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MYCOLOGICAL STUDY ON FRESH AND FROZEN MEAT IN TAIF CITY, SAUDIA ARABIA

(With 3 Tables)

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دراسة فطرية على اللحوم الطازجة والمجمدة في محافظة الطائف بالمملكة العربية السعودية

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أجريت الإختبارات الفطرية على (٢٠٠) عينة مختلفة من اللحوم الطازجة والمجمدة أخذت من ذبائح الأبقار والإبل والأغنام والدجاج التي تباع في محلات الجزارة والأسواق المركزية في محافظة الطائف بالمملكة العربية السعودية. وكان عددعزلات أجناس الأعفان من اللحوم الطازجة (٦٤) عزلة، ومن اللحوم المجمدة (١٠٤) عزلات. وكانت أجناس الأعفان (الفطريات) الأكثر تكراراً هي: نوع كلادوسبوريوم، نوع المكنسية، نوع أكريمونيوم، نوع العفنة، نوع الرشاشية، نوع الثامنيديوم، ونوع المبيضات. لقد أثبتت نتائج البحث أن معدل عزل الأعفان يزداد عندما تخزن اللحوم، وخصوصاً في بيئة ذات درجات حرارة مرتفعة (٣٧ الى٣٨ مئوية) بالمقارنة مع درجات الحرارة المنخفضة(-١ إلى – ٥ مئوية).

SUMMARY

A total of 200 different fresh and frozen meat samples obtained from carcasses of cattle, camel, sheep, and chicken were collected from butcher shops and supermarkets of Taif City, Saudia Arabia and subjected to mycological examination. Mould genera isolates from fresh and frozen meat samples were 64 and 104 respectively. The most frequently isolated genera were *Cladosporium spp.*, *Penicillium spp.*, *Acremonium spp.*, *Mucor spp.*, *Aspergillus spp.*, *Thamnidium spp.*, and *Monilia spp.*,. The obtained results proved that the recovery rate of moulds increased by storage, particularly at high temperate 37-38 °C than lower temperatures and from (-1 to –5 °C) Consecutively.

Key words: *Meat, beef, camel, sheep, broilers, Saudi Arabia*

INTRODUCTION

Moulds grow under a wide range of conditions but are most likely to spoil acidic foods like fruits, or low- moisture foods like bread. Under these conditions mould outgrow bacteria (Frazier & Westhoff, 1978). Moulds are microscopic fungi that live on plant or animal matter. No one knows how many species of fungi exist, but estimates range from tens of thousands to perhaps 300,000 or more (unpublished data from USDA's Meat and Poultry Hotline, April 2002). Most moulds are filamentous (threadlike) organisms and the production of spores is characteristic of fungi in general. These spores can be transported by air, water, or insects (Collins & Lyne, 1976; Finegold & Baron, 1986). Also, slime moulds are protozoa – related organisms of poly-phyletic origin. The class Myxomycetes (true slime moulds) is the most important group. During their trophic stage they form spore- producing structures, the sporocarps (or "sporangia") (Dorfelt *et al*., 2003). 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 food animals (Laciakova & Laciak, 1994). The reason for an increasing interest is the ability of moulds to produce secondary metabolites – mycotoxins – that have public health, i.e carcinogenesis, mutagenicity, and high thermostability (Mizakova *et al*., 2002).

There is essentially no fungus – free environments in our daily lives (Chao *et al*, 2002). 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 (Jesenska, 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).

There is seldom any doubt about the fitness of meat for consumption when it shows evidence of decomposition or putrefaction, and as such it is definitely described as spoiled. But spoilage does not necessarily imply decomposition or putrefaction. This is particularly

pertinent in the case of meat, because spoilage is not due solely to microbial action but to such factors as insects and intrinsic enzymatic and oxidative reactions as well (Forrest *et al*, 1975). Actually, some of the more obvious chemical and physical changes attributable to microorganisms, especially moulds were described by quite a few of workers (Forrest *et al*, 1975; Frazier and Westhoff, 1978).

Mould is ubiquitions, regarded more or less as contaminants of meat and its products which may lead to spoilage and/ or food poisoning.

Growth of mould on meat substance is suitable for its available water, pH, nitrogenous compounds besides some fermentable carbohydrates and minerals, therefore most food are susceptible to fungal invasion during stages of production, processing, transport and storage (Stoloff, 1984).

Because of the varied sources, the kinds of microorganisms likely to contaminate meats are many. Moulds of many genera may reach the surfaces of meats and grow there. Especially important are species of the genera *Cladosporium*, *Sporotrichum*, *Geotrichum*, *Thamnidium*, *Mucor*, *Penicillium*, *Alternaria* and *Monilia* (Frazier & Westhoff, 1978).

The purpose of our investigation was to define the occurrence of different mould genera in different fresh and frozen meat. In a sort, the effects of incubation temperatures on the growth of moulds kinds were also assessed in different fungal culture media.

MATERIALS and METHODS

Samples:

A total of 200 random samples of fresh and frozen meat (25 samples of each kind) taken from carcasses of sheep, camels, cows and chicken were collected during the period of 1-6, 2003 from different butcher shops and supermarkets at Taif City, Saudia Arabia.

Sample preparation:

Ten grams were taken aseptically from each sample of fresh and frozen meats, and 90 ml of diluent (physiological saline) were added to the sample in a sterile homogenizing vessel with 10000 rev. was used for homogenization; the homogenization time was 2.5 min. The fresh and frozen samples were prepared in the same way according to standard procedures described previously (Sharf, 1966; Collins & Lyne, 1976;

Harrigan & McCance, 1976) and subjected to the following mycological examinations:

I- Cultivation and total mould count:

It was done by direct spreading on the surface of the following mycological culture media Sabouraud Dextrose Agar (Oxoid), Brain Heart Infusion Agar with penicillin and streptomycin may be incorporated in the media to final concentration of 20 units and 40 units per ml respectively (HiMedia, India), Blood Agar Base No.2 (Oxoid) +7% Sterile Blood, Potato Dextrose Agar (Oxoid), of Czapek –Dox Agar (prepared according to Hartigan & McCance description, 1976) in which the acidity has been adjusted to around pH 3.5 by use of sterile lactic acid or Citric acid immediately prior to pouring the plates. All these media were applied effectually, using a sterile pipette, which were suitable culture media for estimation of total mould count (Sharf, 1966; Harrigan & McCance, 1976), especially for mould species which could not grow on the first medium. Then the inoculated plates were incubated at varied temperatures as follows:

- A group of inoculated plates were incubated at temperatures from (-1 to -5) °C for 7 days.
- A group of inoculated plates were incubated at temperatures from (21-25) °C for 4 days.
- A group of inoculated plates were incubated at temperatures from (37 - 38) °C for 1-2 days.Then the number of moulds was determined in one g of the sample, according to Sharf, (1966); Harrigan & McCance, (1976).

Somewhat, few inoculated plates required additional incubation for a few days, in order to observe the colonies of moulds, if plates are likely to be hypogrown.

II - Identification of the isolated mould:

The mould genera and species were identified according to Collins & Lyne (1976), Harrigan & McCance (1976) and Finegold & Baron (1986). Moulds are differentiated on the basis of morphological and cultural characteristics so that it is necessary to employ methods of culture and examination that, as far as possible, avoiding distortion of sporing structures.

RESULTS

Results are presented in Tables 1- 3.

Table 1: Total mould count per gram of fresh and frozen meat samples

Samples	Total mould count per gram of sample	
	Minimum	Maximum
Fresh meat:		
Beef	2×10^2	9×10^2
Camel's meat	1.1×10^2	2×10^2
Mutton	3×10^2	6.3×10^3
Chicken meat	2×10^2	3.1×10^2
Frozen meat:		
Beef *	3×10^2	7×10^3
Camel's meat	2×10^2	9×10^2
Mutton *	4.2×10^3	18×10^4
Chicken meat *	2.1×10^2	6.1×10^2

(*) Most of these meats were imported.

Table 2: Incidence of the isolated mould genera in fresh meat samples (No – 100)

Incubation temperature	Kind of meat	Species of isolated microscopic filamentous fungi	No. of isolates and the total isolation percentage
37 – 38 ° C	Beef	- *Aspergillus fumigatus*	3
		- *Cladosporium sp.*	1
		- *Penicillium chrysogenum*	2
		- *Acremonium sp.*	1
	Camel's meat	- *Mucor sp.*	2
		- *Aspergillus fumigatus*	2
	Mutton	- *Penicillium fellutanum*	1
		- *Acremonium sp.*	2
		- *Scopulariopsis brevicaulis*	3
		- *Aspergillus fumigatus*	2
		- *Mucor sp.*	2
	Chicken meat	- *Aspergillus fumigatus*	3
			24 (37.5 %)
(-1)to (-5) °C	Beef	- *Thamnidium sp.*	1
		- *Acremonium sp.*	1
		- *Mucor sp.*	4
	Camel's meat	- *Cladosporium sp.*	1
	Mutton	- *Aspergillus niger*	4
		- *Thamnidium sp.*	2
		- *Acremonium sp.*	5
		- *Penicillium chrysogenum*	2
	Chicken meat	- *Candida albicans*	1
		- *Aspergillus niger*	1
			22 (34.38 %)
21 – 25 °C	Beef	- *Cladosporium sp.*	2
		- *Aspergillus flavus*	2
	Camel's meat	- *Mucor sp.*	3
		- *Sterile mycelia*	1
	Mutton	- *Mucor sp.*	2
		- *Penicillium chrysognum*	3
		- *Cladosporium sp.*	2
		- *Aspergillus fumigatus.*	1
	Chicken meat	- *Aspergillus fumigatus*	2
			18 (28.12 %)
Total number of the isolates			64 (100 %)

Table 3: Incidence of the isolated mould genera in frozen meat samples (No = 100)

Incubation temperature	Kind of meat	Species of isolated microscopic filamentous fungi	No. of isolates and the total isolation percentage
37 – 38 °C	Beef	- *Cladosporium sp.*	2
		-*Penicillium acseicolum*	3
		- *Sterile mycelia*	1
	Camel's meat	- *Acremonium sp.*	3
		-*Penicillium acseicolum*	4
	Mutton	- *Mucor sp.*	2
		- *Acremonium sp*	3
		- *Penicillium camemberti*	1
		- *Sporendonema sebi*	1
		- *Scopulariopsis sp.*	3
	Chicken meat	- *Aspergillus fumigatus*	4
		- *Monilia sitophila*	2
			29 (27.88 %)
(-1)to (-5) °C	Beef	- *Aspergillus fumigatus*	2
		- *Cladosporium sp.*	1
		- *Sporotrichum(Aleurisma) sp.*	2
	Camel's meat	- *Thamnidium sp.*	6
	Mutton	- *Aspergillus fumigatus*	4
		- *Aspergillus niger*	2
		- *Aspergillus flavus*	5
		- *Thamnidium sp.*	1
		- *Sporotrichum(Aleurisma) sp.*	3
		- *Penicillium fellutanum*	2
		- *Penicillium chrysogenum*	3
		- *Acremonium sp.*	3
		- *Mucor sp.*	2
		- *Cladosporium sp.*	2
	Chicken meat	- *Aspergillus fumigatus*	4
		- *Monilia sitophila*	2
			44 (42.31 %)
21 – 25 °C	Beef	- *Penicillium chrysogenum*	4
		- *Cladosporium sp.*	2
		- *Aspergillus flavus*	1
	Camel's meat	- *Penicillium chrysogenum*	3
		- *Cladosporium sp*	4
	Mutton	- *Aspergillus flavus*	4
		- *Mucor sp.*	3
		- *Penicillium camemberti*	5
		- *Fusarium sp.*	2
	Chicken meat	- *Aspergillus fumigatus*	3
			31 (29.81 %)
Total number of the isolates			**104 (100 %)**

DISCUSSION

It is evident from the results recorded in Table (1) that the maximal mould counts per gram for fresh meat were 9×10^2, 2×10^2, 6.3×10^3, and 3.1×10^2 in beef, camel's meat, mutton and chicken meat respectively.

The maximal mould counts per gram for frozen meat were 7×10^3, 9×10^2, 18×10^4 and 6.1×10^2 in beef, camel's meat, mutton and chicken meat consecutively.ICMSF (1974) recommend that the general viable count at 35 °C (or at 20 °C in the case of chilled meats) should be less than 10^7 per gram. Presence of high mould count in fresh and frozen samples of mutton may be attributed to composition structure, air pollution and contamination of water, handlers, knives and utensiles (Frazier & Westhoff, 1978). As seen from the Tables 2 and 3, various moulds were detected in fresh and frozen meats. The most frequently isolated genera were *Aspergillus spp.*, *Cladosporium spp.*, *Penicillium spp.*, *Acremonium spp.*, *Thamnidium spp.*, *Scopulariopsis spp.*, *Mucor spp.*, and *Monilia spp.* The results shown in Table (2) cleared that the numbers of the isolated mould genera detected in fresh meat samples at incubation temperatures from (37 –38 °C, -1 to –5 °C and 21- 25 °C were 24 (37.5 %), 22 (34.38%) and 18 (28.12 %) respectively .

Table (3) showed that the number of isolated mould genera could be detected in 100 samples of frozen meats identified by cultural and morphological properties were 29 (27.88%), 44 (42.31% and 31(29.81%) at incubation temperatures from (37 – 38 °C, -1 to –5 °C and 21 –25 °C) consecutively. These results indicate clearly, that the frozen meat especially those imported meats at hand, are more contaminated than the raw meats in view of its infections.

These moulds find the chance in the products to cause more or less a public health hazard or spoilage of such products. Mould spoilage of the cut surfaces of refrigerated or frozen meat can occur at temperatures down to about –5 °C. At the lower temperature mycelial growth may occur without spore production, and this will give rise to a white fluffy appearance caused by. e.g. *Mucor*, Rhizopus. Other types of mould spoilage which may be obvious on inspection are "white spot" – caused by. e.g. *Sporotrichum*; "black spot" – caused by. e.g. *Cladosporium*; and green patches – caused by. e.g. *Penicillium* (Harrigan & McCance, 1976), and "whiskers" due to *Mucor* and *Thamnidium spp* (Collins & Lyne, 1976).

In dry chill conditions, mould spoilage is possible in chilled beef (-1 °C) and frozen mutton (-5 °C or less) but moulds do not grow below-10 °C (Collins & Lyne, 1976). Much more studies pointed out that the *Aspergillus* species are the most frequent cause of invasive mould infections in immuno-compromised patients (Henry *et al.* 2000). Benito *et al* (2002) isolated an extracellular protease from *Penicillium chrysogenum* (Pg 222) from dry-cured ham in which has been purified.
In recent 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 spp.*, *Acremonium spp.*, *Mucor spp.*, *Cladosporium spp.*, and *Aspergillus spp.* were the most frequently isolated genera of moulds (Mizakova *et al,* 2002). In this study, the isolated mould strains were differ from one species to another and within the same species as well as on the different media used and different incubation temperatures. The results of this study nearly similar with those reported by Mizakova *et al*, (2002).

Seventy - eight species of moulds have already been isolated from meat and various meat products but the most prevalent types are: *Penicillium spp., Acremonium spp., Mucor spp., Cladosporium spp.*, and *Aspergillus spp.* However, only 50 of them are reported to be toxicogenic as indicated by Ostry, (2001) in Slovak Republic

Currently, numerous studies aimed at the detection of microscopic filamentous fungi and their toxins in plants, food 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 a flatoxigenic moulds (*Aspergillus flavus* and *Aspergillus oryzae*) in luncheon meat. 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.

REFERENCES

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

Benito, M.J; Rodriguez, M; Nunez, F; Asensio, M.A; Bermudez, M.E. & Cordoba, J.J (2002): Purification and characterization of an extracellular protease from Penicillium chrysogenum Pg 222 active against meat proteins. Applied and Environmental Microbiology, 68, 7, 3532 -- 3536.

Chao, H.J; Schwartz, J; Milton, D.K. & Burge, H.A (2002): Populations and determinants of airborne fungi in large office buildings. Environmental Health Perspectives, 110, 777 – 782.

Collins, C.H. & Lyne, P.M. (1976): Microbiological methods. 4 th Edition. Butterworth World Student Reprints; London, Boston.

Cvetnic, Z. & Pepelnjak, S. (1995): Aflatoxin – producing potential of Aspergillus flavus and Aspergillus parasiticus isolated from samples. of smoked – dried meat. Nahrung 39, 302- 307

Dorfelt, H; Schmidt, A.R; Ullmann, P. & Wunderlich, J. (2003): The oldest fossil myxogastroid slime mould. Mycol. Res, 107, 1, 123 - 126.

Finegold, S.M. & Baron, E.J. (1986): Bailey and Scott's Diagnostic Microbiology. 7th Edition. The C.V. Mosby company, St. Louis. Toronto.

Forrest, J.C.; Aberle, E.D.; Hedrick, H.B.; Judge, M.D. & Merkel, R.A. (1975): Principles of Meat Science. A Series of Books in Food and Nutrition. W.H. Freeman and Company., U.S.A.

Frazier, W.C. & Westhoff, D.C. (1978): Food Microbiology. McGraw-Hill Book Company, New York, St. Louis.

Hanssen, H.P. (1995): Mould control in the meat processing industry. Fleischwirtschaft, 75, 52.

Harrigan, W.F & McCance, M.E. (1976): Laboratory Methods in Food and Dairy Microbiology. Academic Press, London, New York.

Henry, T.; Iwen, P.C. & Hinrichs, S.H. (2000): Identification of *Aspergillus species* using internal transcribed spacer Regions 1 and 2. Journal of Clinical Microbiology, 38, 4, 1510 - 1515.

Jesenska, Z. (1987): Mikroskopicke,huby v pozivatinach a.v Krmivach . Alfa, Bratislava, 36 -37.

Laciakova, A. & Laciak, V. (1994): Moznosti eliminacie mikroskopickych vlaknitych hub dezsinfekcnymi prostriedkami. Vet. Med. (Czech), 39, 723-731.

Mizakova, A.; Pipova, M. & Turek, P. (2002): The occurrence of moulds in fermented raw meat products. Czech J. Food Sci, 20, 3, 89-94.

Ostry, V. (2001): Vyskyt plisni v mase a masnych vyrobcich (1).Vliv na zdravi cloveka. Maso, 12, 20 -24.

Reiss, J. (1986): Schimmelpilze. Springer Verlag, Berlin, Heidelberg, New York and Tokyo.

Sharf, J.M. (1966): Recommended Methods For the Microbiological Examination of Foods. 2nd Edition, American Public Health Association, Inc, Washington.

Stoloff, L. (1984): Toxigenic Fungi. In Compendium of Methods for the Microbiological examination of Foods. (Speck, H.L. ed). American Public Health Association, Washington. D.C.

Zaky, Z.M.; Ismail, M.A. & Refaie, R.S. (1995): Aspergillus flavus and a flatoxins residues in luncheon meat. Assiut Veterinary Medical Journal, 33. 114-118.