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Comparative Study of Total Bacterial Count in Fresh and Frozen Sausages From Different Sources

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ABSTRACT

This study was conducted in the College of Animal Production Science and Technology, Sudan University of Science and Technology to investigate the total bacterial count for the three types of beef sausage collected from different sources. The samples were analyzed in three different brands of these raw cuts in duplicate. One of samples was collected from sausage factories (A), the second sample was collected from the local market (B) and the third sample was manufactured in the lab at the college (C). This study showed no significant differences ($P > 0.05$) in the bacterial count of the three samples but there was high significant difference ($P < 0.01$) between the three samples (A, B, C) during storage period. The results showed that average bacterial count of fresh and frozen samples for sausages from sample (A) were ($5.53 \times 10^{5-}$ and $1 \times 10^{5-}$ respectively), sample (B) were ($6.5 \times 10^{5-}$ and $2 \times 10^{5-}$) and sample(C) were ($7.5 \times 10^{5-}$ and $2 \times 10^{5-}$) . The results were showed that the contamination rate was high in sausage from sample B and C sample compared with sample A. The study also revealed that there was a decreased in the number of bacteria with storage period.

INTRODUCTION:

Meat is one of the most important sources of protein-rich food, which contains all the essential amino acids, along with a large group of vitamins, especially vitamin B and minerals. Although there are many other animal products that can replace red meat in human nutrition such as dairy products, eggs, fish and poultry meat. Fresh sausages are highly perishable and serve as substrates for several spoilage and pathogenic microorganisms due to their high water content and abundance of essential nutrients (COCOLIN et al.,

2004). The initial microbial load plays a role in the determination of meat product's shelf-life (Olaoye, and Onilude, 2010). Ray and Bhunia, (2008) and Pesavento, et al., (2010) reported that the contamination of meat is a continuing possibly from the moment of Bleeding until consumption. Manufacturing is a way to expand product range and improve lifespan (Kalalou et.al 2004). FAO (1991) reported that sausage is a meat product specially made from fresh minced meat. The sausage is based on the quality of the mixture for dry sausage and less dry dough**.** The sausages casing are used to give the sausage the known shape through the manufacturing process. There are two types of sausage casings, natural and factory. Natural is the intestines of small ruminants (sheep, goats) and large ruminants (cattle). Industrial is cellulose, collagen or plastic (Judge *et.al* 1990). Dennis (2004) stated that sausage casings are natural from the digestive system of animals. In 1998, there was an increased number of reported cases of illness due to Listeria monocytogenes which the Centers for Disease Control and Prevention as well as state and local health departments in the U.S. attributed to the consumption of cooked hot dogs and deli meats (FSIS, 1999). Shehu and Adesiyun (1990) reported that *Enterotoxigenic Escherichia coli* has been involved in food-borne illness and recovered from various food types, processed or raw (Firstenberg and Sullivan, 1997). The fact cannot be overemphasized that raw or pre-processed foods sold in supermarkets pose a direct health hazard to consumers if they contain an infective dose of pathogens or toxic levels of their toxins. According to Kuku (1985), the presence of bacteria could be as a result of it being a common organism on the skin, hands and boil and hence their presence in sausage may be as a result of contamination due to handling, processing, transportation and storage. Its presence in high numbers is a good indication of poor hygiene and temperature control. Also the presence of bacteria in high numbers in cured meat may indicate the presence of enterotoxin –producing strains of S .aureus (AS/NZS, 1999), thus the data generated are of great importance to inform public health authorities, to detect food-borne diseases outbreaks early and to implement and evaluate food safety programmes. Essien (2003) stated that the addition of excessive amounts of water can reduce the quality of sausages, because of damaged fatty tissues that increase the loss of fat, and lead to the distribution of unequal salt to the final product. Food additives contribute to the improvement and intensification of certain properties of meat and protein and the strength of water binding and emulsification (FAO, 1991). Food hygiene laws determine the maximum level of each of substances and measures of quantity and concentration and methods of testing is not allowed to be used in natural meat, whether natural or chemical additives, but only used in meat manufactured (Jafar

2006). One of the most important additives in processing is the bounding materials which hold the water, dissolve the fat and divide according to the source to the animal (skimmed milk, casein, whey), soybeans which is a non-meat additive used in sausage processing as flour. The sausages filler are materials that catch water but do not metabolize the fat, most of it are carbohydrate, including rice, boiled potatoes, starch, flour, barley, or wheat or crush (bread crumps). Judge *et al.,* (2001) and Kerry *et. al.* (2002) reported that salt is the most common and most important additive in sausage processing. Salt is an essential additive to meat and its products as it is a preservative and taste enhancer. Spices are a source of plant material giving flavor and contain aromatic essential oils that volatilize at normal temperatures**.** Toldra (2002) stated that the characteristics of the flavor that give particular type of a sausage depends on the spices used in processing. Lin *et al*., (1991) found that garlic has anti-bacterial and anti-oxidant effects in meat products. Oxidized compounds are the main ingredients and are responsible for flavor and taste. Skimmed milk is one of the most important additives in sausage processing (John, 1975). Khalifa, (2002) stated that the effect of beef storage on total viable count as (5.75×10^{-4}, 6.2×10^{-4}, 4.25×10^{-5} and 4.25×10^{-5}) for shade dried beef at zero ,one ,two and three month of storage respectively. According to Paulsen *et. al.,* (2006) meat perishable animal product and microbial spoilage of meat has great concern to the food industry. Dyett, *et. al*. (1981) found that food preservation depends mainly on controlling the temperature of storage. Pearson and Tuber (1998) found that spices are very important to give flavor and are also antibacterial. Jay, (1996) stated that the important to keep microorganisms at low for reasons of aesthetics, public health and products shelf-life. Ray and Bhunia, (2008) and Pesavento, *et al*., (2010) reported that the contamination of meat is a continuing possibly from the moment of Bleeding until consumption. Judge *et al.,* (1990) reported that the spoilage of meat was defined as the state at which meat become unfit for human consumption. Spices are usually used to fix or fix a rectified apoptosis and may contain some microbes. Common types of spices used in Sudan include black pepper, garlic, coriander, cinnamon, medicinal nut, Chinese kebab. Stinger *et.al.* (1969) reported that meat contamination occurs from various sources such as environment, equipment, slaughterhouse, manufacturing method, but leather remains the main sources of meat contamination. Jay, (1970) reported that the storage of meat at the refrigerator temperature cause meat spoilage as a result of the growth of microbes. Oregon Department of Agriculture (1973) found that fresh and frozen meat and its bacterial count should not exceed CFUG ($5\ X\ 10^{-6}$). The quality of meat and meat products was found to be dependent on the number and type of polluting bacteria

(Brownlie, 1966). Zhou *et al* (2010) reported that chilling is a critical for meat hygiene, safety, shell life, appearance and eating quality. Siham, (2015) reported that the bacterial count of fresh

The Objectives of this study were:

1. To evaluate bacterial count of sausage from different sources.
2. To study the effect of storage period on the number of bacteria.

MATERIALS AND METHODS:

The study was conducted at the laboratory of Meat Science and Technology, and the laboratory of the microbiology at the College of Animal Production Science and Technology, Sudan University of Science and Technology.

Meat samples: 5 kg of fresh deboned from fresh meat beef (bone-free) was obtained from the Center of animal production research. Each muscle samples were freed from external visible fat and connective tissue.

***Fillers*:** The following materials were used to the sample (C) which processed in the college laboratory:

1. Bread crumbs used as a milled.
2. Potatoes cooked under pressure for 10 minutes and then chopped.

Sausage Preparation: The sausage was manufactured using two types of filler (Bread Crumbs and potatoes). The ingredients were added evenly to the mixture as shown in table (1). The sausage was make from minced meat, salt (sodium chloride), garlic, coriander, cinnamon, black pepper, nutmeg, fat, cold water, skimmed milk and 15% filler. The whole mixture was mixed well after adding the skimmed milk powder to the dough, then the stuffing is done in natural casings using the sausage piston, then braided and placed in nylon bags and placed in the freezer to wait for the next tests according to (Siham, 2008).

Table (1) The Ingredients of the Sausages Recipe per grams:

Ingredient	Gram
Fillers (bread crumbs or sweet potato)	900
Ice water	300
Salt	5
Black pepper	6
Coriander	9
Piper cubeb	3
Garlic	9
powder Skimmed milk	120
Cinnamon	9
Nutmeg	3

Bacteriological Assessment: Total viable bacterial counts of fresh and refrigerated samples of camel, beef and goat meat was done after variable periods of storage (1-15 day). Samples were placed in icebox during transport to laboratory and kept

samples was higher than those stored in the frozen storage at -18 °C. SSMO (2008) reported that total aerobic plate count for fresh sausage should not exceed than 5.25×10^{-5}CFU/gm.

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in a deepfreeze (-18c°). The samples were then blended with 270 ml sterile distilled water by using electric blender (Homogenizer MSE) for 3 minutes. Duplicate samples were taken .Serial dilutions were made for each sample and each dilution was plated in standard plate- count Agar. Duplicates of each sample were incubated at 37 c^0 for 48 hours. To determine total viable counts, 1 ml of each of 10^{-5} and 10^{-7} dilutions were plated on nutrient agar plates in triplicates. The plates were incubated at 37 C^o for 24hours.The same procedure was repeated for Staphylococcus aureus count, enterobacteriaceae count, lactic acid bacterial count on mannitol salt agar, MacConkey agar and De Man Rogosa Sharpe (MRS) agar respectively. Pschrophyllic count done for all samples in Benin-City. They were incubated on nutrient agar plates at 4O C for 48 h. For MRS agar, the plates were incubated at 37O C for 48-72 hours. Anaerobic count was done by incubating plates in an anaerobic jar for 24 h.

Culture Media: The medium was in form of dehydrated powder. It was composed of Bacto-tryptone-yeast extract, Dextrose and agar. It was prepared by dissolving 23 gm of medium in one liter of distilled water. Ten gram of each sausages sample were taken aseptically, cut into small pieces and blended with 90 ml sterile cooled normal saline for 3–4 minutes at high speed. The homogenized suspension was allowed to stand for 10 minutes to allow the foam to subside and heavy particles to settle.

The average bacterial loads of the fresh and frozen samples of A (Sausage from a factory) were (5.53 $x10^{5-}$ and 1 $x10^{5-}$)

Total viable counts: Using sterile pipette 1.0 ml of the supernatant was transferred to a test tube containing 9.0 ml sterile normal solution. The contents were mixed by another sterile pipette and 1.0 ml of the mixture was transferred to a second tube until the fifth tube thus decimal serial dilutions up to 10^{-6} were prepared. Using sterile pipettes 1.0 ml of the dilutions10^{-2}, 10^{-3} , 10^{-4} and 10^{-5} was transferred into duplicate sterile Petri dishes. Fifteen to twenty milliliters of molten plate count agar cooled to 42 –45°C, in a water bath, were poured into each plate containing the inoculums. Plates were then rotated from side to side and then left to dry and incubated in inverted position (Cruickshank, 1975). The dilutions 10^{-3}, 10^{-4} and 10^{-5} were used for storage samples.

Statistical analysis: The data collected were subjected to statistical analysis by using complete randomized design used to analyze the results obtained from this study and subjected to ANOVA followed by Least significant difference test (LSD) using the (SPSS, 2008. version ,17).

RESULTS:

Tables (2; 3) and Figure (1; 2) shows the bacterial count of fresh and frozen samples obtained from different sources. Initially on first day, total bacterial count (TBC) for the samples were significantly higher ($P < 0.05$) compared to treatments on week tow.

respectively. The average load of the fresh and frozen samples B (Sausage from local market) were (6.5$x10^{5-}$ and 2 $x10^{5-}$)

respectively. Whereas, the average load of fresh and frozen samples C (Sausage manufactured in the lab at the college) were (7.5 $x10^{5-}$ and 2 $x10^{5-}$) respectively.

Table (2) Mean values (± SD) of the total bacterial count (TBC) of fresh and frozen samples of sausages (A, B and C) after variable periods of storage (1-15 day) at -18 C°:

Sausage type	Storage period	Sausage TBC× 10^{-5}
A (Sausage from a factory)	1st day	5.53^{a} x 10^{5-}
	7 days	1. 0 x 10^{5-}
	15 days	1. 0 x 10^{5-}
B (Sausage from local market)	1st day	6.5^{b} x 10^{5-}
	7 days	2. 0 x 10^{5-}
	15 days	2. 0 x 10^{5-}
C (Sausage manufactured in the lab at the college)	1st day	7.5^{c} x 10^{5-}
	7 days	5.93 x 10^{5-}
	15 days	2. 0 x 10^{5-}
Meat type × Storage time		
Standard Deviation		0.07
Level of Significant		**

NS = No significant difference between the two means.

* = ($P< 0.05$)

** = ($P< 0.01$)

a, b and c = Means within the same row with different superscripts differ

The results showed that there were no significant differences ($P > 0.5$) in bacterial count between the three sausage samples (A, B, C). Also there were significant differences in the number of bacteria in sausage after one week of storage and after two weeks. The results showed that the percentage of contamination). high in sausage samples from source B and **C** compared to source A**.** In general, there was decreased in the bacterial count in sausage samples with increase of the freezing time. The fresh samples have the higher bacterial count compared to samples that stored at deep-freeze temperature (-18c^{o}

Table 3: Mean values (± SD) of the total bacterial count (TBC) of fresh sausages sample (A, B and C)

The Source of sausage	The Number of bacteria in fresh sample (CFU/gram)
A (Sausage from a factory)	5.53 x 10^{5-}
B (Sausage from local market)	6.5 x 10^{5-}
C (Sausage manufactured in the lab at the college)	7.5 x 10^{5-}
Standard Deviation (S. D.)	0.07
Significant level (S. L.)	N.S

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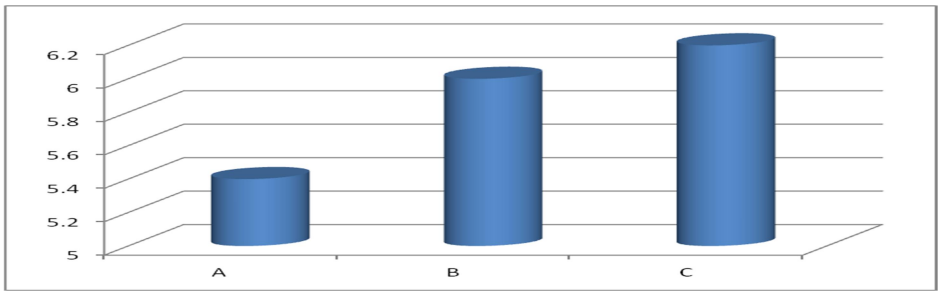

Figure 1: The Total bacterial counts (CFU/gm) for different types of fresh sausages samples (A Sausage from a factory), B Sausage from local market, and C Sausage manufactured in the lab at the college).

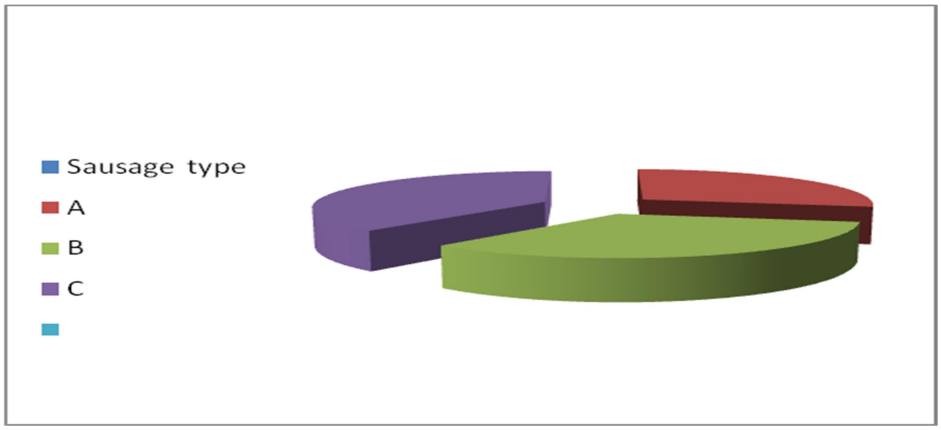

Figure 2: Total bacterial counts (CFU/gm) for different types of sausage after two Weeks of frozen Storage (A (Sausage from a factory), B Sausage from local market, and C Sausage Manufactured in the lab at the college).

DISCUSSION

In this study the average total number of bacteria in fresh and frozen samples were (5.53 $x10^{5-}$ and 1 $x10^{5-}$), (6.5$x10^{5-}$ and 2 $x10^{5-}$) and (7.5 $x10^{5-}$ and 2 $x10^{5-}$) for samples A, B and C respectively, this results agrees with the results of SSMO (2008), who stated that the bacterial number in the sausage is (5.25 x 10^{-5} (logarithm / bacterial colony / unit). The result in this study was not consistent with the results of Siham, (2015) who reported that the average total number of bacteria in beef sausage samples was (2×10^{-6}). This study showed that the average total bacterial count of sausage produced from three different sources after a week of manufacturing (A) 5.2 $x10^{5}$; (B) 6.0 $x10^{5}$ and (C) 5.6 $x10^{5}$, this study agrees to that reported by Mohamed, (1990) who found the total number of bacteria is ranged between (1.0×10^{2} - 7.0×10^{5}). Also this study showed that the average bacterial count in sausage samples after two weeks of storage were 1. 0 $x10^{5-}$; 2. $0x10^{5}$ and 2. 0 $x10^{5-}$ for samples A; B and C respectively. Also the study showed that there was a decrease in the bacterial count with increasing of storage period, this result disagree with the result of Youssef, (1996) and

Siham, (2008) who found that after frozen storage there was an increase in bacterial counts. A high percentage of bacteria in this study may be due to contamination of the outer surface of meat by bacteria. The result in this study agreed with the study of Alamin, (2015) who stated that the total bacterial count was higher in the fresh sausage samples on the first day compared with the samples that was stored in -18c^0 for one week and two weeks. The results in this study are also agrees with the study conducted by Khalifa, (2002) who found that the bacterial count decrease with an increase in storage period. The result in this study is inline with Rajkumar *et al*. (2004), who reported that bacterial count decrease with increase of storage period. It is also agreed with the result of Abass, (2009), who found that the total bacterial count was decreased during storage period. Also the result in this study is agrees with that reported by SSMO (2008), who stated that the total bacterial count in fresh sausage was 5. 25 × 10^{-5} CFU / gm. In relation to total bacterial count counts, it was possible to establish that the sausages Sausage Manufactured in the lab at the college of animal production analyzed was of high quality, this is because the this samples shows the least number of the total bacterial count, which is indicative of good manufacturing practices because the products used were raw and not heat treated. According to Gram *et al.* (2002), the level of microorganisms detected, "total count", can be used to predict the shelf life of the product.

CONCLUSION:

The present study concluded that the total number of bacteria decreased by increase storage period of sausage processed from the three different resources. Also there were significant differences in the number of bacteria in sausage after one week of storage and after two weeks.

REFERENCES

Abbas, M. O., (2009). Effect of adding different levels of citric acid on quality Attributes of fresh beef sausage. M. Sc. U of K 2009.

Abdullah Mohammed Jaafar (2006). Book of preservatives and food additives.

Autralian/ New Zealand Standards Methods for Food Microbiology (AS/NZS). (1999): Guide to determine the equivalence of Food Microbiology nest method. AS/NZS 4659. (1999).

Brownlie, L. E. (1966). Effect of some enviromental factor on psychrophilic bacteria. J. App. Bacteriol. 39: 447.

COCOLIN, L. *et al*. (2004) Study of the ecology of fresh sausages and characterization of populations of lactic acid bacteria by molecular methods. Applied and Environmental Microbiology, v. 70, p. 1883-1894, 2004. P Mid: 15066777 P M Cid: PMC383013. http://dx.doi.org/10.1128/AEM.70.4.1883-1894.2004

Cruickshank, R. (1975). Medial Microbiology, 12th end. Churchill Livingstone, Edinburgh and London 1975.

Dennis, B. (2004). Breaking Neuson prepared food and meat processing .Meat sausage formoula spears meat alternatives.

Dytt, E. E.; Hughes, R.B.; Jones, C.R.V. and Wilson, N. R. D. 9 (1981). Meat and meat products: Factors affecting quality control. Applied Sci., publishers, London, UK 1981. Bulletin No.126.

Essien , E. (2003). Sausages manufacture principles and practice, wood head publishing limited, Cambridge, (2003) England, pp 5-9.

Firstenberg, E. R. and Sullivan, N. M. (1997): EZ Coli Rapid Detection System: a rapid method for the detection of Escherichia coli 0157 in milk and other foods. Journal of Food Protection 60(3):219-225. 1997.

FSIS (1999): FSIS action plan for addressing Listeria monocytogenes. U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, D, C.

Food and Agriculture Organization of the United Nations, FAO, (1991). Guide lines for slaughtering, meat cutting and further processes. Animal production and health paper No. 91 .FAO, Rome. 1991.

GRAM, L. *et. al.* Food spoilage—interactions between food spoilage bacteria. International Journal of Food Microbiology, v. 78, p. 79-97, 2002. http://dx.doi.org/10.1016/S0168-1605(02)00233-7

John C. (1975). Principles of Meat Science (A Series of books in food and nutrition) by John C. Forrest (1975-11-17) Hardcover – 1843.

Judge, M .D . ; Aberle ,E.; Forest, J.; Hedrick, H. and Merkel, R.(2001). Principles of meat sciences (4th ed), Kendall, Hunt, lowa,USA. 2001.

Judge, M.D.; Aberle, E.; Forest, Forest, J.; Hedrick, H. and Merkel, R.(1990). Principles of meat sciences (2th ed), Kendall, Hunt, lowa,USA.

Kalaloui I.,Faid M. and Ahami T.A.(2004). Internataional journal of Agriculture and Biology, 6,447.

Kerry, J.; John, K. and David, L.W., (2002). Meat processing improved quality, Wood head publishing limited, Cambridge, England.

Khalifa , N.B.E.(2002). Microbiological and Biochemical evaluation of traditional dried beef, Shermout, M.Sc. (Agric.) Thesis University of Khartoum.

Kuku, F. O. (1985): Soilage of fruits, vegetable and tuber crop. Nigeria food Journal 2:1-3.

Lin, X.; Li. I.; Zhang, Q. and Mei, X. (1991). The preventive effect of garlic against toxicity of dimethyluitrosamine in rats fed with aminopyrine and nitrite. ACTA Nutrimena Sinica 13(2): 126-132.

Mohamed, F.E.(1990). The effect of freezing on bacteria in processed meat .Ph.D .thesis .University of Khartoum, Sudan.

Olaoye, O.A. and Onilude, A.A. (2010). World Journal of Microbiology and Biotechnology 26: 1445–1454.

Oregon Department of Agriculture (1973). Oregon meat bacterial criteria. Cited by Michael, H.W. In: Attitudes and policies

of state governments. J. Food Technol. (1978) 32, 63-67.

Paulsen, P.; Hiesberr, J.; Gifing S. and Smulders, F. J. M. (2006). Modified atmosphere storage under subatomospheric pressure and beef quality: Microbiological effect. J. Animal Sci. 48: pp.2448-2455.

Pearson, A.M. and Tuber, F.W.(1984). Species and condiments. In: Processed Meats. 2nd e., AVI Publishing Company Inc ., West port, connecticut, p. 256.

Pesavento, G.; Ducci, B.; Nieri, D.; Comodo, N. and Lo Nostro, A., (2010). Food Control 21: 708–713.

Rajkumar, V.; Agnihorti, M. K. and Sharma, N., (2004). Quality and shelf-life vacuum and aerobic packed chevon patties under refrigeration. Asian Australian Journal of Animal Science, 17: 548-553.

Ray, B. and Bhunia, A. (2008). Foodborne infections. In: Fundamental food microbiology, 4th edition, pp. 283–313. Boca Raton: CRC.

Shehu, L. M. and Adesiyun, A. A.(1990): Characteristics of strains of Escherichia coli isolated from locallyfermented milk (Nono) in Zaria, Nigeria.J. Food Protection 53:574-577 .(1990) .

Siham , A.A., (2008). *A comparative study of chemical composition And Eating Quality Attributes of Camel meat and Beef*. M.SC. Thesis Sudan University of Science and Technology.

Siham, A.A., (2015). *A comparative study of chemical composition and quality Attributes of fresh and processed meat of calf, camel meat and goat meat*. PhD. Thesis Sudan University of Science and Technology.

SPSS, (2008). Statistical Package for the social sciences. Version 17.0 SPSS Inc. Chicago.

SSMO,(2008). Sudanese Standards Meteorology Organization Khartoum, Sudan.

Strainger, W.C.; Bilskie, M.E. and Mawnan, H.D. (1969). Microbial profiles of fresh beef. J. Food Techno. Champ. 23: 97.

Toldra, F. (2002). Dry cured meat products, Food and Nutrition press, I.N.C, Trumbull, CT, pp. 63-88.

Yousef Mohammed Partner (1996), Meat Technology and Offal (BOOK) - Department of Food Science - Faculty of Agriculture - University of Cairo.

Zhou, G. H., Xui, X. L. and Liu, Y. 2010. Preservation technologies for fresh meat: a review. Meat Science86 (1): 119-128.