

## Evaluation of Microbial Contamination during Beef Sausage Processing as Biological Hazard

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### Abstract

*The study was conducted to evaluate the microbial contamination during processing of beef sausage in Khartoum state. Samples and swabs were taken from different steps during sausage processing, after deboning, after mincing, after mixing, after stuffing, after freezing, and after storing for one month from different three plants ( A ≡ Modern processing line of sausage; B ≡ Medium processing line of sausage; C ≡ Traditional processing line of sausage). , Good manufacture practice, good hygienic practice consider, The samples subjected for microbiological investigation (total viable count of bacteria , total mould and yeast, staphylococcus aureus, Salmonella stypfi, total Coliform group and E. coli) It is clear from the obtained data, the highest mean value of total viable count of bacteria reported in raw meat, spices and soy bean ( $6.50 \times 10^5$ ), ( $8.40 \times 10^5$ ) and ( $3.37 \times 10^5$ ) respectively, total mould and yeast detected, where gastrointestinal tract (casings) showed the highest mean value of total mould and yeast ( $4.00 \times 10^3$ ). The analysis of the swab samples for the counter surfaces, knife, mincer, cutter, blender and stuffer before the beginning of the processing showed that the level of total viable count of bacteria reported by counter surfaces of ( $5.27 \times 10^4$ ) and mincer ( $5.47 \times 10^4$ ), analysis showed no growth of mould and yeast. Where sausage produced by traditional processing line C reported the highest mean value of TVCB ( $7.00 \times 10^5$ ), followed by sausage produced by medium processing line B ( $6.00 \times 10^5$ ), while the modern processing line A shown the lowest mean value ( $1.80 \times 10^4$ ) of TVCB.*

*The total mould and yeast not detected in sausage produced by modern line A and sausage produced by medium processing line B, while sausage produced by processing line C reported the highest mean value of mould and yeast ( $5.40 \times 10^3$ ). The staphylococcus aureus of sausage produced by modern processing line A found to be nil, while the highest mean value reported by sausage C ( $9.00 \times 10^4$ ) step four during stuffing. The traditional line C of producing sausage revealed highly contamination with Salmonella styphi except in step six after cold storage. The result also showed that the highest mean value of total Coliform group in sausage produced by medium line B (241.00), while the lowest mean value of total Coliform group reported by sausage produced by modern line A (150.00).data revealed that sausage produced by modern processing line A did not showed any E. coli growth even after storage.*

**Keywords:** Microbial Contamination, Beef Sausage processing, biological hazard

## INTRODUCTION

Meat and meat products are perishable, so special care and handling must be exercised. It is necessary to minimize deterioration to prolong the time during which an acceptable level of quality is maintained. A number of methods are employed throughout the meat industry to retard deteriorative changes and extend the length of the acceptability period. In recent years there has been a large increase in the production and consumption of meat and meat products, at the same time there is an increasing consumer demand for a healthy and balanced diet(Ordenez *et al.*, 2000).

Food safety has been of concern to humankind since the drawn of history and many of the problems encountered in our food supply go back to the earliest recorded years. Many rakes and recommendation advocated in religious or historical texts are evidence of the concern to protect people against food-born hazards and food adulteration (Yasmine, 2001).

The hazard analysis critical control point [HAACP] system is a scientific approach to process control. It is designed to prevent the occurrence of problem by assuring that controls are applied at any

point in a food production system where hazardous or critical situations could occur. Hazards include biological, chemical, or physical contamination of food products, as described by (Food safety and inspection service USDA, 1999). The HACCP has become an internationally recognized tool for managing the safety aspects of the production, processing, preparation and distribution, of food. HACCP in relation to microbiological food safety and born pathogens, the concept is recommended as the best insurance policy against undesirable microorganisms.

The traditional approach to food safety assurance is based on applying codes of Good Hygiene Practice (GHP) and Good Manufacturing Practice (GMP) in food processing where confirmation of safety and identification of potential problems are obtained by end – product testing and the check for compliance with the codes by sampling the foods for laboratory analysis.

In Sudan, the meat industry is one of the food processing sectors that are in the process of implementing certain hygienic measures to assure safety (Mohammed, 2007). The objective of this study was to evaluate the microbial contamination during beef sausage processing as biological hazard.

## **MATERIALS**

**Samples:** Fresh meat (After post mortem completed) free of contamination and deterioration or deteriorated part purchased from local slaughter house. The sample processed in three level of processing line plane (Modern plane line, Medium plane line, and Traditional plane line). Samples were taken from different steps in sausage processing plant, after deboning, after mincing, after mixing, after stuffing, after freezing, and after storing for one month. All sanitary and hygienic condition consider, also good manufacture practice, good hygienic practice, and good veterinary practice applied.

**Preparation of sausage:** Deboned meat minced in the mincer for two times, than transfer to mixer for mixing all the ingredients together for homogenization, and finally stuffed and freezes, Prepared according to the method described by (Pearson & Gillett, 1999).

## ANALYTICAL METHODS

### Microbiological analysis

**Media preparation:** All media plate count agar, malt-extract agar, Staphylococcus medium 110, nutrient broth, selenite broth, Bismuth sulphate agar, triple sugar iron agar, McConkey broth, brilliant green bile 2% broth, EC broth medium, eosin ethylene blue agar were prepared according to the methods described by DIFCO and BBL Manual (2003).

**Preparation of serial dilution:** Thirty gram from each sample of sausage processing steps was weight aseptically in sterile bottles and then blended with 270 ml distilled water by using an electric blender. Then an electric shaker was used for shaking to give 1/10 dilution, as described by (Harrigan, 1998) and (Harrigan and Mac cance ,1976).

### Microbial parameters:

Total viable count, Mould and yeast enumeration, Staphylococcus and Salmonella were described by (Harrigan, 1998) and (Harrigan and Mac cance, 1976).

**Coliform test (*Escherichia coli*):** Presumptive coliform test, confirmed coliform test, faecal coliform test and differentiation of faecal coliform (*E. coli*) test were described by (FAO, 1992).

**Statistical analysis:** The experiment was designed using Duncan's Multiple Range test [DMRT]. The data obtained were subjected to Statistical Analysis System [SAS] using Randomized Complete Design [RCD] (Mead and Gurnow, 1985). Significant attributes were further analyzed using Duncan's Multiple Range Test [DMRT] at 5% level of significant, as described by (Duncan, 1955).

### Microbiological properties of sausage during different processing steps

**Microbiology properties of raw material cfu/g:** The term sensitive ingredients may also be applied to materials that are historically associated with known microbial hazards. Therefore, the

sensitive raw materials which used in processing line were defined as water, wheat flour, and dried milk powder (Corlett, 1998).

Table (1) showed that the microbiological load of different raw materials which used in the processing line of sausage products, it is clear from the obtained data, the highest mean value of total viable count of bacteria reported in raw meat, spices and soy bean ( $6.50 \times 10^{5a}$ ), ( $8.40 \times 10^{5a}$ ) and ( $3.37 \times 10^{5a}$ ) respectively, the main sources of contamination during processing come from mention raw material, while water recorded the lowest mean value ( $1.67 \times 10^{2d}$ ) of bacteria. The result agrees with SSMO (2010) limits who state that, the total bacterial count not exceeded ( $2.25 \times 10^5$ ).

Shrestha *et al.*, (2016) who studied hazard analysis critical control point (HACCP) system was conducted in “buff sausage” production plants of two private sausages (i.e. industry A and industry B) and found the total bacterial count of raw meat was found to be  $1.2 \times 10^6$  cfu/gm, sample spices contained  $2.4 \times 10^3$  cfu/g and sample water had total bacterial count of  $4.5 \times 10^3$  cfu/ml, moreover the total bacterial count in industry B showed the raw meat was  $2.2 \times 10^7$  cfu/gm, sample spices contained  $1.9 \times 10^3$  cfu/ml and water sample was  $6.4 \times 10^3$ .

Also data in table (1) showed that there are significant differences total mould and yeast detected, where gastrointestinal tract (casings) showed the highest mean value of total mould and yeast ( $4.00 \times 10^{3a}$ ). While spices reported the lowest mean of value ( $6.20 \times 10^{2c}$ ). Generally the study proved that the raw material increased the microbial load significantly specially spices and bread crumbs.

Shrestha *et al.*, (2016) who studied hazard analysis critical control point (HACCP) system was conducted in “buff sausage” production plants of two private sausages in industry A found the total moulds and yeasts (cfu/g) of the raw meat was  $6.1 \times 10^5$  cfu/gm, sample spices had found to be  $3.4 \times 10^3$  cfu/gm and water sample had contained  $2.7 \times 10^2$  cfu/ml, moreover the total bacterial count in industry B showed the raw meat was found to be  $5.5 \times 10^6$  cfu/gm, sample spices had found to be  $2.5 \times 10^3$  cfu/gm and water sample had contained  $7.4 \times 10^2$  cfu/ml

### **Microbiology properties of equipment cfu/g**

The analysis of the swab samples presented in table (2) for the counter surfaces, knife, mincer, cutter, blender and stuffer before the beginning of the processing showed that the level of total viable count of bacteria reported by counter surfaces of ( $5.27 \times 10^{4a}$ ) and mincer ( $5.47 \times 10^{4a}$ ), while the lowest mean value reported by stuffer ( $6.57 \times 10^{2c}$ ), where knife not showed any growth. Analysis showed no growth of mould and yeast. The study proved that, the prolonged time of the equipment remained without being washed after the working increased microbial load, especially counter surfaces and mixing equipment could allow the initial contamination to increase on the surface of equipment.

### **Microbiology properties of sausage product during processing cfu/g**

Microbiological profiles of sausage as function of processing steps presented in table (3). Results showed that, the total viable count of bacteria on sausage products indifferent line reported significant different, where produced by traditional processing line C reported the highest mean value of TVCB ( $7.00 \times 10^5$ ), followed by sausage produced by medium processing line B ( $6.00 \times 10^5$ ), while the modern processing line A shown the lowest mean value ( $1.80 \times 10^4$ ) of TVCB. The change during storage presented (3). Data showed highly significant differences. Where sausage produced by modern processing line A with ( $3.50 \times 10^2$ ) reported the lowest mean value, while the highest mean value of TVCB recorded by sausage produced by traditional processing line C ( $9.80 \times 10^4$ ). These decreased may due to effect of cold storage during storage. The range of a viable count of beef sausages product, minced beef, beef burger kofta products showed high levels of contamination in ranges more than  $2.5 \times 10^4$  (cfu), which considered as a critical microbiological condition when compared with fresh meat (Ashton, 1981; FAO, 2000; Youssif, 2015).

Normal range is  $10^3$  to  $10^4$  organisms per gram as mentioned by Heinz and Hautzinger, (2007); Ashton, (1981) who stated that it is quite normal and unavoidable to find bacterial counts of viable count of the order of several thousand per  $\text{cm}^2$  on meat surfaces in commercial slaughtering and meat handling. However, viable count numbers exceeding 100,000 per gram ( $10^5$  per  $\text{cm}^2$ ) on fresh meat are not acceptable. However, European Commission Scientific Committee

on Veterinary Measures estimate the infectious dose range to be from  $0.1 \times 10^2$  to  $0.1 \times 10^4$  (cfu). Thus the detected range has clear implications on public health (Youssif, 2015).

Data in table (3) showed the results of total mould and yeast that not detected in sausage produced by modern line A and sausage produced by medium processing line B, while sausage produced by processing line C reported the highest mean value of mould and yeast ( $5.40 \times 10^3$ ), these slight growth may due to cross contamination during analysis, because study showed that no significant effect of mould and yeast in processing lines , and these may due to nature of ingredients and their moisture content and may be effect of spices.

The change during storage did not showed any significant difference except in sausage produced by traditional processing line C reported significant decreasing in mean value of mould and yeast.

Data presented in table (3) revealed that, sausage produced by traditional processing line C ( $4.00 \times 10^4$ ) reported the highest mean value of *staphylococcus aureus*, while not detected in sausage produced by modern processing line A.

The change during cold storage did not showed any growth for *staphylococcus aureus* except in sausage produced by traditional processing line C showed slight growth ( $1.10 \times 10^4$ ), these decreased may due to effect of storage temperature degree.

In various levels, *Staphylococcus aureus* was isolated from the most processed beef products sampl ed. With regard to the potentiality of *Staphylococcus aureus* pathogen, it is commonly found on the skin and mucous membranes of humans and warm blooded animals (Banwart, 1981; Wilks and Humble, 1997). Usually, the symptoms of *staphylococcal* intoxication appear when consuming food contains a toxin of less than 1.0 microgram.

This toxin level is reached when *Staphylococcus aureus* populations exceeds  $10^5$  per gram. However, the study showed that the viable count of *S.aureus* was at the range of more than  $10^4$  was 11.1% in minced beef, 38.9% in beef burger, 27.8% in beef kofta, and 11.1% in beef sausage products samples. Such contamination level is considered hazardous since meat products might not be thermally treated to the degree that is capable of inactivating the heat resistance of enterotoxins which is commonly encountered in this bacterium species. Foodborne illness caused by *S. aureus* enterotoxin is primarily a result of contamination by food

handling personnel and is generally associated with temperature abuse of cooked products (Bryan, 1992; Bergdoll, 1989).

Table (3) revealed that there are not contamination of *Salmonella styphi* in both samples produced by modern processing line A and medium line B, while sausage produced by traditional line C showed growth of *Salmonella styphi*, but immediately disappeared after cold storage, these disappearing may due to the effect of cold storage which may caused cell injured. Three site of microbial load increased, in step one during deboning the main causing of contamination lack of personal hygiene, step three mixing of ingredients the main sources of contamination the raw material and mixing equipment specially spices and step four stuffing during linking and tying and may due lack of personal hygiene, while the are significant decrease in step six cold storage, the accumulation action of microorganism during processing.

**The total mould and yeast during processing cfu/g:** Table (4) revealed the result of total mould and yeast during processing, where showed no contamination during different steps of processing but there contamination In sausage produced by traditional processing line C ( $3.50 \times 10^{3c}$ ) in steps three during mixing of ingredients, reported the lowest mean value these growth may due to initial microbial load of raw material and/or cross contamination during processing.

Data revealed that there are contamination in traditional processing line in step three mixing of ingredients and slight increased during stuffing, these increased may due to lack of personal hygiene during linking and tying, and decreasing again and disappearing at step six during cold storage.

**The contamination with Staphylococcus aureus during processing cfu/g:** Data obtained from table (5) revealed that there were significant differences in staphylococcus aureus of investigated sausage products as a result of processing steps. The staphylococcus aureus of sausage produced by modern processing line A found to be nil, while the highest mean value reported by sausage C ( $9.00 \times 10^4$ ) step four during stuffing, and followed step three in both line B ( $8.20 \times 10^2$ ) and C ( $7.00 \times 10^4$ ), while highly decreased in step six in both line B ( $0.00$ ) and C ( $1.10 \times 10^2$ ), no detected in line A. these decreasing

may due to effect of storage temperature and pressure of ice crystal form during freezing.

Mohammed, (2007) who studied the extent of implementing the hazard analysis critical control point (HACCP) system in Sudanese beef and found that, the occurrence of *Staphylococci* in beef products. *Staphylococci* counts ranged between  $3.9 \times 10^2$  -  $8.0 \times 10^3$  (CFU)/ gram. Workers, hands, equipment and environmental conditions (Shapon and Shapon,1994). The FAO (1992) reported that the presence of *Staphylococci* in beef products indicated the contamination from skin, mouth and nose of the employees.

It is clear data revealed that staphylococcus aureus of different sausage sample affected by cold storage. Also staphylococcus aureus increased in two site in step three mixing of ingredients and step four during stuffing where there direct contact between workers and products through linking and tying of sausage.

**The contamination with Salmonella styphi during processing cfu/g:** Generally data in table (6) revealed that the traditional line C of producing sausage highly contamination with Salmonella styphi except in step six after cold storage. It could be concluded that, the very important concept in the manufacturing of meat product, in addition to mixing step, and stuffing, since ingredients which used in these step contain spices which considered as an essential source of contamination with pathogen Salmonella styphi. Hygienic condition and lack of personal hygiene which clear in step four due to direct contact of water with product during linking and tying after stuffing. Mohammed, (2007) who studied the extent of implementing the hazard analysis critical control point (HACCP) system in Sudanese beef and found that, the occurrence of *Salmonella* in beef products. With the exception of Agwat sausage results showed that *Salmonella* was not detected in all the samples under investigation. Presence of *Salmonella* in beef products is an indication that the plant's system for controlling contamination is not working Tompkin (1995) mentioned that the presence of this organism indicates poor food preparation and handling practices. Consideration may also be given investigating the health status of food handlers on the premises who may have been suffering from Salmonellosis or asymptomatic carriers of the organism.

**The contamination with Coliform group during processing cfu/g:** The result of total Coliform group in table (7) indicates that the treatment samples differ scientifically ( $P \leq 0.05$ ). The result also showed that the highest mean value of total Coliform group in sausage produced by medium line B (241.00), while the lowest mean value of total Coliform group reported by sausage produced by modern line A (150.00). After storage there are highly decreased in load of total Coliform group, where the lowest mean value reported by sausage produced by medium line B (8.87) and the highest mean value in sausage produced by traditional line C (100.00), however sausage produced by modern processing line A not detected, the decreased may due to the effect of cold storage mainly. As table (7) revealed that sausage produced by modern processing line A with mean value (5.00) showed the lowest mean value of total Coliform group in step one, while the highest mean value of total Coliform group reported sausage produced by processing line C (460.00), in step three mixing, the increasing of total Coliform group observed in step four stuffing.

The contamination seen in traditional processing line C and increased step by step till reach the maximum level in step three and then decreased due to the effect of cold storage, while the level of contamination stable with slight increase in step three mixing and step four stuffing, these contamination due to the lack of personal hygiene and poor sanitation and hygienic condition, the medium processing did not showed any odd contamination.

**The contamination with Escherichia coli during processing cfu/g:** Escheriashia coli of different sausage samples presented in table (8). data revealed that sausage produced by modern processing line A did not showed any E. coli growth even after storage, these referred to good hygienic practice [GHP], while sausage produced by medium line B (34.00) showed the highest mean value of Escherichia coli, but there highly decreased after storage (2.67) followed by sausage produced by traditional line C (15.00).

Table (8) showed the contamination with E. coli during processing where the highest mean value of E.coli revealed by sausage produced by medium processing line B(34.00) step four stuffing during linking and tying, these may due to lack of personal hygiene and

training, while the lowest mean value revealed by sausage produced by modern line step five ( 3.00) freezing.

Youssif, (2015) who study the applicability of hazard analysis and critical control points (haccp) system in beef processing factories in Khartoum state and stated that, the higher contamination level by *E. coli* was found in 5.6% of beef sausage product and the bacterium counts was reached more than  $10^4$  gram. Despite *E. coli* is usually used as an indicator of faecal contamination of food or water (Martins and Germano, 2008) however, certain strains are known to be pathogenic and some produce a toxin in the intestine that results in symptoms of abdominal pain and diarrhoea. The result showed that 50.0% of the isolated *E. coli* found grow and multiply for more than  $10^2$  colonies and 11.1% for more than  $10^4$ , this considered spoilage causes growth (Banwart, 1981). Certain strains are enter pathogenic and represent food born infection, and it can be used as an indicator organism of faecal contamination from raw material or during processing, the same method was followed in my previous study in Al Kadar abattoir (Youssif, 2004).

The most unusual result was the presence of *E. Coli* in cooked (smoked) ready to eat mortadella product and the total viable count of *E. Coli* on mortadella product was found more than the infective dose (less than 10) microorganisms per gram was in 87.5%, and from  $10^2$  to less than  $10^3$  was in 12.5% of samples. This unexpected contamination might have been occurred either due to insufficient cooking temperature or after smoking (Youssif, 2015).

The sausage produced by traditional processing lie C showed highly contamination with *E. coli* in different steps, these due to poor hygienic condition and lack of [GHP], while the three are different sausage samples showed high level of contamination in step four stuffing mainly during linking and tying, these due to lack of personal hygiene. It could be concluded that, the very important step in the manufacturing of sausage in addition to deboning, mixing and stuffing, since there direct contact with person which considered as an essential source of contamination with pathogens *E. coli*.

## **CONCLUSION:**

The study proved that the raw material increased the microbial load significantly specially spices and bread crumbs. The study proved

that, the prolonged time of the equipment remained without being washed after the working increased microbial load. Also the study proved that the manufacture of sausage in traditional and medium processing scale exposed to microbiological contamination and absent of inspection and control. Furthermore, all pathogens [Salmonella styphi, staphylococcus aureus and Escherichia coli] appeared in sausage processing in two step especially in step four (stuffing) during linking and tying and step three during ingredient mixing.

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**Table (1): Mean values and their standard errors for raw materials various treatments (interactions)**

| Raw materials                    | Total viable count (cfu/g) | Total moulds and yeasts (cfu/g) |
|----------------------------------|----------------------------|---------------------------------|
| Meat                             | 6.50 X 10 <sup>5a</sup>    | *0.00 <sup>d</sup>              |
| Spices                           | 3.37 X 10 <sup>5a</sup>    | 6.20 X 10 <sup>2c</sup>         |
| Bread crumbs                     | 7.00 X 10 <sup>4b</sup>    | **1.20 X 10 <sup>3b</sup>       |
| Soy bean                         | 8.40 X 10 <sup>5a</sup>    | *0.00 <sup>d</sup>              |
| Water                            | 1.67 X 10 <sup>2d</sup>    | *0.00 <sup>d</sup>              |
| Gastrointestinal tract (casings) | 3.50 X 10 <sup>4c</sup>    | 4.00 X 10 <sup>3a</sup>         |
| SE                               | 0.07071                    | 0.04472                         |
| Lsd <sub>0.05</sub>              | 0.2179                     | 0.1378                          |

Means± SD having different superscripts differ significantly (P≤0.05).

[\* No growth - \*\* No fungi growth- \*\*\* Negligible growth]

**Table (2): Mean values and their standard errors for equipment**

| Equipment           | Total viable count (cfu/g) | Total moulds and yeasts (cfu/g) |
|---------------------|----------------------------|---------------------------------|
| Surfaces            | 5.27 X 10 <sup>4a</sup>    | *0.00 <sup>a</sup>              |
| Knife               | *0.00 <sup>d</sup>         | *0.00 <sup>a</sup>              |
| Mincer              | 5.47 X 10 <sup>4a</sup>    | *0.00 <sup>a</sup>              |
| Cutter              | ***0.00 <sup>d</sup>       | *0.00 <sup>a</sup>              |
| Blender             | 4.40 X 10 <sup>3b</sup>    | *0.00 <sup>a</sup>              |
| Stuffer             | 6.57 X 10 <sup>2c</sup>    | *0.00 <sup>a</sup>              |
| Ambient air         | #                          | #                               |
| SE                  | 0.1472                     | -                               |
| Lsd <sub>0.05</sub> | 0.4536                     | -                               |

Means± SD having different superscripts differ significantly (P≤0.05).

# = Medium microbial growth.

[\* No growth - \*\* No fungi growth- \*\*\* Negligible growth]

**Table (3): Mean values and their standard errors for total viable count (cfu/g) of the various treatments (interactions)**

| Processing steps    | Treatments               |                          |                          |
|---------------------|--------------------------|--------------------------|--------------------------|
|                     | A                        | B                        | C                        |
| Deboning            | 1.8 X 10 <sup>4j</sup>   | 6.00 X 10 <sup>5ef</sup> | 7.00 X 10 <sup>5de</sup> |
| Grinding            | 1.60 X 10 <sup>4j</sup>  | 5.80 X 10 <sup>5ef</sup> | 2.70 X 10 <sup>6c</sup>  |
| Mixing              | 1.40 X 10 <sup>5g</sup>  | 8.60 X 10 <sup>5d</sup>  | 8.00 X 10 <sup>6a</sup>  |
| Stuffing            | 7.00 X 10 <sup>5de</sup> | 3.20 X 10 <sup>6c</sup>  | 6.00 X 10 <sup>6b</sup>  |
| Freezing            | 6.00 X 10 <sup>4i</sup>  | 9.80 X 10 <sup>4h</sup>  | 5.00 X 10 <sup>5f</sup>  |
| Storaging           | 3.500 X 10 <sup>2l</sup> | 4.80 X 10 <sup>3k</sup>  | 9.80 X 10 <sup>4h</sup>  |
| SE                  | 0.04082                  |                          |                          |
| Lsd <sub>0.05</sub> | 0.1171                   |                          |                          |

Means± SD having different superscripts differ significantly (P≤0.05).

A ≡ Modern processing line of sausage; B ≡ Medium processing line of sausage; C ≡ Traditional processing line of sausage

**Table (4): Mean values and their standard errors for total moulds and yeasts (cfu/g) of the various treatments (interactions)**

| Processing steps    | Treatments*        |                    |                         |
|---------------------|--------------------|--------------------|-------------------------|
|                     | A                  | B                  | C                       |
| Deboning            | *0.00 <sup>d</sup> | *0.00 <sup>d</sup> | *0.00 <sup>d</sup>      |
| Grinding            | *0.00 <sup>d</sup> | *0.00 <sup>d</sup> | *0.00 <sup>d</sup>      |
| Mixing              | *0.00 <sup>d</sup> | *0.00 <sup>d</sup> | 3.50 X 10 <sup>3c</sup> |
| Stuffing            | *0.00 <sup>d</sup> | *0.00 <sup>d</sup> | 5.60 X 10 <sup>3a</sup> |
| Freezing            | *0.00 <sup>d</sup> | *0.00 <sup>d</sup> | 4.30 X 10 <sup>3b</sup> |
| Storaging           | *0.00 <sup>d</sup> | *0.00 <sup>d</sup> | *0.00 <sup>d</sup>      |
| SE                  | 0.0001826          |                    |                         |
| Lsd <sub>0.05</sub> | 0.0005237          |                    |                         |

Means± SD having different superscripts differ significantly (P≤0.05).

[\* No growth - \*\* No fungi growth- \*\*\* Negligible growth]

A ≡ Modern processing line of sausage; B ≡ Medium processing line of sausage; C ≡ Traditional processing line of sausage

**Table (5): Mean values and their standard errors for *Staphylococcus aureus* (cfu/g) of the various treatments (interactions)**

| Processing steps    | Treatments         |                          |                         |
|---------------------|--------------------|--------------------------|-------------------------|
|                     | A                  | B                        | C                       |
| Deboning            | *0.00 <sup>i</sup> | 7.00 X 10 <sup>3d</sup>  | 4.00 X 10 <sup>3c</sup> |
| Grinding            | *0.00 <sup>i</sup> | 3.00 X 10 <sup>2fg</sup> | 5.80 X 10 <sup>4b</sup> |
| Mixing              | *0.00 <sup>i</sup> | 8.20 X 10 <sup>2b</sup>  | 7.00 X 10 <sup>4b</sup> |
| Stuffing            | *0.00 <sup>i</sup> | 8.30 X 10 <sup>2e</sup>  | 9.00 X 10 <sup>4a</sup> |
| Freezing            | *0.00 <sup>i</sup> | 4.00 X 10 <sup>2g</sup>  | 5.00 X 10 <sup>2f</sup> |
| Storaging           | *0.00 <sup>i</sup> | *0.00 <sup>i</sup>       | 1.10 X 10 <sup>2h</sup> |
| SE                  | 0.03162            |                          |                         |
| Lsd <sub>0.05</sub> | 0.09070            |                          |                         |

Means± SD having different superscripts differ significantly (P≤0.05).

A ≡ Modern processing line of sausage; B ≡ Medium processing line of sausage; C ≡ Traditional processing line of sausage

**Table (6): Mean values and their standard errors for *Salmonella stypfi*(cfu/g) of the various treatments (interactions)**

| Processing steps | Treatments* |     |     |
|------------------|-------------|-----|-----|
|                  | A           | B   | C   |
| Deboning         | -ve         | -ve | +ve |
| Grinding         | -ve         | -ve | +ve |
| Mixing           | -ve         | -ve | +ve |
| Stuffing         | -ve         | -ve | +ve |
| Freezing         | -ve         | -ve | +ve |
| Storaging        | -ve         | -ve | -ve |

- +ve = positive: Detected.

- -ve = negative: Not detected.

A ≡ Modern processing line of sausage; B ≡ Medium processing line of sausage; C ≡ Traditional processing line of sausage

**Table (7): Mean values and their standard errors for total coliform (cfu/g) of the various treatments (interactions)**

| Processing steps    | Treatments                |                          |                           |
|---------------------|---------------------------|--------------------------|---------------------------|
|                     | A                         | B                        | C                         |
| Deboning            | 5.00±1.00 <sup>g</sup>    | 92.70±1.53 <sup>e</sup>  | 206.70±7.64 <sup>c</sup>  |
| Grinding            | 15.00±1.73 <sup>g</sup>   | 241.00±4.36 <sup>b</sup> | 240.00±8.88 <sup>b</sup>  |
| Mixing              | 36.00±3.61 <sup>f</sup>   | 240.00±2.00 <sup>b</sup> | 460.00±26.46 <sup>a</sup> |
| Stuffing            | 150.00±17.32 <sup>d</sup> | 241.00±4.36 <sup>b</sup> | 160.00±17.00 <sup>d</sup> |
| Freezing            | 9.00±1.73 <sup>g</sup>    | 43.00±2.65 <sup>f</sup>  | 153.30±10.41 <sup>d</sup> |
| Storaging           | 0.00±0.00 <sup>g</sup>    | 8.67±1.53 <sup>g</sup>   | 100.00±8.00 <sup>e</sup>  |
| SE                  | 5.558                     |                          |                           |
| Lsd <sub>0.05</sub> | 15.94                     |                          |                           |

Means± SD having different superscripts differ significantly ( $P \leq 0.05$ ).

A ≡ Modern processing line of sausage; B ≡ Medium processing line of sausage; C ≡ Traditional processing line of sausage

**Table (8): Mean values and their standard errors for *E. coli* (cfu/g) of the various treatments (interactions)**

| Processing steps    | Treatments*              |                         |                          |
|---------------------|--------------------------|-------------------------|--------------------------|
|                     | A                        | B                       | C                        |
| Deboning            | *0.00 <sup>h</sup>       | *0.00 <sup>h</sup>      | 20.00±2.00 <sup>d</sup>  |
| Grinding            | *0.00 <sup>h</sup>       | *0.00 <sup>h</sup>      | 23.00±2.65 <sup>bc</sup> |
| Mixing              | 11.00±6.95 <sup>f</sup>  | *0.00 <sup>h</sup>      | 20.33±1.53 <sup>cd</sup> |
| Stuffing            | 23.00±3.61 <sup>bc</sup> | 34.00±3.00 <sup>a</sup> | 24.00±1.00 <sup>b</sup>  |
| Freezing            | 3.00±0.00 <sup>g</sup>   | 14.00±2.00 <sup>e</sup> | 20.00±1.73 <sup>d</sup>  |
| Storaging           | *0.00 <sup>h</sup>       | 2.67±0.58 <sup>gh</sup> | 15.00±1.00 <sup>e</sup>  |
| SE                  | 0.9494                   |                         |                          |
| Lsd <sub>0.05</sub> | 2.723                    |                         |                          |

Means±SD having different superscripts differ significantly ( $P \leq 0.05$ ).

A ≡ Modern processing line of sausage; B ≡ Medium processing line of sausage; C ≡ Traditional processing line of sausage