MICROBIAL AND CHEMICAL QUALITY OF RETAILED MINCED MEATS

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ABSTRACT 50 random locally produced minced meat samples (25 each of packaged frozen Received at: 25/3/2015 and chilled) were collected from supermarkets and meat markets in Damanhour City to assess their microbiological and chemical quality. The obtained results of Accepted: 7/10/2015 frozen minced meat showed that the logarithmic means \pm S.E. of aerobic plate counts (APC), anaerobic, enterobacteriacae, coliforms, Staphylococcus aureus and mould and yeast counts were : 6.15 ± 5.78 , 4.60 ± 3.89 , 4.40 ± 4.11 , $3.93\pm$ 3.64, 4.40 \pm 3.88 , 2.72 \pm 2.18 and 3.91 \pm 3.64 \log_{10} cfu/g, respectively, while in chilled mince were : 6.83 ± 6.04 , 4.86 ± 4.04 , 4.76 ± 4.49 , 4.57 ± 4.26 , 5.26 ± 4.71 , 3.59 ± 3.49 and $3.65\pm3.04 \log_{10}$ cfu/g, respectively. This study mentioned that the maximum permissible limit was exceeded by 36% and 88 % for total aerobic counts (6 log₁₀ cfu/g), 72% and 84% for Staph.aureus according to Egyptian standard specifications (ESS) No. 1694/2005 of Egyptian Organization for Standardization and Quality Control. Also, samples of both products were not be completely free from coagulase positive Staphylococcus aureus but were completely free from Escherichia coli, Shigellae and Salmonellae as pathogenic microorganisms. The chemical examination revealed that the hydrogen ion concentrations(pH) of frozen samples was ranged from 6.10 to 6.71, with a mean \pm S.E. of 6.26 \pm 0.03 while in chilled ones was ranged from 6.12 to 6.86, with a mean \pm S.E. of 6.34 \pm 0.03, while total volatile basic nitrogen (TVB-N) in frozen mince was ranged from 6.72 to 13.44, with a mean \pm S.E. of 9.52 \pm 0.37 mg N/100g and was ranged from 6.72 to 24.19, with a mean \pm S.E. of 13.38 ± 0.96 mg N/100g in chilled mince. These values of TVB-N were not exceeded the maximum permissible limit, (20 mg N/100g) according to ESS No.1694/2005 except 12% of chilled minced samples. The fat oxidation criteria determined by thiobarbituric acid value (TBA) in both products were very low and did not exceeded the permissible limit in foods (0.9 mg malonaldhyde/kg). These results indicated that the microbiological quality of retailed minced meat samples were unsatisfactory, and have health hazards for consumers as it may be lead to food poisoning.

Key words: Minced meat, Microbiology, Chemical quality, pH, TVB-N, TBA.

INTRODUCTION

Minced beef meat has very high economic value because of its nutritive quality and practically usage in almost every type of meat products meals and as a food of animal origin is highly susceptible to microbial invasion and food poisoning (Velzen *et al.*, 2008).

Retail shop minced meat contain higher microbial load of the large exposed surface area, more readily available water, nutrient and greater oxygen penetration (Forest *et al.*, 1985). Extrinsic parameters (e.g., temperature and packaging atmosphere) can

affect meat spoilage. Microbial spoilage leads to the development of off odors and slim formation which product undesirable for human makes the consumption (Djenane et al., 2005; Ercolini et al., 2006 and Koutsoumanis et al., 2006). Microbial flora of spoiled meat is very complex and this makes the spoilage very difficult to prevent and it is a limiting factor which determines the shelf-life of meat. The initial microflora of meat is mesophilic and after carcass evisceration microbial load reaches to $10^2 - 10^4$ bacteria per 1cm². This load can vary with storage conditions (Saucier et al., 2000). It has been established that microbial levels of 6-7 log cfu/g are critical spoilage of meat (Djenane et al., 2005).

The surface of meat cut support the growth of a large number of microorganisms and ground meats offer not only ample and desirable surfaces but also a through inoculation of the meat during grinding (Banwart, 1987).

Enterobacteriaceae family includes facultative anaerobic gram negative straight bacilli, motile employ peritrichous flagella. Most members of this family are mainly mesophilic while some strains can grow at 0°C (Downes and Ito, 2001). Enterobacteriaceae family include some of foodborne genera as *Escherichia*, Salmonella, Klebsiella, Serratia, Enterobacter, Citrobacter, Yersinia, Proteus, Providencia, Shigella and Erwinia that pose a great health risks for consumers (Holt et al., 1994).

Among food poisoning microorganisms of most concern with meat are *Staph.aureus*, *Salmonella* and enteropathogenic *E.coli*. Growth of *Staph.aureus* is restricted by the rapid drop in pH. For products heavily infected with *Staph. aureus*, there may be a risk after storage above 15°C, as staphylococci can multiply to 10⁶/g even at pH of 5.6 to 6.8 (ICMSF, 1980). *Staph.aureus* is resistant to drying and may grow and produce enterotoxins in products having a water activity as low as 0.85 (Merson, 1973).

Spoilage is commonly detected by sensory and/or microbiological analysis, in addition to an alternative method involves the measurement of chemical changes associated with the growth of specific organisms spoilage in meat and meat products (Dainty, 1996). Lipid oxidation is one of the main factors used to estimate meat quality due to the susceptibility of meat and meat products to oxidative degeneration (Morrissey et al., 1998). The control of lipid oxidation in fresh and further processed meat products is a goal of food scientists and food processors (Sheldon et al., 1997). The changes in quality incurred by lipid oxidation are manifested by adverse changes in colour, flavour, and nutritive value, and also by the possible production of toxic compounds (Jensen et al., 1998).

The objective of this study was to evaluate the microbial and chemical quality of minced meat (frozen and fresh) usually retailed in Damanhour City, Egypt. The hygienic quality was appreciated using a standardized sampling method and enumeration of bacteria of hygienic importance, also with screening of some pathogens as Staphylococcus aureus, Salmonella spp. and Shiglla spp.

MATERIALS and METHODS

I - Collection of samples:

A total of 50 random samples of about 500g of locally produced minced meat (25 each of frozen

polyethylene packaged and unpackaged chilled type) were collected from different meat markets of Damanhour City. Each sample was wrapped separately in sterile polyethylene bag and transferred directly to the laboratory without delay in an ice box. In the laboratory, the following examinations were performed at once.

II- Bacteriological examination:

1. Preparation of samples according to ISO 6887 / 2 / 2003:

Ten grams of the prepared sample were transferred to a sterilized homogenizer flask containing 90 ml of 0.1% sterile peptone water. The contents were homogenized at 14000 r.p.m. for 2.5 minutes to provide a dilution of 10^{-1} . The homogenate was allowed to stand for 5 minutes at room temperature, then 1 ml of homogenate was transferred with a sterile pipette into a sterile test tube containing 9 ml of 0.1% sterile peptone water to obtain a dilution of 10^{-2} . Then further decimal ten fold serial dilutions up to 10^{-6} were prepared.

2. Total viable aerobic bacterial count (APC) according to APHA, 2001:

One ml from each dilution was transferred into duplicate sterile Petri dishes and mixed with about 10 ml of sterile plate count agar medium "melted and kept at 45°C. After solidification, cultivated plates as well as control one were incubated at 37°C for 48 hours in an inverted position. Average count was calculated as a total aerobic count per gram of sample.

3. Total viable anaerobic bacterial count (Gudkov and Sharpe, 1966):

0.1ml of each dilution was seeded evenly into each of duplicate plates of reinforced clostridial agar medium. The plates were overlodged with an additional layer of agar medium at 50-55°C. All solidified plates were incubated anaerobically using gas pack anaerobic jar at 37 °C for 48hr. Anaerobic bacterial counts were calculated.

4. Enumeration of total Enterobacteriaceae count (ISO, 2004):

0.1 ml from each of the previously prepared serial dilutions was aseptically transferred and evenly distributed over a dry surface of violet red bile glucose (VRBG) agar medium by a bented glass rod, After setting, a thin layer of (VRBG) agar was overlaid. Inoculated plates as well as control one was incubated at an inverted position at 37°C for 24-48 hrs. All purple colonies which surrounded by a purple zone were counted and the average number of colonies was determined. The Enterobacteriaceae count cfu/g was calculated.

5. Total Coliforms count (FDA, 2002):

0.1 ml from each of the previously prepared serial dilutions was aseptically transferred and evenly distributed over a dry surface of duplicate plates of sterile violet red bile agar media (VRB). by a bented glass rod, After setting, a thin layer of (VRB) agar was overlaid. After setting, the inoculated plates were incubated at 37°C for 24 hrs. All dark red colonies measuring 0.5 m.m or more in diameter of uncrowed plates were then counted and the average number of colonies was determined and so the Coliform count per gram was calculated. Suspected colonies were stabbed in semi-solid agar for further identification.

6. Detection and isolation of *Salmonellae* was carried out according to the methods outlined by ISO 6579 / 2002.

25 g of sample were aseptically added to 225 mL of preenrichment medium, buffered peptone water and incubated for 18h at 37°C. The preenriched culture, 0.1 and 1 mL, respectively, was transferred to Rappaport -Vassiliadis broth and Selenite broth and incubated at 42 and 37°C, respectively. After 24 and 48 h of incubation, a loopful from each of the enriched broths was streaked onto plates of *Salmonella Shigella* agar and Xylose Lysine Deoxycholate (XLD) agar and incubated at 37°C for 24 h.

7. Detection and isolation of Shigellae (Feng et al., 2007).

8. Total Staphylococcus aureus count (FAD, 2001):

0.1 ml from each decimal dilution was spread over the surfaces of duplicate dried Baired Parker agar plates. The inoculated plates were incubated at 37°C for 48 hours in an inverted position. The black shiny colonies with narrow white margines and surrounded by a clear zone were counted. Suspected colonies were stabbed in semi-solid agar for further morphological and biochemical identification (catalase, mannitol, coagulase, thermostable nuclease production and oxidation-fermentation of glucose).

9- Total mould and yeast count:

The total mould and yeast counts was done by using Sabourad's dextrose agar medium, supplemented with chloramphenicol and chlortetracycline (100 mg of each) as described by koburger (1970).

III-Chemical examination:

1- pH (Hydrogen ion concentration): according to ESS 63-11 / 2006.

2- Determination of total volatile basic nitrogen "TVB-N" (FAO, 1980):

by Conway microdiffusion method.

3-Determination of thiobarbituric acid value (TBA): according to ESS 63-10 / 2006.

Table 1: Statistical analytical results of different microbial counts $(\log_{10} \text{ cfu/g})$ of the examined minced meat samples (n=25 of each)

RESULTS

| | | Froz | en | | Chilled | | | | | |
|---------------------------|----------|---------|------|-------------|------------|---------|------|------|--|--|
| Parameter | Positive | Samples | M GE | | Positive S | Samples | Maaa | | | |
| | No. | % | Mean | 5.E. | No. | % | Mean | 5.E. | | |
| APC | 25 | 100 | 6.15 | 5.78 | 25 | 100 | 6.83 | 6.04 | | |
| T.anaerobic count | 25 | 100 | 4.60 | 3.89 | 25 | 100 | 4.86 | 4.04 | | |
| T.enterobacteriaece count | 10 | 40 | 4.40 | 4.11 | 19 | 76 | 4.76 | 4.49 | | |
| T. coliform count | 6 | 24 | 3.93 | 3.64 | 18 | 72 | 4.57 | 4.26 | | |
| Staph. aureus count | 18 | 72 | 4.40 | 3.88 | 21 | 84 | 5.26 | 4.71 | | |
| T.mould count | 18 | 72 | 2.72 | 2.18 | 19 | 76 | 3.59 | 3.49 | | |
| T.yeast count | 19 | 76 | 3.91 | 3.64 | 22 | 88 | 3.65 | 3.04 | | |

- S.E.= Standard error of mean .

| | - | Fr | ozen | | Chilled | | | | | |
|--------------------|------|-------|------|-------|---------|-------|-------|------|--|--|
| parameter | Min. | Max. | Mean | S.E.M | Min. | Max. | Mean | SEM | | |
| рН | 6.10 | 6.71 | 6.26 | 0.03 | 6.12 | 6.86 | 6.34 | 0.03 | | |
| TVB- N(mg/100g) | 6.72 | 13.44 | 9.52 | 0.37 | 6.72 | 24.19 | 13.38 | 0.96 | | |
| TBA-RS(mg/kg) | 0.22 | 0.63 | 0.36 | 0.02 | 0.21 | 0.69 | 0.45 | 0.03 | | |

Table 2: Statistical analytical results of chemical quality parameters in examined minced meat samples (n=25 of each).





| | | Total aerobic plate count | | | | | Total Colifor | l ms | Т | otal Si | taph.au | reus o | coun | t | | | |
|-------------------------------------|-------------------|---------------------------|---------------|--------------------------|----|-------------------|------------------|-------------------|----|---------|----------|--------|-----------------|-----|-----|------|----|
| | | | | | | San | nples | | | | | | | | Sam | ples | |
| Type of Range minced (Log cfu/g) | No. of samples | % | P.L. * | accepted Non accepted | | No. of samples | % | No. of samples | % | P.L.* | accepted | | Non accepted | | | | |
| incat | | | | | No | % | No. | % | - | | | | | No. | % | No. | % |
| | 0 - ≤ 2 | 0 | 0 | | | | | | 19 | 76 | 7 | 28 | | | | | |
| | >2 - ≤ 3 | 0 | 0 | - | | | | | | 0 | 0 | 0 | 0 | - | | | |
| | >3 - ≤ 4 | 0 | 0 | cfu/g | | | | 9 36 | 2 | 8 | 3 | 12 | cfu/g | 7 | 28 | 18 | 72 |
| Frozen | >4 - ≤ 5 | 3 | 12 | | 16 | 64 | 9 | | 4 | 16 | 13 | 52 | | | | | |
| | >5 - ≤ 6 | 13 | 52 | | | | | | 0 | 0 | 2 | 8 | | | | | |
| | > 6 - ≤ 7 | 8 | 32 | | | | | | 0 | 0 | 0 | 0 | | | | | |
| | >7 - ≤ 8 | 1 | 4 | | | | | | 0 | 0 | 0 | 0 | | | | | |
| | $0 - \leq 2$ | 0 | 0 | log | | | | | 7 | 28 | 4 | 16 | log | | | | |
| | >2 - ≤3 | 0 | 0 | 9 | | | | | 1 | 4 | 0 | 0 | 2 | | | | |
| | >3 - ≤ 4 | 0 | 0 | - | | | | | 10 | 40 | 1 | 4 | - | | | | |
| Chilled | >4 - ≤ 5 | 1 | 4 | - | 3 | 12 | 22 | 88 | 5 | 20 | 7 | 28 | - | 4 | 16 | 21 | 84 |
| | >5 - ≤ 6 | 2 | 8 | - | | | | | 2 | 8 | 12 | 48 | - | | | | |
| | >6 - ≤ 7 | 13 | 52 | _ | | | | | 0 | 0 | 1 | 4 | _ | | | | |
| | >7 - ≤ 8 | 9 | 36 | _ | | | | | 0 | 0 | 0 | 0 | _ | | | | |

Table 3: Frequency distributions of bacterial counts of the examined minced meat samples.

*P.L.= maximum acceptable limit according to Egyptian Standard Specification "E.S.S.", No. 1694 / 2005 for minced meat.

Table 4: Frequency distributions of chemical quality paramaters of the examined minced meat samples.

| | en minced m | Chilled minced meat | | | | | | | | | |
|-----------|-------------|---------------------|--------|-----|-----|----------|-------|-----|--------|-----|-----|
| pH TVB-N | | | | I | н | | TVB-N | | | | |
| Range | No. | % | Range | No. | % | Range | No. | % | Range | No. | % |
| >5.0-6.0 | 0 | 0 | >5-10 | 17 | 68 | >5.0-6.0 | 0 | 0 | >5-10 | 7 | 28 |
| > 6.0-6.5 | 24 | 96 | >10-15 | 8 | 32 | >6.0-6.5 | 22 | 88 | >10-15 | 7 | 28 |
| > 6.5-7.0 | 1 | 4 | >15-20 | 0 | 0 | >6.5-7.0 | 3 | 12 | >15-20 | 8 | 32 |
| > 7.0 | 0 | 0 | >20-25 | 0 | 0 | >7.0 | 0 | 0 | >20-25 | 3 | 12 |
| | 25 | 100 | | 25 | 100 | | 25 | 100 | | 25 | 100 |

Table 5: Incidence of coagulase positive Staph. aureus strains in examined minced meat samples (n=25 of each)

| Frozen | | Chilled | | | | |
|--------------------|------|--------------------|------|--|--|--|
| No. of +ve strains | % | No. of +ve strains | % | | | |
| 15 | 83.3 | 19 | 90.5 | | | |

| | Coliform group | Moulds | Yeasts |
|---------|---|---|---|
| Product | Citrobacter freundii Citrobacter diversus Enterobacter aerogenes Escherichia blattae Klebsiella oxytoca Klebsiella pneumoniae subsp. ozaenae subsp. pneumoniae | Aspergillus flavus Aspergillus niger Pencillium sp. Fusarium sp. Mucor sp. Cladosporium sp. Curivlaria sp. Geotricum sp. | Candida albicans Candida tropicalis Cryptoccus sp. Rhodotroula sp. |
| Frozen | 0 0 0 3 0 2 1 | 4 2 0 3 4 8 0 1 | 15 0 0 6 |
| Chilled | 3 1 2 6 2 1 3 | 6 3 2 2 3 5 3 0 | 18 4 2 4 |

Table 6: Isolated microorganisms from examined minced meat samples

DISCUSSION

The data recorded in Table (1) and Fig.(1) revealed that the means of aerobic (APC) and anaerobic bacterial counts (\log_{10} cfu/g) of packaged frozen and unpackaged chilled minced meat samples were 6.15 ± 5.78 & 4.60 ± 3.89 and 6.83 ± 6.04 & 4.86 ± 4.04, respectively.

These higher levels of contamination may be due to unhygienic practices during meat production in slaughter houses, using of low quality frozen meat or non selected meat trimmings.

Higher results were obtained by Isigidi *et al.* (1985) who reported a mean total viable count of 7.47 log cfu/g in minced beef. Mousa *et al.* (1993) reported that the mean values of total mesophilic and enterobacteriacae counts were 7.2 X 10^8 and $4.3X10^5$, respectively. Ouf, Jehan (1997) was recorded the means of total viable counts as $9.6 \times 10^7 \pm 4.0 \times 10^7$ and $1.9 \times 10^6 \pm 5.9\times 10^5$ cfu/g in packed chilled and frozen minced meat, respectively.

Nearly similar result for APC was reported by Skrökki, 1997 (1.4 $\times 10^{6}$ cfu/g), while lower result was obtained by Grunspan *et al.* (1996) (1.7 to 8.8 $\times 10^{4}$ cfu/g).

Table (3) showed that 36% and 88% of packaged frozen and chilled unpackaged minced meat samples were exceeded the Eygptian permissible limit ($\leq 6 \log$ cfu g⁻¹). According to ICMSF (1986), 4% and 36% were exceeded the value of 7 log cfu/g which was considered the upper acceptability limit of APC, respectively.

Egyptian Standard Specification "ESS" (1694/2005) set a standard maximum limit of 10^6 cfu/g for total aerobic count and 10^2 for anaerobic bacteria in either minced beef and stated that the

chilled mince should be stored maximum period of 4 - 7 days at $4 - 0^{\circ}$ C.

Butchers and meat sellers working in outlets of retail minced meat shops pay little attention to their personal hygiene and serve meats with dirty hands and clothings. They lack knowledge regarding the importance of disinfecting and sanitizing; consequently, they clean their shops once daily with detergent and water, no sanitizer medium was used. Cenci-Goga *et al.* (2005) stated that mincers are difficulte to be cleaned thus accumulate large amount of organic matters which favoring microbial growth and reducing the efficacy of their sanitation procedures.

Table (1) showed that the means of enterobacteriacae counts of frozen and chilled minced meat samples were 4.40 ± 4.11 and 4.76 ± 4.49 and they were not differ significantly (p>0.05). The incidence of enterobacteriacae in examined samples was higher (76%) in chilled samples than in frozen ones (40%) (Table, 3).

Kammenou *et al.* (2003) examined minced meat retailed in butcher's shops and supermarkets and found that the \log_{10} counts of aerobic plate count and enterobacteriaceae were ranged from 6.20 to 6.84 and 3.15 to 4.5, respectively.

Aerobic plate counts often are chosen as an indicator of the effectiveness of hazard analysis and critical control point (HACCP) plans, because data for all aerobic bacteria are more easily collected than data for pathogens of concern or other indicator et organisms (Chong-Hae al.. 2008). Enterobacteriaceae are very useful as indicators of bad hygiene or bad treatment of food products, and their presence in large numbers indicated a big possibility their multiplication, implying of multiplication of other pathogens (Nissen et al., 2001).

Table (1) showed that the means coliform counts $(\log_{10} \text{cfu/g})$ of frozen packaged and chilled unpackaged minced meat samples were 3.93 ± 3.64 and 4.57 ± 4.26 and they were not significantly differ. The coliform count was decreased by nearly one log in air packed and freezed samples (figure,1).

According to the guidelines from the meat hygienic manual (Canadian Food Inspection Agency), the counts of coliform group in all examined samples of either frozen and chilled minced meats were exceeded the maximum permissible limit, $3 \log_{10} \text{ cfu/g}$ (Saucier *et al.*, 2000).

The incidence of contaminated samples by coliform microorganisms was higher in chilled mince (72%) than in frozen packaged one (24%) (Table,1), as the same trend of family Enterobacteriacae. Uzunlu and Yildirim (2003) stated that the decrease in coliform count during storage is related with the used ingredients in meat patties and storage in refrigeration conditions (4 ± 1 °C) between 1.8 °C and 4.4 °C that are the minimum growth temperatures for this bacterial group.

Because the growth of aerobic microorganisms is usually the best indicator of both the spoiling process and the hygiene after slaughter, in particular, the increase in the numbers of coliform bacteria, these were used in this study as an indicator when testing the hygienic quality of minced meat.

Salmonellae, Shigellae and Escherichia coli failed to be detected in any of examined samples. Quick freezing is done by lowering the temperature of foods to about -20 °C within 30 minutes. Small intracellular ice crystals are formed. Microorganisms undergo a rapid thermal shock (with no time for low temperature adaptation or blocking of suppression of metabolic activity), and there is only a brief exposure to adverse concentrations of solutes. Growth of microorganisms is generally prevented at freezing temperatures, although some of them can grow slightly below 0°C but at an extremely slow rate (Jay, 1996). Gram-positive microorganisms survive frozen storage better than Gram-negative ones (ICMSF, 1980).

Table (1) and Fig.(1) showed that the means of *Staphylococcus aureus* counts of frozen packaged and chilled minced meat samples were 4.40 ± 3.88 and $5.26 \pm 4.71 \log_{10}$ cfu/g respectively. These counts were higher than the maximum accepted limit of ESS $1694 / 2005 (10^2$ cfu/g), so that 72% and 84% of examined samples were not accepted, respectively (Table, 3).

Lower results were registrated by Attala and Kassem (2011) as $3.94 \pm 0.16 \log_{10}$ cfu/g. They stated that the higher counts of *Staph. aureus* in worker's

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hands indicates ignorance of workers and butchers by the personal hygiene rules. Moreover, a condition like injured hands or having abscess greatly enhance *Staph. aureus* contamination. *Staph. aureus* can be isolated from meat grinders, knives, saw blades and cutting boards or tables (Downes and Ito, 2001).

Table (5) concluded that coagulase positive *Staph. aureus* could be isolated by 83.3% and 90.5% from frozen and chilled samples, respectively. Altabari (1984) stated that food poisoning by *Staph. aureus* enterotoxin could be occurred when minced meat already contaminated by large numbers of bacterium during processing and is preserved at temperatures higher than 14°C. To avoid this, attention should be given to the initial bacterial contamination and meat should be kept at temperatures lower than 9 °C.

Hassanin, Fatein *et al.* (2001) concluded that the using of nisin in combination with potassium sorbate at low concentrations in minced meat appeared to have synergistic effect for killing *Staph.aureus* contaminated it.

Table (1) and Fig.(1) showed that the logarithmic means ($log_{10}cfu/g$) of mould and yeast counts in frozen samples were 2.72 ± 2.18 and 3.91 ± 3.64 and in chilled ones were 3.59 ± 3.49 and 3.65 ± 3.04 , respectively.

Growth at temperatures below 0°C is more likely for yeasts and molds than for bacteria (Jay, 1996). These microorganisms are also lipolytic (Comi and Cantoni, 1980) but they were found in low numbers when compared with bacteria. It is suggested that yeast and mould play an important role in causing spoilage of meat (Deak, 1991 and Fleet, 1992).

From Table (6) it was evident that the isolates numbers of members of coliform group in frozen and chilled samples were *Citrobacter freundi* (0&3), *Citrobacter diversus* (0 & 1), *Entrobacter aerogenes* (0 & 2), *Escherichiablattae* (3 & 6), *Klebsiella oxytoc* a (0, 2), *Klebsiella pneumoniae* subsp.ozaenae (2 & 1) and *Kl.pneumoniae* subsp.pneumoniae (1 & 3). Mould species isolates were as follow: *Aspergillus flavus* (4 & 6), *Asp.niger* (2 & 3), *Pencillium sp.* (0 & 2), *fusarium sp.* (3 & 2), *Mucor sp.* (4 & 3), *Cladosporium sp.* (8 & 5), *Curivlaria sp.* (0 & 3) and *Geotricum sp.* (1 & 0). Also, numbers of isolates of yeast species were *Candida albicans* (15&18), *Candida tropicalis* (0 & 4), *Cryptoccus sp.* (0 & 2) and *Rhodotroula sp.* (6 & 4).

The presence of coliform in minced meat is an indication of faecal contamination of fresh meats during slaughtering and processing. The isolation of *Enterobacter spp*, may be as a result of poor environmental conditions due to dust and

contamination of water used during slaughtering (Talaro and Talaro, 2006). Banwart (1981) stated that species of *Klebsiella* are potential health hazard, as members of coliform group, act as indicator organisms and can cause food spoilage.

Chemical freshness tests:

The suitability of minced meat for consumption also depends on its pH. When spoiled, minced meat can be dangerous to human health, especially if the meat is not heated before eating or if the heating time is very short (Skrökki, 1997).

Table(2) declared that pH values of frozen minced meat samples were ranged from 6.10 to 6.71 with a mean of 6.26 ± 0.03 , while these of chilled ones were ranged from 6.12 to 6.86 with a mean of 6.34 ± 0.03 .

In a study designed to observe the hygienic practices of butchers and assess the microbiological quality of beef, the pH measures of the various samples of meat were between 6.50 and 6.90 and this is within the normal for meat (5.6-7.0) (Soyiri et al., 2008). According to Ronald et al. (1999), glycogen, the stored carbohydrate in animal tissue is converted into lactic acid in the aging process of meat. This lactic acid produced tends to lower the pH of the muscle from about 7.0 in the living animals to 5.6 in the carcasses after a period of time. The protein units in the meat however tends to make the pH neutral (Lechowich, 1971) and this condition favour the growth and survival of bacteria. It is known (Claus and Berkeley, 1989 and Cowan and Steel, 1975) that at a certain minimum pH the growth of the following bacteria is limited E.coli (5.0), Salmonella spp. (4.6), S. aureus (4.9), and B. cereus (5.0). This implies fresh meat and beef in particular have a high risk of harbouring a lot of bacterial pathogens. Proteolysis may have produced nitrogenous compounds caused increase in the pH values (Aksu and Kaya, 2005).

According to the Finnish unofficial recommendations, minced meat is not suitable for consumption when the pH is over 6.4 or below 5.6 (Naukkarinen and ja Ketola, 1987; Hyvönen, 1991 and Skrökki, 1993). For many years, pH values of 5.6 - 6.2 have been considered to be good, and pH values of 6.3 - 6.4 to be tolerable, in Finland.

Total volatile basic nitrogen (TVB-N) measurement is the traditional chemical mean most widely used for evaluation of the degree of meat spoilage. Table (2) and Fig.(2) showed TVBN (mg/100g) content of the examined frozen minced meat samples was ranged from 6.72 to13.44, with a mean value of 9.52 ± 0.37 while of the chilled mince was ranged from 6.72 to 24.19, with a mean of 13.38 ± 0.96 .

Higher results in minced meat were registrated by Mohammed (2011); 19.46 \pm 1.44 and by Salem,

Amani (2012) in control non treated samples was the highest, $20.1 \pm 2.2 \text{ mg}/100\text{g}$. The means of TVN in both frozen and chilled mince were lower than those that obtained by Pearson (1973), who determined that fresh beef meat could contain maximum values of 17 mg N/100 g.

Table (4) illustrated that the chilled minced samples (12%) only was exceeded the acceptable lemit of TVB-N ($\leq 20 \text{ mg/100g}$) according to ESS 1694 / 2005.

Ammonia, determined as TVN, may be used as an index of meat quality under chill temperature according to the scheme of Pearson (1975) showing meat's fresh TVN to be 13 and its acceptable value to be ≤ 17 .

It was evident that beef storage for 4 days at $1-2^{\circ}$ C and barely 3 days at 5-7°C is acceptable for human consumption. However, in four days TVN values of 23.8 at $1-2^{\circ}$ C with bacteria log number 8.1/g and in 2 days TVN of 20.9 with bacteria log no 7.7/g are adjudged fit for human consumption being odourless, non-slimy and non-putrid. Both TVN and degree of alkalinity accurately measure meat spoilage as they consistently increased as spoilage advanced and bacteria log number increased with increasing storage time (Agunbiade *et al.*, 2010).

Ndaw et al. (2008) stated that TBA is a good indicator for the assessment of quality of meat and degree of lipid oxidation. Table (2) recorded that thiobarbituric acid (TBA) value of frozen minced meat samples was ranged from 0.22 to 0.63 with a mean of 0.36 \pm 0.02 mg MDA /kg, where in chilled ones was ranged from 0.21 to 0.69 with a mean of 0.45 ± 0.03 MDA/kg. The increased in TBA values may be due to lipid hydrolysis and secondary products formation under low temperature (Forrest et al., 1975). Lipid peroxidation is a complex process occurring in aerobic cells and reflects the interaction between molecular oxygen and polyunsaturated fatty acids. Free radical are known to take part in lipid peroxidation, which cause food deterioration, aging organisms and cancer promotion (Ashook and Ali, 1999). It has been proposed that a maximum TBA value indicating the good quality of minced meat is 0.9 mg MDA/ Kg (ESS, 1694/2005).

The results indicated that, the microbiological quality of retailed minced meat samples was unsatisfactory, and the product pose a high risk for consumers as it may be lead to food poisoning. Preventative measures include warning consumers of the health risks associated meats and encouraging them to thoroughly cook ground meat and to apply food handling guidelines. In addition, good manufacturing practices (GMP) for slaughtering and processing of minced

meat should be accepted as strategies to control pathogenic bacteria.

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الجودة الميكروبية والكيميائية للحوم المفرومة المعدة للبيع

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أجريت هذة الدراسة على عدد ٥٠ عينة عشوائية من اللحوم المفرومة المجمدة المعبأة والمبردة (٢٥ عينة من كل نوع) المتداولة في أسواق مدينة دمنهور وذلك لتقييم جودتها الميكر وبية والكيميائية. أظهرت نتائج الفحص الميكر وبيولوجي أن اللوغارتم العشري لمتوسطات العد الكلى للبكتريا الهوائية واللاهوائية ومجموعة العصيات المعوية والقولونية والميكروب العنقودي الذهبي بالإضافة إلى الفطريات والخمائر في مفروم اللحم المعد المعداً كانت كالآتي ٢.١٠ ٢٠ ٥، ٧، ٤، ٤ ٤، ٣، ٤، ٤ ٤، ١، ٤، ٤، ٣. ٣ ± ٣.٣، ٤.٤ ± ٢.٧٢ ، ٢.١٨ ± ٢.٧٢ أو ٢.١٠ لو ٢. خلية بكتيرية/ جرام من العينة على التوالي. بينما كانت تلك المتوسطات في m اللحم المفروم المبرد m ، ϵ ، m± ٣.٠٤ لو., خلية بكتيرية/ جرام من العينة على التوالي. وقد أظهرت الدراسة أن ٣٦٪ و٨٨٪ من العد الكلي للبكتريا الهوائية و ٧٢٪ و ٨٤٪ من العد الكلي للميكروب العنقودي الذهبي تجاوزت الحد المسموح به في اللحم المفروم المجمد والمبرد وفقا للمواصفة القياسية المصرية رقم ١٦٩٤ لسنة ٢٠٠٥ . كما قد خلت جميع العينات من التلوث بالسالمونيلا والشيجلا والإشيريكية القولونية بينما تم عزل الميكروب المكور العنقودي الذهبي المفرز لأنزيم coagulase بمعدل٨٣.٣٪ و ٥٠.٩٪ في كل من اللحم المفروم المجمد والمبرد على التوالي. كما بينت الأختبارات الكيميائية الموضحة لطَّزاجة اللحوم أن الأس الهيدروجيني للحم المفروم المجمد تراوح بين ١٠٠ و ١.٧٦ بمتوسط ٢.٢٦ ± ٠.٠٣ بينما في اللحم المفروم المبرد تراوح بين ٢.١٢ و ٢.٨٦ بمتوسط ٢.٣٤ ٢.٠٣ وأن تركيزات النتروجين القاعدي المتصاعد تراوحت في العينات المجمدة بين ٢.٧٢ و ١٣.٤٤ بمتوسط ٩.٥٢ ± ٣٧.٠، بينما في اللحم المفروم المبرد تراوحت بين ٢.٧٢ و ٢٤.١٩ بمتوسط ١٣.٣٨ ± ٩٦. • . هذا وقد سجلت قيما منخفضة لم تتجاوز الحد المسموح به ٩. • مجم مالونالدهيد/كجم) في الأغذية لقيمة حامض الثيوبربتيورك الدال على أكسدة الدهون في تلك المنتجات. وبذلك تكون الجودة الميكروبية لهذة المنتجات غير كافية وتناولها يشكل خطورة على صحة المستهلكين لما تسببه من تسمم غذائم.