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Effect of Some Food Preservatives on the Lipolytic Activity of Beef Luncheon Fungi

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Beef luncheon meat is one of the most popular meals in several countries in the world including Egypt. Thirty one fungal species and 3 species varieties were recovered from 30 samples of beef luncheon meat collected from different supermarkets in Qena. *Alternaria*, *Aspergillus*, *Emericella*, *Mucor*, *Mycosphaerella*, *Penicillium* and *Rhizopus* were the most common genera on the two types of media. From the above genera, the most prevalent species were *Alternaria alternate*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Emericella nidulans*, *Mucor racemosus*, *Mycosphaerella tassiana*, *Penicillium chrysogenum* and *Rhizopus stolonifer*. Screening of fungi for their abilities to produce lipase enzyme showed that, ten isolates represented 32.26% of total isolates appeared high lipase production, while sixteen isolates (51.61%) were moderate and 5 isolates (16.13%) were low producers. *Aspergillus niger*, *Fusarium oxysporum* and *Nectria haematococca* produced the highest amount of lipase enzyme, so these fungi were used in further studies. The incorporation of five food preservatives (Disodium phosphate, sodium benzoate, citric acid, potassium sorbate and sodium citrate) individually in the culture medium of lipase production exhibited an inhibitive effect on the mycelial growth and enzyme production by *Aspergillus niger*, *Fusarium oxysporum* and *Nectria haematococca*.

KEYWORDS : Beef luncheon fungi, Food preservatives, Lipase

Meat is a rich nutrient matrix that provides a suitable environment for proliferation of meat spoilage microorganisms and common food-borne pathogens, therefore adequate preservation technologies must be applied in order to preserve its safety and quality (Aymerich *et al*., 2008). Microscopic filamentous fungi often contaminate vegetal and animal products, becoming a source of diseases for human and slaughter animals (Laciakova and Laciak, 1994). The conditions of the environment in the manufacturing rooms, stores, refrigerators and shops sometimes are very suitable for the development of moulds inside the products, more frequently on the surface of various sorts of meat and meat products (Jesenska, 1987). Feeds and foods are often contaminated with various 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 (Andersen, 1995). *Aspergillus*, *Cladosporium* and *Penicillium* 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 *Aspergillus*, *Scopulariopsis* and *Penicillium* species (Grazia *et al*., 1986; Hwang *et al*., 1993). Recently, Mizakova *et al.* (2002) studied the occurrence of mould in pork and beef used as raw materials, in salami emulsions as well as in five kinds of fermented raw meat products. *Penicillium*, *Acremonium*, *Mucor*, *Cladosporium* and *Aspergillus* were the most frequently isolated genera of mould.

Lipases (triacylglycerol acylhydrolases) catalyze the hydrolysis and the synthesis of esters formed from glycerol and long chain fatty acids. Microbial lipases are commercially significant. The applications of lipases include organic synthesis, hydrolysis of fats and oils, modifications of fats, flavor enhancement in food processing, resolution of racemic mixtures and chemical analysis (Martinelle *et al*., 1995; Sharma *et al*., 2001). In eukaryotes, lipases are involved in various stages of lipid metabolism including fats digestion, absorption, reconstitution and lipoprotein metabolism (Balashev *et al*., 2001). Because of their wide ranging significance, lipases remain a subject of intensive study (Alberghina *et al*., 1991; Bornscheuer, 2000).

To prevent the development and spread of spoilage and pathogenic microorganisms via meat foodstuffs, antimicrobial packaging materials could be a potential alternative solution (Quintavalla and Vicini, 2002; Coma, 2008). The most common antimicrobials used by researchers are the various organic acids. Organic acids have characteristic sensitivities to microorganisms, sorbic acids and sorbates are very strong antifungal agents (Han, 2005).

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Acetic acid, lactic acid or potassium benzoate leads to a reduction of *Listeria monocytogenes* populations in smoked sausage (Geornaras *et al.*, 2006; Coma, 2008). This article was conducted to determine the occurrence and distribution of moulds in beef luncheon meat as well as the effect of five food preservatives on the activity of fungal lipases and biodegradation of food.

Materials and Methods

Collection of beef luncheon samples. Thirty beef luncheon meat samples were collected randomly from different supermarkets in Qena during the period from May to July 2007. The samples were collected in sterilized polyethylene bags and transferred directly to the Mycological laboratory for fungal analysis.

Determination of beef luncheon fungi. The baiting technique was used for determination of fungi on two types of media, Glucose-Czapek's and Malt extract agar at 25°C. Beef luncheon meat was cut into equal segments (1 cm^2 each). Four segments were placed on the surface of the agar medium in each plate. Five replicates were prepared for each sample and each type of cultivation media. Cultures were incubated at 25°C for 7 days. At the end of the incubation period, the developing fungi were counted, identified and calculated/20 segments.

Media used for isolation of fungi. Glucose-Czapek's agar medium (g/*l*; sodium nitrate, 3.0; potassium dihydrogen phosphate, 1.0; Magnesium sulphate, 0.5; potassium chloride, 0.5; ferrous sulphate, 0.01; glucose, 10.0; agar 15.0) was used for isolation of glucophilic fungi. Malt extract agar medium is favorable for the isolation of different genera and species of zygomycetes with chemical composition: 25.0 g Malt extract and 15.0 g agar per liter. Chloramphenicol (0.5 mg/*ml*) was used as bacteriostatic agent (Al-Doory, 1980).

Screening of fungi for lipase production. The lipolytic activity was measured using tributyrin as a lipid substrate (Cardenas *et al.*, 2001). The medium used contained (g/*l*) peptone, 10 g; Mg $SO_4 \cdot 7H_2O$, 2 g; $CaCl_2 \cdot 2H_2O$, 0.2 g; tributyrin emulsion, 10 (w/v); agar, 15 g with pH 6. Tributyrin was sterilized separately by autoclaving for 15 minutes at 1.5 atm and 1 *ml* was added to 100 *ml* of sterile basal medium before solidification. Using a sterile cork borer (10 mm diameter) inoculum discs bearing myclelia from a previously prepared agar culture were obtained. Discs in triplicates were placed in cavities made in the assay agar medium. The cultures were incubated for 48 hours at 25°C. The diameter of clear zone around each disc was measured and the average was calculated in mm.

Effect of food preservatives on lipase production. *Aspergillus niger*, *Fusarium oxysporum* and *Nectria haematococca* were selected since these species were found to be active lipase producers. Fifty ml aliquots of modified Czapek's liquid medium (g/*l*: $NaNO_3$, 3.0; KH_2PO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; KCl, 0.5; $FeSO_4$ 0.01 and Olive oil emulsion, 10 *ml*) were individually dispensed in 250 *ml* Erlenmeyer conical flasks. PH of the medium was adjusted to 6.5 using citrate buffer. The flasks were autoclaved for 15 minutes at 1.5 atm. Five types of food preservatives (sodium citrate, potassium sorbate, disodium phosphate, citric acid and sodium benzoate) which are most common in food industries in Egypt, were added under aseptic conditions to the sterilized liquid medium at rates of 100, 200, 400 and 800 ppm. Media without preservatives served as control. Each flask was inoculated with two agar mycelial discs (10 mm diameter) obtained from 7 days old cultures. Inoculated flasks were incubated for 7 days at 25°C, then filtered. The filtrate was centrifuged and the clear supernatents were used for assaying of lipase activity. Mycelial dry weights of the tested fungi were also determined.

Assay for lipase activity. The method described by Mateos Diaz *et al.* (2006) was employed. This method was designed to calculate the amount of free fatty acids (FFA) in m mole liberated by the enzyme action. 10 ml of olive oil emulsion solution (0.1% olive oil) was diluted by 20 *ml* distilled water and added to 0.25 *ml* of sodium taurocholate and 5 ml of the crude enzyme (fungal filtrate), pH was adjusted to 8.8. After 20 minutes of incubation at 30°C with shaking, the mixture titrated with 0.05 M NaOH until pH 8.8 (the original pH). The consumed volume of NaOH was determined and the volume activity calculated. Samples with 5 *ml* distilled water (replaced the enzyme solution) was used as a blank.

Results and Discussion

Thirty one fungal species and 3 species varieties were recovered from 30 samples of beef luncheon meat on glucose-Czapek's (18 Genera + 30 species + 3 variety) and Malt extract (19 Genera + 31 species + 3 variety) agar at 25°C. *Alternaria* (1 species), *Aspergillus* (8+2 var.), *Emericella* (1 + 1 var.), *Mucor* (2), *Mycosphaerella* (1), *Penicillium* (5) and *Rhizopus* (1) were the most common genera on the two types of media. From the above genera, the most prevalent species were *Alternaria alternate*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Emericella nidulans*, *Mucor racemosus*, *Mycosphaerella tassiana*, *Penicillium chrysogenum* and *Rhizopus stolonifer* (Table 1). These species were isolated with different numbers and frequencies from various types of meat products in different places of the world by several workers (Grazia *et*

Table 1. Total counts (TC, calculated per 20 segments in all samples), number of cases of isolation (NCI, out of 30), occurrence remarks (OR) and the relative importance values (RIV) of fungal genera and species recovered from beef luncheon meat on glucose-Czapek's and Malt extract agar at 25°C

Fungal genera & species	Glucose-Czapek's agar			Malt extract agar		
	TC	NCI & OR	%	TC	NCI & OR	%
Acremonium strictum	2	2 R	0.25	4	2 R	0.52
Alternaria alternata	43	17 H	5.35	27	14 M	3.52
Aspergillus	469	30 H	58.33	466	30 H	60.68
A. flavus	96	27 H	11.94	93	28 H	12.11
A. flavus var. *columnaris*	11	4 L	1.37	8	3 R	1.04
A. fumigatus	137	25 H	17.04	118	23 H	15.36
A. niger	157	29 H	19.53	180	28 H	23.44
A. ochraceus	10	6 L	1.24	4	3 R	0.52
A. sydowii	3	2 R	0.37	6	6 L	0.78
A. tamarii	6	3 R	0.75	7	3 R	0.91
A. terreus	35	10 M	4.35	43	11 M	5.60
A. terreus var. *aureus*	7	3 R	0.87	4	1 R	0.52
A. ustus	7	3 R	0.87	3	1 R	0.39
Cladosporium cladosporioides	9	3 R	1.12	9	3 R	1.17
Cochliobolus spicifer	7	3 R	0.87	5	2 R	0.65
Cunninghamella echinulata	--	--	--	2	1 R	0.26
Curvularia ovoidea	2	1 R	0.25	2	1 R	0.26
Emericella	35	15 H	4.35	25	11 M	3.26
E. nidulans	28	13 M	3.48	21	10 M	2.73
E. nidulans var. *lata*	7	4 L	0.87	4	2 R	0.52
Epicoccum nigrum	8	7 L	1.00	5	4 L	0.65
Fusarium oxysporum	7	4 L	0.87	4	2 R	0.52
Gibberella fujikuroi	10	5 L	1.24	2	2 R	0.26
Humicola grisea	2	1 R	0.25	1	1 R	0.13
Mucor	18	11 M	2.24	42	11 M	5.47
M. hiemalis	10	5 L	1.24	8	4 L	1.04
M. racemosus	8	6 L	1.00	34	10 M	4.43
Mycosphaerella tassiana	75	16 H	9.33	54	16 H	7.03
Nectria haematococca	3	2 R	0.37	6	4 L	0.78
Penicillium	85	21 H	10.57	73	22 H	9.50
P. camemberti	3	2 R	0.37	3	2 R	0.39
P. chrysogenum	62	16 H	7.71	54	18 H	7.03
P. duclauxii	2	1 R	0.25	3	2 R	0.39
P. funiculosum	7	3 R	0.87	10	4 L	1.30
P. oxalicum	8	3 R	1.00	4	2 R	0.52
Phoma herbarum	3	2 R	0.37	4	2 R	0.52
Rhizopus stolonifer	24	8 M	2.99	35	10 M	4.56
Stachybotrys chartarum	2	1 R	0.25	2	1 R	0.26
Total counts	804	100%		768	100%	
Number of genera	18			19		
Number of species	30 + 3 varieties			31 + 3 varieties		

H = High occurrence, 15~30, M = Moderate occurrence, 8~14, L = Low occurrence, 4~7, R = Rare occurrence, 1~3.

al., 1986; Casado *et al*., 1991; Hwang *et al*., 1993; Mizakova *et al*., 2002). Recently, Mizakova *et al*. (2002) isolated 78 species of moulds from meat and various meat products. The most frequently isolated genera were *Penicillium*, *Acremonium*, *Mucor*, *Cladosporium* and *Aspergillus*. Most of them are reported to be toxicogenic (Zaky *et al*., 1995; Ostry, 2001; Mizakova *et al*., 2002).

Screening of fungal isolates for their abilities to produce lipase enzyme. Thirty one fungal isolates representing 16 genera, 28 species and 3 varieties were tested for their ability to produce lipase enzyme. The result obtained indicated that, all fungi tested are able to produce lipase enzyme, but with variable degrees (Table 2). In this respect, ten isolates represented 32.26% of total

Table 2. Screening of 31 fungal isolates recovered from beef luncheon meat for their abilities to produce lipase enzyme

Isolates	Lipase activity
	Clear zone (mm)
Acremonium strictum	18 L
Alternaria alternata	25 H
Aspergillus flavus	23 M
A. flavus var. *columnaris*	24 M
A. fumigatus	21 M
A. niger	31 H
A. ochraceus	29 H
A. sydowii	19 M
A. tamarii	27 H
A. terreus	20 M
A. terreus var. *aureus*	21 M
A. ustus	21 M
Cladosporium cladosporioides	19 M
Cochliobolus spicifer	25 H
Curvularia ovoidea	19 M
Emericella nidulans	25 H
E. nidulans var. *lata*	25 H
Fusarium oxysporum	30 H
Gibberella fujikuroi	22 M
Mucor hiemalis	24 M
M. racemosus	27 H
Mycosphaerella tassiana	20 M
Nectria haematococca	32 H
Penicillium camemberti	21 M
P. chrysogenum	21 M
P. duclauxii	17 L
P. funiculosum	17 L
P. oxalicum	19 M
Phoma herbarum	18 L
Rhizopus stolonifer	23 M
Stachybotrys chartarum	13 L

High > 24 mm Moderate = 19~24 mm Low < 19 mm

isolates appeared high lipase production, while sixteen isolates (51.61%) were found to be moderate and 5 isolates (16.13%) were low producers. *Aspergillus niger*, *Fusarium oxysporum* and *Nectria haematococca* were on the top for enzyme production, so these fungi were selected for further studies. Nagy *et al.* (2006) screened thirty-eight filamentous fungi cultivated under solid state fermentation conditions for lipase production. Many of these fungi yielded good activities of lipase enzyme. *Gliocladium roseum* and *Trichoderma harizianum* produced the highest amount of lipase.

Effect of food preservatives on growth and lipase production by fungi. The incorporation of five food preservatives (Disodium phosphate, sodium benzoate, citric acid, potassium sorbate and sodium citrate) individually in the culture medium of lipase production exhibited an

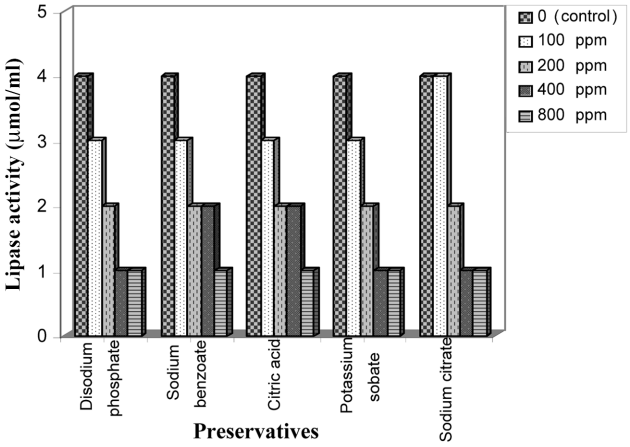

Fig. 1. Effect of food preservatives on lipase production by *Aspergillus niger*.

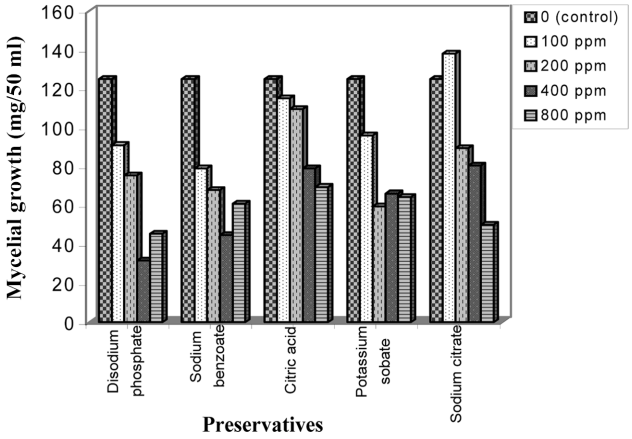

Fig. 2. Effect of food preservatives on mycelial growth of *Aspergillus niger*.

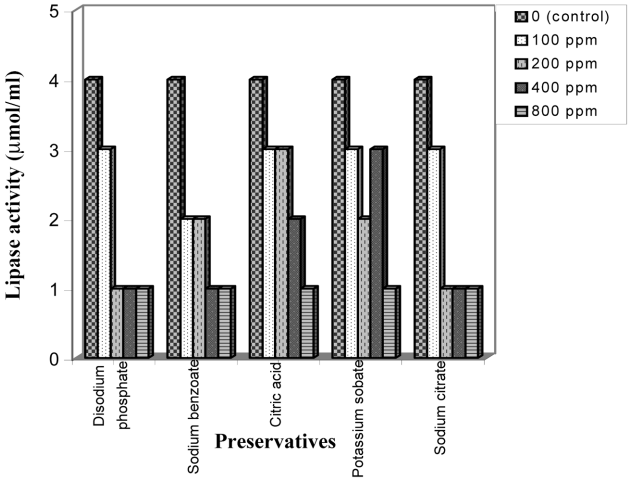

Fig. 3. Effect of food preservatives on lipase production by *Fusarium oxysporum*.

inhibitive effect on the mycelial growth and enzyme production by *Aspergillus niger*, *Fusarium oxysporum* and

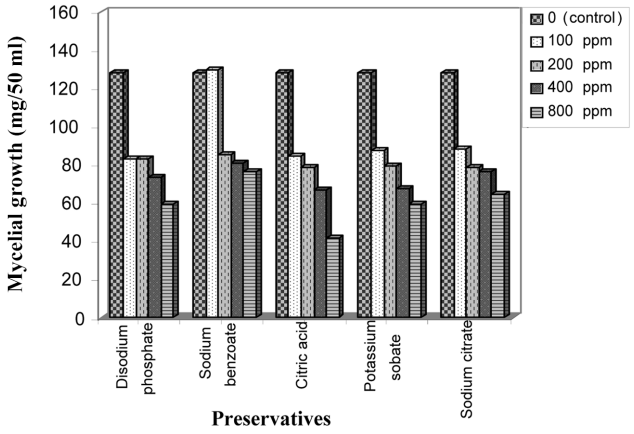

Fig. 4. Effect of food preservatives on mycelial growth of *Fusarium oxysporum*.

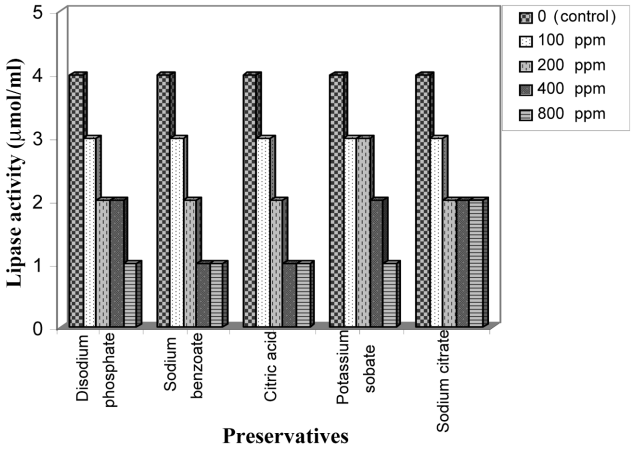

Fig. 5. Effect of food preservatives on lipase production by *Nectria haematococca*.

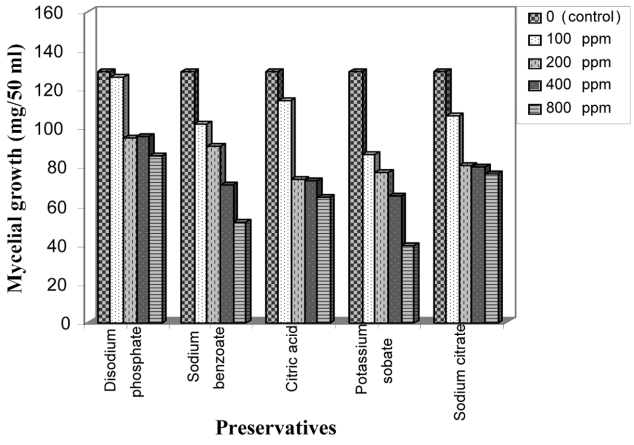

Fig. 6. Effect of food preservatives on mycelial growth of *Nectria haematococca*.

Nectria haematococca, except in few cases there is no effect by the low dose. Generally, the inhibition degree was correlated with the type and the concentration of preservatives in the culture medium for lipase (Figs. 1~6). Beuchat (1981) reported that potassium sorbate and sodium benzoate acted synergistically with heat to inactivate ascospores or vegetative cells of four molds including *Aspergillus flavus*, *Penicillium puberulum*, *Byssochlamys nivea* and *Geotrichum candidum*. The two preservatives had varied degrees of effectiveness on molds. Potassium sorbate was clearly more inhibitory than sodium benzoate to colony formation by *A. flavus*, and the presence of sucrose and sodium chloride enhanced this inhibition. Organic acids have characteristic sensitivities to microorganisms, sorbic acids and sorbates are very strong antifungal agents (Han, 2005). Recently, Geornaras *et al.* (2006) and Coma (2008) reported that acetic acid, lactic acid or potassium benzoate leads to a reduction of *Listeria monocytogenes* populations in smoked sausage.

References

Alberghina, L., Schmid, R. D. and Verger, R. 1991. Lipases: structure, mechanism and genetic engineering. Wiley-VCH, Weinheim.

Al-Doory, Y. 1980. Laboratory medical mycology. Lea and Febiger, Philadelphia Kimpton publishers, London.

Andersen, S. J. 1995. Compositional changes in surface mycoflora during ripening of naturally fermented sausages. *J. Food Protect*. 58:426-429.

Aymerich, T., Picouet, P. A. and Monfort, J. M. 2008. Decontamination technologies for meat products. *Meat Science* 78:114-129.

Balashev, K., Jensen, T. R., Kjaer, K. and Bjornholm, T. 2001. Novel methods for studying lipids and lipases and their mutual interaction at interfaces: Part I. Atomic force microscopy. *Biochimie* 83:387-397.

Beuchat, L. R. 1981. Combined effects of solutes and food preservatives on rates of inactivation of and colony formation by heated spores and vegetative cells of molds. *Appl. Environ. Microbiol.* 41:472-477.

Bornscheuer, U.T. 2000. Enzymes in lipid modification. Wiley-VCH, Weinheim.

Cardenas, J., Alvarez, E., De Castro-Alvarez, M. S., Sanchez-Montero, J. M., Valmaseda, M., Elson, S. W. and Sinisterra, J. V. 2001. Screening and catalytic activity in organic synthesis of novel fungal and yeast lipases. *J. Mol. Catal. B: Enzym.* 14:111-123.

Casado, M. J. M., Borras, M. A. and Aguilar, R. V. 1991. Fungal flora present on the surface of cured Spanish ham. Methodological study for its isolation and identification. *Fleischwirtschaft* 71:1300-1302.

Coma, V. 2008. Bioactive packaging technologies for extended shelf life of meat-based products. *Meat Science* 78:90-103.

Geornaras, I., Skandamis, P. N., Belk, K. E., Scanga, J. A., Kendall, P. A., Smith, G. C. and Sofos, J. N. 2006. Post-processing application of chemical solutions for control of *Listeria monocytogenes*, cultured under different conditions, on commercial smoked sausage formulated with and without potassium lactate-sodium discetate. *Food Microbiol.* 23:762-771.

Grazia, L., Romano, P., Bagni, A., Roggiani, D. and Guglielmi, G. 1986. The role of moulds in the ripening process of salami.

Food Microbiol. 3:19-25.

Han, J. H. 2005. Antimicrobial packaging systems. In: Innovations in Food Packaging. pp. 81-107. Ed. J. H. Han, Elsevier Academic Press, Amsterdam.

Hwang, H. J., Vogel, R. F. and Hammes, W. P. 1993. Entwicklung von Schimmelpilzkulturen für die Rohwurstherstellung Charakterisierung der Stämme und toxikologische Bewertung. *Fleischwirtschaft* 73:89-92.

Jesenska, Z. 1987. Mikroskopické huby v požívatinácha v krmivách. Alfa, Bratislava: 36-37.

Laciakova, A. and Laciak, V. 1994. Možnosti eliminácie mikroskopických vláknitých húb dezinfekčnými prostriedkami. *Vet. Med. (Czech)* 39: 723-731.

Leistner, L. 1986. Mold-ripened foods. *Fleischwirtschaft* 66: 1385-1388.

Martinelle, M., Holmquist, M. and Hult, K. 1995. On the interfacial activation of *Candida antarctica* lipase A and B as compared with *Humicola lanuginosa* lipase. *Biochim. Biophys. Acta* 1258:272-276.

Mateos Diaz, J. C., Rodriguez, J. A., Roussos, S., Cordova, J., Abousalham, A., Carriere, F. and Baratti, J. 2006. Lipase from the thermotolerant fungus *Rhizopus homothallicus* is more thermostable when produced using solid state fermentation than liquid fermentation procedures. *Enzyme Microb. Technol.* 39:1042-1050.

Mizakova, A., Pipova, M. and Turek, P. 2002, The occurrence of moulds in fermented raw meat products. *Czech J. Food Science* 20:89-94.

Nagy, V., Toke, E. R., Keong, L. C., Szatzker, G., Ibrahim, D., Omar, I. C., Szakacs, G. and Poppe, L. 2006. Kinetic resolutions with novel, highly enantioselective fungal lipases produced by solid state fermentation. *J. Mol. Catal. B: Enzym.* 39:141-148.

Ostry, V. 2001. Výskyt plísní v mase a masných výrobcích (I). Vliv na zdraví člověka. *Maso* 12:20-24.

Quintavalla, S. and Vicini, L. 2002. Antimicrobial food packaging in meat industry. *Meat Science* 62:373-380.

Sharma, R., Chisti, Y. and Banerjee, U. C. 2001. Production, purification, characterization and applications of lipases. *Biotechnol. Adv.* 19: 627-662.

Zaky, Z. M., Ismail, M. A. and Refaie, R. S. 1995. *Aspergillus flavus* and aflatoxins residues in luncheon meat. *Assiut Vet. Med*. 33:114-118.