Saudi Journal of Pathology and Microbiology
Scholars Middle East Publishers
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

ISSN 2518-3362 (Print)
ISSN 2518-3370 (Online)

Microbiological quality of fresh and frozen ground meat, Alexandria city, Egypt

Aiada Daw Mohamed[1*], Ramadan Daw Mohamed[2], haow Mohamed madi[3], Sumayyah Ahmed abdullah[4]
[1,3,4]Laboratory Department, Medical Technology College, Bani Waleed University, Libya
[2]Animal production Department, Agriculture College, Bani Waleed University, Libya

Original Research Article

***Corresponding author**
Aiada Daw Mohamed

Article History
Received: 27.09.2017
Accepted: 10.10.2017
Published: 30.10.2017

DOI:
10.21276/sjpm.2017.2.9.1

Abstract: This study was carried out during a 4 months period from December 2012 to March 2013. A total of 140 fresh and frozen ground beef samples were purchased from local butchers and supermarkets in Alexandria. Each of the ground beef sample was analyzed for its microbiological quality (total plate count, total coliform count and *E. coli* count)**.** Regarding the type of meat, and according to their APC, fresh meat proved to be unsatisfactory in a higher percentage than fresh meat. However according to the TCC parameter, frozen meat samples were a little higher than the fresh meat samples, but in *E. coli* parameter the frozen meat percentage much higher than fresh meat.

Keywords: Fresh , frozen ,ground meat, microbiological quality, APC, TCC and *E. coli*

INTRODUCTION

In fact, tissues from healthy animal are sterile [1], however many factors can influence which microbes are present on certain meat. After slaughtering, meat can be contaminated with bacteria from the water, air, and soil as well as from the workers and the equipments involved during the manufacturing process. Animal products, including carcasses and fresh meat, are easily contaminated with microorganisms and support their growth if not properly handled, processed and preserved [2,3].

The commonest method of prolonging the shelf life of meat is by cooling [4]. Two methods of preserving meat through low temperatures, namely chilling and freezing, can be applied. For chilling, meat is stored at a temperature of 0°C to 4 °C and for freezing -18°C. The colder the temperature, the slower the enzyme action and the growth and development of bacteria. Thus it can be said that meat can be stored longer at freezing temperatures than at chilling temperatures [5].

Meat is first chilled after slaughter. It may be kept chilled if there is only a short period of time for distribution. The shelf life of all types of unpackaged meat held at chilling temperature, 0°C to or even better -1°C to +4°C, is only between a few days and one to two weeks - depending on the cut of meat, temperature, bacterial load and relative humidity [4]. Also fresh meat products are commonly marketed at refrigerated temperatures (2–5°C). However, many undesirable changes of the products can occur during refrigeration due to microbial growth and lipid oxidation, which give rise to quality reduction, meat spoilage, and economic loss [6].

For longer storage periods, the shelf life is much longer at freezing temperatures and depends on circumstances such as whether or not it is packaged and type of packaging temperature [4].

Grau (1979), mentioned that meat surface temperatures remains in the growth range for Escherichia and Salmonella flora for a considerable period and Enterobacteriaceae counts of spay chilled carcasses increase during chilling[7].This explains the fact that although the initial microbial contamination of meat contains both mesophilic and cold tolerant bacteria, only the latter will compete successfully at chill temperatures.[8] Ray mentioned that chilled meat has mesophiles, such as Micrococcus, Enterococcus, Staphylococcus, Bacillus, Clostridium, Lactobacillus, coliforms, and other Enterobacteriaceae, including enteric pathogens [9].

While freezing and frozen storage has some impact on bacteria, frozen foods are not sterile and are not sterilized by prolonged freezing [10]. Survival of bacteria on meat is related to the rate of freezing. Slow freezing has been shown to be more lethal than rapid freezing [10].During slow freezing (e.g. a reduction of 1°C/min), most microorganisms move into the unfrozen fraction of water in the food[11]. As extracellular ice forms in this fraction, the solutes become more concentrated in the unfrozen water. This causes increased water loss from the bacterial cells and exposes them to osmotic stress over a prolonged period [12].Osmotic stress causes a change in the intracellular pH and ionic strength, which inactivates enzymes, denatures other proteins, and subsequently interferes with metabolic processes. The membranes and membrane transport systems may also be irreparably damaged, and the bacteria can become more sensitive to oxidative stress [12].

The species of bacteria present in the frozen product depends on the initial population [11]. Some are killed, while others are only sublethally damaged and can recover upon thawing, particularly if frozen storage is above -10°C (below -10°C sublethally damaged bacteria tend to die over time, hence the recommendation that frozen meat be stored at or near -18°C). Usually the process of freezing, rather than frozen storage, is more lethal to bacteria [13].

In general, Gram negative bacteria are more susceptible to freezing injury than Gram positive organisms [11]. Fresh meat productions are marketed after having been cooled freshly or frozen in a particular way. In both situations the meats need to be marketed without getting sour and been corrupted and without any change in the quality and appropriate packaging. The microorganisms are very good indicators concerning whether the food has been processed in hygienic conditions. The number of aerobic /Coliforms bacteria is a good criterion in determining the hygienic quality of meat [14].

MATERIAL AND METHODS

Sample collection

One hundred grams of ground meat were obtained from worker in collection sites and placed in the food collection bags. Sample source and sample number was identified on sample form. Refrigeration of sample was used during transport. Frozen samples were kept frozen. A submission form for each sample was filled out including: type of meat and its color; date and locality of the collection site; name of butcher and its personal hygiene and sanitary condition of the shop.

Sample preparation

Ten gram of each sample were weighted and transferred to a stomacher bag under aseptic conditions. The sample was then diluted to a 10^{-1} dilution with 90ml of peptone water and homogenized for 2 min by using a Stomacher. Following homogenization, ten-fold serial dilutions for each sample were made in sterile peptone water up to 10^{-4}, by transferring 1 ml of previous dilution to 9 ml of peptone water**.**

Microbiological analysis

Aerobic Plate Count

One ml of each dilution was pipette into separate, appropriately marked Petri dish and to each plate, 12-15 ml plate count agar (cooled to 45 ± 1°C) was added within 15 min of the original dilution. After Petri dishes solidified were incubated promptly for 48 ± 2 h at 35°C. Plates with colony number between 30-300 colony-forming units (CFU) were selected, coun++ted and multiplied by the dilution factor to calculate CFU/gram of ground meat.

Determination of coliform and E. coli using the Most Probable Number (MPN) method

One ml of each dilution was cultured in the Lauryl Sulphate Tryptose broth (LST) tubes for determining the presence of coliform and the positive LST tubes showing gas were subcultured into Brilliant Green Bile (BGB) broth and EC broth. All positive BGB tubes recorded as confirmed MPN of coliform bacteria per gram of ground meat, however gas production in the EC tubes was considered a confirmed test for fecal coliform organisms and subcultured onto Eosin Methylene Blue (EMB) agar plates which incubated for 24h at 35°C and examined for green metallic sheen colonies and then transfered to IMViC (Indole ,Methyl red ,Vogues-Proskauer and Citrate) tubes. MPN of *E*. coli per gram were computed considering producing ++-- or-+-- IMViC patterns.

RESULT AND DISCUSSION

The results of this study were recorded according to the Commission of the European Communities[15] and the Egyptian guidelines[16,17] as satisfactory, acceptable and unsatisfactory.

Tables 1 a b and c show the type of ground meat samples in relation to their microbiological quality according to their APC, TCC and *E. coli* count.

Regarding the microbiological quality of the140 examined ground meat samples, 98(70.0%) were fresh meat. Out of these, 35(35.7%) were satisfactory according to their APC parameter, while 36(36.7%) were acceptable and 27(27.6%) were unsatisfactory. The corresponding figures for the 42(30.0%) frozen meat were 22(52.4%),12(28.6%) and 8(19.0%) respectively. (Table 1 a).

The collective means of the APC of fresh and frozen meat were 4.6×10^6 cfu\g and 2.9×10^6 cfu\g respectively. Table 1 b shows the results of the previous samples according to their TCC. The corresponding figures for these samples were 28(28.6%), 33(33.7%) and 37(37.8%) respectively in the fresh meat. Those of the frozen meat were 9(21.4%), 16(38.1%) and 17(40.5%) respectively.

The collective means of the TCC of the fresh and frozen meat were 9.3×10^4 cfu\g and 1.2×10^4 cfu\g respectively.

Table 1 c shows the results of the previous samples according to their *E. coli* count. The corresponding figures for these samples were

53(54.1%) 32(32.7%) and 13(13.3%) respectively in the fresh meat. Those of the frozen meat were 14(33.3%), 19(45.2%) and 9(21.4%) respectively.

The collective means of the *E. coli* count of the fresh and frozen meat were 1.5×10^3 cfu\g and 3.1×10^3 cfu\g respectively.

It was calculated that the meat type did not affect statistically the microbiological quality of the examined ground meat samples according to their APC, TCC or *E. coli* count (P ranged from 0. 075 to 0. 180).

Table 1: Type of the ground meat samples in relation to their microbiological quality according to their parameters

a. APC parameter

Type of Meat	Microbiological quality of ground meat								
	Satisfactory (n=57)		**Acceptable (n=48)**		**Unsatisfactory (n=35)**		**Total (n=140)**		**Mean**
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**CFU\g**
Fresh	35	35.7	36	36.7	27	27.6	98	70.0	4.6×10^6
Frozen	22	52.4	12	28.6	8	19.0	42	30.0	2.9×10^6

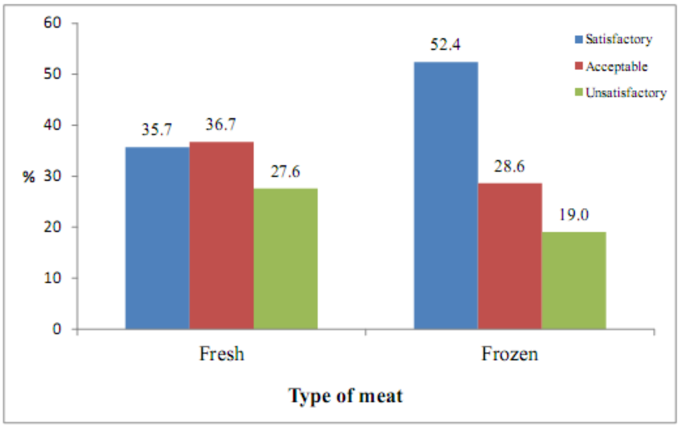
b. TCC parameter.

Type of Meat	Microbiological quality of ground meat								
	Satisfactory (n=37)		**Acceptable (n=49)**		**Unsatisfactory (n=54)**		**Total (n=140)**		**Mean**
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**CFU\g**
Fresh	28	28.6	33	33.7	37	37.8	98	70.0	9.3×10^4
Frozen	9	21.4	16	38.1	17	40.5	42	30.0	1.2×10^4

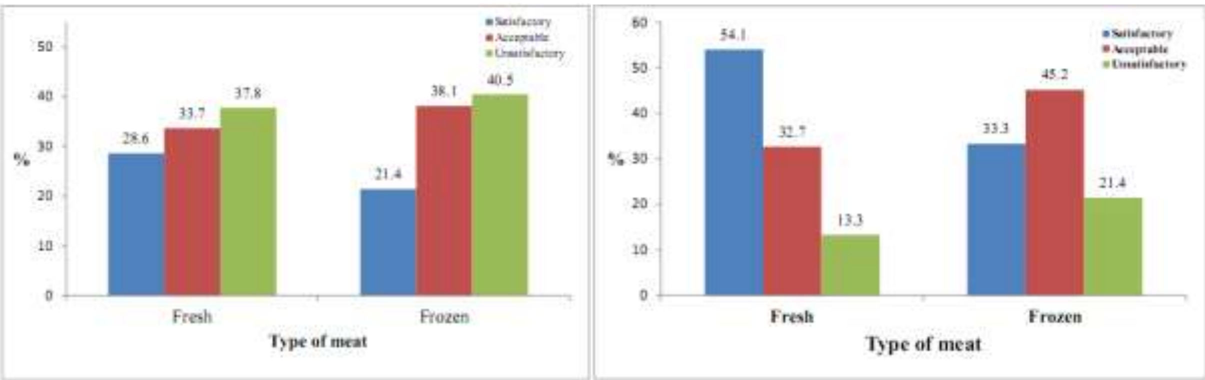
c. *E. coli* parameter.

Type of Meat	Microbiological quality of ground meat								
	Satisfactory (n=67)		**Acceptable (n=51)**		**Unsatisfactory (n=22)**		**Total (n=140)**		**Mean**
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**CFU\g**
Fresh	53	54.1	32	32.7	13	13.3	98	70.0	1.5×10^3
Frozen	14	33.3	19	45.2	9	21.4	42	30.0	3.1×10^3

Figures 1 a, b and c show the same results as a bar chart.



a)APC parameter



b)TCC parameter **c) *E. coli* parameter**

Fig-1: Type of the ground meat samples in relation to their microbiological quality according to their parameters

The need for microbial assessment of fresh meats and other meat products processed and packaged for human consumption is emphasized and recommended to reduce possible contamination [18].

When meat is frozen the number of the microorganisms decreases. However the melted frozen meat creates suitable conditions for the reproduction of bacteria and increases the potential of food to get spoiled [14]. Indeed, and according to the APC parameter, the percentage of fresh meat samples of the present study showed unsatisfactory results (27.6%) higher than the 19.0% of the frozen meat samples. This results of frozen meat samples was comparable to the 21.2% of the frozen meat pies reported by Gunderson *et al* [19]. However, the frozen meat samples studied by Kumar *et al* [20] were unsatisfactory in a much higher percentages ranging from 38.91% to 84.34%**.**

Since the number of coliform bacteria is a good criterion in determining the hygienic quality of meat [21]. so the high level of TCC in this study may indicate poor hygienic processing, handling or storage of the studied frozen and fresh meat, where 40.5% were recorded as unsatisfactory frozen meat and 37.8% fresh meat. These results are considered high when compared with the study reported in Australia by Vaderline *et al* [22], who found coliform number of >1000 g in only 15 samples (1.9%) out 790 frozen meat samples.

Irrespective of the presence of Gram negative organisms in fresh meat analyzed, it is believed that cooking processes and hygiene could greatly reduce the microbial load to harmless level. Thorough cooking as well as good hygiene is the order to prevent contamination of food eaten raw are therefore essential[23]. The studied samples were examined for their *E. coli* count and revealed 21.4% of the frozen meat samples to be unsatisfactory while only 13.3% of the fresh one. These results are comparable to the 20% reported by Plavsic *et al* [24]. However Abid *et al* [21] in Pakistan found *E. coli* absent in all 20 frozen meat examined samples.

CONCLUSION

From the previous results it can be concluded, that the APC decreased from fresh to frozen meat however little increase in frozen meat was recorded according the TCC as well as a higher increase in *E. coli* count; but in general there was no significant difference between the two types of meat.

REFERENCES

1. Lawrie, R. A. (1984). The preservation effect of smoke on meat. Meat Science Pergaman Press Inc. *Maxwell House Fair view park –Elmford: New York*, 49-52.
2. Ray, B., & Bhunia, A. (2008). Control by reduced water activity. In: Ray B, Bhunia A, editors. Fundamental Food Microbiology. London: *CRC Press*, 385-90.
3. Mead, G. C., & Thomas, N. L. (1973). Factors affecting the use of chlorine in the spinchilling of eviscerated poultry. *British Poultry Science, 14*, 99-117.
4. Meat and meat products in human nutrition in developing countries, [Internet], available from: http://www.fao.org/docrep/t0562e/T0562E01.htm#Acknowledgments [accessed 2013 May 8].
5. Johanna, E. (2005). Crobiological quality of raw fresh beef postharvesting. [Master Thesis]. Faculty of Health Sciences Tshwane: *University of Technology*.
6. Gheisari, H. R. (2011). Correlation between acid, TBA, peroxide and iodine values, catalase and glutathione peroxidase activities of chicken, cattle and camel meat during refrigerated storage. *School of Veterinary Medicine, Shiraz University, Shiraz, Iran, Veterinary World, 4*(4), 153-7.
7. Samuel, J. L., O' Boyle, D., Mathers, W. J., & Frost, A. J. (1979). Isolation of *Salmonella* from mesenteric lymph nodes of healthy cattle at slaughter. *Research in Veterinary Science, 28,* 231-41.
8. Strydom, P. E., & Buys, E. M. (1995). The Effects of spray chilling on carcass mass loss and the surface associated bacteriology. *Meat Science, 39* (2), 265-76.

9. Ray, B. (2005). *Fundamental Food Microbiology*. 3rd ed. Taylor and Francis e-library, 44-5.
10. Ayres, J. C., Mundt, J. O., & Sandine, W. E. (1980). Microbiology of foods. San Francisco: W.H. Freeman and Company.
11. Gill, C. O. (2002). Microbial control with cold temperatures. In: Juneja VK, Sofos JN, editors. Control of foodborne microorganisms. *New York: Marcel Dekker*, 55-74.
12. Farkas, J. (1997). Physical methods of food preservation. In: Doyle MP, Beuchat LR, Montville TJ, editors. Food Microbiology: Fundamentals and frontiers. *Washington D.C: ASM Press*, 497-519.
13. Roberts, T. A., Pitt, J. I., Farkas, J., & Grau, F. H. (2012). Microbial Ecology of Food Commodities. In Microorganisms in Foods. Volume 6. *Blackie Academic & Professional; Chapter 10*, 379-389.
14. Ismail, K., & Belma, D. (2002). Microbiological investigation on some of the commercial frozen meat in Izmir. *Turk Electronic J Biotechnol, Special issue*, 18-23.
15. Kyprianou, M. (2005). commission of The European Communities, microbiological criteria for foodstuffs, Brussels, C Available from: http://www.food.gov.uk/multimedia/pdfs/microcriteria2005reg.pdf. [Accessed 2013 May 3].
16. Egyptian organization for standardization and quality control. Egyptian standards frozen meat, Egypt, E.S 1522_2005, [Internet]. Available from; http://www.eos.org.eg/public/ar-eg/, [Accessed 2013 May 13].
17. Egyptian organization for standardization and quality control. Egyptian standards fresh meat, Egypt, E.S 4334_2004, [Internet]. Available from; http://www.eos.org.eg/public/ar-eg/, [Accessed 2013 May 13].
18. Okonko, I. O., Ogun, A. A., Adejoye, O. D., Ogunjobi, A. A., Nkang, A. O., & Adebayo-Tayo, B. C. (2009). Hazards analysis critical control points (HACCP) and Microbiology qualities of Sea-foods as affected by Handler's Hygiene in Ibadan and Lagos, Nigeria. *African Journal of Food Science*, *3*(1), 035-050.
19. Kereluk, K., & Gunderson, M. F. (1959). Studies on the Bacteriological Quality of Frozen Meat Pies. *Appl. Environ. Microbiol*, *7*(5), 320-323.
20. Kumar, A., Etsay, K., & Enquabaher, K. (2010). Evaluation of quality of beef produced and sold in parts of Tigrai region of Ethiopia. *Trop Animal Health Product*, *42*(3), 445-9.
21. Abid, H., Ali, J., Hussain, F., & Rehman, Z. (2010). Microbiological quality evaluation of frozen food available in Peshawar city, Pakistan. *Pak J Biochem Mol Biol*, *43*(1), 29-32.
22. Vanderline, P. B., & Shay, B. M. J. (1998). Microbiological quality of Australian beef. *Carcass Meat and Frozen Protection*, *61*, 437-43.
23. Amann, R. L., Ludwing, W., & Schleifer, K. H. (1995). Phylogenetic Identification and Situ detection of Individuals microbial cell without cultivation. *Microbial Rev, 59* (91), 4369.
24. Plavsic, D. V., Saric, L. C., Cabarkapa1, I. S., Gubic, J. M., & Popovic, M. L. (2009). Microbiological safety of minced and grill meat in the year 2009. *Food Processing, Quality and Safety*, *3-4*, 49-52.