Quality Evaluation of Beef Sausage Incorporated with Bee Honey

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DEDICATION
To my parents,
For their everlasting love, care, hope and dedication for their patience and perseverance in assuring my every success.
To my sisters and brothers.
To my loyal teachers,
Past, present and future.
To my lovely Faculty.
To my colleagues, of the tenth classmates graduates.

With my deep love and respect

Rabaa
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>i</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>iii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>viii</td>
</tr>
<tr>
<td>ARABIC ABSTRACT</td>
<td>x</td>
</tr>
<tr>
<td>CHAPTER ONE INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>CHAPTER TWO LITERATURE</td>
<td>4</td>
</tr>
<tr>
<td>2.1. Definition of Meat</td>
<td>4</td>
</tr>
<tr>
<td>2.2. Meat and Human Nutrition</td>
<td>5</td>
</tr>
<tr>
<td>2.3. Structure and Composition</td>
<td>6</td>
</tr>
<tr>
<td>2.3.1. Structure</td>
<td>6</td>
</tr>
<tr>
<td>2.3.2. Composition</td>
<td>8</td>
</tr>
<tr>
<td>2.4. Quality Attributes of Meat</td>
<td>9</td>
</tr>
<tr>
<td>2.4.1. Color</td>
<td>10</td>
</tr>
<tr>
<td>2.4.2. Tenderness</td>
<td>10</td>
</tr>
<tr>
<td>2.4.3. Juiciness</td>
<td>10</td>
</tr>
<tr>
<td>2.4.4. Firmness and Texture</td>
<td>11</td>
</tr>
<tr>
<td>2.5. Meat Microbiology</td>
<td>11</td>
</tr>
<tr>
<td>2.6. Microorganisms in Meat</td>
<td>12</td>
</tr>
<tr>
<td>2.6.1. Staphylococci</td>
<td>12</td>
</tr>
<tr>
<td>2.6.2. <em>Escherichiu coli</em></td>
<td>14</td>
</tr>
<tr>
<td>2.7. Spoilage of Meat and Meat Products</td>
<td>15</td>
</tr>
<tr>
<td>2.8. Meat Processing</td>
<td>16</td>
</tr>
<tr>
<td>2.9. Sausage as Meat Product</td>
<td>17</td>
</tr>
<tr>
<td>2.9.1. Classification of Sausage</td>
<td>18</td>
</tr>
<tr>
<td>2.9.2. Ingredients in Sausage Making</td>
<td>19</td>
</tr>
<tr>
<td>2.9.3. Sausage Casing</td>
<td>22</td>
</tr>
<tr>
<td>2.10. Meat Preservation</td>
<td>23</td>
</tr>
<tr>
<td>2.10.1. Chemical Preservatives</td>
<td>24</td>
</tr>
<tr>
<td>2.11. Honey as Preservative</td>
<td>25</td>
</tr>
<tr>
<td>2.12. Definition of Honey</td>
<td>26</td>
</tr>
<tr>
<td>2.13. Chemical Properties of Bee Honey</td>
<td>27</td>
</tr>
<tr>
<td>2.14. Quality Attributes of Honey</td>
<td>27</td>
</tr>
<tr>
<td>2.15. Uses of Bee Honey</td>
<td>28</td>
</tr>
</tbody>
</table>
2.16. Safety of Honey .......................................................... 29
2.16.1 Reaction Following Topical Use ................................. 29
2.16.2. Honey Allergies .................................................... 30
2.17. Antimicrobial Properties of Honey .............................. 30
2.17.1. Explanation of Antimicrobial Activity ....................... 32
2.17.1.1. Osmotic Effect ............................................... 32
2.17.1.2. Acidity ....................................................... 32
2.17.1.3. Hydrogen Peroxide ........................................ 33
2.17.1.4. Photochemical Factors .................................... 34
2.18. Honey as Antioxidant ............................................. 35

CHAPTER THREE MATERIAL AND METHODS .......................... 37
3.1. Materials ............................................................... 37
3.1.1. Food Materials .................................................... 37
3.1.2. Casing .............................................................. 37
3.2. Methods ............................................................... 37
3.2.1. Raw Materials Preparation .................................... 37
3.2.1.1. Meat Preparation ........................................... 37
3.2.1.2. Bee Honey Preparation .................................... 38
3.2.2. Sausage Preparation ........................................... 38
3.2.3. Methods of Analysis .......................................... 38
3.2.3.1. Moisture Content ........................................... 38
3.2.3.2. Crude protein Content .................................... 41
3.2.3.3. Fat Content .................................................. 41
3.2.3.4. Ash Content .................................................. 42
3.2.3.5. Peroxide Value (PV) ....................................... 42
3.2.3.6. pH Measurement ............................................ 43
3.2.3.7. Microbial Analysis ......................................... 43
3.2.3.7.1. Preparation of Serial Dilution ......................... 43
3.2.3.7.2. Determination of Microbial Load of Beef Sausage 43
3.2.3.7.3. Determination of Coliform Bacteria .................. 44
3.2.3.7.3.1. Presumptive Coliform Test ......................... 44
3.2.3.7.3.2. Confirmed Coliform Test .......................... 44
3.2.3.7.3.3. Faecal Coliform Test ............................... 44
3.2.3.7.3.4. Differentiation of Faecal Coliform Test .......... 45
3.2.3.7.4. Staphylococcus aureus ................................. 45
3.2.3.7.4.1. Tube Coagulase Test .............................. 45
3.2.3.7.5. Psychtrophic Bacteria ................................. 45
3.2.3.8. Sensory Evaluation ....................................... 46
3.2.3.9. Statistical Analysis ....................................... 46
CHAPTER FOUR RESULTS AND DISCUSSION ........................................ 47
4.1. Proximate Composition ............................................................. 47
4.2. pH Measurement ....................................................................... 49
4.3. Peroxide Value (PV)................................................................. 49
4.4. Microbial Analysis ................................................................. 54
4.4.1. Total Viable Count .............................................................. 54
4.4.2. Total Coliform ................................................................. 57
4.4.3. E. coli .................................................................................. 57
4.4.4. Staphylococcus aureus ....................................................... 63
4.4.5. Psychotrophic Bacteria ....................................................... 63
4.5. Sensory Evaluation ................................................................. 63

CHAPTER FIVE CONCLUSIONS .................................................. 71

CHAPTER SIX RECOMMENDATIONS .......................................... 72

References ..................................................................................... 73

Appendix -1 Sensory Evaluation Form ............................................ 84
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Proximate analysis and pH of raw beef meat and bee honey.</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>Basic sausage formula</td>
<td>39</td>
</tr>
<tr>
<td>3</td>
<td>Sausage formulation for all treatments</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>Effect of bee honey on the proximate composition of beef sausage</td>
<td>48</td>
</tr>
<tr>
<td>5</td>
<td>Effect of the storage period on proximate composition of beef sausage.</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>Effect of bee honey on the sensory evaluation of beef sausage</td>
<td>68</td>
</tr>
<tr>
<td>7</td>
<td>Effect of storage period on the sensory evaluation of beef sausage.</td>
<td>70</td>
</tr>
</tbody>
</table>
### LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figures</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Effect of bee honey on the pH of beef sausage.</td>
<td>51</td>
</tr>
<tr>
<td>2</td>
<td>Effect of storage period on the pH</td>
<td>52</td>
</tr>
<tr>
<td>3</td>
<td>Effect of bee honey on the PV of beef sausage.</td>
<td>53</td>
</tr>
<tr>
<td>4</td>
<td>Effect of storage period of the PV of beef sausage.</td>
<td>55</td>
</tr>
<tr>
<td>5</td>
<td>Effect of the bee honey on the TVC (log&lt;sub&gt;10&lt;/sub&gt; cfu/g) of beef sausage.</td>
<td>56</td>
</tr>
<tr>
<td>6</td>
<td>Effect of the storage period on the TVC (log&lt;sub&gt;10&lt;/sub&gt; cfu/g) of beef sausage.</td>
<td>58</td>
</tr>
<tr>
<td>7</td>
<td>Effect of bee honey on the Total coliform count of beef sausage.</td>
<td>59</td>
</tr>
<tr>
<td>8</td>
<td>Effect of the storage period on the Total coliform count of beef sausage.</td>
<td>60</td>
</tr>
<tr>
<td>9</td>
<td>Effect of bee honey on the <em>E. coli</em> of beef sausage.</td>
<td>61</td>
</tr>
<tr>
<td>10</td>
<td>Effect of storage period on the <em>E. coli</em> of beef sausage.</td>
<td>62</td>
</tr>
<tr>
<td>11</td>
<td>Effect of bee honey on the <em>Staphylococcus aureus</em> of beef sausage.</td>
<td>64</td>
</tr>
<tr>
<td>12</td>
<td>Effect of the storage period on the <em>Staphylococcus aureus</em> of beef sausage.</td>
<td>65</td>
</tr>
<tr>
<td>13</td>
<td>Effect of bee honey on the Psychotrophic bacteria of beef sausage.</td>
<td>66</td>
</tr>
<tr>
<td>14</td>
<td>Effect of the storage period on the Psychotrophic bacteria of beef sausage.</td>
<td>67</td>
</tr>
</tbody>
</table>
Quality Evaluation of Beef Sausage Incorporated with Bee Honey
M.Sc. thesis dissertation

By: Rabaa Ahmed Mohammed.

Abstract: The objective of the current study was to evaluate the effect of bee honey addition on the safety and storability of beef sausage. Beef sausage was processed by the addition of different concentrations (0.0%, 2.5%, 5.0% and 7.5%) of bee honey. The processed beef sausage was packaged in poly ethylene bags and refrigerated at 4°C ±2 for up to 9 days. Several variables were determined, using subjective and objective measurements, to evaluate the effects of concentration of bee honey and storage periods on the quality attributes of the processed sausage. These included proximate composition, pH, peroxide value (PV), total viable count, total coliform, *Escherichia coli*, *Staphylococcus aureus* and psychotrophic bacteria as well as sensory attributes of the sausage. The evaluations were carried out immediately after processing, three, six and nine days post processing.

Among all treatments, the control sausage (0% bee honey) had the highest (P<0.05) moisture, pH, PV, total viable count, total coliform, *Escherichia coli*, *Staph aureus* and psychotrophic bacteria. However, it had lower (P<0.05) color and texture scores.

Sausage sample with 7.5% bee honey had the highest (P<0.05) protein content and over all acceptability, color, aroma, taste and texture scores.
But it had the lowest (P<0.05) PV, total viable count, total coliform, *Escherichia coli*, *Staph aureus* and psychotrophic bacteria.

Generally and regardless of storage period PV, total viable count, total coliform, *Escherichia coli*, *Staphylococcus aureus* and psychotrophic bacteria decreased with the increase of bee honey concentrations added to the sausage. Incorporation of bee honey in sausage formulation led to substantial improvement in the sensorial and keeping qualities of beef sausage.
الهدف: تأثير دراسة تقييم لزيادة تقييم اللحوم بزيادة السائل وزيادة الكفاءة في إنتاج اللحوم.  

البحث تشمل تجارب مختلفة تمت على 120 حيواناً، حيث تم تقسيمهم إلى 3 عشاقب (نسبة ضخمة). وشملت التجارب زيادة السائل (0، 2.5، 5، و7.5%) على بيئة التخزين، حيث تم تخزين الحيوانات في درجة حرارة سنة تحت 9 أيام.

النتائج: تبينت أن زيادة السائل في الشحذة كتب تزيد من جودة اللحوم، حيث أن زيادة السائل (7.5%) كانت تزيد من جودة اللحوم بشكل كبير. كما أن زيادة السائل (7.5%) كانت تزيد من نسبة المواد الغذائية في اللحوم، حيث أن زيادة السائل (7.5%) كانت تزيد من نسبة المواد الغذائية في اللحوم بشكل كبير.

الخلاصة: يمكن أن يكون زيادة السائل (7.5%) في الشحذة كتب تزيد من جودة اللحوم، حيث أن زيادة السائل (7.5%) كانت تزيد من نسبة المواد الغذائية في اللحوم بشكل كبير.
CHAPTER ONE
INTRODUCTION

Nutritionally, meat is a very good source of essential amino acid, to lesser extent of certain mineral, vitamins and essential fatty acids. Also meat provides calories from proteins, fats and limited quantities of carbohydrates (Judge et al., 1990). Sausage is one of the old meat products in which fresh comminuted meat are modified by various processing methods to yield desirable organoleptic and keeping properties. In addition to minced meat, seasoning, and other ingredient; added in sausage making include curing agent, corn syrup and smoke.

Meat and meat products are highly perishable materials so sanitation and cooling is essentials in handling, marketing and processing of meat. The sanitation in the Sudan, in general is very poor with regard to slaughtering, handling, marketing and processing of meat, except for very few meat plants and slaughter houses.

The assurance of inventory and the shelf life of meat and meat product represent an important challenge for the meat industry.

The spoilage of refrigerated meat caused by microbial agent such as bacteria which are responsible for the off odors, off flavors, discoloration, gas production and slime production such problem necessitated the usage of artificial preservatives to prolong the shelf life of meat and insure its safety. In earlier times food was preserved with salt, sugar, spices and wood smoke. With the development of new products, chemical antimicrobial agents and many organic acids were
relied on to achieve a longer shelf life and greater assurance of protection from microbial spoilage.

With growing concern over the presence of chemical residues in foods the demand for nontoxic natural preservatives is increasing.

Many natural substances in honey bee species with different plant origins may play an important role in functional properties such as anti oxidative and anti bacterial activities. Honey is nectar collected from many plants and processed by honey bees (*Apis mellifera*). This natural product is widely appreciated as the only concentrated form of sugar available worldwide (FAO, 1996). and is also used as food preservative (Cherbuliez *et al.*, 2003).

Honey has been reported to contain about 200 substances (complex mixture of sugar, but also small amounts of other constituents such as minerals, proteins, vitamins, organic acids, flavonids, phenolic acids and other phytochemicals) Its also contains a number of components to act as preservatives; these include tocopherol, ascorbic acids flavonids, other phenolics and enzymes such as glucose oxidase, catalase and peroxidase (Crane 1975; Ferreres *et al.*, 1993 and Loyrish, 1974). It is suggested that any of these substances owe their preservative properties to their anti oxidative activity (Cerutti, 1994).

Development of natural preservatives with both antioxidants and antibacterial activities, that prolong the shelf life of meat and prevent food-borne illness, is desirable. I suggest that honey can act as a natural
antibacterial and anti oxidant which is important with the recent emphasis on decreasing the use of artificial preservation in food.

**Objectives:**

- The effect of bee honey on safety and storability of beef sausage.
- The effect of bee honey on physicochemical and organoleptic properties of beef sausage.
Increasing population and increased demand per capital together with moderately rapid income growth lead not only to an increased demand for staple foods but also for preferred foods including, particularly, meat and meat products and this will create large food deficits. An increased production of animal protein will make an important contribution towards filling this deficit (Bender, 1992).

Throughout recorded history, the consumption of meat has indicated a position of social and economic prestige. It is noteworthy that meat consumption is often an indicator of economic status of a country or individual (Judge et al., 1990).

2.1. Definition of meat
Meat is defined as those animal tissues, which are suitable for use as food. All processed or manufactured products, which might be prepared from those tissues, are included in this definition (Judge et al., 1990). Also meat is defined as the flesh of animals used as food, and it is often widened to include, as well as musculature, organs such as liver and kidney, brains and other edible tissues (Lawrie, 1990).

Gerrard (1977) and Lawrie (1991) define meat as “The postmortem aspect of the three hundred or so anatomically distinct muscles of the body together with the connective tissue in which the muscle fibers are deposited and such intramuscular fat as cannot be trimmed off without
breaking up the muscle as a whole. Intramuscular fat is anatomically included, being physically inseparable.”

Beef cattle are raised to produce animals which will be slaughtered for meat production. Meat is composed of lean muscle, fat and connective tissue, and bones. In addition there are blood and lymphatic vessels and glands (Judge et al., 1990)

2.2. Meat and human nutrition
Meat, and other animal foods such as milk, can make a valuable contribution to the diets in developing countries. It is less nutritional importance in industrialized countries where wide varieties of food of all kinds are available. Many diets in developing countries are based on cereals or root crops and relatively bulky, especially where fats are in short supply and this can limit the total energy intake. This is especially true of infants after weaning and young children (Bender, 1992).

The nutritive value of meat is attributed to it is, proteins, fats, carbohydrates, vitamins, and minerals, whereas meat does provide calories from the proteins, fats and the limited quantities of carbohydrates that are present. Its more vital contributions to the diet are derived from the high quality and quantity of its protein. The available supply of B vitamins and certain minerals, and the presence of essential fatty acids (Judge et al., 1990).

The importance of meat in the diet is as a concentrated source of protein, which is not only high biological value but its amino acid composition, complements that of cereal and other vegetable proteins.
Beef appears to have a somewhat higher content of leucine, lysine and valine than lamb, and lower content of threonine. The amino acid content may be affected by processing (e.g. heat, ionizing radiation), but unless processing conditions are both severe and prolonged such as destructions is minimal rather more important is the possibility that certain amino acids may become unavailable(Bender, 1992). Meat is generally an excellent source of iron and zinc, several B-vitamins, and liver is a very rich source of vitamin A, but its a very poor source of the water soluble vitamin C, and of the fat soluble vitamin A, D, E, and K, that are found primarily in the body fat and variety meat, liver, kidney, heart etc (Judge et al., 1990).

Meat is a very poor source of water soluble vitamin C, except when ascorbate has been added to processed meat products (Judge et al, 1990).

On a nutritional basis a lone, meat is vital to the diet. It is one of the few foods which provide complete protein, as well as being rich source of such essential nutrients as iron, niacin and vitamin B, (National live stock Board, 1975).

2.3. Structure and composition of meat

2.3.1. Structure

The flesh from any meat producing animal is composed of muscle fibers (myofibrils), connective tissue and adipose (fatty) tissue, bone too is an essential part of the gross structure of the meat animal (Hirai et al., 1973).

Muscle tissue: Bundles of fibers or muscle cells held together with connective tissue make up the lean portion of meat. The thickness of the
muscle fibers, the size of fiber bundles, and the amount of connective
tissue binding them together determine the grain of the meat. When the
fiber and bundles are small, the meat is fine and top quality. The
individual muscle fiber is specialized, multinucleated, elongated cell,
varying in size with function and use. The muscle cell has an outer
covering of membrane and an inner filling of small rod-like structure
called fibrillae. The fibrillae are dense protoplasm enmeshed in semi
muscle material (Hirai et al., 1973).

Connective tissue: Although the muscle tissue gives meat its
characteristic appearance and some extent it is flavor and texture, it is the
connective tissue of meat that determines tenderness. Connective tissue
in meat forms the walls of muscle fibers, binds them into bundles,
surrounds the muscles as a membrane and makes up the tendon and
ligaments which attach the muscle to the bones. Some connective tissue
is loose and some is very compact (Hirai et al., 1973).

Fat: Fat is distributed throughout meat in small particles or in large
masses. The pattern formed by uniform distribution of fat in small
“lakes” throughout the muscle or lean flesh is called marbling and
considered an important factor in contributing tenderness and flavor to
muscle tissue. An exterior layer of fat is known as cover fat and serves to
retain the moisture of the muscle or lean tissue and to product the flesh
from the action of microorganisms. Although it is generally agreed that
the presence of fat adds to flavor and possible to the tenderness of meat.
2.3.2. Composition

The chemical and biochemical contents of the muscle are affected by intrinsic and extrinsic factors. The most important intrinsic factors are species, sex, age, and anatomical location of muscle. The extrinsic factors are nutrition, fatigue, fear, pre-slaughter manipulation and environmental conditions before, during and after slaughter. Generally the composition of meat is 75% water, 18% protein, 3.5% soluble non-protein substances and 3% fat (Lawrie, 1991).

The composition of lean meat approximated as 75% water, 18% protein, 4% soluble none protein substances including minerals components and 3% fat (Lawrence and Dekker, 1984).

Meat is made up of protein, fats, minerals (phosphorus, iron and calcium etc.) some carbohydrate, nitrogenous and non nitrogenous extractives, pigments, enzyme, vitamins and water (Hirai et al., 1973).

Protein: The amount of protein in any particular cut of meat is directly related to the amount of lean tissue in it. Hence, the amount of protein in a cut of meat decreases as the fat and bone content increase. The principal proteins in meat are the muscle – cell or protoplasmic protein, actin and myosin, and the extra cellular protein, collagen and elastin which are abundant in connective tissue (Hirai et al., 1973).

Carbohydrates: Two forms of cryohydrates are found in meat, glycogen which is stored mainly in liver and glucose which is found in blood (Hirai et al., 1973).

Pigments: Myoglobin and hemoglobin are two pigments which contribute to the red color of meat. Hemoglobin transports oxygen in
blood stream, but myoglobin holds it in the muscle for contraction. The organ meats have more hemoglobin than skeletal muscles have because of their greater blood supply (Hirai et al., 1973).

Enzymes: Protein – splitting enzymes may be responsible for increasing the tenderness of meat during ripening or aging (Hirai et al., 1973).

Minerals: phosphorus and iron are the chief minerals found in meat. Both are found in different combination of muscle tissue. Potassium is also in muscle fiber or dense material and sodium is more concentrated in fluids (Hirai et al., 1973).

2.4. Quality attributes of meat

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

Variation in beef quality is large and is due to many factors, such as differences in genetic background, sex, age, management, nutrition.

The consumer’s decision to purchase beef is guided by the perception of healthiness and a variety of sensory traits including color, tenderness, juiciness, and aroma or flavor (Verbeke and Viaene, 1999). It is therefore worthwhile considering differences in meat quality at the consumer level, with respect to both sensory traits and health aspects. Meat palatability depends upon such qualities as aroma and flavor, color or appearance, tenderness and juiciness (Hirai et al., 1973).
2.4.1. Color
Color of meat is an important quality attributes that influences consumer acceptance of meat and meat products. (Glitsch, 2000). Consumers prefer bright-red fresh meats. Meat color depends on the relative amounts of three pigments: bright-red oxymyoglobin, purple deoxymyoglobin, and brown metmyoglobin. Fresh meat color is short-lived, so that meat discoloration is inevitable during storage in the presence of oxygen (Zhu and Brewer, 1998).

2.4.2. Tenderness
Tenderness is the most important palatability characteristic. In general, muscles containing the least connective tissue are more tendered; this correlation is not always full proof. Tenderness of meat involves physical, physiological and biochemical factors of muscles that may be broadly divided into ante – mortem and post-mortem factors. The later group may further be separated into ante-rigor mortis and post-rigor mortis. A number of processor has been devised to influence these factors so that an optimum tenderness results. Naturally occurring meat proteolytic enzymes may be utilized under controlled conditions to age or tenderized meat (NIIR, 2004).

2.4.3. Juiciness
This is a very difficult character to assess since it is related not only to the initial impression of wateriness , due to release of meat fluid, but also to the longer lasting effects of fat in the meat stimulating the salivary glands. As a result one might expect some relation between fatness and
juiciness and this in the facts the case. Young animal will give juicy meat (Cooper and Willis, 1984).

The principal sources of juiciness in meat, as detected by the consumer are the intramuscular lipids and the water content, the marbling that are present also serves to 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 a barrier to moisture lost during cooking (Judge et al., 1990).

2.4.4. Firmness and texture

Firmness of the flesh associated with pre and post mortem treatment of cattle and may be connected with water holding capacity. Firmness does not seem to be associated with fatness and well marble carcasses are unlikely to suffer from watery muscle texture and hence coarse texture meat will be tougher to eat (Cooper and Willis, 1984).

2.5. Meat microbiology

There are various forms of meat such as fresh meats, cured meats, sausage products, canned meats, pickled meats and fermented meat products. Because of difference in production, processing and composition of these products different microbiology problems are encountered in them.

The microbiology of meat is highly dependent on the conditions under which animals are reared, slaughtered and processed. Any additional handling such as the preparation of individual cuts may increase the
bacterial load, resulting primarily from contact with contaminated equipment and utensils. Bacteria are still essentially confined to the surface of cuts of meat, and other comminuted meats, such as ground beef which invariably have higher number of microorganisms than non-comminuted meats due to distribution of microorganisms throughout the meat, increased surface area, and release of tissue fluids and use of equipment which are often inadequately cleaned and sanitized (Brown, 1982). It is important to keep microorganisms at low for reasons of aesthetics, public health and product’s shelf – life (Jay, 1996).

2.6. Microorganisms in meat
Meat being a good material for bacterial growth, its quality depend on the initial bacterial contamination. This contamination causes meat deterioration, lower quality, and some time illness may be caused by bacterial pathogens or their toxins.

In Sudan there are studies on the genera of aerobic bacteria included in fresh meat *Bacillus spp.*, *Staphylococcus spp.*, *Pseudomonas spp.*, *Acinetobacter spp.*, *E. coli.*, *Proteus spp.*, and *Salmonella spp.* (Hussiein, 1987; Mohammed 2000).

2.6.1. Staphylococci
Staphylococci may be expected to exist, at least in low numbers in any or all food products of animal origin or in those that are handled directly by humans, unless heat-processing steps are applied to affect their destruction. They have been found in large numbers of commercial foods (Jay, 2000).
*Staphylococcus aureus* is a major pathogen for humans, ranging in severity from food poisoning or minor skin infections to severe life-threatening infection (Jawetz *et al*., 2001). It forms a fairly large yellow colony on rich media and is hemolytic on blood agar, grows at temperatures 15-45°C and at NaCl concentration as high as 15%, produces the enzyme coagulate and should always be considered as a potential pathogen. It is found on the anterior nasal mucosa of 40-50% of healthy adults, in the throats of many of them, in feces of about 20% and on the skin of 5-10% (Duerden *et al*., 1992).

*Staphylococcus aureus* was recognized with five enterotoxin i.e. A, B, C, D and E (Mathieu *et al*., 1992). Staphylococcus enterotoxin A and B, prove to be of public health concern and this calls for an improvement of human hygiene in the food industry (Isigidi *et al*., 1992, Jawetz and Adel, 1990). *Staphylococcus aureus* causes food poisoning, toxic shock syndrome and toxic skin exfoliation and others diseases (Jawetz and Adel, 1990; Cheesbrough, 2000).

Food poisoning due to staphylococcal enterotoxin is characterized by a short incubation period (1-8h) violent nausea, vomiting, diarrhea, rapid convalescence and no fever (Jawetz *et al*., 2001).

The presence of *Staphylococcus aureus* in food is usually taken to indicate contamination from the skin, mouth or nose of workers handling the food, but inadequately cleaned equipment may also be a source of contamination, in Nairobi it was isolated from beef carcasses and minced beef (Ombui *et al*., 1992). In Sudan it was isolated from sausage, minced meat and burger meat (Esmail, 1997).
2.6.2. *Escherichia coli*

*E. coli* is gram negative, lactose fermenting, facultative aerobic short rod. First documented outbreak of *E. coli* food-borne gastroenteritis occurred in the U.S. in 1971 (Jay, 2000). The first outbreaks of food-borne hemorrhagic colitis in the U.S. was in 1982 (Jay, 2000).

Riley *et al.* (1983), *E. coli* O157H7 was found to be the cause of two severe outbreaks characterized by hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). The first case of *E. coli* O157 infection in Italy was reported in 1988 (Caprioli *et al.*, 1990). *E. coli* O157H7 is one of the enterohemorrhagic *E. coli* (EHEC) serotypes that produce verocytotoxins (VTEC). These pathotypes were identified in 1977 and have been associated with several diseases in both humans and animals (Conedera *et al.*, 1995).

*E. coli* O157H7 is able to produce toxins which can cause very serious illnesses in humans, such as HC and HUS (Wang *et al.*, 1996).

The largest recorded food borne outbreak was associated with ground beef, and all raw meat should be considered a possible vehicle for hemorrhagic colitis (Jay, 2000).

Vero cytotoxin producing *Escherichia coli* O157H7 have been isolated from raw beef-burger obtained from a retail source linked to a small community outbreak of O157H7 Vero cytotoxin *E. coli* infection in Wales (Willshaw *et al.*, 1994; Czajka and Batt, 1996) *E. coli* strains are of importance as potential food-borne pathogens and have a wide distribution in food environments in low numbers. As indicator, the presence of *E. coli* in food in sufficient number is taken to indicator the
possibility of fecal contamination and the possible presence of other entero-pathogens such as salmonellae (Jay, 2000).

Enterotoxigenic *E. coli* causes watery diarrhea due to the production of plasmid mediated toxin in infants and adult. It is often referred to as travelers’ diarrhea, whereas enterotoxigenic *E. coli* causes vomiting, fever, and prolonged diarrhea mainly in infants.

2.7. Spoilage of meat and meat products
Meat and meat products represent an important part of the human diet. During the manufacture of these meat products, apart from microbiological changes, other chemical and physicochemical modifications occur, especially dehydration, fermentation of carbohydrates and acidification, development of color, lipolysis and auto oxidation of lipids and proteolysis. These changes are responsible for the organoleptic characteristics of the final products.

Meat spoilage has been defined as the state at which meat become unfit for human consumption (Judge *et al.*, 1990).

Meat spoilage is not always evident and consumers would agree that gross discoloration, strong off-odors, and the development of slime would constitute the main qualitative criteria for meat rejection. In general, spoilage is a subjective judgment by the consumer, which may be influenced by cultural and economic considerations and background as well as by the sensory acuity of the individual and the intensity of the change.
Post-mortem spoilage occurs either by chemical breakdown, for instance the oxidation of fats, lipid oxidation, resulting in rancidity, is one of the most important quality defects of meat or meat product during storage.

Oxidative processes in meat lead to the degradation of lipids and proteins which, in turn, contribute to the deterioration in flavor, texture and color of displayed fresh retail meat (Decker et al., 1995). These biochemical changes in meat limit the shelf life of fresh meat products.

2.8. Meat processing
The original basis for meat processing was preservation by inhibiting or suppressing microbial decomposition. In addition to preventing spoilage, preservation also results in flavorful and nutritious products. Meat processing has now taken on the additional aspects of providing both convenience and variety (Kramlich et al., 1982).

Processed meat means a meat product containing no less than 300 g/kg meat, where meat either singly or in combination with other ingredients or additives, has undergone a method of processing other than deboning, slicing, dicing, mincing or freezing, or includes manufactured meat and cured and/or dried meat flesh in whole cuts or pieces.

Meat was processed as early as prehistoric times, probably drying in the sun and later by smoking over wood. Today, meat is processed with salt, color fixing ingredients, and seasonings in order to impart desired palatability traits to intact and comminuted meat products. Intact meat product includes bacon, corned beef, ham, smoked and pork hocks.
Comminuted meat products include all types of sausage items (NIIR, 2004).

Sausage is one of the oldest known forms of processed meat products and is very popular in many areas. Fresh sausages, e.g. fresh pork sausage, country-style pork sausage, fresh kielbasa (Polish), korr (Swedish), Italian sausage, bratwurst, bockwurst, chorizo (fresh) and thuringer (fresh), are some common examples (Romans et al, 1994).

The cite authors indicate that fresh sausage is a sausage “made from selected cuts of fresh meat (not cooked or cured) and must be stored in a refrigerated (or frozen) state prior to being consumed.” Therefore, adding ‘curing agents” (mainly nitrites and nitrates) to a formula, or not, is the major criterion used to judge whether the product belongs to “fresh sausage” or cured sausage. Also, raw materials of fresh sausage should not be cooked. No typical thermal treatments, such as drying, smoking or cooking, should be applied when making fresh sausages.

2.9. Sausage as meat product

The term sausage is derived from the Latin word (salsus) meaning salt or literally translated, refers to chopped or minced meat preserved by salting. In ancient time, the sausage mixtures were encased in animal intestines and consequently were more or less cylindrical in shape. Sausage is a form of specially prepared, ground or chopped meat in most cases cured; this is enveloped in casing and smoked, fermented or cooked before eating. Sausages are made in numerous forms all around the world, using various types of meat or even fish or combination of the two. (Isidor et al., 1972).
The history of sausage making being some thousands of years ago. It is believed that sausages were made by Chinese in ancient time. And there are reports that Babylonians made and consumed sausage some 1,500 years ago (Pearson and Gillett, 1999).

Europeans have produced many kinds of sausages, particular types originating in specific areas in conformation to climatic conditions. For example, Germany and Northern European countries characterized by periods of cold weather, fresh sausages were developed, while the Mediterranean climate, in Italy and southern France encourages the preparation of dry sausage that kept well (Isidor et al., 1972).

2.9.1. Classification of sausage

Sausage may be roughly divided in to two general groups: raw sausage and heat processed sausage. According to the methods applied in their manufacture, raw sausages may further be subdivided in to two categories: fresh sausage and fermented sausage. Similarly, heat processed sausage are classified in smoked precooked, sausage, emulsion- type sausages and cooked sausages.

a) Fresh sausages are made from fresh meats.

b) Fermented sausages are made from cured or uncured, fermented and often smoked meats but they are not heat processed in any way, they are divided into semidry and dry sausages.

c) Smoked precooked sausages are mostly cured, non fermented products, their shelf life is increased by heating due to partial reduction of their moisture content; they are usually finally cooked before consumption.
d) Emulsion type sausages comprise ready-to-eat products made from comminuted and well-homogenized cured meats, fatty tissue, water and seasonings, usually smoked and slightly cooked.

e) Cooked sausages are ready-to-serve products, basically made from previously cooked fresh or exceptionally cured raw materials, subjected to final cooking after stuffing, with or without additional smoking (FAO, 1985).

2.9.2. Ingredients in sausage making

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

Beef: Although all types of beef are suitable for sausage making; one of the most difficult problems for a sausage maker is to choose satisfactory beef at a reasonable price. Many sausage products can be 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).

Beef quality is, in general, determined by a large number of interdependent extrinsic and intrinsic factors such as breed, condition, sex, exercise, pre-slaughter, treatment, slaughtering conditions and finally, methods of handling, chilling, and degree of aging (Isidor et al., 1972).

Fat: Although beef fat is a valuable sausage material, it requires special care, and precautions must be taken against undesirable changes of fat. Beef fat has quite particular properties. It easily becomes sour or rancid if
improperly handled or if kept under improper conditions it’s far preferable to use the beef fat as fresh as possible without freezing or storing. If, however, the fat must be stored, the storage temperature should not exceed 5°C. 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 fat of aged cows is more suitable for dry sausage making. The amount of added fat depends on the type of sausage and on the fat content of meat used in sausage manufacturing. In general, the total content of fatty tissue should not exceed 25% (Isidor et al., 1972; FAO, 1985).

Seasoning: Seasonings are any ingredients which improve flavor and include spices, herbs, vegetables, nuts, and other substances (monosodium glutamate) etc, while enhancing favor, 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 care and selection of proper spices for sausages are important. The taste of spice generally depends on the flavor of the oil it contains. Spices are usually ground before adding to meat (Isidor et al., 1972).

The most common sausage spice is pepper. Other usual seasonings including spices are: all- spice, bay leaves, cardamom seeds, cayenne pepper, celery seeds, lemon peel, chili pepper, cinnamon, cloves, coriander, and curry powder, garlic, ginger, onion and pimenton sage. In many factories liquid spices are used with considerable success.
Binders: Binders are ingredients used to bind together different sausage components or to hold original or added water in meat. Usually binders have a low nutritive value compared to meat. They are especially used in fresh, cooked, and smoked sausage production (Isidor et al., 1972).

Materials commonly used as sausage binders include special types of potato, rice, corn or other flours. As well as rusk bread, etc. the most widely used protein binders and emulsifiers are milk powder, sodium caseinate, soy protein, etc. powdered skimmed milk gives smooth texture to the product (Isidor et al., 1972).

Sugar: Dextrose, or corn sugar, is best for use in sausages. Dextrose prevents fading in the finished products and does not possess the sweetening characteristic of corn sugar. Sugar is used in some countries as a preservative of meat and for fish. The sugar flavor in meat products is unusual for the average American or European, but it is acceptable or pleasing to many Asians compared to the other people. The range of sugar normally used in beef sausage –making from 0.5 to 2% (Isidor et al., 1972).

Sugar in the curing of meat is common. However, in most instances, sugar is used as adjunct to provide flavor, mask the salt flavor or to provide reservoir from an acid-forming substance (Pearson and Gillett, 1999).

Ice or water: Many products would be dry and unpalatable if only the moisture contained in the meat ingredient were present in the final product. Additional water improves their tenderness and juiciness. Water added as ice also to keep product temperature down during
emulsification. The water added to the burger formulation also serves to replace water that will be lost during processing operations. Thus, by adding water, the yield of finished product can be improved (Forrest et al., 1975).

Coloring: Artificial coloring, particularly of beef sausages cannot be technologically justified. But it is usually permitted. Meat contains enough natural red pigment, and any addition of artificial color to sausage is only a sign of a low technological knowledge or an attempt to cover the use of low quality ingredients. When added, it must be a certified food color and must be declared on the package (Isidor et al., 1972).

2.9.3. Sausage casing
Casings are used to make most sausage as well as some other processed meat. They determine sausage size and shapes (Pearson and Gillett, 1999).

Animal casing/product in animal casing (animal intestine), cost more, but certain products require them. Animal casing are usually edible so that the consumer generally eat the casing along with products.

However, animal casing are less uniform in size tend to be more fragile, and require more care in stuffing. 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 Gillett, 1999).

Cellulose casing include those from cotton bags and those derived from processed cotton linters or wood pulp (Pearson and Gillett, 1999)
2.10. Meat preservation

All foods are once living tissues and are of organic origin. Some food, such as meat and fish, are killed before being distributed to the consumer. Other foods, such as fruits and vegetables, may be stored and distributed in the living state. Because of it is organic nature, food is susceptible to deterioration or spoilage by saprophytic and parasitic microorganisms (Gaman and Sherrington, 1998).

The main aims of food preservation are to prevent loss, autolysis and microbial growth. Methods of preservation, particularly of meat, as indicated by Lawrie (1990), however, different superficially, are alike in that they employ environmental conditions which discourage the growth of microorganisms. They may be grouped in three broad categories based on control by temperature, by moisture, and more directly, by lethal agencies (bactericidal, bacteriostatic, fungicidal, fungistatic and radiation). Thus preservation by some means is absolutely essential for prolonging shelf life, and for the storage of all fresh meat and most processed meat products (Judge et al., 1990).

All the principles of processing and preservation methods of meat are based on some of the factors affecting microbial activity in meat. They include extrinsic factors: such as temperature, relative humidity, oxygen availability, and the physical state of meat; and intrinsic factors: such as water, pH, oxidation-reduction potential of meat, nutrient requirement, and the presence or absence of inhibitory substances and protective tissues.
Judge et al. (1990) and Lawrie (1991) classified the storage and preservation of meat on three basic principles: Temperature controls that by using refrigeration above and below the freezing points, and thermal processing (pasteurization-sterilization). The storage and preservation based on moisture control, which include, dehydration, freeze-dehydration and curing. The last principle based on direct microbial inhibition, such as ionizing radiation, antibiotics and chemical preservatives.

2.10.1. Chemical preservatives

Chemical preservatives, such as potassium sorbate (PS) and sodium benzoate (SB), have been used to extend the shelf-life of processed meats in some Asian countries including South Korea. Nitrite is a reactive chemical and must be used with caution. It is lethal to humans in a dose of approximately 1 g (Fassett, 1973). It acts as a nitrosating agent and under appropriate conditions produces nitroso compounds, some of which are specific and potent carcinogens. Nitrite is converted to nitric oxide, an active nitrosating agent which can react with secondary amines to form carcinogenic nitrosamines.

During the 1970s the safety of cured meats was strongly debated (Krol and Tinbergen, 1974). At issue was the question if preformed nitrosamines were present at all or at levels of concern and if the known levels of residual nitrite represented a risk to human health. The potential problem was recognized and dealt with by the meat processing industry. The use of nitrate was essentially eliminated, the levels of nitrite used
were lowered and much tighter control of manufacturing processes was instituted.

In meat studies, the antioxidants ascorbate and vitamin E has been investigated (Lee et al., 1998). However, the direct application of ascorbate to fresh meat is banned and the potential benefit of a dietary supplement of ascorbic acid to animals, as a means of improving the oxidative stability of meat, is questionable (Morrissey et al., 1998).

Food grade organic acids or their salts have been used to reduce the microbiological load on carcasses and thus preserve quality of fresh meat (Ogden et al., 1996). Nisin has been used to inhibit spoilage of cheese products and vegetable fermentation (Breidt, et al., 1995). However, use of these preservatives may cause outbreaks of allergies or side effects in consumers if they consumed more than certain amounts in their foods. Currently, consumers prefer to take “healthier foods” which are low-fat, low-sodium, functional food products. These foods will require a reduction in the use of chemical preservatives, and more use of natural ingredients in the processing of food products.

2.11. Definition of honey

Honey is complex substance made when the nectar and sweet deposits from plants and trees gathered, modified and stored in the honey comb by honey bees. Honey is a complex biological mixture that consists mostly of inverted sugars, primarily glucose and fructose. It has antibacterial and antifungal properties and will not rot or ferment when stored under normal conditions. However, honey can crystallize with time. Crystallized honey is not damaged or defective in any way, for human
use, but bees will automatically remove crystallized honey from their hive and discard it, since they can only use liquid honey. According to the Encyclopedia Britannica (1991) honey is a sweet viscous liquid food, dark golden in color, produced by bees from the nectar of flowers and plant honey dew. Honey contain water (13-20%), two chief sugars: fructose (40-50%) and glucose (32-37%), small amounts of sucrose (<2%) and mineral constituents (ash less than, 1%). Honey also contains numbers enzymes, vitamins in small amounts, free acids, various plant coloring materials and trace elements (Fe, Cu, Zn, Sn, etc). Honey is one of the most easily assimilated foods. It was almost the only source of sugars available to the ancients and was valued for its medical benefits. In Egypt it was employed as an embalming material. In many countries it was and still used to preserve fruit and to make different kinds of cakes, sweet meats and also alcoholic drinks. Honey is mentioned in the Bible and Koran and in Greek mythology; nectar was treated as drink of the gods. So honey is a natural products derived from plants and elaborated by the honey bees. It is of great importance and high merit for humans (Braziewicz et al., 2002).

2.12. Chemical properties of bee honey
Honey is basically composed of carbohydrates like monosaccharide and oligosaccharide (60-85%) which vary in regular forms with moisture content (13-20%), low proportions of inorganic and organic material such as protein and polysaccharide, and high floral pollen contents (White, 1969). Moisture content has an influence on honey color, viscosity flavor, density and refractive index and its Sone of the most
important physicochemical parameters for the analysis, conservation and stability of foods in general (Mateo and Bosch-Reig, 1997; Sancho et al., 1991).

### 2.13. Uses of bee honey

Honey is most commonly in its unprocessed state, i.e. liquid, crystallized or in the comb. In these forms it is eaten as food, incorporated as an ingredient in various food recipes or taken as a medicine. However, honey is considered a food only in a few societies such as those of the industrialized countries in Europe, North America, Latin America, North Africa, the Near East and increasing in Japan. In most parts of Africa it is used for brewing honey beer and to a much lesser degree, as medicine. In most of Asia it is generally regarded as a medicine or at most an occasional sweet. High per capita consumption in industrialized nations does not reflect the consumption of unprocessed honey per person but includes a very large quantity of honey used in industrial food production, i.e. as a food ingredient. Honey is largely used on a small scale as well as at an industrial level in baked products, confectionary, candy, marmalades, jams, spreads, breakfast cereals, beverages, milk products and many preserved products enhancing their quality characteristics (Krell, 1996).

### 2.14. Honey as preservative

Traditionally, its use in food has been as sweating agent. However, several aspect of its use indicates that honey also functions as a food preservative. Honey contain a number of components to act as preservatives these include $\alpha$-tocopherol, ascorbic acid, flavonoids, and
other phenolics and enzymes such as glucose oxidase, catalase and peroxidase (Crane, 1975; Ferreres et al., 1993; Loyrish, 1974) its suggested that any of these substance owe their preservative properties to their anti oxidative activity (Cerutti, 1994).

Food processors are known to use honey in many different food products thus: sweetness, functional advantages (viscosity, flavor hygroscopic miscibility spread ability and color) and as natural appeal (Wilson, 1976).

Numerous workers (Dawson, 1998; Antony, 2006) have implicated honey in meat and meat products such as hams, bacon and sausages. Dawson (1998) reported that application of honey improved meat and spice flavors when mixed with brine. Honey can bind ingredients and also a culture substrate in cured products. The authors also reported that honey improved the cooking yield in poultry meats since meat products are sold on it is weight hence, yield is vital to processors.

2.15. Quality attribute of honey

Honey samples that are available commercially, differ in quality on account of various factors like geographical, seasonal and processing conditions, floral source, packaging and storage period, moisture content varied from 17 to 22.6% Brix from 76 to 81.5%, pH from 3.62 to 5.46, apparent viscosity from 1.76 to 13.8 Pascal sec. acidity from .03 to .15 total reducing sugars from 61.3 to 72.6 and sucrose from 1.2 to 5.7% (Anupama et al., 2003).

The sensory properties such as color, aroma and taste of honey vary according to the geographical honey in Indian generally of assorted type
as it is collected from regions having different climatic condition and very often from forests or wild flower source. Hence, they have varied sensory and physico-chemical properties (Anupama et al., 2003). Another quality issue in the honey industry is the authentication of honey based on its floral origin. Many researchers have used pollen analysis to distinguish honey types based on its floral origin (Singhal et al., 1997)

2.16. Safety of honey

2.16.1. Reaction following topical use

Effem’s African studies (1988 and 1993) on the treatment of serious ulcers with daily application of honey did not reveal any allergies or other adverse effects over the several weeks of treatment.

Phuapradit et al. (1997) report of the use of honey in abdominal wounds did not reveal any adverse effects, such as allergies from the use of honey.

Nadyisaba et al (1993) studied the topical use of honey in 40 patients with major wounds. They reported only one patient discontinued treatment due to a burning sensation on application of the honey.

Wood et al (1997) also reported that some patients with leg ulcers experienced a burning sensation when honey was applied.

Emarah (1982) reported that when honey was used to treat eye conditions, patients experienced stinging and redness the eye, which was not severe enough to warrant cessation for the treatment. No reasons for the burning sensation were reported by the authors of these reports.
2.16.2. Honey allergies

While apparently uncommon, allergies to honey have been reported and can involve reactions varying from cough to an anaphylaxis (Kiistala et al., 1995).

The incidence of honey allergy was reported by Bauer et al. (1996). To be 2.3% of a group of 173 patients with food allergies among patients with confirmed honey allergy, 17% had suffered anaphylaxis and 30% asthma (Bauer et al., 1996).

The authors reported that the protein responsible for honey allergy is derived from proteins secreted by bees and from proteins derived from plant pollens. Individuals with inhalation allergies to particular plant (e.g. members of the composite family) many times demonstrate allergies to honey produced by bees for aging on these plants (Bousquet et al., 1984 and Hebbling et al., 1992).

2.17. Anti microbial properties of honey

The anti microbial properties of honey have been known for thousands of years (Zumla et al., 1989). Honey has been studied to clarify which components are responsible for antagonistic activity against pathogenic microorganisms including Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus (Cooper et al., 2002 and Willix et al., 1992).

The anti microbial activity of honey has been attributed to hydrogen peroxide, osmolarity, acidity aromatic acids and phenolic compounds (Molan, 1992 a, b) the high osmolarity of honey is due to high content of sugars (average over 85% of honey) including fructose, glucose, maltose, sucrose and other types of carbohydrate (White, 1975). Hydrogen
peroxide, by glucose oxidase originating from the honey bees (White et al., 1963), honey was tested against six food borne pathogens by (Taomina et al., 2001) it was shown that varying levels of anti microbial activity of honey were present depending on the variety of honey. The activity of honey was attributed to not only hydrogen peroxide but also anti oxidant compounds in honey. Recently, propolis, a mixture of viscous compounds including herbal poly phenolies found in honey and honey combs, showed anti staphylococcus activity (Lu et al., 2005). It was reported that poly phenolies of propolis inhibited glucosyl transferase activity and the growth of *streptococcus mutans*, which is associated with dental caries (Koo et al., 2002 and Park et al., 1998).

In New Zealand, Manuka honey has been consumed as a medicinal product due to it is high level of anti microbial activity (Allen et al., 1991). Manuka honey has been reported to exhibit anti microbial activity against pathogenic bacteria including multi- drying resistant *S. aureus* strains and *Helicobacter pylori* from gastric ulcers (Alsomal et al., 1994; Cooper et al., 2002; Willix et al., 1992). An alternative to traditional antibiotic therapy, gama irradiated manuka honey is commercially available as a topical ointment for burned or wounded skin to protect from opportunistic bacterial infections or cure chronic wounds (Cooper et al., 2002). The unusually high antimicrobial activity of manuka honey has been attributed to aromatic acids or phenolic compounds derived directly from the honey sources, the manuka bushes (Weston et al., 1999).
2.17.1. **Explanation of anti bacterial activity**

The numerous reports of investigations which have established the nature of the anti bacterial honey are cited in a comprehensive review of this subject. Brief summary of established is given here:

2.17.1.1. **Osmotic effect**

Honey is saturated or super-saturated solution of sugar, 84% being mixture of fructose. The water content is usually only 15.21% by weight. The strong interaction of these sugar water molecules leaves very few of the water molecules available for microorganisms. This is what is measured as the water activity (aw): mean values for honey have been reported from 0.562 to 0.620. Although some yeasts can live in honeys that have high water content causing spoilage of honey, the aw. of ripened honey which below 17.1% is too low to support the growth of honey bacteria, occurring in the water content. Many species of bacteria have their growth completely inhibited if the aw. is in the range 0.49-0.99 these values correspond to solution of a typical honey (aw. of 0.6 undiluted) of concentration from 12% down to 2% (v/v). On other hand, so many microorganism have their maximum rate of growth when the aw is 0.99 so inhibition by the osmotic effect (water- with drawing) so the effect of diluted solutions of honey obviously depends on the species of bacteria (Molan, 1992).

2.17.1.2. **Acidity**

Honey is characteristically quite acidic and its pH being between 3.3 and 4.5 which is low inhibiting to many animal pathogens. The optimum pH for growth of these species normally 7.2 and 7.4 the minimum pH values
for growth of some common wound–infecting species is: *Escherichia coli* 4.3, *Salmonella spp* 4.0 *Pseudomonas aeruginose* 4.4, *Streptococcus pyogenes* 4.5 thus in undiluted honey the acidity is significant antibacterial factor. But if honey is diluted by the body fluids which are well buffered, the pH will not be so low and the acidity of honey will not be effective inhibitor of many species of bacteria (Molan, 1992).

2.17.1.3. Hydrogen peroxide

The major antibacterial activity in honey has been found to be due to hydrogen peroxide produced enzymically in the honey. The glucose oxidase enzyme is secreted from the hypopharyngeal gland of bee into the nectar assist in the formation of honey from the nectar (Molan, 1992). The hydrogen peroxide and acidity produced by the reaction

Glucose +H₂O + O₂ →↑gluconic acid + H₂O₂; which serve to preserve the honey. The hydrogen peroxide produced would be of effect as only during the ripening of honey.

Full strength honey has a negligible level of hydrogen peroxide because this substance is short-lived in the presence of the transition metal ions and ascorbic acid in honey which catalyze the decomposition of oxygen and water. The enzyme has been found to be practically inactive in full strength honey; it gives rise to hydrogen peroxide only when honey is diluted. This is because the acidity produced in the action of the enzyme drops the pH to a point which is too low for the enzyme to work any more. On dilution of honey the activity increase by a factor of (2.500-50.000) thus giving a “slow release” anti septic at a level which is anti
bacterial but not tissue damaging (Adock, 1962; White et al., 1963; Cohen and Hoehstein, 1962).

2.17.1.4. Photochemical factors
The evidence for the existence of other anti bacterial factors is mainly that, the peroxide system does not account for all of the observed anti bacterial activity. But there have reports of isolation of anti bacterial substance from honey that are not hydrogen peroxide, it has been found that heating honey which inactivates the glucose oxidase causes loss of activity against some species whilst it is relained against others. Although the stability of the enzyme varies in different honeys, there have been reports of honeys with stability well excess of this variation showing, that there must be on additional anti bacterial factor involved. The most direct evidence for the existence of non- peroxide anti bacterial factors in honey is seen in the reports of activity persisting in honey treated with catalase to remove the hydrogen peroxide activity.

Several chemicals with anti bacterial activity have been identified in honey by various researchers: pinocembrin, terpenes, benzyl, alcohol, hydroxyl benzoic acid (syringic acid), methyl 3.5 dimethoxy-4- hydro benzoate, 3.4- methyl trimethoxy benzoic acid, 2- hydroxyl -3- phenyl propionic acid and 14-dihydroxyl benzene. However, the quantities of these present were far too low to significant amount of activity (Molan, 1992).
2.18. Honey as Antioxidant

Honey as a source of antioxidant has been proven to be effective against deteriorative oxidation reactions in food, caused by light, heat and some metals (Mckibben and Engeseth, 2002), such as enzymatic browning of fruit and vegetables (Chen et al., 2000), lipid oxidation in meat (Nagai et al., 2006), and inhibit the growth of food borne pathogens and food spoilage (Taomina et al., 2001). Over all honey serves as source of natural anti oxidants (Al-Mamary et al., 2002; Aljadi et al., 2004; Antony et al., 2000; Berette et al., 2005; Cheldof et al., 2002; Kücük et al., 2007; Nagai et al., 2001) which play an important role in food preservation and human health by combating damage caused by oxidizing agents e.g. oxygen, namely reducing the risk of heart disease, cancer, immune system decline, cataracts, different inflammatory processes, etc (The national Honey Board, 2003).

The antioxidants present in honey include both enzymatic: catalase (Schepartz, 1966) glucose oxidase, peroxidase (Loyrich, 1974) and non enzymatic substances: ascorbic acid, α-tocopherol (Crane, 1975), carotenoids, amino acids, proteins, organic acids, Millard reaction products (Al-Mamary et al., 2002; Aljadi et al., 2004; Baltrusaityte et al., 2007; Cheldof et al., 2001; Cheldof et al., 2002; Schramm et al., 2003; The National Honey Board 2003) and more than 150 poly phenolic compounds including flavonols, flavonoids phenolic acids, catechins and cinnamic acid derivatives.

In the literature, several studies for the identification and quantification of anti oxidant components of honey bee products have been reported (Buratti et al., 2007; Ferreres et al., 1994; Gheldof et al., 2002).
Many methods for determining the anti oxidative activities in honey have been used, e.g. determination of total phenolic content (Beretta et al., 2005), radical formation and following scavenging as in 2,2-diphenyl-1-picryl hydrazyl (DPPH) and super oxide radical – scavenging activity measurement (Aljadi et al., 2004; Chen et al., 2000; Gheldof and Engeseth, 2002; Gheldof et al., 2002) the ferric reducing anti oxidant power (FRAP) assay (Aljadi and Kamaruddin, 2004) and enzymatic or non enzymatic measurements of lipid per oxidation inhibition (Chen et al., 2000; Mekibben and Engeseth, 2002; Nagai et al., 2001).

Although it has already been demonstrated that it has anti oxidant activity and different anti oxidant compounds nothing is reported about the different contributions of the entire honeys and their phenolic extracts to those properties.
CHAPTER THREE  
MATERIAL AND METHODS

3.1. Materials
3.1.1. Food materials
The fresh beef meat was obtained from Khartoum North butcher shops. The beef meat was transferred immediately stored frozen at -18 °C in freezer Food Research Center, Shambat.

Bee honey was obtained from Department of Crop Protection, Faculty of Agriculture, University of Khartoum and stored at room temperature. Spices, salt, sugar, potato were obtained from local markets.

The additional fat needed for the sausage formulation was obtained from the butcher shops. However, for ease of the calculation, only uniform rendered fat, free of protein and moisture content was used.

3.1.2. Casings
Animal casings were obtained from Khartoum North butcher shops.

3.2. Methods
3.2.1. Raw materials preparation
3.2.1.1. Meat preparation
Stored beef was allowed to thaw over night in a refrigerator at 4 ±2 °C and sliced then ground through an 0.375 inch, plate using a meat grinder. and stored refrigerated at 4 ±2°C.
3.2.1.2. Bee honey analysis

Bee honey was taken and analyzed for protein, ash and moisture content according to A.O.A.C. (1995). Bee honey and raw meat were analyzed for protein, moisture, fat, and ash according to A.O.A.C. (1995) the values tabulated in table (1) were needed for formulation of the different sausage treatments recipes.

3.2.2. Sausage preparation

A basic sausage formula shown on table (2) was used in the preparation of sausage. Minced meat, salt, minced fat, potato, spices and half of calculated ice water were chopped using a Meat Chopper for about 4 minutes there after the rest of the ingredients were added. The mixture was well homogenized. Then the entire mass was divided in to four equal parts. Each part was assigned randomly to one of the bee honey treatments i.e. 0, 2.5, 5, and 7.5% (Table 3). After homogenization the mixture was transferred to a manual stuffer to be stuffed into animal casing, linked at lengths of 10cm, packed in poly ethylene bags and stored refrigerated for up to 9 days and assessed immediately after processing (0 day) and at an intervals of 3, 6 and 9 days post storage.

3.2.3. Methods of analysis

3.2.3.1. Moisture determination

The moisture content was determined according to the method of A.O.A.C. (1995). The moisture content was calculated as shown below:

\[
\text{Moisture content}\% = \frac{W_1 - W_2 \times 100}{W_s}
\]

Where:
- \(W_1\) = weight of sample before drying.
- \(W_2\) = weight of sample after drying.
- \(W_s\) = weight of sample
Table 1: Proximate composition (%) and pH of raw beef meat and bee honey

<table>
<thead>
<tr>
<th>Material</th>
<th>Moisture</th>
<th>Protein</th>
<th>Fat</th>
<th>Ash</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef meat</td>
<td>75.0</td>
<td>19.07</td>
<td>2.03</td>
<td>1</td>
<td>6.8</td>
</tr>
<tr>
<td>Bee honey</td>
<td>13.4</td>
<td>0.80</td>
<td>-</td>
<td>.38</td>
<td>3.3</td>
</tr>
</tbody>
</table>

- Trace

Table 2: Basic sausage formula.

<table>
<thead>
<tr>
<th>Component</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>14</td>
</tr>
<tr>
<td>Fat</td>
<td>10</td>
</tr>
<tr>
<td>Moisture</td>
<td>63</td>
</tr>
<tr>
<td>Starch</td>
<td>8</td>
</tr>
<tr>
<td>Salt</td>
<td>1.5</td>
</tr>
<tr>
<td>Spices</td>
<td>1.4</td>
</tr>
<tr>
<td>Binder</td>
<td>2.0</td>
</tr>
</tbody>
</table>
Table 3: Sausage formulation for all treatments.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Level of bee honey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>Beef meat</td>
<td>1125</td>
</tr>
<tr>
<td>Bee honey</td>
<td>0</td>
</tr>
<tr>
<td>Potato</td>
<td>125</td>
</tr>
<tr>
<td>Fat</td>
<td>125</td>
</tr>
<tr>
<td>Water/ice</td>
<td>250</td>
</tr>
<tr>
<td>Salt</td>
<td>23</td>
</tr>
<tr>
<td>Sugar</td>
<td>4.5</td>
</tr>
<tr>
<td>Black pepper</td>
<td>4.5</td>
</tr>
<tr>
<td>Nut meg</td>
<td>1.25</td>
</tr>
<tr>
<td>Garlic</td>
<td>5</td>
</tr>
<tr>
<td>Milk powder</td>
<td>32.25</td>
</tr>
<tr>
<td>Coriander</td>
<td>4.5</td>
</tr>
<tr>
<td>Chinese kabab</td>
<td>1.25</td>
</tr>
</tbody>
</table>

* Wight in gram
3.2.3.2. Crude protein determination

The protein content of the samples was determined by the micro kjedahl technique according to the A.O.A.C. method (1995). 0.2g of sample was weighed accurately into micro-kjedahl flask, two hundreds milligrams of catalyst mixture and 3.5 ml of concentrated sulphuric acid were added, the sample content were heated on an electric heater for about 2 hr until the digestion was completed. and cooled, then the content was placed into the distillation apparatus. Twenty milliliters of 40% NaOH were added the ammonia evolved was received in 10 ml of 2% boric acid solution. The trapped ammonia was titrated against HCl (0.02N) using universal indicator (methyl red + bromo cresol green), the total nitrogen and protein were calculated using the following equation.

\[
N\% = \frac{\text{Volume of HCl} \times N \times 14 \times 100}{\text{Sample Weight} \times 1000}
\]

\[
CP\% = N\% \times 6.25
\]

Where:
CP\% = crude protein
N\% = crude nitrogen.
N= normality of HCl.
14= equivalent weight of nitrogen.

3.2.3.3. Fat determination

Total fat was determined according to the A.O.A.C. methods (1995). Three grams of sample was extracted with petroleum ether (BP 60-80°C) for 8hr. in soxhlet apparatus. The fat content was calculated according to the following equation.
\[
\text{Fat\%} = \frac{(W_2 - W_1) \times 100}{\text{Sample weight}}
\]

Where:
- \( W_1 \) = weight of empty flask
- \( W_2 \) = weight of flask with oil

3.2.3.4. Ash determination

The ash content of sample was determined according to the A.O.A.C. method (1995). Three grams of sample was weighed into a clean and dry porcelain crucible and placed in a temperature controlled furnace at 600\(^\circ\)C for complete ashing. The crucible with ash was transferred directly to desiccator, cooled, weighed and the ash content calculated as shown below:

\[
\text{Ash \%} = \frac{(W_1 - W_2) \times 100}{\text{Sample weight}}
\]

Where:
- \( W_1 \) = weight of crucible with ash.
- \( W_2 \) = weight of empty crucible.

3.2.3.5. Peroxide Value (PV)

Peroxide value of the beef sausage sample was determined according to A.O.A.C. (1995). One gram of extracted was accurately weighed into 250 ml conical flask. Thirty ml of a mixture of glacial acetic acid and chloroform (3:2) were added to the conical flask. One gram of saturated solution of potassium iodide was added. The flask was vigorously shaken for 1 min. and kept away from the light for exactly 5 min. then titrated with accurately standardized solution of 0.01N sodium thiosulphate. Titration continued until the yellow color almost disappeared. A 0.05 ml of starch indicator solution was added. Titration was performed with continuous shaking till the end point. A drop of thiosulphate was
added until the blue color has just disappeared. PV was calculated as shown below:

\[ PV \% = \frac{(A-B) \times N \times 1000}{S} \]

Where:
- B = reading of blank in mls
- A = reading of sample mls.
- S = weight of oil sample.
- N = normality of sodium thiosulphate.

### 3.2.3.6. pH Measurement

Five grams of the sample were placed in a blender jar and 50 ml of distilled water were added. The mixture was blended at high speed for 1 min. The pH of the mixture was measured using a digital pH meter (model 210, HANNA instruments microprocessor pH meter). The pH meter was calibrated with standard buffers (4, 7) before pH measurement was taken.

### 3.2.3.7. Microbiological Analysis

#### 3.2.3.7.1. Preparation of Serial Dilution

Ten grams of meat sample were weighed aseptically and added to conical flask containing 90 ml sterile 0.1% peptone water and sufficiently shaken for homogenization. This dilution was referred as stock solution (dilution 10^{-1}). One ml of stock solution was pipetted aseptically, with sterilized pipette into 9 ml sterile peptone water (dilution 10^{-2}) and serial decimal dilutions up to 10^{-6} were prepared as described by (Harrigan 1998).

#### 3.2.3.7.2. Determination of Microbial Load of Beef Sausage

Total viable count was carried out using the pour-plate methods as described by Harrigan (1998). One ml of each dilution was transferred
aseptically sterile Petri dish. To each dilution 10-15ml of melted and cooled (45°C) Plate Count Agar were added. The inocula were mixed with medium and allowed to solidify. The plates were then incubated aerobically in an incubator at (37°C) for 48hr. A colony counter was used to count the viable bacterial colonies. The count was expressed as colony-forming unit (cfu) per gram.

3.2.3.7.3. Determination of coliform bacteria
3.2.3.7.3.1. Presumptive coliform test
One ml of each of three first dilutions (10⁻¹, 10⁻² and 10⁻³) was inoculated aseptically in triplicates of 9 ml sterilized MacConkey broth using the three-tube technique with Durham tubes. The tubes were incubated at 37°C for 48 hours. Positive tubes gave acid and gas in the Durham tubes.

3.2.3.7.3.2. Confirmed coliform test
All tubes of the dilutions showing gas fermentation in 24 hours were subjected to the confirmation test using brilliant green bile lactose broth fermentation tubes with Durham tubes, and then incubated at 37°C for 48 hours. The most probable number (MPN) was recorded. The most probable number (MPN) Tables were used to record the coli forms number (FAO, 1992).

3.2.3.7.3.3. Faecal coli form test
At least 3 loops full of each confirmed positive tube were sub-cultured into EC broth medium and then incubated at 44.5°C for 24 hours. Tubes showing any amount of gas production were considered positive. The most probable number (MPN) was recorded.
3.2.3.7.3.4. Differentiation of faecal coli form test
For further confirmation of faecal coliform, tubes giving positive reaction at 44.5°C for 24 hours were streaked on Eosin Methylene Blue (EMB) agar. Colonies with green metallic sheen confirmed a positive test.

3.2.3.7.4. *Staphylococcus aureus*
0.1 ml. was transferred from suitable dilutions by means of sterile pipette and spread on solidified manitol salt agar plates. The plates were incubated at 37°C for 36 hours. Then the plates were examined. Presumptive coagulase- positive *Staphylococci*. Produce colonies with bright yellow zones whilst coagulase – negative *Staphylococci* are surrounded by a red or purple zone. Suspect colonies were picked off and were sub cultured in a Nutrient Broth media to be ready for carry out the coagulase test.

3.2.3.7.4.1. Tube coagulase test
Half ml of diluted fresh human plasma was added to small test tube, and then 0.5 ml of an 18-24hrs broth culture was added and incubated at 37°C. Coagulase was examined after an hour then at intervals up to 24 hrs.

3.2.3.7.5. Psychotropic bacteria
One ml was transferred aseptically from every dilution to sterile Petri dish. Fifteen milliliter of sterile nutrient agar were added to every Petri dish after the incoulement was mixed with the medium and allowed to be
solid. The plates were incubated for 4°C at 2-3 days. Then by using a colony counter the viable bacterial colonies were counted.

3.2.3.8. Sensory evaluation
Ten member sensory panel consisting of M.Sc. and B.Sc. student of food science and technology Department, Faculty of Agriculture, University of Khartoum, semi-trained according to the procedure of (Cross et al., 1978). The panel evaluated the cooked sausage sample of the different treatment for color, aroma, taste, texture, juiciness and overall acceptability. A hedonic scale of 1-7 (7=extremely like, 1= extremely dislike), was used. Panelists received samples which were randomly numbered. Water at room temperature was made available for the panel to clean their palate from the previous sample taste.

3.2.3.9. Statistical analysis
The data collected were subjected to analysis of variance and whenever appropriate the mean separation procedure of LSD was employed (Steel and Torrie, 1980). The SAS program (SAS institute, 1988) was used to perform the GLM analysis.
4.1 Proximate compositions

The effect of bee honey on the proximate composition of beef sausage is shown on table (4). The highest moisture content was reported for the beef sausage without bee honey (0%). Obviously as the level of bee honey added to beef sausage increased the moisture content of the beef sausage decreased. The result was consistent with the report of Tuley (1989) who found a reduction in the moisture content of meat products treated with honey. This assertion was also confirmed by Antony et al. (2006), Belewu and Morakinyo (2009) that the reduction of the moisture content could be due to the effect of high osmotic pressure and low water activity of the bee honey.

The 7.5% treatment had the highest protein content and the least value was reported for the control (0%). The 2.5 and 5% treatments had similar protein contents. The result of Dawson and Mathew (1998) showed that the increase in protein content of meat extended with honey may be due to the enzyme present in the bee honey. This was also supported by the result of Antony et al. (2006) who reported that the primary dilution of meat and turkey products by the addition of honey improved the protein content.

Honey treated and untreated beef sausage samples showed no significant difference (P>0.05) in the fat content. This was supported by the result of Haffjee and Mossa (2001) that nutritionally, honey does not add any fat
Table 4: Effect of bee honey on the proximate composition (%) of beef Sausage.

<table>
<thead>
<tr>
<th>Bee honey levels</th>
<th>Moisture</th>
<th>Protein</th>
<th>Fat</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>72.43A±1.22</td>
<td>17.55B±2.20</td>
<td>7.79A±2.36</td>
<td>2.02A±0.33</td>
</tr>
<tr>
<td>2.5%</td>
<td>70.77AB±2.51</td>
<td>19.00BA±1.05</td>
<td>9.30A±2.26</td>
<td>2.10A±0.33</td>
</tr>
<tr>
<td>5%</td>
<td>65.43BC±5.48</td>
<td>19.46A±0.38</td>
<td>8.91A±2.76</td>
<td>2.20A±0.31</td>
</tr>
<tr>
<td>7.5%</td>
<td>64.22C±6.74</td>
<td>20.55A±0.66</td>
<td>8.60A±2.53</td>
<td>2.30A±0.40</td>
</tr>
</tbody>
</table>

*A-C= means bearing different superscript capital letters in the same column are significantly different (P<0.05).
* n=6
to the meat and turkey products studied. The total ash content of the all treatment showed no significant difference (p> 0.05). This was supported by Mouteria (2006) that due probably to the poor content of ash in bee honey.

Initially and throughout the storage period table (5) tested there were no significant difference (P>0.05) in moisture, fat, ash, and protein contents.

4.2 pH measurement
The effect of bee honey on the pH is shown on fig. (1). There was no significant difference (P>0.05) between all treatments. The pH from the different treatment fall with in the pH range (5.32- 5.40).

Gerrard (1977) reported that the meat from freshly killed cattle will usually have pH 6.5 to 6.8 (slightly acid); but it falls to the lowest level, around 5.5 with in 48 hrs post mortar. Generally, pH values reported in this study agree with that reported by peter et al (2005) who found the all ground beef patties showed pH values in the range 5.3-5.6.

The effect of storage period on the pH of beef sausage is displayed on fig. (2). The pH of beef sausage showed a slight change with the storage period tested. Except for day 6, the pH of beef sausage for the other storage period i.e. 0, 3 and 9 days all were similar pH.

4.3 Peroxide Value (PV)
PV value of treated and untreated beef sausage is presented in fig. (3). PV value of the untreated (0%) beef sausage was higher than all the treatments tested (P<0.05).
Table 5: Effect of the storage period on proximate composition (%) of beef sausage

<table>
<thead>
<tr>
<th>Days of storage</th>
<th>Moisture</th>
<th>Protein</th>
<th>Fat</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 day</td>
<td>68.25A±5.56</td>
<td>18.88A±1.55</td>
<td>9.31A±2.96</td>
<td>2.34A±0.17</td>
</tr>
<tr>
<td>6 days</td>
<td>68.19A±5.74</td>
<td>19.40A±1.73</td>
<td>7.98A±1.50</td>
<td>1.99A±0.39</td>
</tr>
</tbody>
</table>

*A-C= means bearing similar superscript capital letters in the same column are not significantly different (p> 0.05).
*n =12
Fig 1: Effect of beef honey on the pH of beef sausage.
Fig 2: Effect of storage period on the pH of beef sausage
Fig. 3: Effect of bee honey on the PV of beef sausage.
However it’s noted that as the level of bee honey added to beef sausage increased the PV value of beef sausage decreased significantly (P<0.05) of the lowest PV (less than 1 meq/Kg) was observed in the beef sausage treated with 7.5% bee honey. The decrease in PV with the increase in bee honey level may be due to antioxidants found in bee honey. This result agree very well with Antony et al. (2002) who reported the effect of dry honey on oxidation of turkey breast meat, and they showed that addition of up to 15% dry honey inhibited the development of oxidation compounds in cooked turkey meat. Also this result is supported by Chen et al. (2000) who concluded that addition of honey reduced lipid oxidation in meat.

The effect of storage period on PV of beef sausage is shown in fig. (4). The highest PV was found on day 0 where as beef sausages stored for 3, 6 and 9 days had similar peroxide values.

4.4 Microbial analysis
4.4.1 Total Viable Count (TVC)

The effect of bee honey on the Total Viable Count is presented in fig. (5). Bee honey, regardless of the level, reduced substantially the TVC of beef sausage. Bee honey on 2.5, 5 and 7.5% level reduced in a 6.82, 6.64 and 4.98 log reduction in TVC respectively fig. (5). Clearly the effect of bee honey increased with the increase in the level of bee honey. Compared with the other treatments (0, 2.5 and 5%) 7.5% treatment gave the lowest TVC. The report of Lee et al. (1998) acknowledges to the results reported here in that honey may be useful for inhibiting bacterial growth in either meat products that are less stable or require longer storage time.
Fig 4: Effect of the storage period of the PV of beef sausage
Fig 5: Effect of the bee honey on the TVC (log 10 cfu/g) of beef sausage.
As shown on fig. (6). The TVC showed similar values over the entire storage period.

4.4.2. Total coli form
As shown on fig (7). The highest total coli form was reported for the beef sausage without bee honey (0%). Obviously as the level of bee honey added to beef sausage increased the total coli form decreased. The poor total coli form growth in the honey treated beef sausage samples (2.5, 5 and 7.5%) supported the findings of Molan (1992) that honey treated meat and poultry products resulted in lower levels of bacterial growth. Also Belewu and Morakinyo (2009) reported that the 15% honey treated cheese sample had no bacterial growth throughout the experimental periods.

The total coli form for all treatment during storage period is shown in fig (8). Initially (0day) the total coli form was reported highest value and with increased storage period the total coli form decreased significantly (P<0.05).

4.4.3 E. coli
The effect of bee honey on the E. coli is shown on fig (9) E. coli decreased significantly (P<0.05) with the increase of bee honey levels. Comparison of untreated and treated beef sausage show that untreated had higher E. coli than treated ones. Regardless of the treated and untreated beef sausage E. coli decreased with the increase in the storage period fig (10). However at day 0 the E. coli was reported the highest value while at day 9 were reported the lowest value with significant difference (P<0.05).
Fig 6: Effect of the storage period on the TVC (log 10 cfu/g) of beef sausage.
Fig 7: Effect of bee honey on the Total coliform count of beef sausage.
Fig 8: Effect of the storage period on the Total coliform count of bee sausage.
Fig 9: Effect of bee honey on the *E. coli* of beef sausage.
Fig 10: Effect of the storage period on the *E. coli* of beef sausage.
4.4.4 *Staphylococcus aureus*

As shown on fig (11). The highest *Staphylococcus aureus* was reported for the beef sausage without bee honey (0%) while the lowest *Staphylococcus aureus* was reported for the 7.5%, however the 2.5 and 5% bee honey treated beef sausage had similar (P>0.05) *Staphylococcus aureus* count.

The effect of the storage period on *Staphylococcus aureus* as shown on fig (12). Obviously there was no significant difference (P>0.05) of all treatments.

4.4.5. Psychotrophic bacteria

As shown on fig (13), obviously the treatment (0%) noted the highest count and 7.5% was reported the lowest count while the 2.5 and 5% treatment was reported the similar count.

Initially and throughout the storage period the psychotrophic count fig (14). Showed similar values over the entire storage period.

4.5. Sensory evaluation

The effect of bee honey on the sensory properties of beef sausage is shown on table (6). Addition of bee honey to beef sausage up to 5% level had no effect on the measured parameters i.e. color, aroma, taste, texture, juiciness and over all acceptability as panelists were notable to detect any changes (P>0.05) in these parameters with the increase in bee honey levels. On the other hand the panelist rated the 7.5% bee honey treatment always superior to the other bee honey treatment i.e. 0, 2.5 and 5% in all the measured parameters.
Fig 11: Effect of bee honey on the *Staphylococcus aureus* of beef sausage.
Fig 12: Effect of the storage period on the *Staphylococcus aureus* of beef sausage.
Fig 13: Effect of the bee honey on the Psychrotrophic bacteria of beef sausage.
Fig 14: Effect of the storage period on the Psychotrophic bacteria of beef sausage.
Table 6: Effect of bee honey on the sensory evaluation of beef sausage.

<table>
<thead>
<tr>
<th>Level of bee honey</th>
<th>Color</th>
<th>Aroma</th>
<th>Taste</th>
<th>Texture</th>
<th>Juiciness</th>
<th>Over all acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>4.94B±0.24</td>
<td>4.81B±0.50</td>
<td>4.83BA±0.74</td>
<td>4.47C±0.46</td>
<td>4.35BC±0.61</td>
<td>4.77B±0.50</td>
</tr>
<tr>
<td>2.5%</td>
<td>5.14BA±0.47</td>
<td>4.77B±0.43</td>
<td>4.87BA±0.55</td>
<td>4.98BA±0.48</td>
<td>4.24C±0.34</td>
<td>4.73B±0.31</td>
</tr>
<tr>
<td>5%</td>
<td>5.23BA±0.34</td>
<td>4.75B±0.59</td>
<td>4.54B±0.43</td>
<td>4.73BC±0.60</td>
<td>4.63BA±0.22</td>
<td>4.54B±0.32</td>
</tr>
<tr>
<td>7.5%</td>
<td>5.38A±0.38</td>
<td>5.30A±0.56</td>
<td>5.11A±0.40</td>
<td>5.18A±0.38</td>
<td>4.66A±0.42</td>
<td>5.23A±0.36</td>
</tr>
</tbody>
</table>

* A-C= means bearing different superscript capital letters in the same column are significantly different (P<0.05).

* n=9
When the effect of storage period on the sensory properties of beef sausage table (7) was examined, the panelists were uncertain; however the general trend was that the ratings of the measured parameters had increased with the increase in storage period. The effect is more pronounced toward the end of the storage period, as the panelists gave all the measured parameters (with few exceptions) the highest score on the 6 day of storage.
Table 7: Effect of storage period on the sensory evaluation of beef sausage.

<table>
<thead>
<tr>
<th>Level of bee honey</th>
<th>Color</th>
<th>Aroma</th>
<th>Taste</th>
<th>Texture</th>
<th>Juiciness</th>
<th>Over all acceptability</th>
</tr>
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* A-B = means bearing different superscript capital letters in the same column are significantly different (P<0.05).
* n=12
Utilization of bee honey in processing of beef sausage usage lead to a significant decrease in PV with the increase of bee honey levels.

Utilization of bee honey resulted in substantial reduction of the Total viable count, total coliform, *E. coli*, *Staphylococcus aureus* and psychrotrophics count according it could improves the quality of beef sausage.
CHAPTER SIX
RECOMMENDATIONS

It is recommended to:

1. Use of bee honey to enhance the keeping quality of beef sausage.

2. Further research is needed to study the application of bee honey on other meat products.

3. Further research is needed to elucidate the mechanism by which bee honey bring about the effect.
REFERENCES


Appendix 1
Sensory Evaluation Form

Date…………………………..
Number…………………………..

7= extremely like
6= moderately like
5= like
4= slightly like
3= slightly dislike
2= dislike
1= extremely dislike

If you have any question please ask.

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<th>121</th>
<th>779</th>
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Key:

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