

PSYCHROTROPHIC SPOILAGE MOULDS IN IMPORTED FROZEN BEEF CUTS

By

N.K. MANSOUR; N.A. YASSIEN; A.M. DARWISH and
A.M. El-SHERIF

Dept. of Food. Control. Faculty of Vet. Med.,
Cairo University, Giza, Egypt

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INTRODUCTION

Imported frozen meat specially frozen beef cuts are used mainly as raw material in meat products in Egypt. Therefore, microbiological qualification of such frozen meat is very important due to the late usage. Some defects as formation of black, green and white spots are formed on the end products, the causative organisms originate mainly from the raw material.

Contamination of imported frozen meat with mould spores can be attributed to the mishandling of meat from time of their arrival until it reached different cold-stores as many authors mentioned that deep freezing (until -18°C) has no significant destructive effect upon mould spores as they can resist cold storage (Monvoisin, 1918; Shonberg, 1954; Frank, 1966; Frazier and Westhoff, 1979; Gill *et al.*, 1981; Gill and Lowery 1982 and Mansour, 1986).

Mycological examination of imported frozen beef and hunted meats affected with black spots revealed that the most predominant moulds were *Cladosporium herbarum*, besides; *Penicillium*, *Mucor*, *Phycomyces*, *Verticillium* and *Cospora* (Berger, 1912 and Silva, 1913).

Bidault (1921 & 1923) examined frozen meat and isolated the following genera; *Penicillium crustaceum*, *Chaetostylum fresenii*, *Thamnidium elegans*, *Hormodenderum cladosporiorides* (*Cladosporium cladosporioides*), *Sty-sanus stemonitis* and *Cladosporium herbarum*.

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Empay and Scott (1939) examined mycologically beef carcass surface and incubated the plates at + 20°C and 1°C. At the first temperature the most predominant moulds were *Fusarium*, *Alternaria* and *Cladosporium* while at the 2nd one (-1°C) were *Penicillium* followed by *Mucor*, *Cladosporium*, *Alternaria*, *Sporotrichum* and *Thamnidium*.

Hadlok (1970 & 1972) isolated the following mould genera from frozen meat and frozen foods: *Penicillium*, *Mucor*, *Thamnidium*, *Cladosporium*, *Monilia*, *Geotrichum*, *Trichothecium* and *Chaetostylum*.

Baxter and Illiston (1976) examined slaughter area, Boning room, Freezer, Chiller, carcasses of sheep, beef and pig for the presence of psychrotrophic fungi and incubated the inoculated plates at + 4°C for 4-5 weeks and isolated the following moulds; *Penicillium expansum*, *Cladosporium herbarum*, *Cladosporium cladosporioides*, *Chrysosporium pannorum*, *Epicoccum purpurascens*, *Rhizoctonia* sps., *Ascomycete*, *Mucor hiemalis* and *Alternaria alternata*.

Beuchat (1978) recorded that some species of *Penicillium* namely *P. brvicompactum*, *P. cyclopium*, *P. ruberulum* and *P. expansum* can grow on cold stored foods at temperatures below -2°C.

Fahmy (1986) mentioned that the most frequent mould genera isolated from frozen meat were: *Aspergillus*, *Penicillium*, *Cladosporium*, *Mucor*, *Trichoderma*, *Alternaria*, *Rhizopus*, *Geotrichum*, *Cephalosporium*, *Fusarium*, *Thamnidium*, *Botrytis*, *Syncephalastrum*, *Pullularia*, *Sporotrichum carnis*, *Monilia*, *Helminthosporium* and other unidentified mould genera.

Gracey (1986) reviewed the moulds responsible for spoilage of chilled and frozen meats, species of *Cladosporium* (black spot), species of *Penicillium* (blue green spots), *Thamnidium*, *Mucor*, *Rhizopus* (Wiskers) and *Chrysosporium* (white spot).

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Refai *et al.* (1991) isolated the following genera from quarters of chilled meat: *Aspergillus*, *Cladosporium*, *Alternaria*, *Curvularia*, *Helminthosporium*, *Penicillium*, *Paecilomyces*, *Scopulariopsis*, *Mucor*, *Rhizopus*, *Fusarium*, *Acremonium* and *Geotrichum*.

The term "Cold-growing microorganisms" has been varied and interferred. The definition of Ingraham and Stokes (1959), Eddy (1960) as well as Gounot (1976) for differentiation between the "psychrophiles" and "psychrotrophs" is the first one in those microorganisms which have an optimum temperature for growth less than 20°C and the second those which have an optimum temperature for growth at 20°C or above.

Morita (1975) suggested that the term "psychrophiles" should be used only for those microorganisms which have an optimal temperature for growth at about 15°C or lower and maximal temperature for growth at about 20°C and a minimal temperature for growth at 0°C or below. The same author added that any microorganism which is able to grow at low temperature but not meeting the aforementioned criteria should be referred to as "psychrotrophs".

The authors herein followed the definition of Morita (1975) and used the term "psychrotrophic" microorganisms "Moulds" which can grow at low temperature. The aim of the present work was to investigate the imported frozen beef cuts for the presence of the psychrotrophic spoilage moulds.

MATERIAL AND METHODS

Collection of samples:

A total of 95 cartons, each containing 25 kg. of imported frozen beef were used in the present study. Such cartons included the following cuts; forequarter (21),

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flank & brisket (29), hindquarter (total 37), namely topside (7), silverside (13), knuckle (9) rump (8) and unidentified cuts (8).

METHODS

Collected frozen beef cuts were subjected to the following investigation:

1. Determination of pH. value, using the digital pH-meter (Hoffman, 1987).
2. Mycological examination using the technique recommended by ICMSF (1978), plating media being acidified Malt extract agar (Oxoid 1982). Inoculated plates were incubated at +4°C for 4-5 weeks according to Baxter and Illiston (1976).
3. Isolation and identification of isolates were according to Raper & Tom (1949) and Samson *et al.* (1976) for genus *Penicillium*, Ellis (1971 & 1976) for genus *Cladosporium* and *Alternaria* and Domsch *et al.* (1980) as well as Samson *et al.* (1981) for other mould genera.

RESULTS AND DISCUSSION

It is evident from the achieved results in table (1) that the mean pH value of the different cuts was 5.69 ± 0.039 . The minimum pH-value was 5.30 and the maximum value was 5.90.

The obtained results in table (2) showed that one gram of frozen meat (in general) contain from $< 10^2$ to 6×10^4 with mean value of 4.4×10^4 psychrotrophic moulds and from $< 10^2$ to 1×10^5 with mean value of 10×10^4 psychrotrophic yeasts. These figures are higher than that obtained by Saudi and Mansour (1990) in ready to eat meals in airline catering.

Table (3) shows that 10 genera of moulds could be

isolated and identified at +4°C. The most commonly isolated moulds were *Cladosporium* species 37 (31.62%) followed by *Penicillium* species 23 (19.66 %) and *Aspergillus* 17 (14.53 %). Other genera of moulds isolated in reasonable percentage were *Ulocladium*, *Alternaria* (*A. alternata*, *A. sonchi*), *Phialophora*, *Rhizopus*, *Fusarium*, *Trichoderma* and finally *Mucor*. Lower figures were reported by Baxter and Illiston (1976), *Cladosporium* (5 species), *Penicillium* (5 species), two species of *Cladosporium* (namely *C. herbarum* and *C. cladosporioides*) and one type of *Penicillium* (*P. expansum*). The most frequent moulds isolated during the present study belonged to genera; *Cladosporium*, *Penicillium* and *Aspergillus* as they constituted over 65 % of the total isolates. Spoilage psychrotrophs and can grow on meat at low temperature (upto -8°C) forming black and blue green spot (Brooks and Kidd, 1921; Gill and Lowery, 1981, and Mansour, 1986). Other moulds as *Rhizopus* and *Mucor* can cause "Wiskers" on frozen meat (Gracey, 1986). *Aspergillus* species cause further hazards in meat products by secretion of mycotoxins under certain environments (Leistner & Eckardt 1981) or cause pulmonary infection and skin lesions (*A. fumigatus* and *A. niger*) in human beings (Adem *et al.* 1965 & Youssef and Refai 1986). Other isolated moulds (the same (Table 1) are food borne fungi (Samson *et al.* 1981).

Table (1): pH value of different examined cuts

Different cuts	No. of Samples	pH - value			
		Min.	Max.	Mean	S.E.+
1) Forequarters (chuck & blade)	21	5.51	5.91	5.67	0.02
2) Flank (plate) & brisket	29	5.30	5.75	5.60	0.02
3) Hindquarter (four cuts)	37	5.51	5.60	5.50	0.042
a. Topside	7	5.51	5.77	5.73	0.043
b. Silverside	13	5.40	5.88	5.65	0.044
c. Knuckle	9	5.51	5.93	5.76	0.05
d. Rump	8	5.60	5.86	5.78	0.04
4) Different cuts	8	5.44	5.90	5.61	0.04
Total	95	5.30	5.93	5.69	0.039

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From the results achieved in table (4) it is evident that the most frequent types of *Cladosporium* are *C. herbarum* and *C. cladosporioides* which constituted 81.08% (30 out of 37 isolates), the other types of *Cladosporium* were *C. sphaerospermum*, *C. tenuissimum* and *C. macrocarpum* (8.10 %, 5.41 % and 5.41 % respectively). *C. herbarum* and *C. macrocarpum* are psychrotrophs (Empey & Scott, 1939 and Baxter & Illiston 1976) and they are incriminated in formation of black spots on chilled and frozen meats (Berger, 1912, Lowry, 1980; Gill *et al.* 1981; Lowry & Gill 1984 and Mansour 1986).

The psychrotrophic penicillia were identified in table (5); *P. expansum* and *P. corymbiferum* constituted 73.90% of the total isolates. Other species as *P. chrysogenum*, *P. citrinum* and *P. cyclopium* were isolated (8.70 % for each). *Penicillium* species cause blue green spots on frozen meat (Gracey, 1986) and *P. corymbiferum* was incriminated with the two famous types of *Cladosporium* (*C. herbarum*, and *C. cladosporioides*) in formation of black spots on frozen meat (Lowry, 1980).

In conclusion, the majority of meat spoilage moulds survive freezing storage of meat and produce their special effect at the favourable temperature and humidity. Contamination of meat with moulds originates generally from slaughter halls and other environment (Mansour *et al.*, 1990 and Hamdy *et al.*, 1990). This contamination can be also caused by handling of meat during deboning or other processes (Refai *et al.*, 1991).

Contamination of meat with moulds leads to spoilage and render it to inferior quality or unfitness for consumption, therefore contamination should be controlled during handling, processing, packaging and transportation of meat. Checking up of the temperatures during transportation of frozen meat and keeping it within the international limits of transportation are very important aspects.

Table (2): Statistical analysis of total psychrotrophic mould and yeast count from frozen meat

	Moulds	Yeasts
Minimum	$< 10^2$	$< 10^2$
Maximum	6×10^4	1×10^5
Mean value	4.4×10^4	10×10^4
St. error \pm	1.4×10^3	3.1×10^3

Table (3): Total isolated psychrotrophic moulds from frozen meat (isolated at +4°C)

Moulds	No.	%
1) Cladosporium	37	31.62
C. herbarum	26	22.22
C. cladosporioides	4	3.42
C. sphaerospermum	3	2.56
C. tenuissimum	2	1.71
C. macrocarpum	2	1.71
2) Penicillium	23	19.66
P. expansum	13	11.11
P. corymbiferum	4	3.42
P. chrysogenum	2	1.71
P. citrinum	2	1.71
P. cyclopium	2	1.71
3) Aspergillus	17	14.53
A. fumigatus	9	7.69
A. niger	6	5.13
A. terreus	2	1.71
4) Ulocladium species	12	10.27
5) Alternaria	7	5.98
A. alternata	5	4.27
A. sonchi	2	1.71
6) Phialophora candida	7	5.98
7) Rhizopus species	5	4.27
8) Fusarium oxysporium	4	3.42
9) Trichoderma species	3	2.56
10) Mucor hiemalis	2	1.71
Total	117	100

Table (4): Frequency of Cladosporium species isolated at +4°C

Cladosporium	No.	%
C. herbarum	26	70.27
C. cladosporioides	4	10.81
C. sphaerospermum	3	8.10
C. tenuissimum	2	5.41
C. macrocarpum	2	5.41
Total	37	100

Table (5): Frequency of Penicillium species isolated at +4°C

Penicillium	No.	%
P. expansum	13	56.52
P. corymbiferum	4	17.38
P. chrysogenum	2	8.70
P. citrinum	2	8.70
P. cyclopium	2	8.70
Total	23	100

SUMMARY

A total of 95 cartons containing imported frozen beef cuts (21) forequarter, 29 flank & brisket, 37 hindquarter and 8 different cuts (were examined for the presence of psychrotrophic moulds. A total number of 117 moulds could be isolated and identified. The most frequent psychrotrophic moulds were *Cladosporium* 37 (31.62%) *Penicillium* 23 (19.66 %), *Aspergillus* 17 (14.53 %) and *Ulocladium* 12 (10.27 %). Other psychrotrophs as *Alternaria*, *Phialophora*, *Rhizopus*, *Fusarium*, *Trichoderma* and *Mucor*. Significance of these psychrotrophic moulds was discussed.

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