicate some level of the relevant mycotoxin contamination.

The present results show a tendency for a higher contribution of mycotoxigenic molds through the analysed samples of wheat flour. However, this does not automatically imply analogous mycotoxin contents in cookies since, besides controllable environmental, i.e. storage conditions, several other factors affect the production of mycotoxins, e.g. strain specificity, strain variation, climate, etc. (Hussein & Brasel 2001). The current Croatian regulations of mycotoxin (i.e. only aflatoxin) contamination are limited to flour, nuts, roasted coffee beans, and cocoa before marketing (Službeni list RH 1994). However, any subsequent inappropriate storage and handling of flour and other raw materials, as well as of cookies after baking, may lead to the mold growth and the accumulation of mycotoxins in the final products. The increasing role of cookies in the Western diet warrants a necessity of a more thorough monitoring of their mycotoxin contents.

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Souhrn

Halt M., Klapec T., Šubarić D., Macura M., Bačani S. (2004): **Plísňová kontaminace trvanlivého pečiva a surovin pro jejich výrobu v Chorvatsku**. Czech J. Food Sci., **22**: 95–98.

Sledovali jsme množství plísní v různých druzích trvanlivého pečiva a v surovinách používaných k jejich výrobě v Chorvatsku. Průměrné množství plísní v konečných výrobcích a ve většině surovin bylo v povolených mezích. Vyšší přítomnost významných toxikogenních plísní byla zjištěna v mouce. Tato okolnost způsobuje větší náchylnost trvanlivého pečiva k akumulaci mykotoxinů.

Klíčová slova: trvanlivé pečivo; plísně; mykotoxiny

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