

MYCOFLORA OF IMPORTED FROZEN BEEF

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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 Mycoflora. A total of 125 moulds could be isolated and identified. The most frequent moulds were *Aspergillus* 53 (42.4) 59, Dematiaceous moulds 30 (24 %), penicillium 22 (17.6%) and *Trichoderma* 5 (4%). Significance of these moulds was discussed.

INTRODUCTION

In Egypt, the quantity of meat produced from native breeds (cattle, buffaloes, camels, sheep, goat and pigs) were 178880, 35 tons according to the data recorded by the General organization of Veterinary Service (GOVS) 1987. Most of that meat are consumed fresh. However, the produced quantity could not fulfil the great want of meat as a raw material in meat processing. Thus, a great quantities of frozen meat are imported to Egypt for that purpose in the form of frozen beef cuts. Such raw material (frozen meat) represented an important source of contamination of meat products.

It is an interesting point of view to study moulds encountered on imported frozen beef cuts from the point of arrival to Egypt.

Moulds could contaminate meat during the different steps of processing in slaughter houses. Intestinal contents, air, walls and equipment play an important role in contamination of meat mycologically (Klare, 1971, Hamdy et al., 1990; Mansour et al., 1990).

Colonization of moulds on frozen meat is in form of black spot, wiskers, white spots and bluish green spots (Gracey, 1986). Important factors for germination of spores on meat are temperature and relative humidity (Mistivec et al., 1975).

Imported frozen beef might be affected with moulds during long trips in ships due mishandlings and irrums. Deep freezing (until -8°C) has no significant effect on moulds (Berger, 1912; Siliva, 1913; Brooks and Hansferd, 1923; Semeniuk and Ball, 1937; Savov and Kovnev, 1974, Lowry and Gill, 1984; Mansour et al., 1991).

Most common mould affection encountered on frozen meat is black spot. Berger (1912) isolated *Cladosporium herbarum* from black spot on meat.

In years 1921 & 1923, Bidault isolated mould species other than *Cladosporium herbarum* and *Cladosporium cladosporioides* as *Penicillium crustaceum*, *Chaetostylum fresenii*, *Thamnidium elegems* and *stysanus stemonitis*.

The main cause of black spot is *Cladosporium herbarum* (Wright, 1923; Semeniuk and Ball, 1937). While Gill et al. (1981) as well as lowry and Ashton (1982) isolated four mould species from black spotted meat; namely *Cladosporium herbarum*, *Cladosporium cladosporioides*, *Penicillium hirsutum* and *Aureobasidium pullulans*.

Responsibility of moulds to form a different types of spoilage on refrigerated meat was recorded by Ayres (1963). *Cladosporium herbarum* causes black spot, *Penicillium* species (Blue green spots), *Thamnidium*, *Mucor* and *Rhizopus* (Wiskers), *Chrysosporium pannorum* (White spots).

Aspergillus niger was the most predominant isolate (48.84%) recorded by Refai and Loot (1969) in cold stored meat. Other moulds could be also isolated as *Penicillium*, *Mucor*, *Rhizopus*, *Pullularia*, *Alternaria*, *Botrytis* and *Streptomyces*.

Hadlok (1970) isolated *Penicillium*, *Mucor*, *Thamnidium* and *Cladosporium* from frozen meat.

Abdel Rhaman et al. (1985) examined imported beef quarters and packed beef mycologically. Results revealed that *Penicillium*, *Cladosporium* and *Aspergillus* were the most predominant isolated genera (23.40%, 22.34% and 9.84% respectively).

Mansour et al. (1991) isolated and identified 117 psychrotrophic moulds from frozen beef cuts. Most frequent isolates were *Cladosporium* (31.62%), *Penicillium* (19.66%), *Aspergillus* (14.53%) and *Ulocladium* (10.27%). Other psychrotrophes as *Alternaria*, *Phialophora*, *Rhizopus*, *Fusarium* and *Mucor* were also isolated. The incubation temperature was +4°C for 4-5 weeks.

Refai et al. (1993) identified 4222 fungal strains belonging to 18 mould genera from swabs taken from the surface of fresh and chilled meat as well as from the surroundings (air, floor, walls and utensils) in modern abattoir in Egypt. *Aspergillus*, dematiaceous moulds and *Penicillium* species were the most common genera isolated representing 29.89%, 25.08% and 18.64% respectively.

This work is planned to throw light on the mycological status of imported frozen beef cuts, particularly at the point of arrival.

MATERIAL AND METHODS

Materials:

A total of 95 random cartons were collected. These represented the lot of imported frozen beef cuts to Egypt. Each carton contained 25 kg of frozen product. That cartons were typed as following: Forequarter (21), flank and brisket (29), topside (7), silverside (13), knuckle(9), rump (8), and other untyped cuts (8). Samples were brought to the

laboratory directly from the port. They were valid for 9 months.

Methods:

The collected samples were investigated as follows:

1. Determination of pH, using the digital pH-meter according to Hoffmann (1987).
2. Mycological examination of samples was done according to ICMSF (1978).
3. Inoculation of plates contained acidified malt extract agar-MEA- (Oxoid, 1982), incubation at 22°C for one week (Samson et al., 1981).
4. Isolation and identification of isolates were performed as recommended by Raper, et al. (1965), Raper and Thom (1968), Samson et al. (1976); Domsch et al. (1980) and Samson et al. (1981).
5. Microscopical examination of isolates was applied by using techniques recommended by Bailey and Scott (1985).

RESULTS AND DISCUSSION

The achieved results in Table (1) showed that the mean pH value of different frozen beef cuts was 5.69 ± 0.039 , with minimum value of 5.30 and maximum value of 5.90. These values indicated that the meat was of good keeping quality.

The number of colony forming unit (cfu)/gm frozen meat (Table 2) ranged from <100 to 4.0×10^4 with mean value of $4.6 \times 10^2 \pm 83.2$. This number was lower than that number/gm frozen meat obtained by Fahmy (1986). However more than the number obtained by Udagawa et al. (1975) who stated that number/gm frozen food was <100 at 20°C. In cold dishes of air line catering the number/gm were nearly similar to the obtained results <100-10⁴, Saudi and Mansour (1990).

A total of 125 strains were isolated identified

Table (1): pH value of different examined cuts

Different cuts	No. of samples	pH value			
		Min.	Max.	Min.	S.E. ±
1 Forequarter (chuck & blade)	21	5.51	5.91	5.67	0.02
2 Flank & brisket	20	5.30	5.75	5.60	0.02
3 Topside	7	5.51	5.77	5.73	0.043
4 Silverside	13	5.40	5.88	5.65	0.044
5 Knuckle	9	5.51	5.93	5.76	0.05
6 Rump	8	5.60	5.86	5.78	0.04
7 Unidentified cuts	8	5.44	5.90	5.61	0.04
Total	95	5.30	5.93	5.69	0.039

Genea, *Aspergillus*, *Penicillium* and *Cladosporium* were the most common isolated strains constituted over 70% of the total isolates (Table 3). These three genera stood on the top of the isolates obtained from frozen meat by different authors (Abdel Rahman et al., 1985; Fahmy et al., 1986).

Concerning *Aspergillus* species (Table 4), they constituted 42.20% of the isolated strains, and agreed with the results obtained by Fahmy (1986). A total of 53 *Aspergillus* species were isolated and identified. *A. niger* constituted over one third and *A. parasiticus* nearly a quarter. Other species of *Aspergillus* as *A. fumigatus*, *A. terreus*, *A. flavus*, *A. oryzae*, *A. nidulans* and *A. ochraceus* were also isolated. Most of the authors recorded that *A. niger* was the most frequent *Aspergillus* in chilled or frozen meat (Refai and Loot, 1969; Fahmy, 1986; Eldaly et al., 1988). Mycotoxins as aflatoxins, patulin and ochratoxins could be isolated by the different types of *Aspergillus* in the presence of favourable conditions of temperature and relative humidity (Purchase, 1971; Lie and Marth, 1968; Leistner and Eckardt 1981).

Penicillium species constituted 17.60% of the total isolates (Table 6). *Penicillium expansum* and *Penicillium corymbiferum* constituted over 60% of the total penicillii. The rest of *Penicillii* were *P. vercosum* var *cyclospium*, *P. corylophilum* and *P. citrinum*. *Penicillium* species produce blue green spots on frozen meat or produce citrinin, patulin

Table (3): Statistical analysis of total mould count/gm frozen meat

	No./ gm
Minimum	$< 10^2$
Maximum	4×10^3
Maximum	4.6×10^2
Mean value	83.2
St. Error ±	

and ochratoxins in foods (Leistner and Eckardt, 1981; Cote and Buck, 1984; Gracey, 1986).

Dematiaceous hyphomycetes (dark moulds) were identified in Table (5). They constituted 24% of the total isolates (Table 3). These dark moulds originated mainly from intestinal contents and soil, which contaminate slaughter halls; floors, walls and of course meat (Klare, 1971; Abdel Rahman, 1981; Mansour, 1986; Mansour et al., 1991). *Cladosporium* species were 46.67% of the total dark moulds, while *Cladosporium* species constituted 11.20% of the isolated strains (Table 3). *Cladosporium herbarum* and *Cladosporium cladosporioides* are psychrotrophic moulds producing black spots on frozen and chilled meat (Masse, 1912; Silva, 1913; Brodes and Kidd, 1921; Ayres, 1963; Gill and Lowry, 1982; Lowry and Gill 1984; Mansour, 1986). Other dark moulds as *Ulocladium*, *Alternaria* and *Phialophora* could colonize on surfaces and be isolated from slaughtered camels and cattle (Frank 1983; Mansour et al., 1991). *Hilmitosporium* and other dark moulds were also isolated from modern abattoirs (Refai et al., 1993). These black moulds contaminate meat directly from air and/or indirectly from contaminated slaughter halls (Mansour 1991).

The results obtained show that mould contamination needs to be rigidly controlled hygienically. Transportation of meat should be at a temperature not less than -18°C . Attention should be paid to the workers, butchers and other people who handle with meat as educational programmes to improve meat hygiene and ensure consumers safety.

Table (3): Moulds isolated from frozen beef cuts

Moulds	No.	%
1) <i>Aspergillus</i>	53	42.40
<i>A. niger</i>	18	14.40
<i>A. parasiticus</i>	13	10.40
<i>A. fumigatus</i>	6	4.80
<i>A. terreus</i>	5	4.00
<i>A. flavus</i>	4	3.20
<i>A. oryzae</i>	4	3.20
<i>A. nidulans</i>	2	1.60
<i>A. ochraceus</i>	1	0.80
2) Dematiaceous moulds	30	24.00
<i>Cladosporium</i>	14	11.20
<i>C. herbarum</i>	9	7.20
<i>C. cladosporioides</i>	4	3.20
<i>C. macrocarpum</i>	1	0.80
<i>Alternaria</i>	10	8.00
<i>A. alternata</i>	6	4.80
<i>A. sonchi</i>	4	3.20
<i>Helminthosporium</i>	3	2.40
<i>Ulocladium</i>	2	1.60
<i>Phialophora</i>	1	0.80
3) <i>Penicillium</i>	22	17.60
<i>P. expansum</i>	9	7.20
<i>P. corymbiferum</i>	5	4.00
<i>P. verrucosum</i> var <i>cyclopium</i>	4	3.20
<i>P. corylophilum</i>	2	1.60
<i>P. citrinium</i>	2	1.60
4) <i>Trichoderma</i>	5	4.00
5) <i>Absidia</i>	4	3.20
6) <i>Mucor</i>	4	3.20
7) <i>Fusarium</i>	2	1.60
8) <i>Geotrichum</i>	2	1.60
9) <i>Verticillium</i>	2	1.60
10) <i>Rhizopus</i>	1	0.80
Total	125	

Table (4): Frequency of *Aspergillus* isolated from frozen meat.

<i>Aspergillus</i>	No.	%
<i>A. niger</i>	18	33.96
<i>A. parasiticus</i>	13	24.53
<i>A. fumigatus</i>	6	11.23
<i>A. terreus</i>	5	9.43
<i>A. flavu</i>	4	7.55
<i>A. oryzae</i>	4	7.55
<i>A. nidulans</i>	2	3.77
<i>A. ochraceous</i>	1	1.89
Total	53	

Table (5): Frequency dematiaceous moulds isolated from frozen meat.

Dematiaceous moulds	No.	%
<i>Cladosporium</i>	14	46.66
<i>C. herbarum</i>	9	30.00
<i>C. cladosporioides</i>	4	13.33
<i>C. macrocarpum</i>	1	3.33
<i>Alternaria</i>	10	33.33
<i>A. alternata</i>	6	20.00
<i>A. sonchi</i>	4	13.33
<i>Helminthosporium</i>	3	10.00
<i>Ulocladium</i>	2	6.67
<i>Phialophora</i>	1	3.33
Total	30	

Table (6): Frequency of *Penicillium* species isolated from frozen meat.

<i>Penicillium</i> species	No.	%
<i>P. expansum</i>	9	40.91
<i>P. corymbiferum</i>	5	22.73
<i>P. verrucosum</i> var <i>cyclopium</i>	4	18.18
<i>P. corylophilum</i>	2	9.09
<i>P. citrinium</i>	2	9.09
Total	22	

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