# MICROBIAL ANALYSIS OF THE MEAT PRODUCTS FOCUSED ON THE OCCURRENCE OF MICROSCOPIC FILAMENTOUS FUNGI

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## SUMMARY

*The study is focused on the microbial analysis of selected soft, heat-processed meat products and raw materials used for their production. A special attention has been paid to the isolation and identification of microscopic filamentous fungi. Five samples (from the surface of beef cuts, ground beef, ground pork, salami emulsion, and final product) were taken each month within a period of one year. The microbial investigation was aimed at the determination of total plate counts, the number of coliforms, Staphylococcus aureus, the presence of Salmonella spp., and the number of moulds. The highest level of microbial contamination (total plate count, counts of coliforms and Staphylococcus aureus) was observed in both kinds of ground meat during summer months. The presence of Salmonella was not determined in any sample. The highest number of microscopic filamentous fungi was found in samples of salami emulsion in summer months. Toxicogenic genera (Aspergillus spp., Penicillium spp., and Fusarium spp.) have also been identified.*

***Key words:*** *meat products, microbial contamination, toxicogenic moulds*

## INTRODUCTION

Raw materials, as well as final meat products are exposed to a high risk of microbial contamination at the time of their production, processing, storage and distribution. Chemical composition of food, properties of the outside environment, and specific growth requirements determine the type of microorganisms and the course of physico-chemical reactions in the contaminated food. Foodstuffs, in general, represent an ideal medium for the persistence and multiplication of toxicogenic filamentous fungi which posses the ability to produce mycotoxins under suitable conditions. More than 64 000 moulds, yeasts, and yeast-like organisms have been found in the environment. Among them, 114 mould and 12 yeast species are of use in the food industry. Sixty-five species of 114 reported are able to synthetize more than 150 kinds of mycotoxins. As to meat and meat products, 78 mould species have already been isolated, 50 of them being potentially toxicogenic. Food contaminated with toxicogenic microscopic filamentous fungi are considered to be a reservoir of the so-called “hidden mycotoxins” (Ostrý i Ruprich, 2001). Therefore, an increased interest in both the quantitative and qualitative microbial examination of the meat products has been noticed worldwide (Leistner, 1986; Jesenská, 1987; Ostrý i Ruprich, 2001).

Meat surface is usually heavily contaminated with a wide range of microorganisms. Due to its beneficial chemical composition (the content of water, proteins, peptides, amino acids, nucleotides, sugars, minerals and vitamins), the meat is a suitable medium for the development of all microorganisms (Steinhauser et al., 1995). Besides various G-negative (*Escherichia* spp., *Enterobacter* spp., *Yersinia* spp., and *Pseudomonas* spp.) and G-positive bacteria (*Bacillus* spp., *Micrococcus* spp. and *Lactobacillus* spp.), psychrotrophic moulds (*Aspergillus* spp., *Cladosporium* spp., *Geotrichum*

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▼ **Table 1.** General characteristics of mould development and the production of mycotoxins in meat and meat products (Ostrý, 2001)
▼ **Tablica 1.** Opće značajke razvitka plijesni i stvaranja mikotoksina u mesu i mesnim prerađevinama (Ostrý, 2001)

| Factors<br>Čimbenici | Growth of moulds<br>Rast plijesni | Production of mycotoxin<br>Stvaranje mikotoksina |
|---|---|---|
| Temperature | from – 12 to 55 ºC | from +4 to +44 ºC |
| pH- value | from 1.7 to 10 | from 2,5; optimum between 5 and 7 |
| Available water, aW | min. of 0.62 | min.of 0.8 – 0.85 |
| Redox potential | aerobic conditions | aerobic conditions |
| Addition of salt, NaCl | up to 20 % NaCl | up to 14 % NaCl |
| Influence of spices<br>Utjecaj začina<br>(eugenol, anethol, thymol ) | inhibition<br>inhibicija | inhibition<br>inhibicija |

spp., *Mucor* spp. and *Rhizopus* spp.) are frequently isolated from the meat surface (Polster et al., 1985). General characteristics and conditions for both the development of moulds and the production of mycotoxins in the meat and meat products are reported in the following table (Ostrý, 2001):

It is evident, that both variability and adaptability of moulds make practically impossible to set some general and stable conditions for their development in food, as well as for the production of mycotoxins. The situation of the meat-processing plants, the slaughter of animals, insufficient cleaning and disinfection of working areas, instruments and other equipment are the most important sources of food contamination by toxicogenic moulds.

As reported by many authors (Wu et al, 1974; Zaky et al., 1974; Jesenská, 1987; Berwal and Dinchev, 1991), numerous strains of microscopic filamentous fungi, isolated from the surface of various meat products, show in vitro the ability to produce toxic substances. In our study (Tab. 2, 3) the most frequently isolated mould genera were as follows: *Penicillium* spp., *Mucor* spp., and *Rhizopus* spp. However, some strains of *Aspergillus flavus* and *Fuzarium moniliforme*, known as potential producers of mycotoxins, have also been isolated.

Currently, there is not enough information about the contamination of food with toxicogenic fungi. Therefore, the aim of this study was to perform microbial analysis of the selected soft heat-processed meat products and raw materials used for their production. All samples were inspected for the presence and counts of different mould genera.

## MATERIAL AND METHODS

In the course of one year, five samples have been taken each month from the surface of beef, ground beef, ground pork, salami emulsion in a beef/pork ratio 20/80% and soft heat-processed meat products. In each of 60 samples, the following parameters were determined: total plate count, the number of coliforms, the presence of *Salmonella* spp., the number and genera of microscopic filamentous fungi.

Ten grams of the sample was diluted with 90 ml of physiological saline and homogenized using the propeller homogenizer (MEZ Náchod, The Czech Republic) at 10 000 rpm for 2.5 min. Decimal dilutions of the sample were further prepared in accordance with the government regulation STN ISO 560102. Specific microbiological investigations followed the procedures set by the following government standards: total plate count - STN ISO 4833, the count of coliforms - STN ISO 4832, the count of *Staphylococcus* aureus - STN ISO 6888, the presence of *Salmonella* spp. - STN ISO, the count of moulds - STN ISO 7954.

## RESULTS AND DISCUSSION

The results of this study are shown in Tables 2 and 3. As seen from both tables, the highest level of microbial contamination was observed in

summer months, when the total plate counts ranged from $10^5$/g to $10^8$/g of the sample. These numbers exceeded limits set by the Slovak *Codex Alimentarius* for soft, heat-processed meat products. As to coliforms, their counts were also significantly higher during summer. On the other hand, the presence of *Staphylococcus aureus* was found in summer, autumn, and winter months in samples of ground beef and pork (Tab.2). The maximum values set by the Slovak *Codex Alimentarius* were exceeded in one sample. The incidence of microscopic filamentous fungi showed the maximum during summer months. The presence of *Salmonella* spp. was not determined in any of samples inspected.

Our results are comparable to those reported in the literature (Andersen, 1995) reffering about the 90 % occurrence of *Penicillium* spp. and the 4 % occurrence of both *Aspergillus* spp. and *Mucor* spp. in raw fermented meat products. The presence of aflatoxin-producing fungi (*Aspergillus flavus* and *Aspergillus oryzae*) was reported in lunch meat. An average incidence of 20 % was reported for

**Table 2.** The average counts of micro-organisms in 1 gram of samples
**Tablica 2.** Prosječni broj mikroorganizama u gramu uzorka

| Period<br>Razdoblje | Samples<br>Uzorak | TPC*<br>UBB* | Coliforms<br>Koliformni mikroorganizmi | *Salmonella* spp. (25g) | Staph. aureus | Moulds<br>Plijesni |
|---|---|---|---|---|---|---|
| Spring months<br>Proljetni mjeseci | 1 | $3.8x10^4$ | 0 | 0 | 0 | 0 |
| | 2 | $2.2x10^7$ | $2.2x\ 10^4$ | 0 | 0 | 0 |
| | 3 | $4.2x10^5$ | $6.2x10^5$ | 0 | 0 | 0 |
| | 4 | $6.4x10^5$ | $6.3x10^2$ | 0 | 0 | 0 |
| | 5 | $3.8x10^5$ | $1.9x10^2$ | 0 | 0 | $7.5x10^1$ |
| Summer months<br>Ljetni mjeseci | 1 | $4.2x10^5$ | 0 | 0 | 0 | $1.5x10^1$ |
| | 2 | $1.9x10^8$ | $1.0x10^5$ | 0 | $2.8x10^4$ | $4.5x10^1$ |
| | 3 | $9.8x10^7$ | $8.2x10^6$ | 0 | $2.2x10^3$ | $5.0x10^1$ |
| | 4 | $9,2x10^6$ | $6.7x10^4$ | 0 | 0 | $4.0x10^2$ |
| | 5 | $4.3x10^5$ | $3.2x10^2$ | 0 | 0 | $1.5x10^2$ |
| Autumn months<br>Jesenski mjeseci | 1 | $2.5x10^3$ | 0 | 0 | 0 | 0 |
| | 2 | $3.1x10^6$ | $8.5x10^4$ | 0 | $1.4x10^2$ | $2.5x10^1$ |
| | 3 | $1.3x10^5$ | $2.1x10^4$ | 0 | $2.2x10^3$ | $4.3x10^1$ |
| | 4 | $2.3x10^5$ | $2.5x10^2$ | 0 | 0 | $2.2x10^1$ |
| | 5 | $2.89x10^5$ | 0 | 0 | 0 | 0 |
| Wintermonths<br>Zimski mjeseci | 1 | $1.8x10^2$ | 0 | 0 | 0 | $6.0x10^1$ |
| | 2 | $1.9x10^6$ | $1.8x10^4$ | 0 | $5.0x10^3$ | $8.5x10^1$ |
| | 3 | $2.8x10^5$ | $3.2x10^3$ | 0 | $6.5x10^3$ | $2.5x10^1$ |
| | 4 | $5.4x10^5$ | $9.2x10^3$ | 0 | 0 | $3.5x10^1$ |
| | 5 | $1.9x10^4$ | 0 | 0 | 0 | $1.2x10^1$ |

Legends: 1 - the surface of beef; 2 - ground beef; 3 - ground pork; 4 - salami emulsion; 5 - soft heat processed meat product
Legenda: 1 - površina govedine; 2 - mljevena govedina: 3 - mljevena svinjetina; 4 - emulzija za proizvodnju salame; 5 - toplinski obrađeni mesni proizvodi; * UBB = Ukupni broj bakterija

*Aspergillus flavus* and *Aspergillus parasiticus* in smoked meat products, pork salami, bacon and ham (Cvetnic et Pepelnjak, 1995). As seen from our results, one must consider that any uncontrollable development of microscopic filamentous fungi in food is undesirable, because it can result in the development of superficial, profound or systemic mycosis, or even mycoalergy.

The presence of moulds in the meat products usually causes a decrease in their biological value (due to the enzymatic degradation of meat components). Moulds often come into metabolic interactions with various bacterial pathogens. Thus, they can participate in an outbreak of food-borne illness. These interactions have already been well documented between moulds and *Clostridium botulinum* or *Staphylococcus aureus* (Polster et al, 1985). Mould metabolic activity results in the neutralization of organic acids, which is accompanied by an increase in pH-value. Under such conditions, the spores of *Clostridium botulinum* are able to germinate and to start with the production of botulinum toxin. Less acidic environment also enables the formation of enterotoxins by *Staphylococcus aureus*.

The development of microscopic filamentous fungi in the meat products must not be negliged. Moulds must be studied and identified permanently. Food producers must follows the principles of good manufacturing practice and take preventive measures in order to reduce the growth of microscopic filamentous fungi and the production of their toxic metabolites in the final products (Čonková et al., 1994).

## CONCLUSIONS

Raw food materials, as well as final products can become contaminated in any stage of their processing, handling and distribution. Foodstuffs represent the best substrate for growth and multiplication of toxicogenic microscopic fungi, which presence can be accompanied by the production of mycotoxins. Based upon the results of this study it can be concluded, that the highest level of microbial contamination (total plate count, the number of coliforms and *Staphylococcus aureus*) was observed in samples of ground beef and pork during summer months. As to microscopic filamentous fungi, the presence of potential mycotoxin producers (*Aspergillus flavus* and *Fuzarium moniliforme*) has also been confirmed in summer months. Therefore, a special attention should be paid to the microbial investigation of ground meat in summer. It must be considered that any uncontrollable development of microscopic filamentous fungi in food can endanger consumer's health by the development of superficial, profound or systemic mycosis, or even mycoalergy.


## SAŽETAK

### NALAZ GLJIVICA TIJEKOM MIKROBIOLOŠKE PRETRAGE MESNIH PROIZVODA

*U radu su prikazani rezultati mikrobioloških analiza odabranih toplinski obrađenih mesnih proizvoda te sirovina u njihovoj proizvodnji. Posebna je pozornost obraćena izolaciji i identifikaciji mikroskopskih nitastih gljivica. Tijekom jednogodišnjega razdoblja svaki je mjesec uzimano pet uzoraka (s površine goveđih trupova, mljevena govedina, mljevena svinjetina, emulzija za proizvodnju salame i finalni proizvod). Cilj mikrobiološke pretrage bio je utvrditi ukupan broj mikroorganizama, broj koliformnih mikroorganizama, Staphylococcus aureus, prisutnost salmonela te broj plijesni. Najviša razina mikrobne kontaminacije (ukupan broj mikroorganizama, broj koliformnih mikroorganizama i S. aureus) uočena je u objema vrstama mljevena mesa tijekom ljetnih mjeseci. Ni u jednom uzorku salmonele nisu izolirane. Najveći broj gljivica utvrđen je u uzorcima emulzije za proizvodnju salame u ljetnim mjesecima. Izdvojeni su i toksogeni rodovi (Aspergillus spp., Penicillium spp. i Fusarium spp.).*


***Ključne riječi:*** *mesne prerađevine, mikrobna kontaminacija, toksogene plijesni*

▼ **Table 3.** The species of microscopic filamentous fungi isolated

▼ **Tablica 3.** Vrste izdvojenih nitastih gljivica

| Period<br>Razdoblje | The species of mould<br>Vrste plijesni |
|---|---|
| Spring months<br>Proljetni mjeseci | *Penicillium* spp., *Mucor* spp. |
| Summer months<br>Ljetni mjeseci | *Penicillium* spp., *Aspergillus flavus, Cladosporium* spp., *Mucor* spp., *Absidium* spp., *Fuzarium moniliforme* |
| Autumn months<br>Jesenski mjeseci | *Penicillium* spp., *Mucor* spp., *Rhizopus* spp., *Scopulariopsis* spp. |
| Wintermonths<br>Zimski mjeseci | *Penicillium* spp., *Rhizopus* spp., *Mucor* spp. |

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# MALENI LOVAČKI SPECIJALITETI - TRČKA SKVRŽULJA (PERDIX PERDIX L.)

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## SAŽETAK

*Trčka je divljač iz skupine poljskih koka. Na teritoriju Republike Hrvatske proširena je po ravničarskim dijelovima unutrašnjosti, Istre i Dalmacije. S obzirom na način prehrane trčke predstavljaju korisnu divljač i ne uzrokuju štete na ratarskim usjevima. Kako bi ih sačuvali u svojim lovištima lovci su osmislili posebne načine prihrane tijekom zime, a u skladu s njihovim načinom ponašanja. Osim toga postoje brojne odrednice u duhu lovačke etike o načinu lova koji će najmanje škoditi populaciji. Trčke se love potragom sa psom, prigonom i pogonom. Prije same kulinarske obrade uputno je ocijeniti dob odstrijeljenih trčki te starije prethodno kuhati. Velik broj recepata za pripremu trčaka osigurava užitak u ovoj cijenjenoj deliciji.*

***Ključne riječi:*** *trčka, divljač, korist, delicija*

## IZGLED I NAČIN ŽIVOTA

Lovci su trčku skvržulju (*Perdix perdix*) zajedno s prepelicama (*Coturnix* spp.), jarebicama (*Alectoris*

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