بسم الله الرحمن الرحيم

Sudan University of Science and Technology

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Effect of Frozen Storage on the Physicochemical, Microbiological and Sensorial Properties of Beef Sausage

تأثير التخزين المجمدفي الخصائص الفيزيوكيميائية والميكروبيولوجية والحسية للسجوك المصنع من لحم البقر

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بسم الله الرحمن الرحيم

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كلية الدراسات العليا

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قسم علوم وتكنولوجيا الأغذية

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أطروحة مقدمة لجامعة السودان للعلوم والتكنولوجيا بالبحث التكميلي لمتطلبات درجة الماجستير في علوم وتكنولوجيا الأغذية

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الآية

قل تعالى:-

أَوَلَمْ يَرَوْا أَنَّا خَلَقْنَا لَهُم مِّمَّا عَمِلَتْ أَيْدِينَا أَنْعَامًا فَهُمْ لَهَا مَالِكُونَ

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ذَلَّلْنَاهَا لَهُمْ فَمِنْهَا رَكُوبُهُمْ وَمِنْهَا يَأْكُلُونَ (72) وَلَهُمْ فِيهَا مَنَافِعُ

وَمَشَارِبُ أَفَلَا يَشْكُرُونَ(73)

صدق الله العظيم

سورة يس

DEDICATION

To my father.

To my mother.

To my brothers.

To my sisters & my friends.

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Thanks at the beginning and at last for Almighty ALLAH. I am deeply indebted to my supervisor Prof. Ahmed Elawad Elfaki. for his continual encouragement and help throughout this work. Thanks also extended for all who help me in finishing this work.

Abstract

The study was conducted to examine the effect of storage period on physicochemical, microbial and sensorial properties of three different types of fresh beef sausage obtained from three sources in Khartoum State (Factory A, factory B and Khartoum North local market C). The Physicochemical, microbial and sensorial properties were made immediately after processing. Then the three sausage types were stored in deep-freezer at –10°C for 6 months. Analyses were carried out at zero time then every two months (2, 4 and 6) months. For type A: moisture ranged (57.32-58.01%), oil (22.25-26.57%), protein (11.67-12.95%), ash (5.09-7.19%) and pH(6.10-6.74), through the storage period. For type B moisture ranged (56.32-59.01%), oil (24.76-27.64%), protein (9.60-12.56%), ash (4.26-9.06%) , and pH(6.10-6.74) through storage period. For type C moisture ranged (56.00-58.72%), oil (26.78-29.54%), protein (12.73-13.63%), ash (4.03-5.27%), and pH (6.02-6.45) through the storage period. Physical properties (weight and length) of all stored samples were almost similar, (17.38-31.79) g and (9.50-10.00) cm. Total viable count of type A showed the lowest number $(4.48\text{-}4.55)^{log}$ cfu/g and type B showed (5.68-6.48) cfu/g while type C showed the highest number of total microbes (6.62-6.73) cfu/g. Sensorial properties showed significant differences between the three types, type B showed the lowest acceptability.

الملخص

أجريت هذه الدراسة لمعرفة اثر التخزين على الخصائص الفيزيكوكيميائية و الميكروبيولجية والحسية لثلاث أنواع مختلفة من السجوك الطازج أخذت من ثلاثة مصادر من ولاية الخرطوم (مصنع A ، ومصنع B ، والسوق البلدي C.

أخضعت العينات للتحليل التقريبي والتحاليل الفيزيكوكيميائية والميكروبيولوجية والحسية عقب التصنيع مباشرة ومن ثم تم تخزين العينات في الثلاجة عند درجة التجميد 18°c- لمدة ستة شهور أجريت التحاليل عقب التصنيع مباشرة ومن ثم كل شهرين(2 , 4 و 6) شهور.

وجد أن التحليل التقريبي للنوع A وهي : الرطوبة (57.32-58.01 %) ، الزيت (22.25-26.57%)، البروتين (11.67-12.95%) الرماد (5.09-7.19%)، الرقم الهيدروجيني (6.10-6.74) على التوالي خلال فترة التخزين، نسبة الرطوبة (56.32-59.01%) ،الزيت (24.76-27.64%) ، البروتين (9.60-12.56%) ، الرماد (4.26-9.06) ، الرقم الهيدروجيني (6.10-6.74) للنوع B خلال فترة التخزين. وللنوع C أظهرت النتائج : الرطوبة (56.00-58.72%)، الزيت (26.78-29.54%) ، البروتين (12.73-13.63%) ،الرماد (4.03-5.27%) الرقم الهيدروجيني (6.02-6.45) خلال فترة التخزين .

كما أظهرت نتائج التحليل الفيزيائي (الوزن والطول) ان هناك تشابه بين العينات الثلاث حيث يتراوح الوزن ما بين (17.38-31.00) جرام والطول ما بين (9.50 – 10) سم

أما الخصائص الميكروبيولجية فقد أظهرت النتائج أن النوع A قد حظيت بأقل عدد من الأعداد الميكروبيولجية أو ينعدم (4.48-4.55 خلية/جم) ، النوع B (5.86-6.48 خلية/جم) أما النوع C (6.62-6.73 خلية/جم) التي كانت اكثر العينات حملاً ميكروبيولجيا.

أيضا وجد أن الخصائص الحسية للعينات أظهرت فروقات معنوية مختلفة حيث حظيت العينة B بأقل قبول .

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CHAPTER ONE

INTRODUCTION

Meat is one of the most popular and nutritious food items which come from flesh of animals that are suitable as food (Forestet *et al*., 2001). Meat and other animal products make valuable contributions to diets of developing countries due to its high nutritional qualities (Olusolaet *et al*., 2010). Meat is defined as those animal tissues, which are suitable for used as food and it is often widen to include, as the musculature, organs such as liver and kidney, brains and other edible tissues (Lawrie, 1991). Different kinds of meat exist as a result of their methods of preparation and preservation. Sausage making began as a means of meat preservation and is one of oldest processed foods. The term comes from chopped lean meat, water, seasonings, salt and fat that is mixed together to form a sausage batter. Although it is not a true emulsion, a dispersed phase of fat globules is embedded in a continuous phase of protein and water. Sausages may be stuffed into natural or artificial casings. There are many sausage varieties including those made from beef, pork, poultry, wild game and veal. Processing techniques can be used to classify sausages into the categories of fresh sausage, uncooked smoked sausage, cooked , semi-dry, dry sausage and cooked meat specialties (Lawrie,1991) .

In the Sudan many factories produce sausages including beef and poultry with different techniques.

Objectives:

- To determine and evaluate the proximate chemical composition and sensory quality of chosen three fresh beef meat sausage.
- To determine and evaluate the microbiological quality and shelf life of chosen three fresh meat sausages.

CHAPTER TWO

LITERATURE REVIEW

2.1 Meat definition

Meat is defined as those animals' tissues, which are suitable for use as food. All processed or manufactured product, which might be prepared from tissues, are included in definition .The processed meat products are defined as those in which properties of fresh meat have been modified by use of one or more procedures, such as grinding or chopping addition of seasoning, alternation of color or heat treatment .Generally, meat processing developed soon after people become hunter (Judge *et al*., 1990)

2.2 Nutritive value of meat

Nutritionally, meat is a very good source of essential amino acids to lesser extent of certain minerals .Although vitamins and essential fatty acids are also present, meat is not usually relied upon for these components in a well-balanced diet (Lawarie, 1991).

With regard to essential amino acids there are significant differences that may exist between animals species, specific muscle location, or the breed and animals age have important effects .The amino acid content may be affected by processing (e.g. .ionizing radiation, heat).But unless processing condition are both sever and prolonged, such destruction is minimal. Rather more important is the possibility that certain amino acids may become unavailable (Bender, 1966).

Meat is generally good source of all minerals except calcium of meat is present in bones and teeth (Judge *et al*., 1990). Meat is also important source of fat vitamins A, D. E and K that are found primary in body fat and variety meat (kidney, liver, heart). Meat is very poor source of water

soluble vitamin C except when a scorbate has been added to processed meat product (Judge *et al*., 1990).

Carbohydrate constitutes less than one percent of weight of meat most of which is present as glycogen and lactic acid thus the liver is a good source of carbohydrate (Judge *et al*., 1990).

Processed meat generally contains less protein and water and more fat than consumed portion of fresh meat .Caloric content of some product is further increased by added cereal or flour .Percentage of some minerals in processed meats are higher than in fresh mat because of added salts and seasoning (Judge *et al*., 1990).

2.3 Physicochemical properties

Quality like beauty is subjective attribute. Various definitions have put forward over the years, but that all have suffered from the lack of any objective approach and have generally concluded that quality of meat was that for which the public was prepared highest price (Cooper and Willis, 1984).

2.3.1 Meat color

Color is perhaps the most critical component of fresh meat appearance, and more importantly a consumer's perception of meat quality is strongly influenced by product appearance. Meat color and eventual discoloration of meat is combined function of (a)muscle PH ;(b) antioxidation status;(c) oxidation of muscle pigments and (d)oxidation of lipids (Chirs and Kerth,1968).The appearance of meat surface to consumer depend on the quality of myoglobin present also on type of myoglobin molecule, on its chemical state and on the chemical and physical condition of other components in meat .In fresh meat ,before cooking the myoglobin is oxymyoglobin which is known as bloom and it represents the bright red color desired by purchasers. The principle pigment of cooked meat is known as globin haemichromgen (Lawarie, 1991).

2.3.2 Tenderness and texture

Inekoronye and Ngoddy (1992) reported that when the meat is heated in the water, the connective tissue is changed to assort of tender gelation and it became more .There have been many attempts to device objective physical methods of assessment by test panel thus ,physical methods have included measuring force of shearing penetrating ,compressing and stretching the meat. Chemical methods have involved determination of connective tissue and enzymes digestion amongst other criteria (Lawarie, 1991).Tenderness is probably the most important factor considered by the consumer in assessing the quality of meat .Two structure component have been shown to determine the tender of the meat, namely the collagen of connective tissue and contractile apparatus of myofiber protein (Zaglul and Cassens ,1987). Kumar *et al*. (1974) Showed that the pre-slaughter and post-slaughter factor effecting meat texture include species, sex, age, feed, pre-rigor factors and processing.

2.3.3 Flavor

Flavor is a complex sensation. It involves odor, taste, texture, temperature, and pH .Of these odor is most important without it, one of the four primary taste sensation –bitter sweet ,sour or saline predomination (Lawarie, 1991).

Lawarie(1979) reported that evaluation of taste and odor still depends mainly on taste panel Judge *et al*., (1990) reported that constituents of the meat tissue become flavor compounds upon being heated. Also some evidence shows that inosinic mono phosphate (IMP) and hypoxanthine enhance flavor meat or aroma. Since IMP and hypoxanthine are break down products of ATP, it is obvious that muscle with large energy stores would have more pronounced flavor. Most of the constituent of meat responsible for the flavor are water soluble component of muscle tissue.

They also reported that some undesirable flavor changes occur during storage could be due to metabolic products.

2.3.4 Water holding capacity

Water holding capacity is ability of meat to retain its water or added water during application of external forces such as cutting ,heating or processing .Many of physical properties of meat including color, texture and firmness of raw meat ,juiciness and tenderness of cooked meat are particularly depend on water holding capacity (Judge *et al*., 1990).The water holding capacity of meat is of obvious importance .This particularly seen in comminuted meat such as burger where the structure of tissue has been destroyed and longer able to the present the release of fluid from protein (Lawrie,1991).

2.3.5 Juiciness

The principle of juiciness in meat ,as detected by the marbling that are present also saves enhance juiciness during the cooking process when the melted fat apparently become translated along the bands of perimysial connective tissue. This uniform distribution of lipids throughout the muscle may act as barrier to moisture cooking (Judge *et al*, 1990). Good quality juicier, the difference being at least partly attribution of lipids to higher content of intramuscular fat in the former. Also, there are some suggestions that juiciness reaches a minimum where the pH level of meat is about six. This possibly reflects the greater ability of muscle protein to bind water pH level (Mohammed, 2005). The degree of shrinking on cooking directly correlate with loss of juiciness and the palatability.

2.4 Chemical composition of meat

In general, meat composed of water, fat, protein, minerals and small proportion of carbohydrate. The most voluble component from the nutritional and processing point of view is protein (FAO, 2007).

2.4.1 Meat protein

Muscle proteins are often classified into three group based on their solubility: sarcoplasmic protein, myofibrillar proteins and stroma proteins. The sarcoplasmic protein which include myoglobin and other heme pigments are water soluble. Myoglobin is very important for meat color but plays only a minor role in meat functionality (Smith,2001). Myosin plays an important role in fat emulsification and water holding capacity of products like sausage(Xiong, 2009). The myofibrillar protein which are soluble protein (1% salt concentration) mainly consist of actin and myosin (Barbut, 1995). Collagen is converted to gelatin when cooked at high temperatures and so a high level of collagen can be detrimental to meat emulsion stability because of protein matrix degradation (Ladwig *et al*., 1989). Raw red muscle meat contains around 20-25g protein/100g. the protein is highly digestible, around 94% compared to the digestibility of 78% in beans and 86% in whole wheat (Bhulla, 1999).

2.4.2 The fat of the meat

Lipids in meat are of three discrete types subcutaneous, inter muscular, intra muscular. Fatty tissue of carcasses usually contains triacylglycerol fat. The amount that accumulates in animals depends on a number of factor, including genetic predisposition, age, gender and sex status, level of nutrition and exercise (Alan and Jane, 1995).

2.4.3 The water of meat

Water is quantitatively the most important component of meat comprising up to 75% of weight. The water content is inversely related to fat content but is un affected by protein content exception young animals. The majority of water is bond between the thick and myofibrils binding is looser than in living animals and some loss, as drips, from freshly cut surfaces is inevitable if undesirable (Alan and Jane, 1995).

2.4.4 pH

After harvest, the loss of circulatory competency requires that the muscle tissue shifts to anaerobic metabolism this results in accumulation of metabolic by-products, especially lactic acid, resulting in pH decline from about 6.8t5.7. The pH is usually determine fresh rather than cooked meat sample (Leo and Nollet, 2012).

2.4.5 Minerals

The mineral contents of meat include calcium, phosphorus, sodium, potassium, chlorine, magnesium with the level of each of these minerals above 0.1% and trace elements such as iron, copper, zinc and many other. Blood , liver, kidney, other red organs and to a lesser extent lean meat in particular beef are good sources of iron. Iron intake is important to combat a anemia which particularly in developing countries is still widespread amongst children and pregnant women. Iron in meat has a higher bio-availability better desorption and metabolism than iron plant products (FAO, 2007).

2.5 Microorganisms in meat

The potential for growth and for toxin production of residue population in finished products depends on the type of organism present and their ability to grow to level of concern under the storage condition applied during the product shelf life (Javadi *et al*., 2011).

Meat products may be contaminated with micro organisms from handler who carry pathogenic micro organism during processes of manufacturing, packaging and marketing. Improper cooking, refrigeration or storage may lead to meat borne illness (Zurea and Rincons, 1988). It is important to keep micro organism at low for reasons of aesthetic, public health products shelf life (Jay,1996).

Meat being a good material for bacterial growth, its quality depend on initial bacterial contamination .This contamination causes meat

deterioration ,lower quality ,and some time illness may cause by their toxins.

In the Sudan there are studies on general of aerobic bacteria included in fresh meat *Bacillus SPP , Staphyloc ocus SPP .,Pseudomonas SPP acintrto bacteria SPP.,E.coli ,Proteus SPP.,Spp, ,Salmonella SPP*. (Hussein,1987; Mohammed, 2000).

Mold species include *Coladosporium ,Sporotrium, Geotrichum ,Penocilluim and mucor* while yeast species include *Candida* SPP ,*Cryptococcus* SPP and *Rhodotorula* SPP (Garica-Lopez *et al*., 1998).

2.6 Deterioration of meat quality

Number of methods are employed through the meat industry to retard deterioration changes and extend length of acceptability period .This depends in mainly on preservative method and inherent properties of specific meat items. The postmortem changes associated with conversion to meat subsequent storage and hand linking are caused by micro organism(bacteria ,mold , and yeast),insect ,indigenous enzymes naturally present in meat ,oxgenous enzymes and physical effects (freezer burn, drop, light ,fading and discoloration) .The microbial sources include equipment ,clothing and hands of personnel, air, water, and doors(Judge *el al*., 1990).

2.6.1 Chemical deterioration changes

Oxidative rancidity is described by the presence of two molecular weight aldhydes, acids and ketone that formed during oxidation and decomposition of fatty acids molecules .The rate of auto –oxidation is enhanced by proxidants such sodium chloride ,some metal ions (e.g Coppr,ions), heat ultraviolet light ,low pH, and numerous other substance oragents ,develop meat of rancidity is related by anidance of roxidanncoe of proxidants by storing meat in refrigerated darkness and minimizing amount of air in container (Judge *et al*., 1990).

2.6.2 Physical deterioration changes

Dehydration is the loss of moisture from the surface that concentrated pigments and due to loss of intracellular water, reduces light reaction ,the meat surface during storage produce dried appeal and acceptability .An excessive loss of moisture from meat surface result in freezer burn which is characterized by cock –like texture and gray to tan color (Judge *et al*.,1990).The loss of weight that results is due to losses of meat moisture during refrigerated storage and known as it is shrinkage .physical changes accompanying shrinkage during prolonged refrigeration storage include surface dehydration and discoloration (Judge *et al*., 1990).Off flavors may occur when meat is storaged in the presence of aromatic compounds such as apples or onions (Judge *et al* ., 1990)

2.7 Preservation of meat

Meat preservation became necessary for transporting meat for long distances without spilling of texture, color, and nutritional value after the development and rapid growth of super markets (Nychas *et al*. 2008).The aims of preservation methods are (a)to inhibit the microbial spoilage and (b)to minimize the oxidation and enzymatic spoilage. Traditional method of meat preservation such as drying smoking, brining, fermentation, refrigeration and canning have been replaced by new preservation techniques such as chemical, bio preservative and non thermal techniques (Zhou *et al*., 2010).Current preservation methods are broadly categorized into three methods (a)controlling temperature (b)controlling water activity (c)use of chemical or bio preservatives (Zhou *et al*. 2010).A combination of these techniques can be used to diminish the process of spoilage(Bagamboula *et al*., 2004).

2.7.1 Low temperature methods

The basic aim of techniques is to slow or limit the spoilage rate as temperature below the microbial growth (Cassen ,1994).Low temperature

methods of storage are used in three level(a)chilling (b)freezing and (c) super chilling .Al these level to inhibit or completely stop bacterial growth (Zhou *et al*., 2010). However, the growth of psychrophilic by all level of refrigeration (Neumeyer *et al*.,1997).And both enzymatic changes will continue at a much slower rate (Barket *et al*.2004).

2.7.1.1 Chilling

Chilling is employed at slaughtering plants immediately after slaughtering and during transport and storage .Chilling is credited for meat hygiene, safety, shelf ,appearance and nutrional quality (Cassen,1994 ; Zhou *et al*.,2010).It is necessary to reduce the temperature of carcass immediately after evisceration to 40 C within 4h or slaughtering (USDC,1995).

2.7.1.2 Freezing

Freezing is an excellent method of keeping the original characteristic of fresh meat .Meat contains about 50-75%by weight water, depending on the species ,and the process of freezing converts most of the water into ice (Heniz and Hautzinger,2007).The preservation capacity of frozen meat is limited because the physical, chemical, or bio chemical reactions that take place in animal tissues after slaughtering do not stop absolutely after cold treatment (Rosmini *et al*., 2004).

2.7.2 Controlled water activity

Microbiological safety of food is directly influenced by the water activity .The term water activity refers to water which is not bound to food molecules and can support the growth of microorganisms. It represents the ratio of the water vapour pressure of the food to the water vapour pressure of pure water under the same conditions (Ghaly *et al* ., 2010).

Water activity in meat products is equivalent to the relative humidity of air in equilibrium with the product (Comaposada *et al* ., 2000)Water activity in meat is control by drying, refrigeration, adding chemicals or a

combination of these methods. Sodium chloride and sugar have been used to control water activity as free water binds up in their presence which results in an osmotic imbalance and finally inhibition of cell growth (Ray, 2004).

2.7.3 Chemical methods for preservation

Traditional methods for preservation of meat by salting and picking are well accepted procedures. Other chemicals have been used as food additives for preservation of meat but every country has drawn its rules and regulations and established limits for the purpose of prevention of harmful effects to human (Cassens, 1994).

Antimicrobial compounds added during processing should not be used as a substitute for poor processing conditions or to cover up an already spoiled product (Ray, 2004). They offer a good protection for meat in combination with refrigeration (Cassen, 1994). Common antimicrobial compounds include: chlorides, nitrites, sulfides and organic acids (Chipley, 2005; Ray, 2004; Archer, 2002).

2.8 Meat products

The assessment of quality in manufactured meat products is still largely subjective and based on the judge ment of either trained taste panels or individual .There are, however, some aspects of quality, in the finished product, and during the actual may be controlled by objective methods of assessment .These methods are being increasingly used and supplemented as newer ones become available. They include the estimation of fat, protein, moisture and dioxide by the accepted and well tried analytical procedures.

In generally terms ,quality control is necessary in order to a ensure that the product composition is uniform does not fall below established stander, b comply with legislation ,and c maintain quality at level to

trances which are acceptable while the cost of production (Herschdoefer, 1968).

2.8.1 Classification of meat products

There is a great effort in developing world to increase the satisfaction and to take care of the health of the consumer (FAO, 2000).There are numerous type of meat products and processes used to manufacture products (Borchert and cassens,1998).These types are frank further, sausages, mortadella, pastrami and minced meat.

2.8.2 Sausages as meat product

Sausages are cylindrically shaped mixture s of various sizes consisting of protein, fat, water, salt, color and flavors. The texture of sausage depends on the type of meat that is mixed and comminuted together with ice, salt, pieces, flavoring, curing agent .and selected meat trimming s to form a sausages emulsion .Exposure to heat then stabilizer or solidifies the emulsion (Joseph, 1960).

The protein content and water content in sausage are 7.5-12.7% respectively in beef sausage (Pearson 1999). Nonmeat binders less than 10% total meat not less than 55% nitrite 200 ppm (SSMO, 2007).

2.8.2.1 Ingredients of beef sausage making

Good sausage cannot be made from unsatisfactory raw material. Formulation for sausages compromises between the desired quality of product and its cost (Isidor and sedky , 1972).

2.8.2.2 Beef

Beef quality is ,in general determined by number of interdependent extrinsic factors such as breed, condition, sexexercise, pre-slaughter, treatment. slaughtering conditions and finally, method or handling, chilling and degree of aging (Isidor *et al*.1972).

Although all type of beef are suitable for sausage making one of the most difficult problems for sausage maker is choose satisfactory beef at

reasonable price, many sausage products can ruined if the sausage maker uses the wrong grade or wrong cut or improper processing, well selected and well prepared beef is essential if the sausage –maker and consumer are to be satisfied (Isidor *et al.*1972).

2.8.2.3 Fat

Beef fat has particular properties. It easily becomes sour or rancid if improperly handled or if kept under condition. It's farprefrable to use the beef fat fresh as possible without freezing or storing if, the best fat for making sausage is from zebu hump and kidney fat .The white fat of younger animals is preferred for fresh frankfurter type sausage while the white making .The amount of added fat depends on the type of sausage and on fat content of meat used manufacturing .In general the total content of fatty should not exceed 25% (Isidor *et at.*1972)

Although beef fat is valuable sausage materials is requires special care, and precaution must be taken against undesirable changes of fat .

2.8.2.4 Sausage casings

After the meat has chopped, it is formed into patties or placed into a container.

The containers such as pans for loaves and casings for links, will hold their shape during cooking .Traditional sausage casing are made from parts of alimentary canal of various animals .These natural casings are largely made up of collagen which has unique characteristic of variation permeability moisture and heat make casings more porous and tend to soften them .Sausage made from natural casings have a"snap" when bitten into that is considered adesirable sensory characteristic (Tronsky, 2003).

The high costs of animal casing couple with a slower rate of stuffing contribute to a higher cost for product in this type of casing (Pearson and Gillet,1999).

Cellulose casing include those from cotton bags or wood pulp (Pearson and Gillett,(1999).

2.8.2.5 Seasoning

Seasoning are any ingredients which improve flavor and include spices, herbs , vegetable ,nuts ,and other substance (monosodium glutamate) etc, while enhancing flavor, they stimulate the secretion of digestive juices (Isidor *et al* .1972)

Some spices have a limited preservative effect and some contribute to the bacterial contamination of sausage . The taste of spice generally depends on the flavor of the oil contains , spices are usually ground before adding to meat (Isidor *et al*. 1972).

CAHPTER THREE

MATERIALS AND METHODS

3.1 Materials

Fresh sausage of beef meat was obtained from three different factories in different locations in Khartoum State (Factory A factory B and local market C).Fresh sausage was stored frozen at -18°C.

3.2 Chemical analysis

3.2.1 Moisture content

The moisture content was determined according to standard methods of association of official analytical chemists (AOAC, 2003).

Principle

The moisture content is a weighed sample removed by heating the sample in an oven under atmospheric pressure at 105 ±1°C. Then the difference in weight before and after drying is calculated as a percentage from the initial weight.

Procedure

A sample of 5g ±1mg was weighed into a pre-dried and tarred dish .Then the sample was placed into an oven (NO.03-822, fn400, Turkey) at 105±1°C until a constant weight was obtained .After that the cover sample was transferred to desiccators and cool to room temperature before reweighing. Triplicate results were obtained for each sample and the mean value was reported.

Calculation

$$Moisture\ content\ \% = \frac{(\mathrm{m2} - \mathrm{m3})}{(m2 - m1)} x100$$

Where:

M1= weight dish+ cover

M2= weight of dish + cover +sample before drying

M3= weight of dish + cover+ sample after drying

The dry matter (DM) was percentage was calculated by subtracting the percentage of moisture from 100%.

3.2.2 Fat content

The crude fat in the product was determined according to the standard method of AOAC (2003)

Principle

The methods determines the substance which are soluble in Hexane (40-60°C) and extractable under specific of Soxhlte Extraction method. The dried Hexane extract is weighted as percentage of dry mater as crude fat

Procedure

A sample of 5g+1mg was weighted into an extraction thimbles (30-100 mm)and covered with cotton that previously extracted with hexane. Then ,the sample and a pre-dried and weighted in Erelenmeyer flask containing about 150 ml hexane (No1622,BDH,England) were attached to the extraction unit (Electrothermal ,England) and the temperature was adjusted to produce about 150 to 200 drops of the condensed solvent per minute for 16 hours .At the end of the distillation period, the flask with was disconnected from the unit and the solvent was redistilled .Later ,the flask with the remaining crude hexane was put in an oven at 105°Cfor 3 hours ,cooled to room temperature in a desiccators ,reweighed and the dried extract was register as crude fat (% DM) according to the following formula :

$$\text{Fat content (\%)} = \frac{(w1 - w2) \times 100}{W3}$$

Where:

W1= weight of flask and ether extract

W2 = weight of empty flask

W3= initial weight of sample

3.2.3 Crude protein

The crude protein was determined in all samples by micro –kjeldahl method using a copper sulphate and sodium sulphate catalyst according to the official method of the AOAC (2003).

Principle

The method consists of sample oxidation and of nitrogen to ammonia, which reacts with the excess amount of sulphuric acid forming ammonium sulphate. The solution is made alkaline and the ammonia is distilled into a standard solution of boric acid (2%) to form the ammonia –boric acid complex , which is titrated against a standard solution of HCL(0.1). Accordingly, the crude protein content is calculated by multiplying thee total N% by 6.25 as a conversion factor for protein.

Procedure

2 gm ± 1mg sample was accurately weighed and transferred together with 2-3 glass pellets ,kjeldahl catalysit (No33064,BDH,Germany) and 30 ml concentrated sulphuric acid into kjeldahi digestion flask .After that ,the flask was placed into a a kjeldahl unit (Tecator, Sweden) for about 3 hours, until a colorless digest was obtained. Following, the flask was left to cool to room temperature .The distillation of ammonia was carried out in 30ml boric acid (2%)by using 40ml distilled water and 60 ml sodium hydroxide solution (%).Finally,the distillate was titrated with standard solution of 0.1HCL in the presence of 2-3 drops of indicator

(Bromocreasol green and methyl red) until a brown reddish colour was observed.

Calculation

$$\text{Crude protein\%} = \frac{(TV \times N \times 14.00 \times F) \times 100\%}{1000 \times \text{sample weight (g)}}$$

Where :

TV= actual volume of HCL used for sample

N= normality of HCL

F= protein conversion factor =6.25

3.2.4 Ash content

The standard analytical method of AOAC (2003) was used for determination of ash content in the samples.

Principle

The inorganic materials which are varying in concentration and composition are customary determined as a residue after being ignited at a specified heat degree.

Procedure

A sample of 2gm ±1mg was weighed into a pre-heated, cooled weighted and tarred porcelain crucible and placed into a muffle furnace (Carbolite ,Sheffeild ,England) at 50 to 600°C until a constant weighted and a white gray ash was obtained. The crucible was transferred to a descanter then allowed to cool to room temperature and weighed after that the ash content was calculated as a percentage based on the initial weight of sample.

Calculation

$$\text{Ash \%} = \frac{(\text{Wt of crucible +ash}) - (\text{Wt of empty crucible})}{\text{Initial weight (Wt)} \times 100}$$

3.2.5 pH measurement

Ten gram of the sample were placed in gar in blender and 100 ml water were added .The mixture was blended at high speed for 1 min. The pH of the mixture was measured by using a pre calibrated PH meter model (HI 8521microprocessor bench PH/MV/°C meter).This has been calibrated with two standard buffers (6.8 and 4.0).

3.3 Microbiological analysis

3.3.1 Preparation of serial dilutions

Ten gram of each sample were weighed aseptically and homogenized in 90 ml of sterile diluents (0.1%peptone water to give (10^{-1}) dilution .Aseptically 1ml from the dilution (10^{-1}) was transferred to a tube contain 9ml sterile diluents .This makes a dilution of (10^{-2}) then the same way the preparation of serial dilutions was continued up to the (10^{-6}) (Harrigan,1998).

3.3.2 Total bacterial count

Total viable count of bacteria was carried out by using the pour plate count method as describe by (Harrigan ,1998). One ml of every dilution was transferred aseptically into sterile Petri dish and to each plate 15 ml of sterile melted plate count agar were added .The inoculums was mixed with medium and allowed to solidify. The plates were then incubate at 37°C for 48 hours . A colony counter machine was used to count the viable bacteria and the result s were presented as cfu/g Test for coli form number (MPN) technique (Harrigan ,1998).

3.3.3 Presumptive test for coliforms

One ml of each of three first dilution (10^{-1}, 10^{-2}. and10^{-3}) was inoculated in triplicates of 9ml of Mac Conkey broth in test tubes with Duraham tubes. Then the tubes were incubated at 37°C for 48 hour .The production of acid together with sufficient gas to fill the concave of the Duraham tubes is recorded as positive presumptive test(Harrigan,1998).

3.3.4 Confirmed test for coliforms

A fermentation tube of brilliant green 2% broth was inoculated by using sterile loop from every showing positive result in presumptive test. Then the tube were incubated at 37°C for 48 hours the most probable number of total coliform (MPN) was recorded by using the table of the most probable number from the combination of positive and negatives tube Harrigan,1998).

3.3.5 Test for *E.coli*

A fermentation tube of E .Coli was inoculated from every tube showing positive result in the presumptive test .the tubes were incubated in water bath at 44.5°C for 4 hours. The presence of *E.Coli* was recorded. For further confirmation of *E. Coli* test a plate of Eosin Methylene Blue A gar (EMB)agar was aseptically inoculated by streaking from a tube of E .coli broth showing positive result .The plates were incubated at 37°C for 48 hours .Colonies with metallic green sheen showing a positive result (Harrigan,1998).

3.3.6 Detection of *salmonella*

Twenty five grams of sample were weighed aseptically and mixed well with 250 ml sterile nutrient broth .This were incubated at 37°C for 24 hours .Then 10 ml were drawn aseptically and added to 100 ml of selenite cystine broth The broth was incubated at 37°C for 24 hours. Then with a loop full streaking was done on solidified bismuth sulphite a gar in plates were then incubated at 37°C for 72 hours. Black metallic sheen colonies indicated the presence of *Salmonella italies*. A confirmatory test was carried out by taking a discrete black sheen colony and sub culturing it in triple sugar iron agar tubes. Production of a black colour at bottom of the tube confirmed the presence of salmonella (Hrrigan,1998).

3.3.7 *Staphylococcus aureus*

Amount of 0.1 ml from every dilution was transferred on to surface of each sterile well solidified Baird parker agar medium in plates and spread all over plates using sterile bent glass rod .Then incubated for 24-36 hours at 37^0 C and the plates were examined for *Staphylococcus aureus* which appeared as black shine convex colonies surround by a clear zone of 2-5m in width (Harrigan,1998).

3.4 Sensory evaluation

Sensory evaluation was done as described by Ranganna (2001). Using the hedonic scoring test method. In this method 20 trained panelists from the Food Science and Technology Dept, College of Agricultural Studies, Sudan University of Science and Technology were asked to evaluate the products with regard of their color, flavor, taste, overall acceptability, using the following hedonic scale:

1= excellent, 2= very good, 3= good, 4= acceptable, 5= unacceptable.

3.5 Statistical analysis

The result were Subjected to Statistical Analysis (SAS) by using two factors completely randomized design. The mean value were also tested and separated by using Duncan's Multiple Range Test (DMRT) as described by Montgonery and Douglas (2001).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Changes in chemical composition of beef sausage

4.1.1 Moisture content

As shown in (Table 1) moisture content of sausage samples slightly increased with increased storage period, this may be due to efficiency of storage. These results disagree with Winger and Fannema (1976) who stated that moisture loss increased with increasing storage period. Also disagrees with Dino (2004) who stated that there was slight decrease in moisture content during frozen storage.

4.1.2 Oil content

The fat content decreased during storage period (Table2), this decrease in frozen stored sausage, may be due to losses of triglycerides. Similar result was obtained by Dino (2004) who found that the fat content of meat decreased during frozen storage. This result is different from with those obtained by Desmond and Troy (2001) who found that sample treated with citric acid had higher oil content when compared with control samples.

4.1.3 Protein content

The protein of beef sausage (Table 3), decreased with increased storage period which may be due slow and little analysis of protein during the frozen storage. This result agrees with that of Lyon (1984) who stated that when freezing cured meat at -34° C, percentage of protein decreased from 19.7%to 18.3%. AL-Aswad (2000) also stated that frozen storage resulted in a decrease in protein of meat. It also agrees with result obtained by Al-Hajo (2008) who stated that protein of chicken meat decreased with increasing storage time.

4.1.4 Ash content

In (Table 4) the ash percentage increased with increased storage time ,because salt ,sugar and spices diffuse in the sausage .This finding is comparable to that of Lyon (1984) and Al-Hajo (2008) who stated that the ash percentage increased during frozen storage in cured poultry meat stored at -18°C for 0,15 and 30 days .

4.1.5 pH values

As shown in (Table 5) increasing the storage period resulted in fluctuation of pH value. This may be due the accumulation of bacterial metabolites and domination of protein. Parrish *et al*. (1969) reported that frozen storage resulted in an increase of meat pH. Also Lester (1996) stated that pH increased during frozen storage. Naveena *et al*. (2006) stated that the pH of chicken patties increased during storage. Banani *et al*.(2006) stated that with the progress of storage time pH of meet samples seem to increase . Muhasin (2009) reported that increased storage time resulted in an increase of sausage pH. Al-Hajo (2009) stated that processed meat stored at –18°C for 30 days resulted in an increased pH.

4.2 Changes in physical properties of beef sausage

4.2.1 Effect of storage period on weight of beef sausage

Table (6) shows significant difference ($P \leq 0.05$) in sample A,B and C with high scores in sample A at zero time of storage period and low scores in sample C at 4 month storage time .

4.2.2 Effect of storage period on length (cm) of beef sausage

Table (7) shows significant ($P \geq 0.05$) difference in sample A,B and C with high scores in sample A at 6 month and low scores in sample B and C at same storage month.

Table (1): Effect of storage period on moisture content (%) of beef sausage

Storage period (month)	Beef sausage samples		
	Factory A	**Factory B**	**Factory C**
0	57.32^{abc} ±0.05	56.32^{bc} ±1.30	58.09^{abc} ±1.64
2	58.01^{abc} ±0.07	59.01^{a} ±0.33	58.72^{ab} ±0.62
4	58.00^{abc} ±1.00	58.28^{abc} ±0.57	56.00^{c} ±3.46
6	57.72^{abc} ±0.55	56.72^{abc} ±0.76	58.255^{abc} ±0.28
$Lsd_{0.05}$	2.128^{*}		
SE±	0.7292		

Values are mean±SD.

Mean values sharing the same super script letter (s) do not differ significantly (P≥0.05) according to DMRT.

Table (2): Effect of storage period on oil content (%) of beef sausage

Storage period (month)	Beef sausage samples		
	Factory A	**Factory B**	**Factory C**
0	26.57^{cde} ±0.18	27.64^{abc} ±2.30	29.54^{a} ±0.56
2	25.92^{cde} ±0.05	27.31^{bcd} ±1.70	28.82^{ab} ±1.02
4	22.25^{f} ±1.93	25.18^{de} ±0.15	28.00^{abc} ±1.00
6	22.75^{f} ±1.10	24.76^{e} ±0.42	26.78^{bcde} ±0.70
$Lsd_{0.05}$	0.1998^{*}		
SE±	0.6846		

Values are mean±SD.

Mean values sharing the same super script letter (s) do not differ significantly (P≥0.05) according to DMRT.

Table (3): Effect of storage period on protein content (%) of beef

Storage period (month)	Beef sausage samples		
	Factory A	**Factory B**	**Factory C**
0	12.95^{abc} ±0.59	12.56^{cd} ±0.41	13.63^{a} ±0.16
2	12.33^{cde} ±0.34	11.73^{e} ±0.12	13.38^{ab} ±0.33
4	11.92^{de} ±0.11	9.75^{f} ±0.57	12.80^{bc} ±0.31
6	11.67^{e} ±0.78	9.60^{f} ±0.11	12.73^{bc} ±0.32
$Lsd_{0.05}$	0.6762*		
SE±	0.2317		

Values are mean±SD.

Mean values sharing the same super script letter (s) do not differ significantly (P≥0.05) according to DMRT.

Table (4): Effect of storage period on ash content (%) of beef sausage

Storage period (month)	Beef sausage samples		
	Factory A	**Factory B**	**Factory C**
0	5.22^{cde} ±0.21	4.26^{e} ±0.05	4.11^{e} ±0.11
2	5.09^{de} ±0.18	4.45^{e} ±1.42	4.03^{e} ±0.07
4	6.00^{cd} ±0.30	6.37^{bc} ±0.28	5.27^{cde} ±0.45
6	7.19^{b} ±1.08	9.06^{a} ±0.09	4.89^{de} ±0.84
$Lsd_{0.05}$	1.097*		
SE±	0.3759		

Values are mean±SD.

Mean values sharing the same super script letter (s) do not differ significantly (P≥0.05) according to DMRT.

Table (5): Effect of storage period on pH-value of beef sausage

Storage period (month)	Beef sausage samples		
	Factory A	**Factory B**	**Factory C**
0	6.27^{cde} ±0.03	6.38^{c} ±0.08	6.34^{cd} ±0.03
2	6.10^{de} ±0.09	6.38^{c} ±0.07	6.02^{e} ±0.02
4	6.74^{b} ±0.21	6.99^{a} ±0.02	6.45^{c} ±0.039
6	6.37^{c} ±0.19	6.42^{c} ±0.05	6.26^{cde} ±0.02
$Lsd_{0.05}$	0.2442*		
SE±	0.08367		

Values are mean±SD.

Mean values sharing the same super script letter (s) do not differ significantly (P≥0.05) according to DMRT.

Table (6): Effect of storage period on weight (g) of beef sausage

Storage period (month)	Beef sausage samples		
	Factory A	Factory B	Factory C
	Scores		
0	31.79^{a}	23.40^{c}	19.00^{e}
2	29.10^{b}	22.80^{cd}	18.33^{e}
4	29.00^{b}	22.60^{cd}	17.20^{f}
6	29.00^{b}	22.40^{d}	17.38^{f}
$Lsd_{0.05}$	0.8510^{*}		
SE±	0.2915		

Values are mean±SD.

Mean values sharing the same super script letter (s) do not differ significantly (P≥0.05) according to DMRT.

Table (7): Effect of storage period on lengths (cm) of beef sausage

Storage period (month)	Beef sausage samples		
	Factory A	Factory B	Factory C
	Scores		
0	10.00[a]	10.00[a]	10.00[a]
2	10.00[a]	10.00[a]	10.00[a]
4	10.00[a]	10.00[a]	9.50[a]
6	10.50[a]	9.50[a]	9.50[a]
$Lsd_{0.05}$	1.379^{NS}		
SE±	0.4726		

Values are mean±SD.

Mean values sharing the same super script letter (s) do not differ significantly (P≥0.05) according to DMRT.

4.3 Changes in microbial properties of beef sausage

4.3.1 Effect of storage period on total viable count of fresh beef sausage

Table (8) shows that total count does not differ significantly (P≥0.05) in sample A and C during storage period and significant of in count of bacteria was observed in sample B between values after 2 month and other values. Similar results were reported by (Kumar *et al.*,2007) in chicken meat.

4.3.2 Effect of storage period on yeasts and moulds count of beef sausage

As shown in Table (9) count of yeasts and moulds do not differ significantly (P≥0.05) in sample C but significant difference (P≥0.05) was observed between zero time and other value in sample B. On the other hand no yeasts and moulds were recorded in all storage time in sample A .The count increased with advancement of storage days this due to post processing contamination and handling, (Das *et al.*, 2013) also reported similar results in chicken nuggets and chicken snakes.

4.3.3 Effect of storage period on *Staphylococcus aureus* count of beef sausage

The Table No.(10) shows lower content of *Staphylococcus aureus* (P≥0.05) in sample A but higher content in sample C with different significance (P≥0.05) at various storage periods within sample A,B and C . Also Molan (1992) agrees with this result.

4.3.4 Effect of storage period on total *Coliforms* of beef sausage

The Table No. (11) Shows the effect of storage period on total *Coliforms* of beef sausage with no different significance (P≥0.05) in sample A and

B. The highest content of total *Coliforms* with different significance (P≥0.05) was found in sample C. Also Molan (1992) reported highest value, and with increased storage period the total *Coliforms* decreased significantly

4.3.5 Effect of storage period on *E.coli* of beef sausage

The Table No.(12) shows no *E.coli* throw 6 month of storage period in sample A. Sample B showed significant difference in *E.coli* count, while sample C which recorded the highest number of *E.coli* did not show significant difference along the period of storage .These results are in agreement with (Dorsa, *et al.,* 1998).

4.3.6 Effect of storage period on *Salmonella* of beef sausage

Table (13) shows detection of *Salmonella* of beef sausage where it is positive in sample C all through the storage period. On the other hand *Salmonella* was not detected in sample A and B in all the storage periods.

4.4 Changes in sensorial properties of beef sausage

4.4.1 Effect of storage period on color

Table (14) shows no significant (P≥0.05) difference in sample A and C but significant (P≥0.05) difference was observed in sample B with lower score at zero month.

4.4.2 Effect of storage period on taste

Table (15) shows significant (P≥0.05) differences in sample A ,B and C during storage period with low scores in sample B at zero storage period and high scores in sample C at the same storage period.

4.4.3Effect of storage period on flavor

Table (16) shows low scores in sample B and high scores in sample C

with different significant (P≥0.05) differences throw the time of storage

.4.4.4 Effect of storage period on general acceptability

Table No.(17) shows no significant (P≥0.05) difference in sample A, but significant difference was observed in sample B and C with low scores in sample B at zero time of storage and high scores in sample C at 2 month of storage time.

Table (8): Effect of storage period on total viable count (cfu/g) of beef sausage

Storage period (month)	Beef sausage samples		
	Factory A	**Factory B**	**Factory C**
0	4.48^{c} ±0.21	5.79^{b} ±0.50	6.62^{a} ±0.16
2	4.52^{c} ±0.22	6.48^{a} ±0.52	6.64^{a} ±0.16
4	4.53^{c} ±0.22	5.68^{b} ±0.52	6.68^{a} ±0.16
6	4.55^{c} ±0.22	5.86^{b} ±0.43	6.73^{a} ±0.47
$Lsd_{0.05}$	0.4459*		
SE±	0.1528		

Values are mean±SD.

Mean values sharing the same super script letter (s) do not differ significantly (P≥0.05) according to DMRT.

Table (9): Effect of storage period on yeast and moulds count (cfu/g) of beef sausage

Storage period (month)	Beef sausage samples		
	Factory A	**Factory B**	**Factory C**
0	0.00^{d} ±0.00	2.15^{c} ±0.09	3.78^{a} ±0.05
2	0.00^{d} ±0.00	2.82^{b} ±0.09	3.85^{a} ±0.06
4	0.00^{d} ±0.00	2.82^{b} ±0.09	3.83^{a} ±0.06
6	0.00^{d} ±0.00	2.86^{b} ±0.09	3.88^{a} ±0.05
$Lsd_{0.05}$	0.5487^{*}		
SE±	0.188		

Values are mean±SD.

Mean values sharing the same super script letter (s) do not differ significantly (P≥0.05) according to DMRT.

Table (10): Effect of storage period on *Staphylococcus aureus* count (cfu/g) of beef sausage

Storage period (month)	Beef sausage samples		
	Factory A	**Factory B**	**Factory C**
0	2.69^{e} ±0.05	2.90^{cd} ±0.07	3.81^{a} ±0.07
2	2.78^{de} ±0.06	2.93^{c} ±0.07	3.84^{a} ±0.08
4	2.78^{de} ±0.06	3.59^{b} ±0.07	3.88^{a} ±0.08
6	2.84^{cd} ±0.06	3.63^{b} ±0.03	3.91^{ab} ±0.06
$Lsd_{0.05}$	0.1305*		
SE±	0.04472		

Values are mean±SD.

Mean values sharing the same super script letter (s) do not differ significantly (P≥0.05) according to DMRT.

Table (11): Effect of storage period on total *coli forms* (MPN/g)of beef sausage

Storage period (month)	Beef sausage samples		
	Factory A	**Factory B**	**Factory C**
0	11.00^{d} ±2.00	18.33^{c} ±3.05	37.67^{b} ±3.78
2	12.00^{d} ±1.00	19.33^{c} ±3.05	39.33^{b} ±3.51
4	12.00^{d} ±1.00	19.33^{c} ±3.05	41.33^{ab} ±2.52
6	13.00^{d} ±1.00	21.67^{c} ±2.52	46.33^{a} ±5.86
$Lsd_{0.05}$	5.071^{**}		
SE±	1.737		

Values are mean±SD.

Mean values sharing the same super script letter (s) do not differ significantly (P≥0.05) according to DMRT.

Table (12): Effect of storage period on *E.coli* (cfu/g)of beef sausage

Storage period (month)	Beef sausage samples		
	Factory A	Factory B	Factory C
0	0.00^{d} ±0.00	5.33^{c} ±2.08	12.67^{a} ±2.08
2	0.00^{d} ±0.00	5.33^{c} ±2.08	13.67^{a} ±2.08
4	0.00^{d} ±0.00	6.67^{bc} ±0.58	15.33^{a} ±3.21
6	0.00^{d} ±0.00	9.00^{b} ±2.00	15.67^{a} ±3.05
$Lsd_{0.05}$	3.127^{**}		
SE±	1.071		

Values are mean±SD.

Mean values sharing the same super script letter (s) do not differ significantly (P≥0.05) according to DMRT.

Table (13): Effect of storage period on *Salmonella* of beef sausage

Storage period (month)	Beef sausage samples		
	Factory A	Factory B	Factory C
0	-ve	-ve	+ve
2	-ve	-ve	+ve
4	-ve	-ve	+ve
6	-ve	-ve	+ve

Table (14): Effect of storage period on color of beef sausage

Storage period (month)	Beef sausage samples		
	Factory A	Factory B	Factory C
	Scores		
0	3.08^{a} ±0.27	1.58^{b} ±0.13	3.00^{a} ±0.25
2	2.67^{a} ±0.34	2.50^{ab} ±0.11	3.25^{a} ±0.29
4	3.08^{a} ±0.27	1.67^{b} ±0.15	2.92^{a} ±0.16
6	2.67^{a} ±0.34	2.50^{ab} ±0.11	3.25^{a} ±0.29
$Lsd_{0.05}$	0.8894*		
SE±	0.3179		

Values are mean±SD.

Mean values sharing the same super script letter (s) do not differ significantly (P≥0.05) according to DMRT.

Table (15): Effect of storage period on taste of beef sausage

Storage period (month)	Beef sausage samples		
	Factory A	Factory B	Factory C
	Scores		
0	3.17^{abc} ±0.24	2.00^{d} ±0.13	3.58^{a} ±0.29
2	2.75^{abcd} ±0.17	2.58^{bcd} ±0.14	3.33^{ab} ±0.27
4	2.58^{bcd} ±0.15	2.33^{cd} ±0.12	2.92^{abcd} ±0.23
6	2.75^{abcd} ±0.17	2.58^{bcd} ±0.14	3.50^{ab} ±0.28
$Lsd_{0.05}$	0.835*		
SE±	0.2985		

Values are mean±SD.

Mean values sharing the same super script letter (s) do not differ significantly (P≥0.05) according to DMRT.

Table (16): Effect of storage period of flavor of beef sausage

Storage period (month)	Beef sausage samples		
	Factory A	Factory B	Factory C
	Scores		
0	3.08^{abc} ±0.27	2.08^{c} ±0.10	3.67^{a} ±0.28
2	3.25^{ab} ±0.31	2.67^{abc} ±0.18	3.25^{ab} ±0.24
4	3.00^{abc} ±0.22	2.75^{abc} ±0.19	2.83^{abc} ±0.19
6	2.58^{bc} ±0.15	2.83^{abc} ±0.25	3.50^{b} ±0.20
$Lsd_{0.05}$	0.8757*		
SE±	0.313		

Values are mean±SD.

Mean values sharing the same super script letter (s) do not differ significantly (P≥0.05) according to DMRT.

Table (17): Effect of storage period on general acceptability of beef sausage

Storage period (month)	Beef sausage samples		
	Factory A	Factory B	Factory C
	Scores		
0	3.33^{ab} ±0.41	1.83^{c} ±0.16	3.33^{ab} ±0.41
2	3.33^{ab} ±0.41	2.92^{ab} ±0.41	3.67^{ab} ±0.52
4	2.92^{ab} ±0.41	2.83^{b} ±0.37	3.33^{ab} ±0.41
6	3.17^{ab} ±0.36	2.92^{ab} ±0.41	3.92^{a} ±0.46
$Lsd_{0.05}$	0.8835*		
SE±	0.3158		

Values are mean±SD.

Mean values sharing the same super script letter (s) do not differ significantly (P≥0.05) according to DMRT.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

1. Chemical and Physical properties values in this study are acceptable and unstable during storage period
2. Sensorial and microbial properties are affected by storage period.
3. Sample A has good quality than sample B, while sample C has higher number of microrganisms and lowest quality.

5.2 Recommendation

1. Deep-freezer must be used to store meat and do not store local market sausage for a long time.
2. More attention and care should be taken to prepare fresh beef meat in factories.
3. Production of sausage should be monitored carefully by the factory owners as well as official authorities'.

REFERENCES

Alan, H.V. and Jane, P.S. (1995). Meat and meat product technology chemistry and microbiology. First edition. Volume 3 food products series.

Al-Aswad, M.B. (2000). Meat Science and Technology. 2nd edn Bagdad. Iraq.

Al-Hajo, N.N.A (2009). Using Different Methods to Tenderize Spent Hens meat. International Journal of Poultry Science, 8(1):99-103.

Al-Hajo, N.N.A. (2008). A Comparative study between some of the local Iraqi methods for curing chicken meat and typical evaluation of fresh and storage meat . International journal of Poultry Science,7(12):1190-1193.

AOAC (2003).Official method of analysis of the association of official Analytical chemists, 17th. Washington, D.C.

Archer, D.L.(2002). Evidence that ingested nitrate and nitrite are beneficial to heath. J. Food protection, 65:872-875.ISSN: 0362028.

Ayo. J, Carballo. J, Serrano. J, Olmedilla-Alonso. B, Ruiz-Capillas, C and Jimenez-Colmenero. F. (2007). Effect of total replacement of pork back fat with walnut on the nutritional proWle of frankfurters. Meat Science 77, Pp 173- 181.

Bagamboula, R. C.; Uyltendaele, F.M. and Debevere, J (2004). Inhibitory effect of thyme and basil essential oils carvacrol, thymo, linalool and p- cymene towards shigella sonnei and S. flexneri. Food microbial 21: 33- 42. ISSN: 0740-0020.

Banani, R.C.; Rabi, S.M.; Runu, C and Utpal, R.C. (2006). Effect of combination pre-treatment on physiochemical, sensory and microbial characteristics of fresh aerobically stored minced goat (black Bengal) meat organs . African Journal of biotechnology, 5(12):1274-1283.

Barbut, U. J. (1995). Importance of fat emulsification and protein matrix characteristic in meat batter stability. J. Muscle Food 6: 161-177.

Barket, B.M and Heijnen, C. (2004). Preservation of fish and meat. Agromisa foundation, Wagemingen. The Nether land. Pp:78-80-ISBN:90-72746-01-9.

Bender, A. E. (1966). Meat and human Nutrition. Cited by Lawaric, R. A. (1991). Meat Science 5th edn.

Bhulla, Z.(1999). Protein Digestibility and Viability Encyclopedia of Human Nutrition. M Sadler, J Strain and B. Caballero (Editors). San999- P. 1646- 1656.

Borchel, L.L and Cassens, R.G.(1998). Chemical hazard analysis for sodium nitrite in meat Curing; University of Wisconsin.

Cassen, R.G. (1994). Meat preservation, preventing losses and assessing safely. 1st Edn., Food and Nutrition Press. Inc. Trumbull, Connecticut, USA, Pp: 79- 92. ISBN: 0917678346.

Chipley, J.R.(2005). Sodium Benzoate and Benzoic acid antimicrobials in food. 3rd Edn. Daviddson, P.M.,J.N. Sofos and

Chirs, R and Kerth (1968). The science of meat quality/ edited by Chirs R. Kerth, Animal Science Department, Texas A 8, M. University of USA.

Comaposda, J.P and Gouand, J.A. (2000). The effect of sodium chloride content and temperature on pork meat isotherms. Meat Sci, 55:291-295. Dol: 10.10161/So 309-1740(99)00154-0.

Copper and Willis M. B.(1984). Profitable beef production, 4th ed. Farming press Ltd Suffol K pp 185 - 191.

Das A,Nath DR,Kumari s,aha R, Effect of fermented bamboo shoot on the quality and shelf life of nuggets prepared from spenthen Vet worled.(2013);6(7):419-423-doi.

Desmond, E.M. and Troy, D.J. (2001). Effect of lactic and citric acid on low - value beef used for emulsion – type meat products. Lebensmittle- wissenschaft und Technologie, Dublin.

Dino, R. (2004). Basko (traditional Indonesian Meat ball) properties with post mortem condition and frozen storage. M.Sc. Thesis, the Graduate Faculty of the Lousiiana State University.

Dorsa, W. J., Cutter C. N. & Siragusa G. R. (1998). Bacterial profile of ground beefmade from carcass tissue experimentally contaminated with pathogenic andspoilage bacteria before being washed with hot water, alkaline solution, ororganic acid and then stored at 4 or 12 ˚C. J. of Food Protection, 61(9), 1109–1118.

FAO (2000). Production yearbook, livestock numbers and production Rome Italy 54, pp 210-213.

FAO (2007). Food and Agriculture Organization of the United Nations Reigonal Office. Asia and the Pacific Bangkok (2007). (c) FAO (2007). ISBN: 978- 974- 7946- 99-4.

Forester, J.C.; Aberle, E.O.; General, D. E.; Midls , W.E.; Heric,H.B.; Judge, M.D and Markets,R.A. (2001). The principle of meat science.4th edition Kendall/ Hunt Publishing company, USA.

Garcia- Lopez, M. L.; Prieto, M and Otero, A. (1998). The physiological attributes of gram negative bacteria associated with spoilage of meat and poultry, Davies, A and Board, R. (Eds) London: Blackie Academic and Professional, Pp: 1- 34. ISBN: 0- 7514-0398-9.

Ghaly, A.D and Budge, S. (2010). Fish spoilage mechanisms and preservation techniques. Review. Am .J. Applied Sci. 7:846-864,ISSN1546-9239.

Harrigan, W. F. (1998). Laboratory Methods in Food Microbiology, 3ed Academic press, San Digo, London, Boston, New York, Sydney, Tokyo and Toronto.

Heinz, G. and Hautzinger, P. (2007). Meat processing technology. For small to medium scale products. Food and Agriculture Organization of the United Nations Regional Office for Asia and the Pacific. Retrieved on 1st June 2010, from hp://ptp.fao.org/docrep/fao/010/ai407e00.pdf.

Herschdoerfer, S.M.H. (1986). Food science and technologyseries of quality conlred in the food industry volume 2.

Hussein, H. A. (1987). Aerobic Bacteria in Fresh and Refrigerated Beef. M. Sc.Thesis, University of Khartoum. . -

Inekoronye, A. I. and Ngoddy, P. O. (1992). Integrated Food Science and Technology for Tropics, The Macmillan press Ltd., London and Basingstoke.

Isidor,S and Sedky,A.(1972). Principle and method of beef sausage manufacturing, processing . 27th Ann. Recip. M Wat conf. 37.52-57.

Javadi, A.; Safarmashaei, S and Dehbandi, A. (2011). Study on microbial properties of franfurtors during shelf life. Annuals of biological Research, 2- 407- 412.

Jay.j.m.(1996).Modern Food Microbiology, 5thed.International Thomason publishing Hentuchv.

Joseph, M. (1960). Food flavoring composition, manufacture and use.

Judge, M. D.; Aberle, E.; Forrest J.; Hedrick, H. and Merkal, R. (1990). Principles of meat science (2nd edn). Kendall/ Hunt, Lowa. USA.

Kumar, D.; Sharm, T. R. and Nalh, H. (1974). Factors Affecting Meat Texture. Indian Food Packer 28 (6):17 -25.

Kumar RR, Sharma BD, Kumar M, Chidanandaiah, Biswas AK. Storage quality and shelf life of vacuum-packaged extended chicken patties. J Muscle Foods. 2007;18(30):253–263. doi: 10.1111/j.1745-4573.2007.00080.x.

Ladwig, *K;*, Knipe, C and Sebranck, T. (1989). Effect of collagen and alkaline phosphate on time of chopping, emulsifying stability and protein solubility of fine cut meat system. J. Food Sci. 54: 541 -544.

Lawarie, R. A. (1990). Meat Science (5th edn), Pergamon Press.

Lawarie, R. A. (1991). Meat science (5th edn) Program on Press Ltd., Heading ton Hill Hall, Oxford.

Lawrie, R.A., (1979). Meat Science (3rd edn). Progamon Press Ltd, Heading ton Hill Hall, Oxford.

Leo, M.L. and Nollet, E. (2012). Poultry and Sea food quality. second edition. Association Editory Terr Boy Liston, Fengchen, Patti.

Lester, E.J. (1996). Freezing Effects on Food Quality, publisher Marcel Dek, CRC press.

Lyon, B.G. (1984). Effect of Sodium Chloride and Sodium poly phosphate treatments properties of canned fowl.Poult.Sci.63: 664-672.

Mohammed, A. E. (2000). Aerobic Bacteria Isolated from Meat at Different Stage of Processing. M. v. Sc. Thesis, University of Khartoum.

Mohammed, R. I. (2005). Physiochemical and sensorial properties of burgers produced from camel and beef (comparative study). A dissertation submitted to university of Khartoum in partial Fulfillment of requirementS for the Degree of Master of science in Food Science and Technology.

Molan, P.C., (1992). The antibacterial activity of honey: 1. the Nature of the Antibacterial Activity. Bee World, 73: 5-28.

Montyomery and Douglas, C. (2001). Design and analysis of Experiments (5th Ed). New York, John Wiley Sons, P.Section 3-2 ISBN 978047131697.

Muhasin, A.El. (2009).Effect of adding different levels of camel meat on physiochemical properties of fresh sausage. M.Sc.Thesis, U of K.

Naveena, B.M.; Muthukumar, M.; Sen, A.A.; Babji, Y. and Murthy, T.R.K. (2006). Quality characteristics and storage stability of chicken patties formulated with finger Millet Flour (Eleusine Coracana), Journal of Muscle Foods 17:92-104.

Neumeyer, K. T.; Ross, G.; Thomason and McMeeken, T. A. (1997). Validation of a model describing the effect of temperature and water activity on the growth of psychrotrophic *Pseudomonas*. Int. J. Food microbiology, 38: 55- 63. Dol: 10: 1016/S0168-1605 (57) 00090-1.

Nychaset, G.J.E.; Skandamis, C.C.T. and Koutsoumani, K.P.(2008). Meat spoilage during distribution. Meat sci.,78:77-89.Dol:10-10161.

Olusolatet, F.T and Mojola, A.B. (2010). Relevance at deried meat product (Kundi). International moisture meat (I M M , for food security).

Parrish, F.C.; Goll, D.E.; Newcomb, W.J.; de Lumen, B.O.; Chaudhry, H.M. and Kline, E.A. (1969). Molecular properties of post mortem muscle.Changes in nonprotein nitrogen and free amino acids of bovine muscle.J.Food Sci.34,196-202.

Pearson, A. M. and Gillett, T.A. (1999). Processed Meat. 3^{ed} ed. Aspen Publishers, Gaithers Burg , Mary land, Vol, U.S.A.

Ranganna, (2001).Hand book of analysis and quality control for fruit and vegetables products:2nded. Taka. M.C. GrowHil published company limte, New Delhi.

Ray,B.(2004). Fundamental food microbiology (3rd Edition). CRC Press. FL, PP. 439-534.ISBN:0-8493-1610-3.

Rosmini, M. R.; Perez- Awarez, J. A. and Fernandez- Lopez, J. (2004). Operational processes for frozen red meat. Int: Hand Book of Frozen Foods. Hun, Y. H. P. and W. Kit Nip (Eds) Marcel Dekkon. Inc. Ny, Pp" 177- 179. ISBN: 0- 247- 4712-7.

Smith, D. M. (2001). Functional properties of muscle proteins in processed poultry products. Pages 181-194 in : A. R. Samsed. Poultry meal processing CRC Press, Boca Raton, FL.

SSMO (2007). Sudanese standards and metrology organization.

Tronsky, I.J.Ehr,T.L Kinsman,D.R. Rice, D.M. Faustman, C. (2003), International Sausage Casings Association. Abrif History of Natural Casings Department of Animal Science, University of Connecticut Home Sausage Making.

USDC (1995). Final rule on pathogen reduction, hazard analysis and critical control points (HACCP) systems. US Department of Commence of Agriculture, Washington, DC.

Winger, R.J. and Fennma. (1976). Tenderness and water holding properties of beef muscle as influenced by freezing and subsequent storage at -3 or -15°c. J.Food Sci.41: 1433.

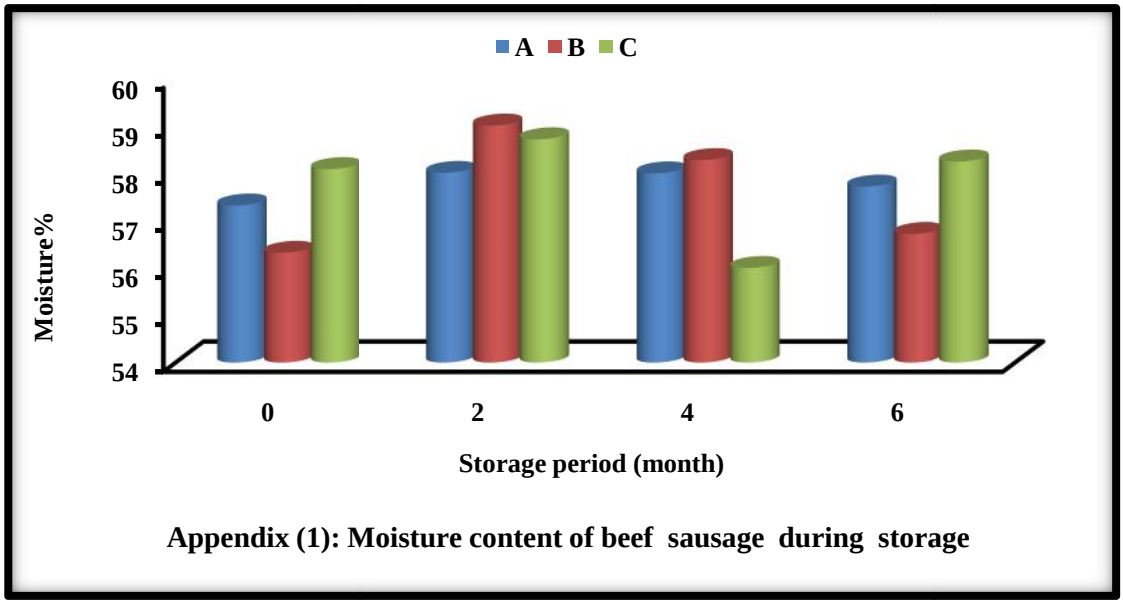
Xiong, Y. L. (2009). Dairy Proteins. Ingredient in meat products 131-141.

Zaglul, J.A. and Cassens, R.G.(1987). Postmortem Processing of export beef Costrica. J. Meat science, 20-87.

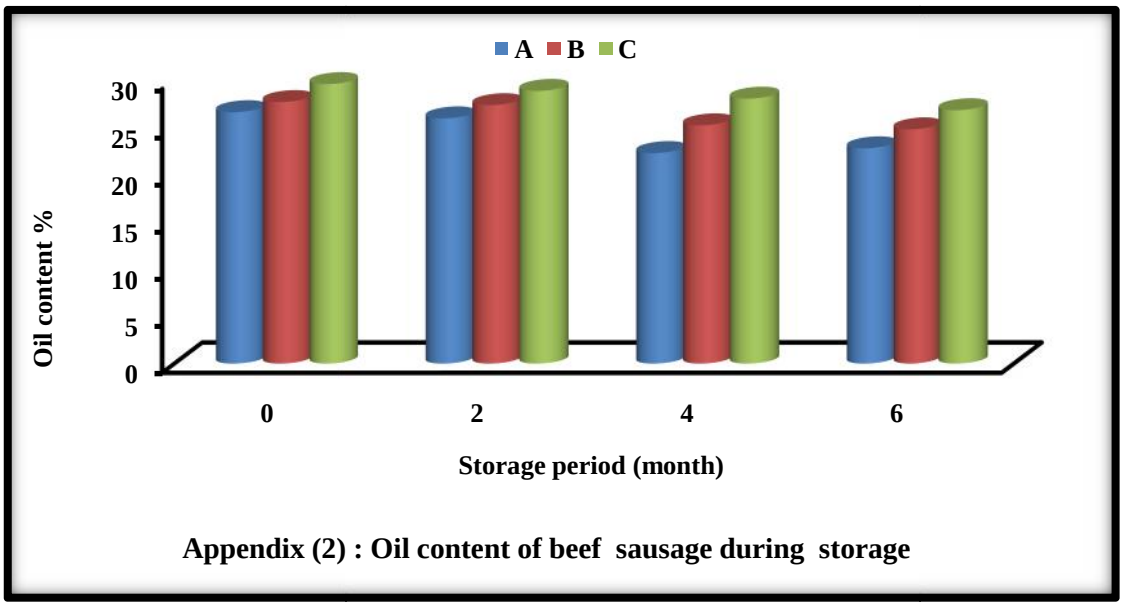
Zhou, G. H.; Xu, X. L. and IU, Y. (2010). Preservation technologies for fresh meat review. Meat Sci., 86: 119- 128.

Zurea- Cosano, G. and Rincons, F. (1988). Microbial growth in vacuum packaged frank furthers produced in Spain, Food Microbiology, 5: 213- 2 18.

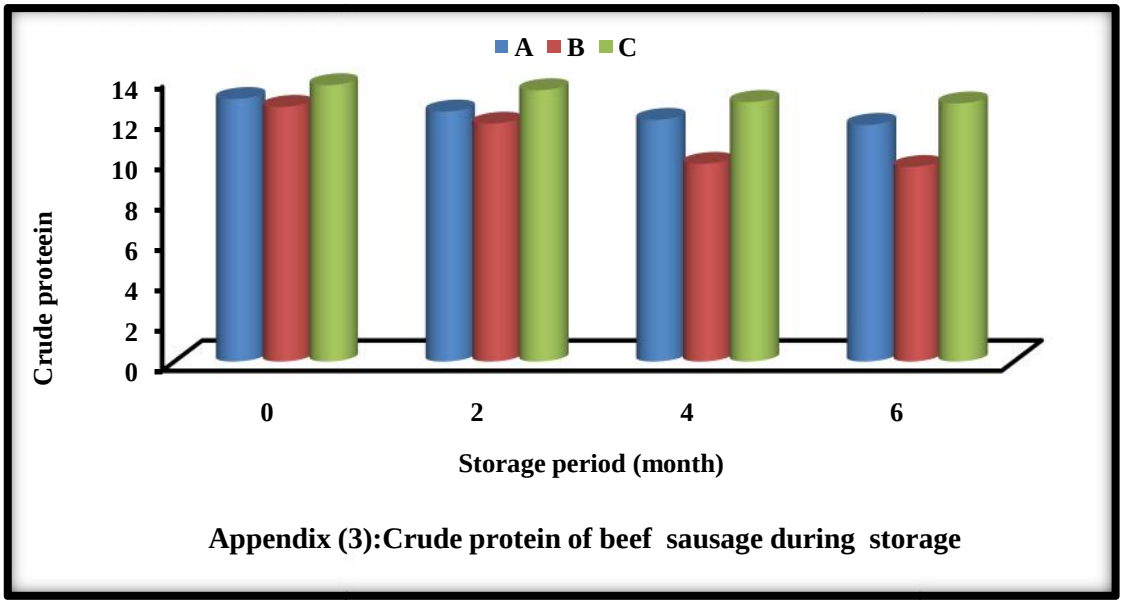
APPENDICES



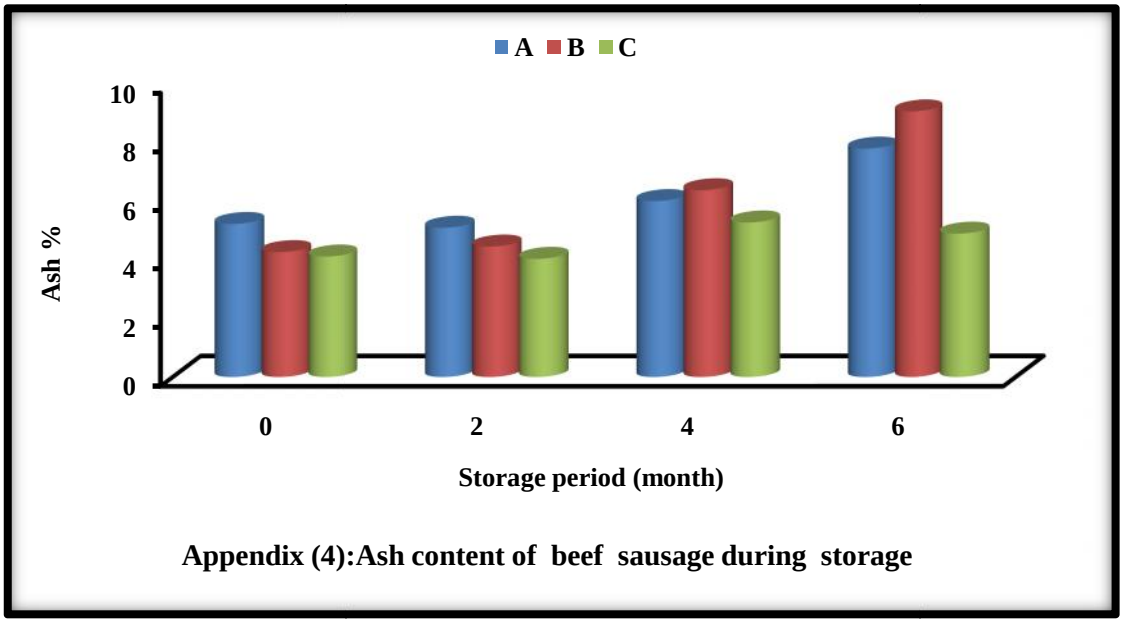
Appendix (1): Moisture content of beef sausage during storage



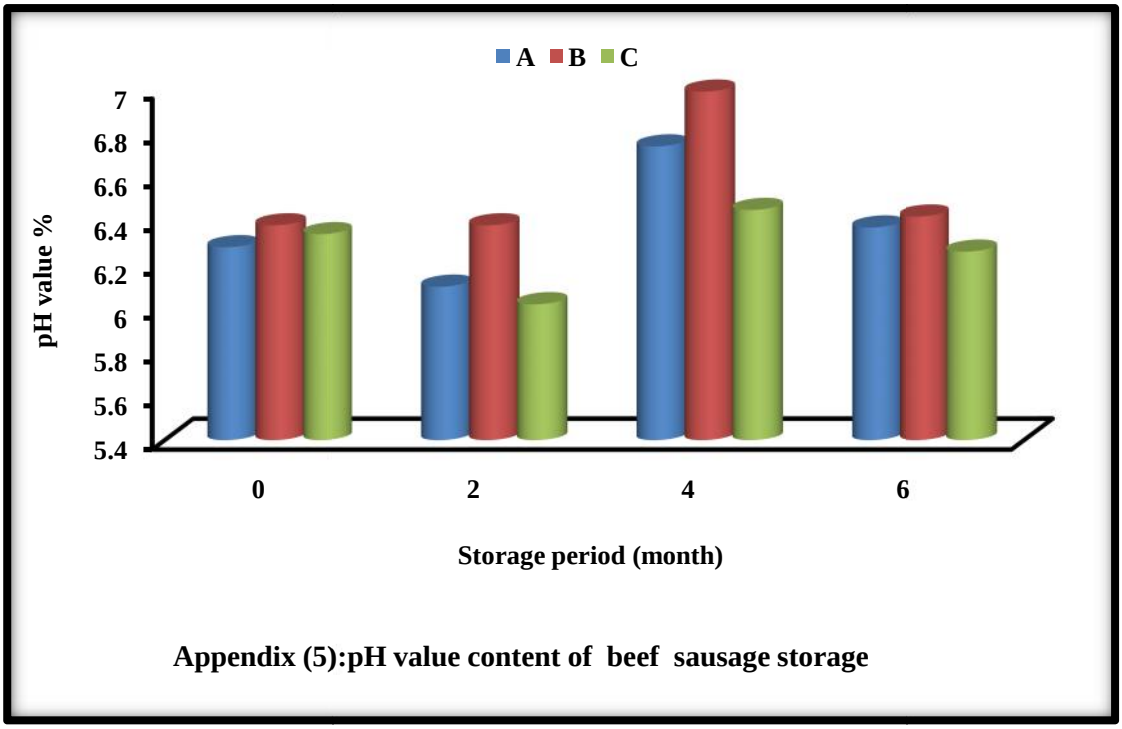
Appendix (2) : Oil content of beef sausage during storage



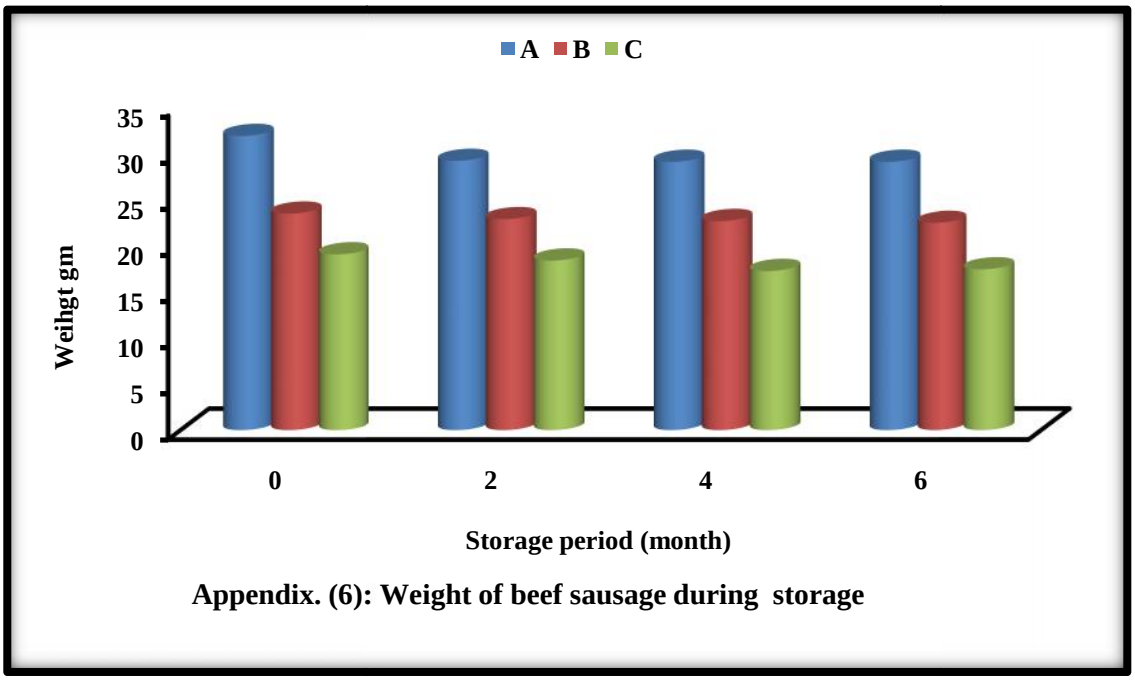
Appendix (3):Crude protein of beef sausage during storage



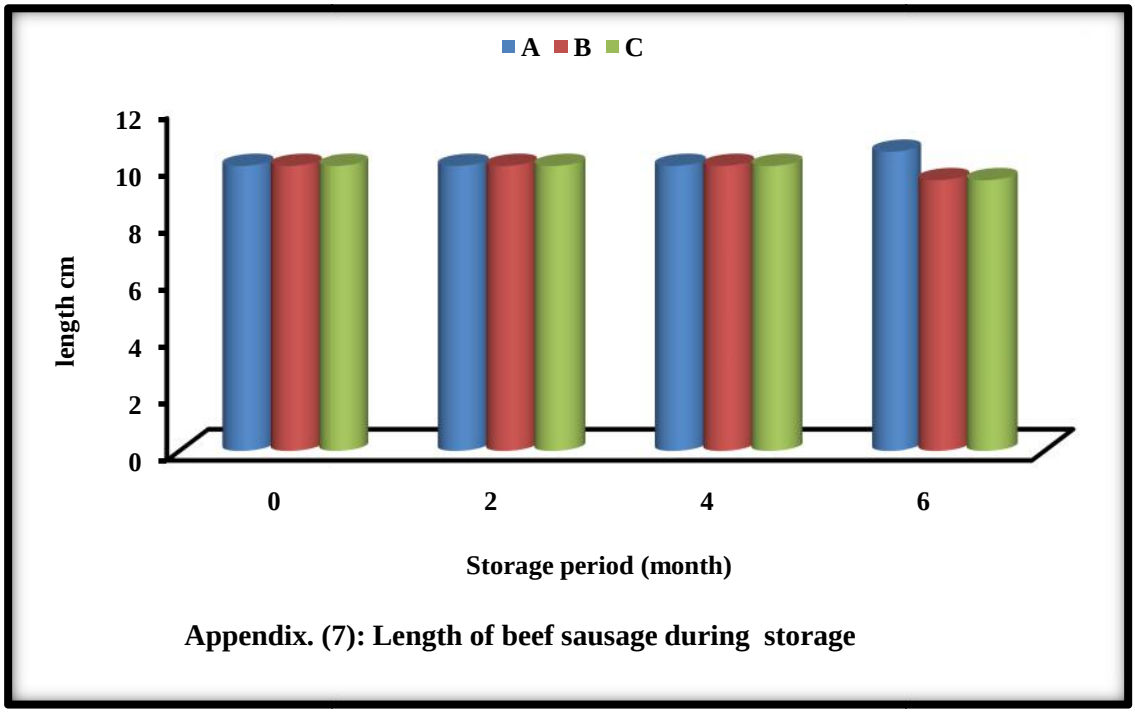
Appendix (4):Ash content of beef sausage during storage



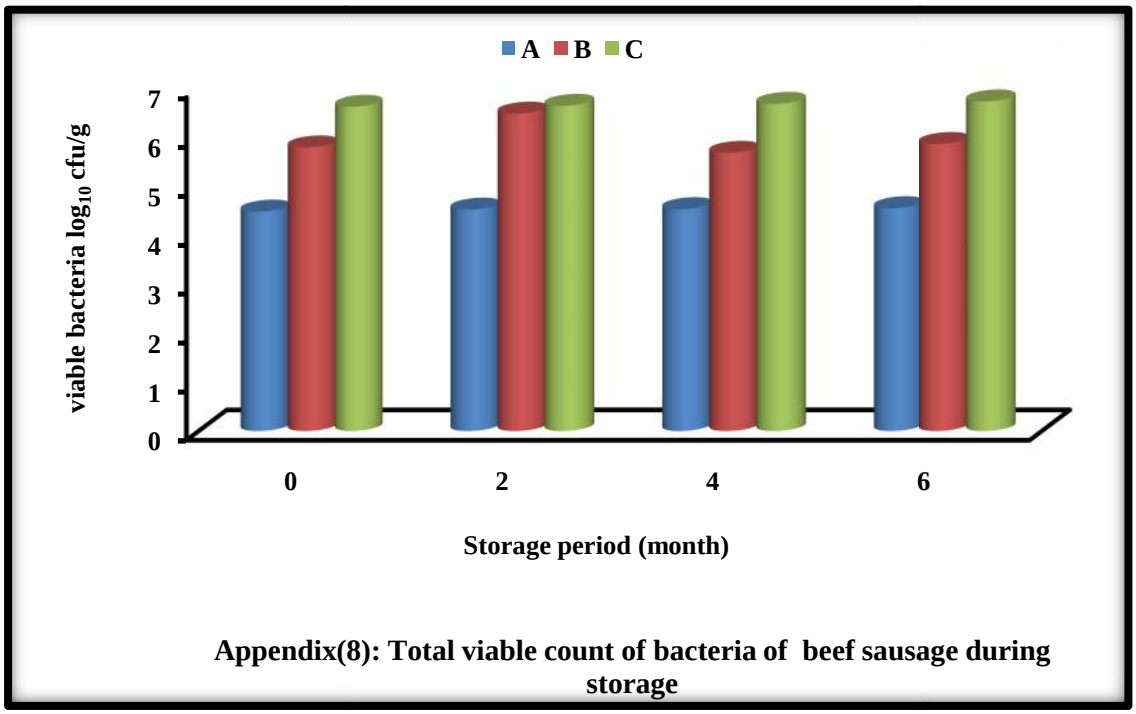
Appendix (5):pH value content of beef sausage storage



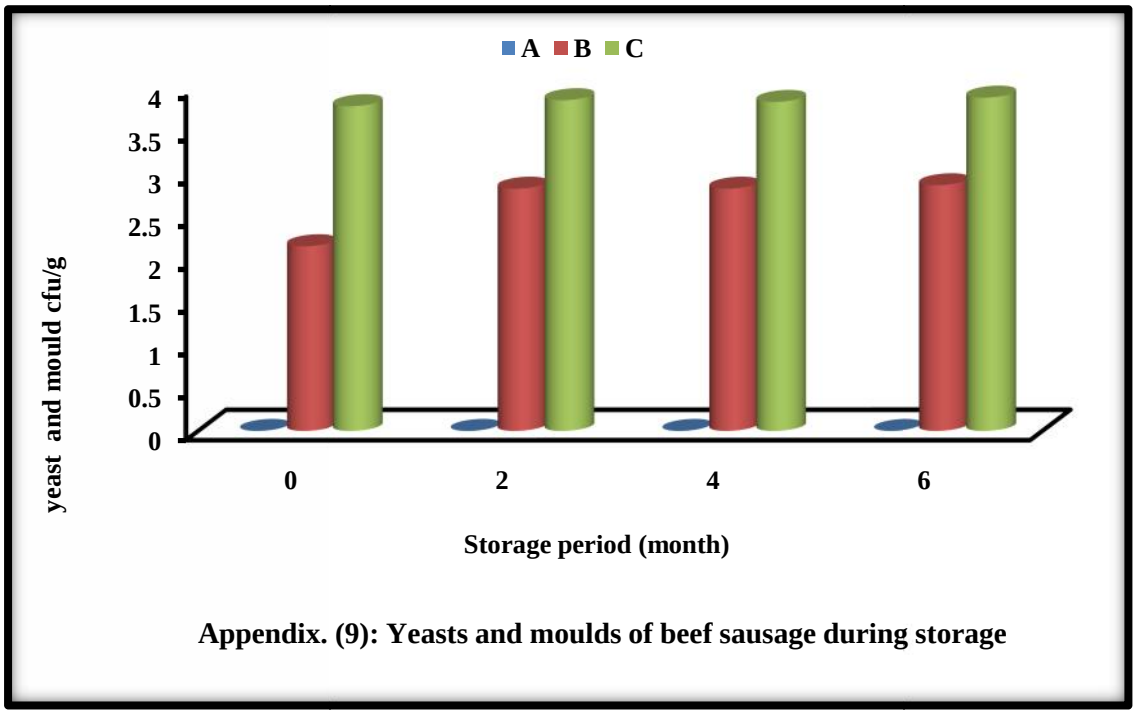
Appendix. (6): Weight of beef sausage during storage



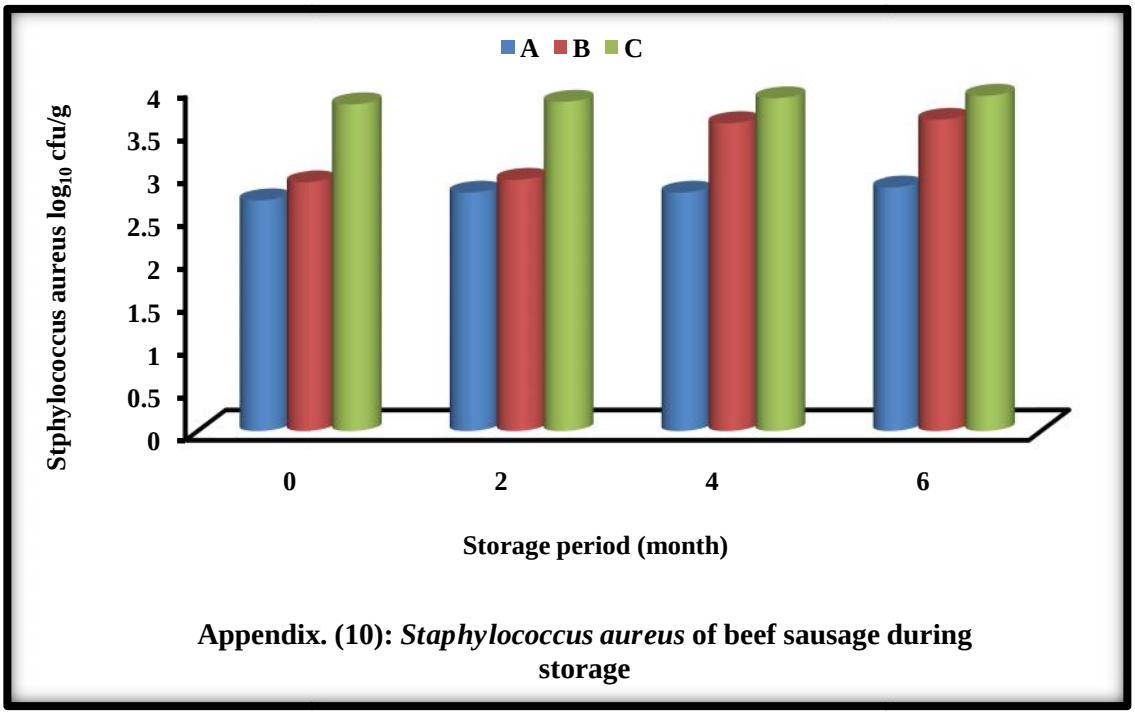
Appendix. (7): Length of beef sausage during storage



Appendix(8): Total viable count of bacteria of beef sausage during storage



Appendix. (9): Yeasts and moulds of beef sausage during storage



Appendix. (10): *Staphylococcus aureus* of beef sausage during storage

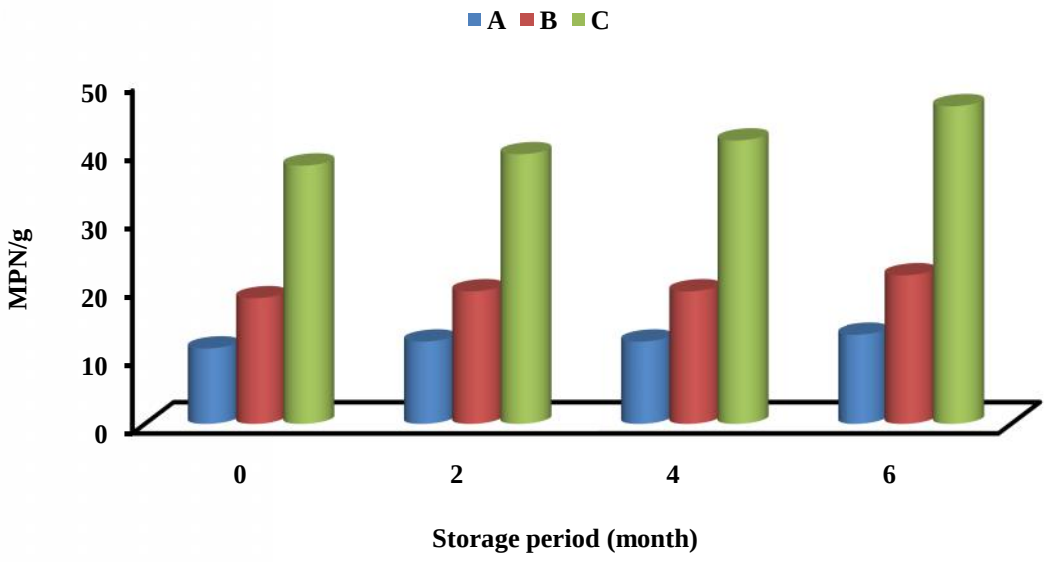
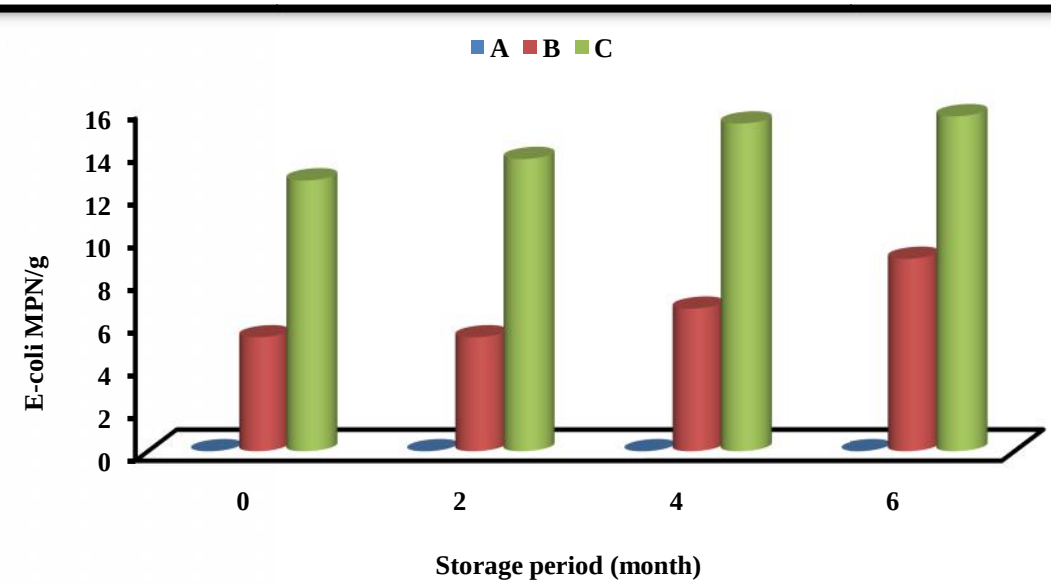
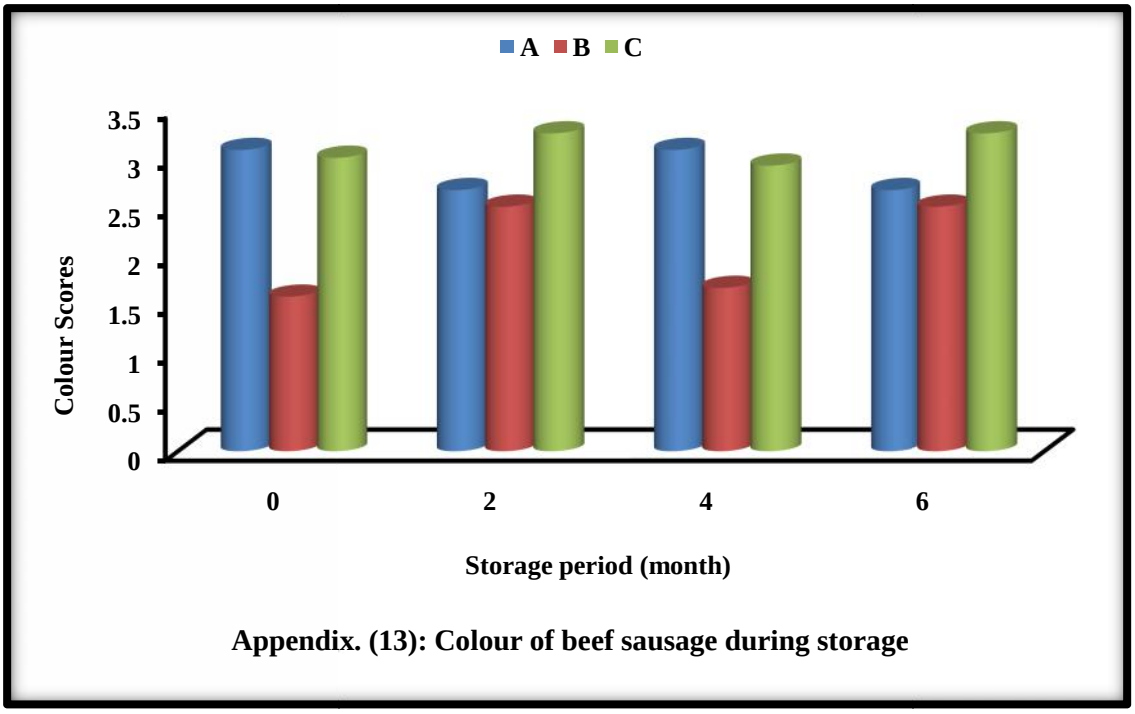


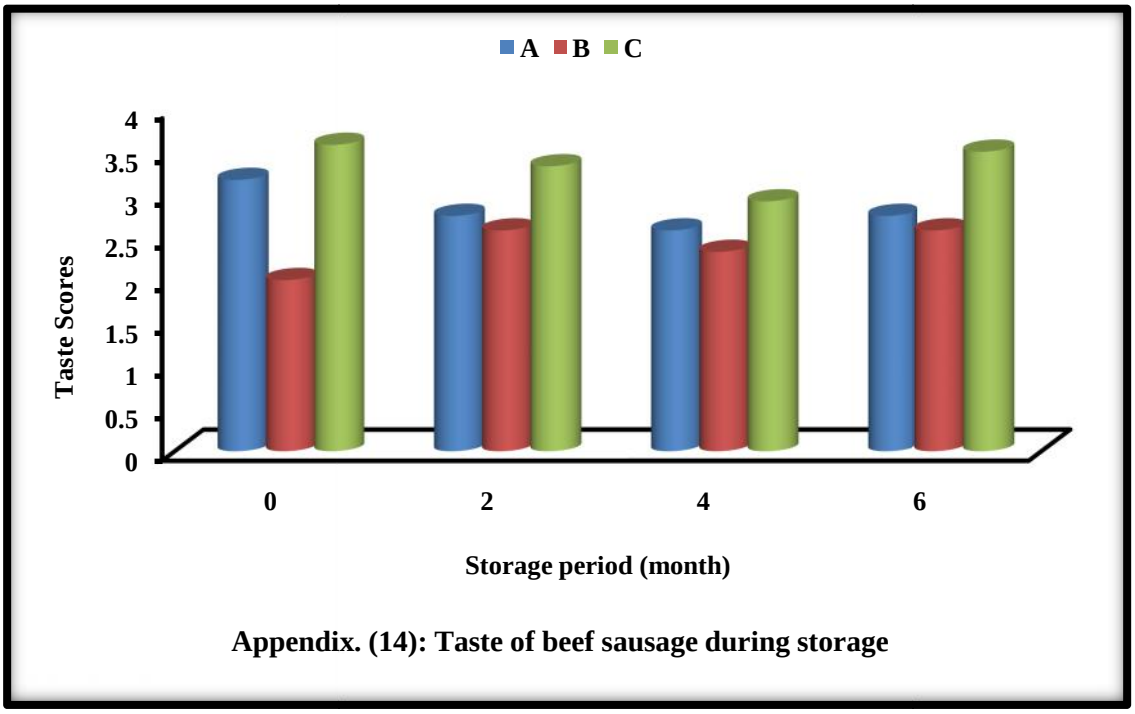
Fig. (11): Coliforms of beef sausage during storage



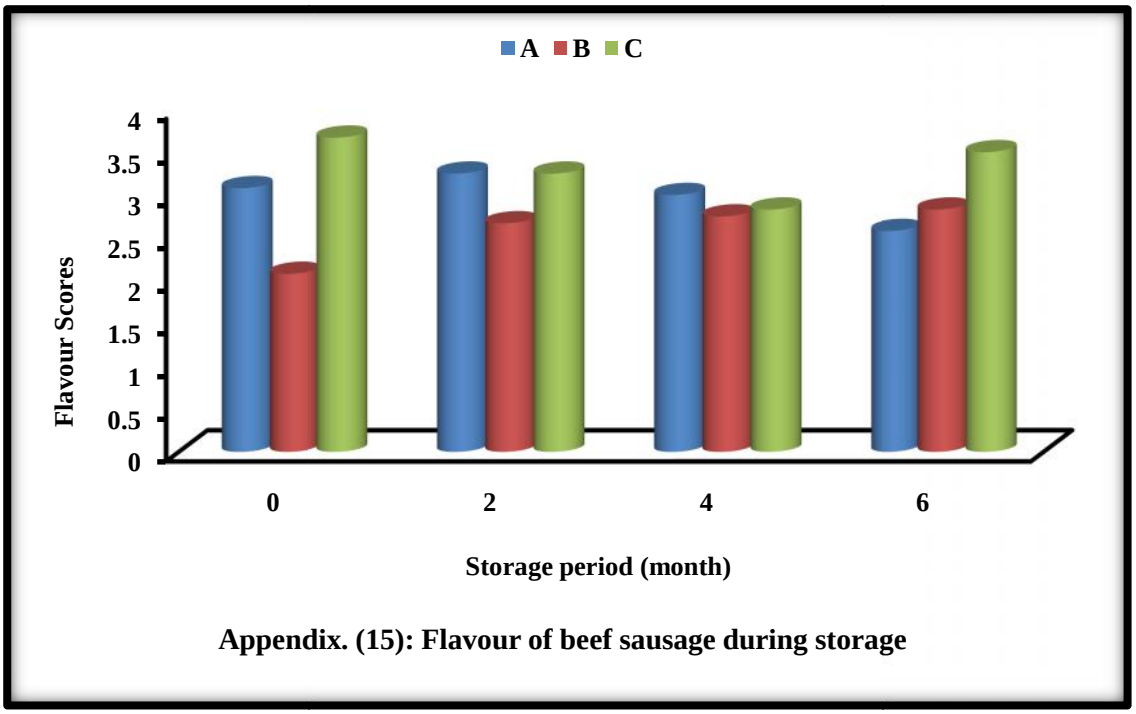
Appendix. (12): *E. coli* of beef sausage during storage



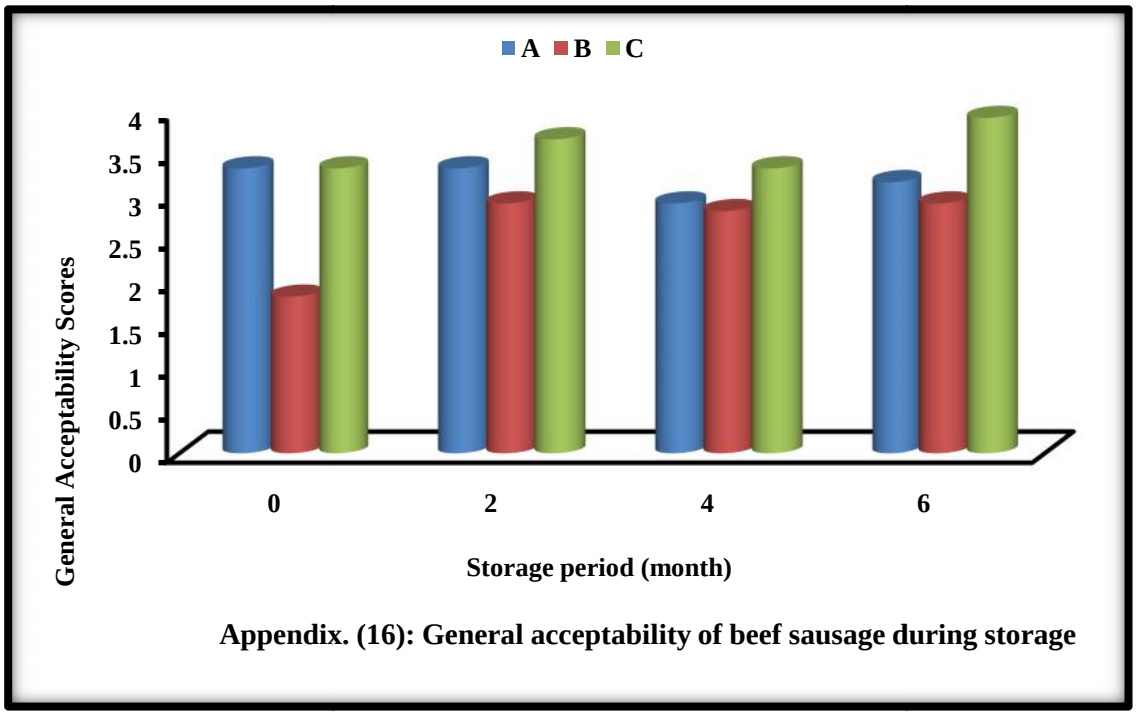
Appendix. (13): Colour of beef sausage during storage



Appendix. (14): Taste of beef sausage during storage



Appendix. (15): Flavour of beef sausage during storage



Appendix. (16): General acceptability of beef sausage during storage