5. SUMMARY AND CONCLUSSION

Meat is considered a some of the best source available protein, minerals and vitamin. Sandwiches are the most common popular foods in Egypt. Such foods are important sources for nutrients supplying, energy and growth. The most commonly consumed from meat are sausage and burger sandwiches. Such foods may be exposed to contamination during processing and handling and this causing health hazards to the consumer specially with increasing popularity of dining a way from the home. At food service (restaurant) the workers are responsible for transfer *Staph. aureus* to the sandwiche which were the primary repute of transmission for *E. coli* O157:H7. Both microorganisms were considered as food borne pathogen. *E. coli* O157:H7 was caused hemorrhagic colitis. The organism has received considerable attention recently because of its implication in several outbreaks of food poisoning in the United States. Also *Staph. aureus* was considered important may be causative agent of food borne illness, many strains of it produce enterotoxin which cause food poisoning if ingested. Burger sandwiches were prepared by meat patties which grilled before. So the meat patties should grilled to a certain internal temperature and held there for proper time period to avoid microbial hazard. Determined thermal resistance of *E. coli* O157:H7 and *Staph. aureus* are very important due to common organisms capable of producing several toxins that cause intoxication symptoms. All meats used to prepare a sandwiches usually keeping in refrigerator or freezing. So the preservation of meat by freezing is retardation of microbial growth to the point

where microbial decomposition does not occur and consequently prolongation the useful life time of meat but refrigeration meats were spoilage by microbiological and chemical activity during refrigerated storage.

Thus this study was planned to study the following points:

1. To study the microbiological quality and chemical composition of sausage and beef burger sandwiches collected from different restaurants.
2. Determination of D and Z values for some strains of *E. coli* O157:H7 and *Staph. aureus* in broth media.
3. To study the lethality of heat on *E. coli* O157:H7 and *Staph. aureus* strains in burger.
4. To study the effect of refrigeration at 7°C and freezing storage at -18°C on the microbiological load, chemical composition, freshness test and some physical properties for the preservation of frozen minced meat.
5. To study the effect of refrigeration at 7°C and freezing storage at -18°C on the growth of *E. coli* O157:H7 strains and *Staph. aureus* strains in minced meat.

The obtained results were summarized as follows:

1. Survey of sandwiches (sausage and burger) from some restaurants in Cairo:

1.1. Sausage sandwiches:

1.1.1. Total bacterial count (TBC):

The log number of total bacterial count was variation from 4.22 to 5.12 in all restaurants samples during 2003 and 2004.

1.2. Psychrotrophic bacterial count:

The least log number was 4.1 in restaurant (D) in the morning during 2003 while large log number was 4.94 in the morning during 2004.

1.1.3. Moulds and yeasts:

The sausage contained moulds and yeasts approximately in the same average in the two years. But the highest count was 2.94 found in restaurant (D) in the morning and lowest number was 2.24 found in restaurant (A) during 2004. There were significance difference in restaurant (D) only in the afternoon but there were no difference for restaurants (A, B and C).

1.1.4. Aerobic sporeformer:

All restaurants samples had normal numbers of sporeformer in two years. All the counts ranged from 2.14 in restaurant (A) to 3.38 in both restaurants (A and D).

1.1.5. Lipolytic bacteria:

All the sausage restaurants samples contained lipolytic bacteria ranged from log 2.46 to 3.51, while restaurant (D) had High number.

1.1.6. Proteolytic bacteria:

There were variation in the log number of proteolytic bacteria between all restaurants ranged from 2.39 to 3.56 during two years.

1.1.7. Total coliform:

Some restaurant had low log number ($\leq$0.48) but restaurant (B) had high log number (2.94).

1.1.8. *E. coli*:

The restaurant A, B were free from *E. coli* at morning in two years, while restaurant (D) contained *E. coli* in some samples in year 2003. But in year 2004 *E. coli* was detected in the samples for all restaurants.

1.1.9. *Staph. aureus*:

Restaurant (A) was free from *Staph. aureus* comparison with another restaurants during two years (2003 and 2004) while restaurant (D) have high number of *Staph. aureus* in the morning.

1.1.10. *Salmonella sp.*:

All restaurants samples were free from salmonella.

1.1.11. Chemical analysis

The moistures contents ranged from 47.45% to 54.45% during two years. While, crude protein content from 10.90% to 12.63% in sausage sandwiches at year 2003 and 2004. Ether extract content was ranged from 9.19% (A) to 11.79% at two years (2003 and 2004).

1.2. Beefburger sandwiches:

1.2.1. Total bacterial count (TBC):

The best results was 4.44 in restaurant (D) in the morning while a bad sample was 5.04 in restaurant (C) also in the morning.

1.2.2. Psychtrophic bacteria:

The log number of psychrotrophic bacteria were ranged from 4.03 to 4.87 during two years.

1.2.3. Mould and yeast:

The log number was 3.43 in the restaurant (D) in the afternoon. While a best result was 1.7 in restaurant (B).

1.2.4. Aerobic sporeformer:

The log number was ranged from 2.46 to 3.59 for All restaurants. That's mean there were contaminated during preparing or handling.

1.2.5. Lipolytic bacteria:

The log number of lipolytic bacteria ranged from 2.69 to 3.38.

1.2.6. Proteolytic bacteria:

The log number ranged from 3.02 to 3.35. All the results in this table illustrated a less proteolytic number. From the results there were no and revisable changes demonstrate that protein hydrolysis by microorganisms.

1.2.7. Total coliform:

The obtained data indicated that there were significant difference in some restaurant samples especially in restaurant (D), while in another restaurant there were no significant difference in restaurant samples.

1.2.8. *E. coli*:

Normally for any sandwiches not allow positive *E. coli* due to contaminated by fecal waste to avoid additional out breaks of food borne illness by such organisms. Restaurant (A) had the best results due to free from *E. coli* during two years.

1.2.9. *Staph. aureus*:

The results indicated that restaurant (A) free from *Staph. aureus* during 2003. While all restaurants contained it during 2004. The worst sample was 1.95 in restaurant (D) in the morning. There were significant differences during two years between restaurants.

1.2.10. Chemical composition of burger sandwiches

Chemical composition of beefburger sandwiches in each of restaurants A, B, C and D as regards moisture content % ranged from 47.55% to 51.87% in two years (2003 and 2004). While, crude protein content ranged from 11.25 to 12.67%. Ether extract content ranged from 8.79 to 11.35%. Also, ash content ranged from 1.33 to 1.53% during two years.

2. Heat resistance parameters of *E. coli* O157:H7 and *Staph. aureus* in broth medium :

D values for *E. coli* strains were between 0.34-2.25. While Z value for *E. coli* strains were (13.92°C) and (19.26°C). D values for Staph. aureus were from 0.78-3.70, with Z value 14.93-20.28 at 55, 60 and 65°C in broth medium.

Z value for *E. coli* strain B were the lowest Z value (13.92°C) but *E. coli* strain A had high Z values (19.46°C) and Z values for *Staph. aureus* strains A and B were 14.93 and 20.28°C, respectively.

3. Thermal destruction of *E. coli* O157:H7 and *Staph. aureus* in beefburger:

After 0.5 min grilling of inoculated beefburger patties the inhibition processing *E. coli* strain was decrease to 70.59% in

strain A, while 44.44% in strain B. But after 2.5 min the percentage was decreased and reached to 99.99 and 99.999%, for the two strains, respectively.

Also, *Staph. aureus* was inhibited by 86.94% in strain A, while 95.68% in strain B after 0.5 min grilling. But after 2.5 min the percent decreasing reached to 99.999 for strain A. While strain B was increased to 99.999% after 2.0 min and not detected after 2.5 min grilling.

4. Effect of refrigerated storage at 7^{o}C for 11 days on microbiological flora, chemical composition and freshness tests in raw minced meat and inoculation minced meat:

4.1. Microbiological examination:

4.1.1. Total bacterial count (TBC):

Total bacterial initial count in raw minced meat was $2.5x10^{5}$ cfu/g, while after inoculation by *E. coli* and *Staph. aureus* reached to $2.8x10^{8}$ cfu/g. TBC increased to $5.2x10^{7}$ cfu/g in the end of storage period, the TBC was not acceptable after 5 days but in inoculated samples increased from $2.8x10^{8}$ to $4.7x10^{10}$ cfu/g after 11 days storage.

4.1.2. Psychrotrophic bacteria:

Psychrotropic bacterial count in raw minced meat was $1.8x10^{4}$ reached to $4.6x10^{6}$ cfu/g after 11 days, while in inoculation minced meat was $1.8x10^{6}$ cfu/g before refrigeration storage and reached to $4.8x10^{8}$ cfu/g at the end of storage period (11 days).

4.1.3. Total colifom:

The count of total coliform was slightly decreased during storage period for 11 days.

4.1.4. *C. perfringens:*

The count of *C. perfringens* was not clearly decreased during the refrigeration storage for minced meat without and with inoculation, respectively.

4.1.5. Lipolytic bacteria:

Lipolytic bacterial count in raw minced meat without inoculation was $3.4x10^4$ cfu/g and had slowly decreased during refrigeration storage to $2.2x10^4$ cfu/g in end storage period. While in raw minced meat with inoculation the count was $4.2x10^4$ cfu/g and decreasing clearly started after five days of storage. Then reached to $1.5x10^4$ cfu/g at the end of storage period.

4.1.6. Proteolytic bacteria:

Proteolytic bacteria count was $2.0x10^4$ cfu/g in raw minced meat and decreased slowly through refrigerated storage period. Also, it was $2.2x10^4$ cfu/g in raw inoculation minced meat and reached to $0.8x10^4$ cfu/g in the end cold storage.

4.1.7. *E. coli*:

E. coli O157:H7 was not detected in raw minced meat. While in inoculation minced meat was $3.4x10^7$ cfu/g. The count of *E. coli* was decreased slowly during refrigerated storage period which reached to $3.1x10^7$ after 11 days.

4.1.8. *Staph. aureus*:

Staph. aureus count was 8.8x10 cfu/g in raw minced meat without inoculation. While, *Staph. aureus* count in inoculation minced meat was $2.0x10^7$ cfu/g and decreased slowly with increasing refrigerated storage period. *Staph. aureus* was $1.5x10^7$ cfu/g at the end of refrigerated storage (11 days).

4.2. Chemical composition:

Moisture content was 69.98% in raw minced meat. It reached to 69.90 and 69.85% in minced meat without inoculation and with inoculation, respectively at the end of refrigerated storage period (11 days).

Crude protein content was decrease from 20.92% to 20.75 and 20.55% for minced meat without inoculation and with inoculation, respectively. Ether extract content was 7.25% in raw minced meat was decreased to 7.10 and 6.91% for minced meat without inoculation and with inoculation, respectively.

Ash content was 0.85% in raw minced meat, while reached to 0.84 and 0.84% in without and with inoculation, respectively. While total carbohydrate were ranged from 1.00% to 1.41% and 1.85% in without and with inoculated minced meat, respectively.

4.3. Freshness tests:

Total volatile nitrogen (TVN) content was 2.8 mg/100 g for raw minced meat. At the end of refrigerated storage period, it reached to 25.2 mg/100 g for minced meat without inoculation, and 27.95 mg/100 g for minced meat with inoculation.

The TBA value was 0.33 mg malonaldehyde /kg in raw meat and reached to 0.98 and 1.34 mg malonaldehyde/kg for minced meat without and with inoculation, respectively at the end refrigeration storage period (11 days).

5. Effect of freezing storage at -18°C for 42 days on microbiological flora, chemical composition and freshness tests in raw minced meat and inoculation minced meat:

5.1. Microbiological examination:

Total bacteria, total coliform, lipolytic bacteria and proteolytic bacterial counts were decreased by freezing and prolonged frozen storage period up to 42 days.

5.1.1. Psychrotrophic bacteria:

Psychrotrophic bacteria was decreased after freezing compared with raw minced meat in two samples. It was $1.1x10^4$ and $1.1x10^5$ cfu/g in zero time for frozen minced meat without and with inoculation, respectively. No slightly change in count during frozen storage period.

5.1.2. *C. perfringens*:

C. perfringens was affected by freezing treatment. The number of *C. perfringens* was 9.0x10 and 8.0x10 cfu/g for frozen minced meat without and with inoculation sample, respectively at zero time. In the end of frozen storage time it was 6.5x10 and 6.0x10 cfu/g, respectively.

5.1.3. *E. coli*:

E. coli O157: H7 not detected in raw and during storage of frozen minced meat. After inoculation and freezing the count decreased from $3.4x10^7$ to $2.2x10^7$ cfu/g. The number was

decreased with increasing storage periods (42 days) and reached to $6.0x10^6$ cfu/g.

5.1.4. *Staph. aureus:*

The initial number of *Staph. aureus* before inoculation was 8.8x10 cfu/g, while directly after freezing was 5.2x10. But after inoculation the initial number was $2.0x10^7$ and by freezing was $1.2x10^7$. During freezing storage at -18°C for 42 days of both samples (without and with inoculation) were gradually decreased in numbers to 4.0x10 and $9.0x10^5$ These may be due to a long time of storage under freezing temperature during frozen storage.

5.2. Chemical composition:

Moisture content was 69.98% in raw minced meat. It reached to 69.90 and 69.84% in frozen minced meat without and with inoculation, respectively at the end of frozen storage period (42 days).

Crude protein content was decreased from 20.92% in raw minced meat to 20.85 and 20.78% for frozen minced meat without and with inoculation, respectively at the end frozen storage period (42 days).

Ether extract content was 7.25%. During frozen storage 42 days was decreased to 7.17 and 7.08% for frozen minced meat without and with inoculation, respectively.

Ash content was 0.85% in raw minced meat, while reached to 0.83 and 0.82% in frozen minced meat without and with inoculation, respectively at the end of frozen storage period (42 days).

5.3. Freshness tests:

Total volatile nitrogen (TVN) content of raw minced meat was 2.8 mg/100 g increased with increasing frozen storage period in all samples. At the end of frozen storage period, it reached to 13.4 mg/100 g for without inoculation, while reached to 20.41 mg/100 g for frozen minced meat with inoculation.

TBA value was 0.33 mg malonaldehyde/kg in raw meat. TBA value was increased with increasing storage period. TBA values reached to 0.79 and 1.26 mg malonaldehyde /kg for minced meat without and with inoculation, respectively at the end of frozen storage period (42 days).

6. Sensory evaluation and physical properties:

6.1. Sensory evaluation and physical properties of refrigerated storage raw minced meat at 7°C for 11 days:

6.1.1. Color:

The color go down with increasing the time of storage. There are significant differences were found after three days. The same results were showed when the color of meat measured by psychrotrophic bacteria.

6.1.2. Odor:

Odor attribute scores at zero time the panelists characterized new odor and increased during the storage period was 19.2 these is due to rapidly effected by cold storage (7^{o}C). There are significant difference after the second day of storage due to dutrative of odor were increased by increasing storage period and the scores became to 4.8 at the end of storage period

in addition raw minced meat had spoiled aromatic impression after 5 days of cold storage.

6.1.3. Overall acceptability:

At zero time overall acceptability was 49.6 the score was decreased with increasing storage time.

Generally from the obtained results of color, odor and overall acceptability it is clear to mention that the minced meat without inoculation remain as acceptable up to five days storage as maximum storage time in refrigerated.

6.2. Sensory evaluation and physical properties of frozen minced meat storage at-18°C for 42 days.

6.2.1. Color:

The color of frozen minced meat during storage period of raw minced meat for 42 days at –18°C. The color had significant difference during the first three days storage. While after three days up to 35 days there were no significant differences in the same attribute. Also from 21 days of storage to end storage period there were no significant difference. The lost scores of color attribute reached to 16.2 after 42 days.

6.2.2. Odor:

Odor was decreasing slightly until 7 days but there are significant difference was found after 14 days storage. These results mean that the change of odor increased by prolonged frozen storage period, but in slow rate From the obtained results no change in the odor up to 35 days.

6.2.3. Overall acceptability:

Overall acceptability was no significant difference from zero time up to 7 days of frozen storage in the same attribute that mean there were no clear change in that sample up to 7 days frozen storage. And also there were no significant difference from 28 days up to 42 days in the same attribute.

7. Texture evaluation for raw minced meat during refrigeration and frozen storage period:

The instrumental texture indicated that the cold meat sample was the softest and showed the lowest value for all texture attributes. The control sample was highest in all texture attributes than other samples. But the frozen minced meat samples were better than the refrigerated samples and showed a best values for all texture attributes.