Frequency Rates of Fungal Contaminants in Imported Meats from Alexandrian Retail Markets

Hassan A. M. Samaha

Assistant Professor of Microbiology and Immunology; Department of Microbiology and Immunology; Faculty of Pharmacy; Al-Azhar University; Cairo; Egypt
E-Mail: samaha1964@yahoo.co.uk

Abstract: Secondary metabolites excreted by fungal contaminants of foods can lead to fatal health problems. This study investigated one hundred samples of imported frozen meat collected from different Alexandria markets both quantitatively and qualitatively for fungal burden. Both different molds and yeasts were detected with (mean ± SD) and range (48.4± 2.01) x 10^3 and (2 – 9) x 10^4 CFU/gm for molds, besides (51.5 ± 1.99) x 10^3 and (2 - 9) x 10^4 CFU/gm, respectively. Molds were detected in 92% of samples with the following decreasing order: *Aspergillus* species (73.9%), *Penicillium* species (56.5%), *Cladosporium species* (51.1%), *Rhizopus species* (44.6%), *Mucor species* (39.1%), *Alternaria species* (34.8 %), *Trichoderma species* (26.1%), *Helminthosporium species* (22.9%), *Phialophora species* (18.5%), *Geotrichum species* (15.2%), *Fusarium species* (13.0%), *Epidermophyton species* (9.8%), *Paecilomyces species* (8.7%) and *Trichophyton* species (4.3%), respectively. In addition, yeasts were found in tested meat samples in the following descending order: *Candida species* (64.9%), *Torulopsis species* (24.5%) and *Rhodotorulla species* (17.0 %), respectively. Details of the above mentioned results and its ramifications on meat qualities and public health will be discussed in the text.

[Hassan A. M. Samaha. **Frequency Rates of Fungal Contaminants in Imported Meats from Alexandrian Retail Markets**. *Life Sci J* 2013; 10(4):158-165]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 20

Keywords: Mycotoxins. Aflatoxin

1.Introduction:

Disease outbreaks due to the consumption of contaminated food and feedstuff are a recurring problem worldwide. The major factor contributing to contamination are microorganisms, especially fungi, which produce low-molecular-weight compounds as secondary metabolites, with confirmed toxic properties referred to as mycotoxins. Several mycotoxins reported to date are cosmopolitan in distribution and incur severe health-associated risks including hepatic, gastrointestinal and carcinogenic diseases **Frederick *et al.* (2004)** at low level and neurological disorders **Bhat *et al.* (2010).** At moderate levels, immune system function is suppressed, increasing susceptibility to infectious disease and slowing child development. At higher levels, aflatoxicosis can result in liver failure and death **Strosnider *et al.* (2006).** The combination of high hepatitis B prevalence and chronic aflatoxin exposure is thought to be responsible for 75 percent of the approximately 200,000 annual deaths due to liver cancer in sub-Saharan Africa **Hainaut and boyle (2008).** Imported frozen meat is a necessity in order to solve national meat demand in Egypt. It is used mainly as a raw material in manufacturing meat products. Inadequate hygienic measurements during loading, disloading, handling, preparation and processing from the time of slaughtering till reaching to the consumer may lead to a significant increase in fungi burden making imported meat unfit for human and animal consumption. This study is a microbiological of fungal load in imported meat in Egypt.

2.Material and methods:

A random 100 frozen meat samples were collected aseptically from different Alexandria markets in sterile bags, cooled with ice in insulated ice box during its transfer to laboratory for mycological examination. Then, 10 g. of each sample was aseptically transferred to homogenizer flasks containing 90 ml of 0.1% sterile peptone water and homogenized at 1,400 r.p.m. for 2.5 minutes to provide 10^{-1} dilution from which serial dilutions were made up to 10^{-9} (APHA, 1985). Each dilution was spread over two plates of sabouraude's dextrose agar supplemented with chloramphenicol and chlorotetracycline **(Koburger and Norden, 1975)** and first inspected after 3 days incubation at 25°C for fungal growth degree with final report on the 5^{th} day. All moulds were identified according to standard procedures as macroscopical and microscopical examination using slide cultures (**Larone, 1976; Frey *et al.*, 1979 and Samson *et al.*, 1995**), whereas all yeast isolates were identified by the relevant methods **(Lodder *et al.*, 1970; Kreger Van-Rij, 1984 and Koneman *et al.*, 1992)**, as morphological and microscopically studies, ascospore formation and vegetative reproduction on rice agar, germ tube test for *Candida albicans*, Sugar fermentation besides sugar assimilation (FAO, 1992).

3.Results and discussion:

Imported frozen meat especially frozen beef cuts are used mainly as raw material in meat products in Egypt. Therefore, mycological qualification of such frozen meat is very important due to the late usage. This study reported 92% of meat samples, collected from different retail markets in Alexandria were contaminated with molds with average mean ± SD equal to (48.4± 2.01) x 10^3 CFU/gm and a range of mold burden (2 – 9) x 10^4 CFU/gm meat (Figure 1). In addition, 94% of tested meat samples were concurrently contaminated with (51.5 ± 1.99) x 10^3 CFU/gm yeasts and a range of yeasts load (2 – 9) x 10^4 CFU/gm meat (Figure 2). Similar results were reported by **(Samaha and El-Gohary, 1992**; **Hassan *et al.*,1996; Hassanien, 1996).** The mycological evaluation of imported frozen meat gives an index about the hygienic quality of the examined samples. High count of mold and yeast in this study may be due to poor sanitary measures during production, transportation, handling and storage of imported frozen meat. When meat is frozen correctly and maintained at the optimal freezing temperature, it should stay good forever. Contamination of imported frozen meat with mold spores can be attributed to the mishandling of meat from time of their arrival sea port until it reached different retail cold stores as many investigators mentioned that deep freezing (until 18°C) has no significant destructive effect upon mold spores as they can resist cold storage **(Frank, 1966 ; Gill and Lowry, 1982 and Mansour, 1986).** In addition, it is hard to guarantee particularly with home freezing that the meat will remain safe and of good quality for eating but once frozen meat is thawed the microbes come back to life and continue their life's work, which is to multiply and consume **(Wallace, 2003).** Economically, mold and yeast by rendering meat carcasses of inferior quality, unmarketable and unfit for consumption can lead to severe financial losses **(Jay, 1978).** It should be mentioned that Egyptian organization for standardization and quality control (2005) mandates that meat suitable for human consumption must be mold and yeast free.

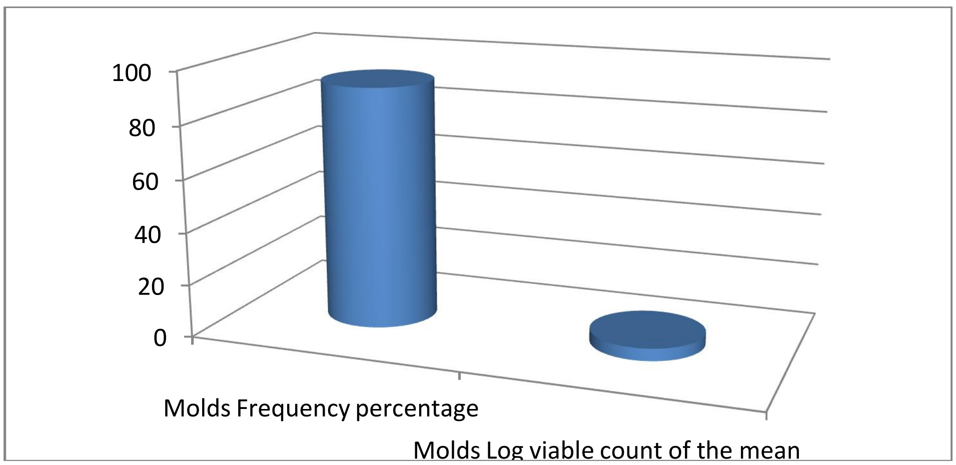


Figure (1): Frequency percentage of molds in meat samples and log of the mean of the viable count.

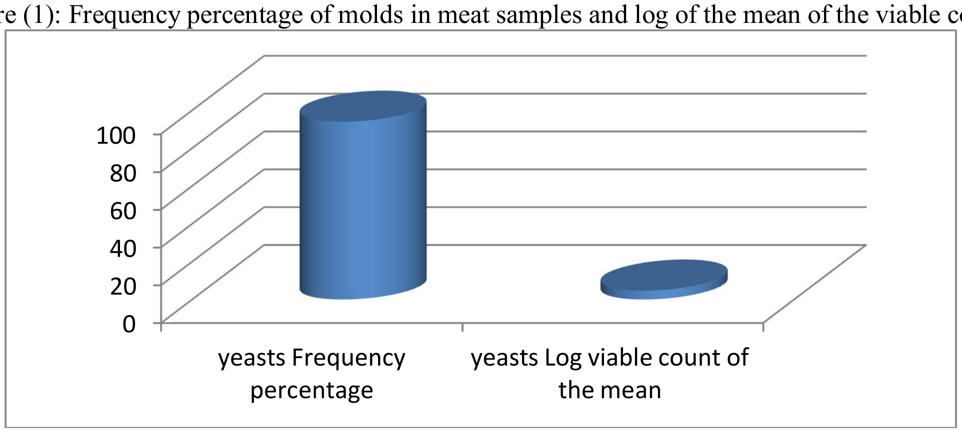
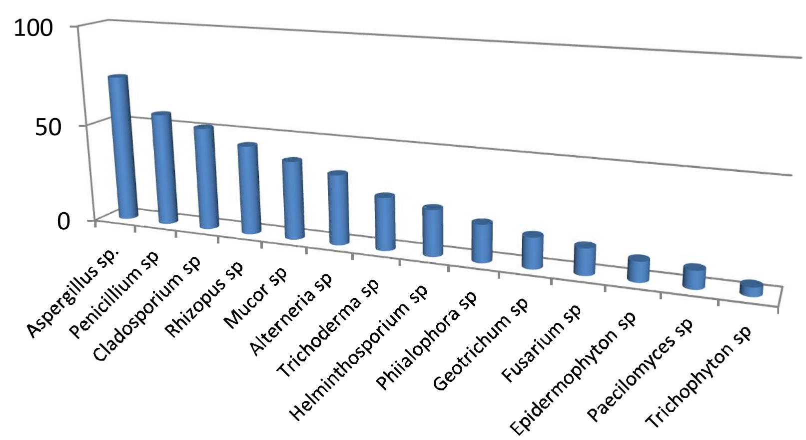


Figure (2): Frequency percentage of yeasts in meat samples and log of the mean of the viable count

This investigation reported imported frozen meat contamination with many fungal genera and species. The most predominant species were *Aspergillus* and *Penicillium* where they were isolated in high frequencies of occurrence from all the examined imported frozen meat. Frequencies percentages of different mold genera could be arranged in descending order, as exhibited in Figure (3), as follows: *Aspergillus spp.* (73.9 %) followed by *Penicillium spp.* (56.5 %), *Cladosporium spp.* (51.1 %), *Rhizopus spp.* (44.6 %), *Mucor spp.* (39.1 %), *Alternaria spp.* (34.8 %), *Trichoderma spp.* (26.1 %), *Helminthosporium spp.* (22.9 %), *Phialophora spp.* (18.5 %), *Geotrichum spp.* (15.2 %), *Fusarium spp.* (13.0 %), *Epidermophyton spp.* (9.8 %), *Paecilomyces spp.* (8.7 %) and *Trichophyton spp.* (4.3 %). Previous investigators reported similar results **(Hadlok, 1970; Nickerson *et al.,* 1972; Torrey *et al.*,1977; King *et al.*,1979; Hechelmann, 1981; Mansour, 1986; Mansour *et al.*,1990; Al - Talhi *et al.*,2004)**.

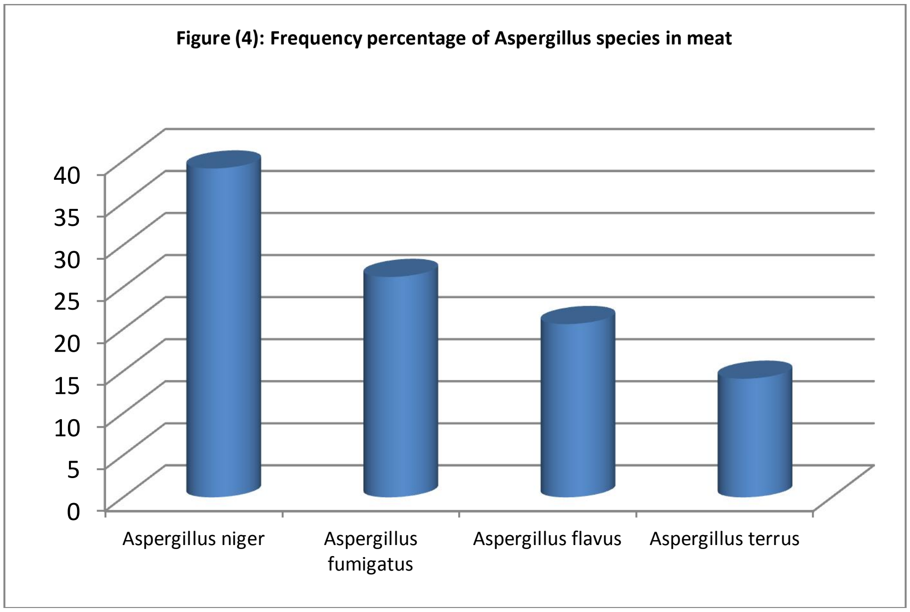
When the most frequent mold genus, i.e. *Aspergillus spp* recovered from 68/ 92 samples (73.9%) of the total fungi, was investigated in detail for their different species, the frequency distribution of *Aspergillus species* isolated from imported frozen meat could be arranged in the following descending order *Aspergillus niger, Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus terrus* in the following percentages 39.1 %, 26.2 %, 20.6 % and 14.1 %, respectively, as had been demonstrated by several researchers **(Abd El - Rahman *et al.*,1985; Fahmy, 1986; El - Khateib and abd El-Rhman, 1989; Zohri, 1990; El – Gazzar, 1992; Samaha *et al.*,1992; Badawy, 2008)**. Some strains of *Aspergillus* such *as A. fumigatus, A. niger* and *A. nidulans* were potential producers of mycotoxins in meat and thereby establish a real public health hazard **(Hanssen and Hagedorn, 1969)**.

Figure (3):Frequency percentage of molds species

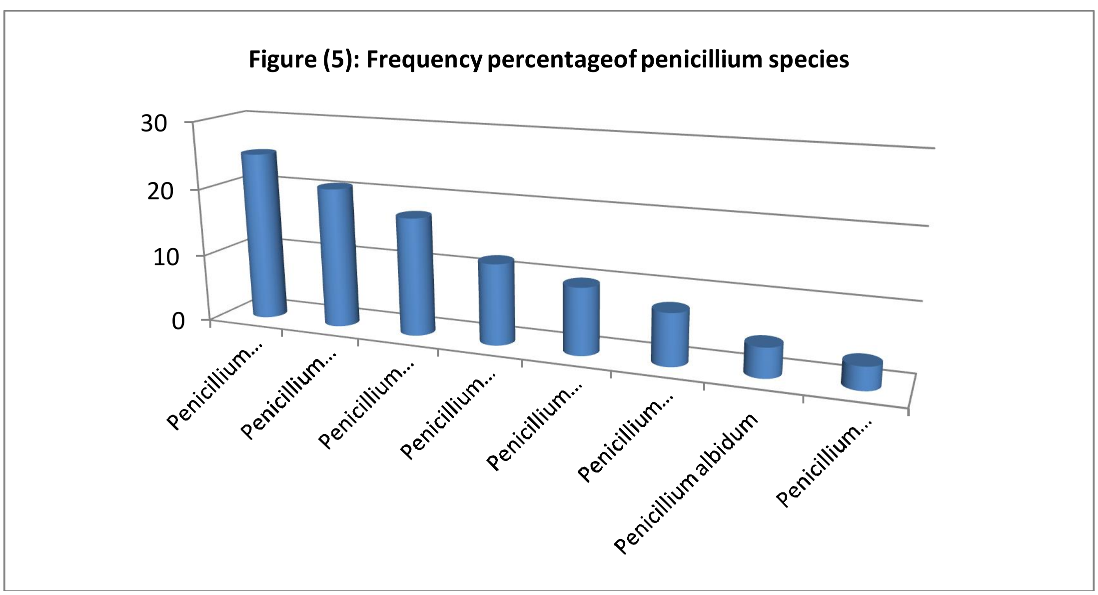


As shown in Figure (4), *Aspergillus niger* was the most frequent isolate, 36/ 92 (39.1 %), as found by **(Mansour *et al.*,1990; Hamdy *et al.*,1993; Refai *et al.*,1993; Ismail *et al.*,1995)**. *Aspergillus niger* could have toxigenic strains with both dangerous effects on human and animal health **(Kamel *et al.*,1976)**. Frequency percentage of *Aspergillus niger* was followed by *Aspergillus fumigatus* and *Aspergillus flavus*. *Aspergillus fumigatus* was detected in 24/92 (26.2 %), as demonstrated by **(Abd El - Rahman *et al.*,1985; Zohri, 1990)**.

Aspergillus flavus was detected in 19/ 92 (20.6 %) samples, similar to those recorded by others **(Zohri, 1990; El – Gazzar, 1992).** It is known that some strains of *Aspergillus flavus* are capable of producing aflatoxins B_1 and B_2. Finally, *Aspergillus terrus* was detected in low frequency percentage, i.e. 13/ 92 (14.1%), as found by **(El – Khateib *et al.*,1989; Ismail *et al.*,1995).** It should be mentioned that Aspergillous spp. are considered as opportunistic pathogen. It may induce pulmonary aspergillosis, allergy, skin infection, sinusitis, as well as nail and ear infection (external otitis). Some species of *Aspergillus* produce aflatoxins, while others can produce sterigmatocystin, patulin and ochratoxin. Aflatoxins proved to have a carcinogenic effect in human in addition to chronic bone damage **(Onions *et al.*,1981and Deger, 1976)**.



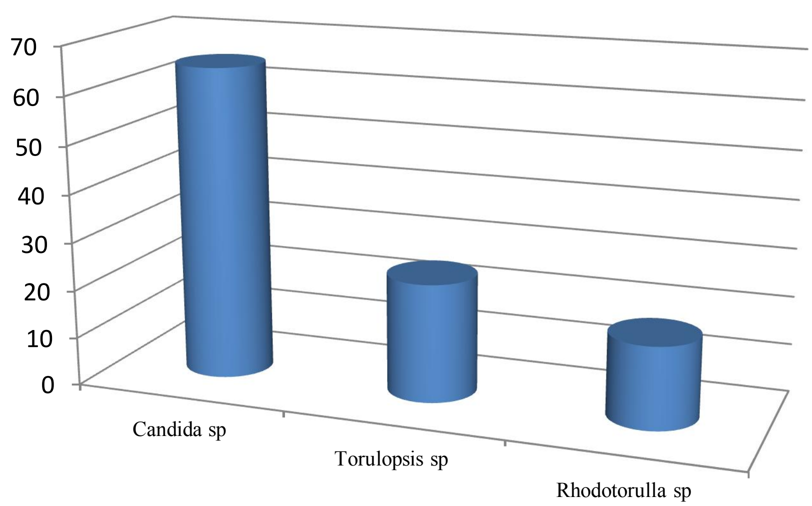
Penicillium occupied the second rank in the total frozen meat samples contaminated with. It was recovered from 52/92(56.5%) of the total fungi contaminated samples. Some species of *Penicillium* have been associated with pulmonary infection, urinary tract infections and yellow rice disease syndrome which are responsible for several cases of death in man **(Banwart, 1980)**. Also, some isolates of genus Penicillium may induce endocardities, external otomycosis, mycotic keratitis and pulmonary infection in immunocompromised patient **(Washington, 1981)**. For example, penicillic acid and sterigmatocystin are mycotoxins produced by some isolates of Penicillium species had a carcinogenic effect **(Mossel, 1982)**. As shown in Figure (5), frequency percentage of *Penicillium species* could be arranged in the following descending order as follows: *Penicillium verrucosum var. cyclopium* (25%), *Penicillium nigricans* (20.7%), *Penicillium oxalicum* (17.4%), *Penicillium grisofulvum* (11.9%), *Penicillium roqueforti,* what (9.8%), *Penicillium expansum* (7.6%), *Penicillium albidum* (4.3%) and *Penicillium multicolor* (3.3%), respectively (Figure 5), similar to those reported by others **(Abd El – Rahman *et al.*,1984; Abd El - Rahman *et al.*,1985; Fahmy, 1986; Abd El – Latif, 1988; Samaha *et al.*,1992; Mansour *et al.*,1994)**.



Besides *Aspergillus* and *Penicillium* species, frozen meat samples existence of *Cladosporium*, *Rhizopus, Mucor, Alternaria, Trichoderma* and *Helminthosporium Phialophora, Geotrichum* and *Fusarium species* at the following frequency percentages, (51.1%), (44.6%), (39.1%), (26.1%), (22.9%), (18.5%), (15.2%), (13%), (9.8%), (8.7%) and (4.3 %), respectively (Figure 3), as previously reported by several investigators **(Fahmy, 1986; Mansour *et al.*,1990; Hassanien, 1996).** Several reports showed that these molds could be implicated in several diseases in immunocompromised patients as chromatomycosis, brain abscess, induction of lesions in rhinofacialcarnial area, lungs, gastrointestinal tract, skin, intraocular infection, external otomycosis, orbital cellulitis, deep wound infection conjunctivitis, endocarditis, hyper sensitive pneumonia and palatitis, bronchitis, colitis, thrush, mycotic keratitis, skin infection in burn patients as well as mycotoxins producion **(Mackie and McCartney, 1956; Jawetz *et al.*,1974; Al-Doory, 1980; Onions *et al.*,1981 and Washington, 1981).**

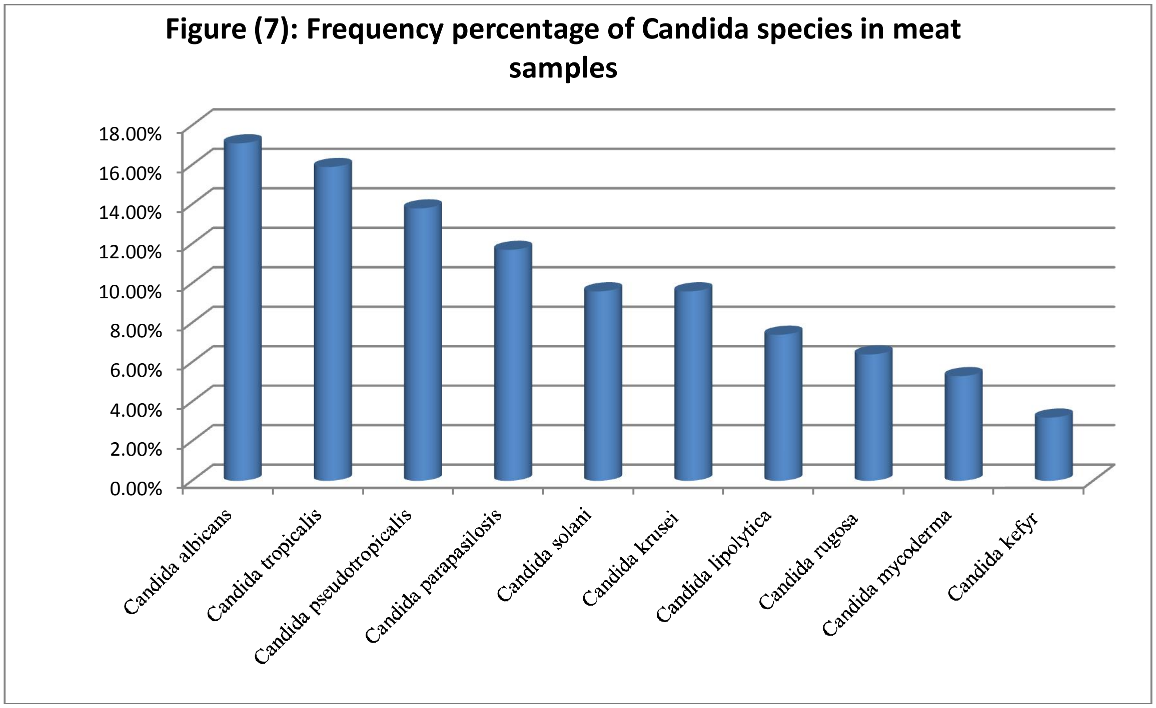
Different yeast genera were isolated from the imported frozen meat. These were *Candida* (64.9%), *Torulopsis* (24.5%) and *Rhodotorulla* (17%) (Figure 6), as listed by several reporters **(Ayres, 1960**; **Badawy, 2008).**

Figure (6): Frequency percentage of yeasts genera

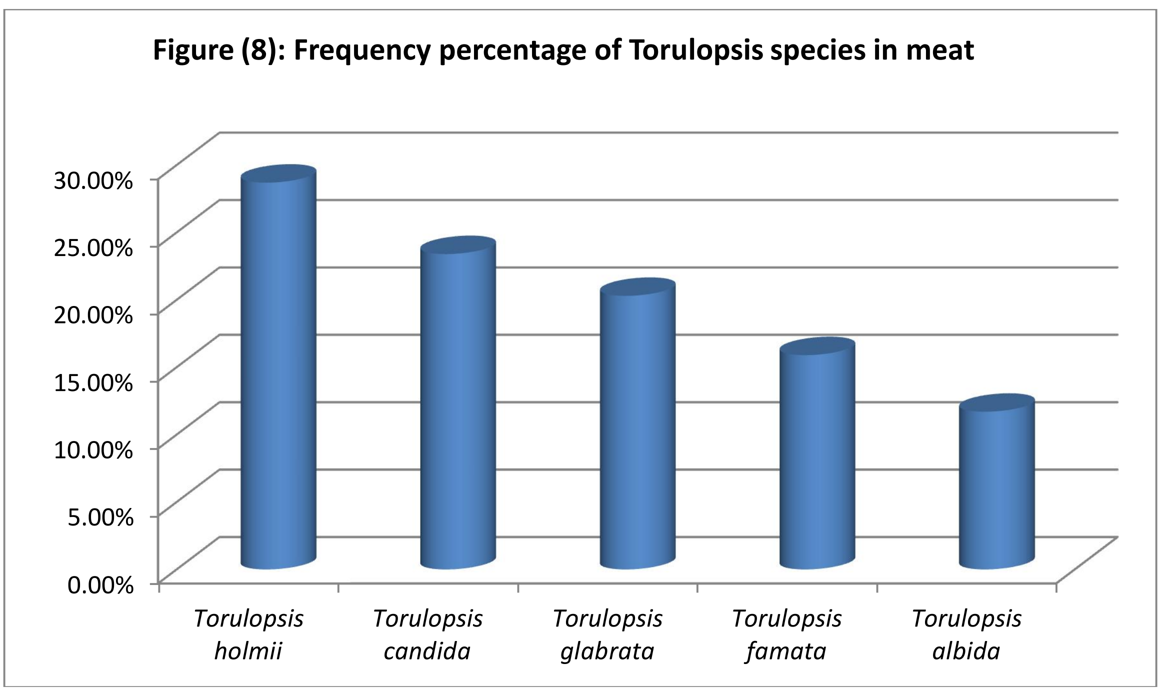


Candida species was the most frequent yeast species isolated from imported frozen meat samples, i.e. 61/94 (64.9%) of the total yeasts. *Candida species* was presented by ten species of which, *Candida albicans* was the most frequent. Frequency percentage distribution of *Candida species* isolated from imported frozen meat were *Candida albicans* (17.1%), *Candida tropicalis* (15.9%), *Candida pseudotropicalis* (13.8%), *Candida parapasilosis* (11.7%), *Candida solani* (9.6%), *Candida krusei* (9.6%) *Candida lipolytica* (7.4%), *Candida rugosa* (6.4%), *Candida mycoderma* (5.3%),, and *Candida kefyr* (3.2%), respectively (Figure 7), similar to others **(Hechelmann, 1981; Fahmy, 1986).**

Some members of *Candida* are incriminated in cases of pulmonary infection, urinary tract infection, vaginitis, thrush, arthritis, osteomyelitis, dermatitis, endocarditis, meningitis and eye infection **(Mackie and McCartney, 1956; Jawetz *et al.*,1974; Al-Doory, 1980 and Washington, 1981).** *Candida albicans* is one of the etiological agents of thrush and pathological condition of white patches in the mouth, throat, esophagus and dermatitis of palms and soles after prolonged antibiotic treatment (**Wilson *et al.*,1981)**. *Candida* may cause gastrointestinal disturbances, vulvovaginitis, endocarditis, pulmonary infection and occasionally fetal systemic disease **(Jesenska and Hrdinova, 1981).** *Candida albicans* is responsible for meningitis, ophthalmitis, osteomyelitis in immunocompromised patients **(Rippon, 1982).** Lipolysis types of yeast as *Candida lipolytica*, may be the main source of fat rancidity accompanied by off odour due to aldehydes and acids production with a main economic losses **(Jay, 1978).**



Torulopsis species was the second rank in the frequency percentage of yeast species isolated from imported frozen meat samples (Figure 8), i.e.23/94 (24.5%) of the total yeast. *Torulopsis species* were five species, where *Torulopsis holmii* was the most frequent, i.e. 27/94 (28.7 %). Frequency distribution of *Torulopsis species* isolated from imported frozen meat was arranged in a descending order as follows: *Torulopsis holmii* (28.7%), *Torulopsis candida* (23.4%), *Torulopsis glabrata* (20.3%), *Torulopsis famata* (15.9%) and *Torulopsis albida* (11.7%), respectively (Figure 8), as previously recorded by **(Baxter and Illston, 1977; Fahmy, 1986).**



8. References

1. **Abd El-Latif, S.M.F. (1988):** Sanitary status of meat serving establishments in Assiut Ph.D.Sc. Thesis meat hygiene Fac. of Vet. Medicine, Assiut University.
2. **Abd El-Rahman, H.; Youssef, H.; and Hefnawey, Y. (1984):** Mycological quality of meat products in Egypt. Assiut vet. Med. J., 12,24 : 153-159.
3. **Abd El-Rahman, H.; Darwish, A. and Hamdy, M. (1985):** Mold affections of meat cold store. Assoc. Vet. Med. J., 14,27 : 131-133.
4. **Al-Doory, Y. (1980):** Laboratory Medical Mycology. Lea & Febiger.
5. **Al-Talhi, A. and Al-Bashan, M. (2004):** Mycological study on fresh and frozen meat in Taif City, Saudi Arabia, Assiut Vet. Med. J., 50, 102 : 22.
6. **APHA (American Public Health Association) (1985):** Standard Methods for the Examination of Dairy Products. 13th ed., Hausher, W.J., Washington, D.C.
7. **Ayres, J.C. (1960):** Temperature relationships and some other characteristics of microbial flora developing on refrigerated beef. Food Res., 25 : 1-18.
8. **Badawy, M. (2008):** Further studies on imported frozen meat. Ph.D.Sc. Thesis, Meat Hygiene. Fac. Vet. Med., Alex. Univ., Egypt.
9. **Banwart, G.J. (1980):** Basic Food Microbiology. A bridged textbook ed. AVI publishing company, INC, Westport, Connecticut, USA.
10. **Baxter, M. and Illston, G.M. (1977):** Environmental reservoirs of psychrrotrophic meat spoilage fungi. New Zealand. Vet. J., 25,6 : 165-167.
11. **Bhat, R.; Rai, R.V. and Karim, A.A. (2010):** Mycotoxins in food and feed : present status and future concern. Comprehensive Reviews in Food Science and Food Safety. 9,1 : 57-81.
12. **Deger, G.E. (1976):** Aflatoxin-human colon carcinogenesis. Ann. Intern. Med. 774-776.
13. **El-Gazzar, M.M. (1992):** Prevalence of mold on the cattle carcasses slaughtered at Zagazig abattoir M.V.Sc. Thesis. Meat Hygiene. Fac. of Vet. Med. Zagazig University.
14. **El-Khateib, T. and Abd El-Rahman, H. (1989):** Mold and yeast hazard in frozen ground beef. Assiut Vet. Med. J., 21,41 : 123-128.
15. **FAO (Food and Agriculture Organization of the United Nations) (1992):** Manual of food quality control and microbiological analysis, Rome. part 4 : 131.
16. **Fahmy, A.M.A. (1986):** Mycological evaluation of frozen meat and chicken. Ph.D.Sc. Thesis. Meat Hygiene. Fac. of Vet. Med., Alex. University.
17. **Frank, H. (1966):** Ein Beitrag Zur taxonomic der Gattung Penicillium link. Habil. Munchen.
18. **Frederick, F. and Richard, F.C. (2004):** Health effect of mycotoxins: a toxicological overview. Clinical Toxicology 2004. 42,2 : 217-234.
19. **Frey, D; Old-Field, R.J. and Bridjer, R.C. (1979):** A colour atlas of pathogenic fungi. Wolfe Medical Public. Ltd. Holland.
20. **Gill, C.O. and Lowry, P.D. (1982):** Growth at sub-zero temperatures of black spot fungi from meat. J. of Appl. Bacteriol. 52 : 245-250.
21. **Hadlok, R. (1970):** Untersuchungen ueber vorkmmen, Herkunft und Bedeutung von Schimmelpilzen insbesondere der Gattung Aspergillus. Micheli Ex Fries, Bei Fleisch Produkten. Heb., Giesen, Germany.
22. **Hainaut, P. and Boyle, P. (2008):** Curbing the liver cancer epidemic in Africa. The Lancet. 371,9610 : 367-368.
23. **Hamdy, M.M.; Mansour, N.K.; Awad, H. and Biomy, M. (1993):** A study on isolated molds with special preference to Aspergillus sp. From surface of fresh and cold stored meat. Vet. Med. J. Giza. 41,2 : 115-120.
24. **Hanssen E.H. and Hagedorn, G. (1969):** Lebensmittel sunter schung 141 : 129- 145, (cited after Kamel et al., 1976, Castellania. 4,8 : 159-161).
25. **Hassan, A.A and Ragheb, R.R. (1996):** Identification of some fungi and mycotoxins in sausage, Vet. Med. J., Giza. 44, 2 : 215-220.
26. **Hassanien, Fatin, S. (1996):** Mycological aspects of some marketed meat products. Zag. Vet. J. 24,1 : 60-64.
27. **Hechelman, H. (1981):** A general discussion of yeasts and molds. Fleischerei. 32,2 : 107-110.
28. **Ismail, M.A.; Abu El-Ala, A.H.; Nassar, A. and Michail, D.G. (1995):** Fungal contamination of beef carcasses and the environment in a slaughter house. Food Microbial. 12 : 44-445.
29. **Jay, J.M. (1978):** Modern food microbiology. 2nd ed. D. Van Nostrand Company.
30. **Jawetz, E.; Melnick, J.L. and Abd El-Berg, E.A. (1974):** Review of medical microbiology. 11th ed. Lance Publications.
31. **Jesenska, Z. and Hrdinova, I. (1981):** Moulds in food in Czechoslovakia. Zeitschrift fur lebensm. Unters. Forsch., 173,1 : 16-20 Dairy Science Abstract 44,38 (1982).
32. **Kamel, S.H.; Refai, M. and Loot, A. (1976):** Studies on the toxins of Aspergillus niger

isolated from meat. Castellania. 4,8 : 159-161. (cited after Zohri, 1990).
33. **King, A.D.; Hocking, A.D. and Pitt, J.I. (1979):** Dichloran rose bengal medium for enumeration and isolation of molds from foods. Appl. Environ. Microbial. 37 : 959-964.
34. **Koburgher, J.A. and Norden, A.R. (1975):** Fungi in foods. VII. A comparison of the surface, pour plate and most probable number methods for enumeration of yeast and mould. J. Milk and Food Technology. 38,12 : 745-746.
35. **Koneman, E.V.; Allen, S.D.; Dowell, V.R. and Summers, H.W. (1992):** Colour atlas and textbook of diagnostic microbiology. 4th ed. J.B. Lipp. Co., New York, London.
36. **Kreger Van Rij, N.J.W. (1984):** The yeasts, a taxonomic study. 3rd ed. Elsevier. Amsterdam.
37. **Larone, D.H. (1976):** Medically important fungi, a guide to identification. Hagerston, Mary Land, USA, London, Harper and Row. Vol. 2.
38. **Lodder, J. and Kreger Van-Rij, N.J.W. (1970):** The yeast a taxonomic study. North Holland publishing company, Amsterdam.
39. **Mackie T.J. and McCartney, J.E. (1956):** Hand book of bacteriology 9th ed. E. and S. Livingstone Ltd. Edinburgh and London.
40. **Mansour, N.K. (1986):** Zum Vorkommen von Schimmelpilzen der Gattung Cladosporium Mlink. Ex. Fries auf Schaffleisch. Diss. Munchen.
41. **Mansour, N.K.; Hamdy, M.; Yassien, N. and Refai, M. (1990):** Dematiaceous hyphomycetes in slaughtered camels, cattle and surroundings at Cairo abattoir. Assoc. vet. Med. J., 24 : 47.
42. **Mansour, N.K.; Yassien, N.A. and El-Sherif, A.M. (1994):** Mycoflora of imported frozen beef. Vet. Med. J. Giza. 42,1 : 89-94.
43. **Mossel, D.A.A. (1982):** Microbiology of food. 3rd ed. Univ. of Utrecht, The Netherland.
44. **Wallace (2003):** How Long Will Frozen Meat Stay Fresh?. Written by O. Wallace Ads by Google copyright © 2003 – 2009. Conjecture corporation.
45. **Onions, A.H.S.; Allsopp, D. and Eggins, H.O.W. (1981):** Smith's introduction to industrial mycology. 7th ed. Edward Arnold.
46. **Refai, M.; Mansour, N.; El-Naggar, A. and Abd El-Aziz, A. (1993):** Fungal flora in modern Egyptian abattoirs. Fleischwirtsch. 73,2 : 172-174.
47. **Rippon, T.W. (1982):** Medical mycology, the pathogenic fungi and the pathogenic actinomyces. 2nd ed. Philadelphia. WB Saunders.
48. **Samaha, I.A. and El-Gohary, A.H. (1992):** Isolation of some mold and yeast from the frozen ground beef of zoonotic importance. Assiut Vet. Med. J., 27,54 : 130-136.
49. Strosnider, H.; Azziz-Baumgartner, E.; Banziger, M. and Bhat, R.V. (2006): Workgroup report: public health strategies for refusing aflatoxin exposure in developing
50. **Washington, J.A. (1981):** Laboratory procedures in clinical microbiology. Springer Verlag, Inc., New York.
51. **Zohri, A.A. (1990):** Mycoflora and mycotoxins of some meat products. Ph.D.Sc. Thesis. Botany Department, Fac. of Sci., Assiut University.

9/25/2013