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**Effect of Adding Different Levels of Citric Acid on Quality Attributes Of Beef
Burger**

أثر إضافة مستويات مختلفة من حمض الستريك على جودة البرجر البقري .

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الآية

قَالَ تَعَالَى:

﴿ وَقُلْ أَعْمَلُوا فَسِرِّي اللَّهُ عَمَلِكُمْ وَرَسُولُهُ، وَالْمُؤْمِنُونَ وَسَتُرَدُّونَ إِلَىٰ عِلْمِ

الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُمْ بِمَا كُنْتُمْ تَعْمَلُونَ ﴿١٠٥﴾ ﴾

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DEDICATION

*I dedicate this work to
my father and my
mother's soul for raising
me to be the person I am
today.*

Huwaida

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I would first and foremost like to thank my Almighty God, the creator and designer for the wisdom and knowledge he has bestowed on me, and for all things in my life.

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Abstract

The study was conducted to evaluate the effect of adding citric acid on the shelf life and quality of beef burger. Different levels of citric acid were added, (0% control, 0.8 and 1 %). Six kilograms of beef were divided into three groups with 3 replicates. Chemical composition, Cooking loss, water holding capacity (WHC), pH, total bacterial counts and sensory evaluation were determined. The chemical analysis showed significant differences in moisture content, protein, fat, ash and pH as citric acid levels increased. The moisture content significantly decreased from (70.43 ± 0.32) to (69.95 ± 1.1) at ($P < 0.05$), crude protein significantly decreased from (17.6 ± 0.27) to (17.47 ± 0.42) at ($P < 0.05$), ash significantly decreased from (2.36 ± 0.04) to (2.07 ± 0.096) at ($P < 0.01$), pH significantly decreased from (5.56 ± 0.02) to (4.00 ± 0.02) at ($P < 0.01$) whereas fat content significantly increased from (1.38 ± 0.07) to (1.58 ± 0.07) at ($P < 0.01$). Physical analysis showed significant differences in water holding capacity (WHC), cooking loss and shrinkage as citric acid levels increased. Water holding capacity (WHC) significantly decreased from (0.73 ± 0.17) to (3.01 ± 0.32) at ($P < 0.01$), cooking loss increased from (19.90 ± 0.59) to (23.75 ± 0.49) at ($P < 0.01$) and shrinkage significantly increased from (15.28 ± 0.28) to (17.33 ± 0.54) at ($P < 0.05$). For all treatments sensory evaluation were significantly affected, The color as evaluated by panelists showed significant increased at ($P < 0.01$) with the increased level of citric acid, 0% level showed the lowest color score (2.05 ± 0.76), while 1% level showed the highest score (3.85 ± 2.16), the texture showed significant increased at ($P < 0.01$) 0% level showed the lowest texture score (2.1 ± 0.64), while 1% level showed the highest score (3.7 ± 1.99), the flavor showed significant increased at ($P < 0.01$), 0% level showed the lowest flavor score (1.85 ± 0.99), while 1% level showed the highest score (4.4 ± 2.35) and the juiciness showed significant increased at ($P < 0.05$), 0% level showed the lowest score (2.2 ± 0.89), while 1% level showed

the highest score (3.3 ± 2.0). Citric Acid leads to significant decrease in total viable bacterial count, total viable bacterial count in level (0%) was ($7.03 \log 10$) it decreased to ($6.90 \log 10$) in level (1%). The maximum desired level of citric acid to be added to burger recipe is 0.8% which is acceptable to the consumer. Future experiments are needed to explain the effect of citric acid on beef burger quality, for future application of citric acid treatments in the meat industry.

الملخص

أجريت الدراسة بغرض تقييم أثر إضافة حمض الستريك على جودة البرجر البقري. تمت إضافة مستويات مختلفة من حمض الستريك (صفر % كنترول ، 0.8% و 1%). تم تصنيع ستة كيلو من اللحم البقري ، قسّمت الى ثلاث مجموعات بثلاثة مكررات ، المجموعة الاولى تحتوي على حمض الستريك بمستوى (صفر %) ، المجموعة الثانية تحتوي على حمض الستريك بمستوى (0.8 %) والمجموعة الثالثة تحتوي على حمض الستريك بمستوى (1%). تم إجراء التحليل الكيميائي والفيزيائي والعد البكتيري والتقييم الحسي للعينات المصنعة ، وقد أوضحت نتائج التحليل الكيميائي فروقات معنوية في كل من الرطوبة، البروتين ، الدهن ، الرماد ودرجة الاس الهيدروجيني بزيادة مستوى حمض الستريك ، وقد نقص محتوى الرطوبة من (0.32 ± 70.43) عند المستوى (صفر %) من حمض الستريك إلى (1.1 ± 69.95) عند المستوى (1 %) من حمض الستريك بمستوى معنوية (0.05) ، كما نقص البروتين من (0.27 ± 17.6) إلى (0.42 ± 17.47) بمستوى معنوية (0.05) ، كما كان هناك نقص في الرماد من (0.04 ± 2.36) إلى (0.096 ± 2.07) بمستوى معنوية (0.01) ، كما نقص درجة الأس الهيدروجيني من (0.02 ± 5.56) إلى (0.02 ± 4.00) بمستوى معنوية (0.01) ، بينما كان هنالك زيادة معنوية في محتوى الدهن من (0.07 ± 1.38) % إلى (0.07 ± 1.58) بمستوى معنوية (0.01). أظهرت نتائج التحليل الفيزيائي زيادة في كل من فاقد الطبخ والإنكماش، ونقصان في قوة الحفظ المائي للبروتين بزيادة حمض الستريك. كان هناك زيادة في فاقد الطبخ من (0.59 ± 19.90) عند المستوى (صفر %) من حمض الستريك إلى (0.49 ± 23.75) عند المستوى (1 %) من حمض الستريك بمستوى معنوية (0.01) وكذلك زيادة في الإنكماش من (0.28 ± 15.28) إلى (0.54 ± 17.33) بمستوى معنوية (0.05) ، بينما نقصت قوة الحفظ المائي للبروتين من (0.17 ± 0.73) إلى (0.32 ± 3.01) بمستوى معنوية (0.01). أظهرت نتائج التقييم الحسي تأثير ملحوظ لحمض الستريك في كل من اللون ، القوام ، النكهة و العصرية . بالنسبة للون فقد أظهر مستوى (صفر %) من حمض الستريك أقل درجة (0.76 ± 2.05) بينما أظهر مستوى (1 %) أعلى درجة (2.16 ± 3.85) بمستوى معنوية (0.01) ، بالنسبة للقوام فقد أظهر مستوى (صفر %) من حمض الستريك أقل درجة (0.64 ± 2.1) بينما أظهر مستوى (1 %) أعلى درجة (1.99 ± 3.7) بمستوى معنوية (0.01) ، كذلك الحال بالنسبة للنكهة ، فقد أظهر مستوى (صفر %) من حمض الستريك أقل درجة (0.99 ± 1.85) بينما أظهر مستوى (1 %) أعلى درجة (2.35 ± 4.4) بمستوى معنوية (0.01) ، وكذلك العصرية فقد أظهر مستوى صفر % من حمض الستريك أقل درجة (0.89 ± 2.2) بينما أظهر مستوى (1 %) أعلى درجة (2.03 ± 3.3) بمستوى معنوية (0.05) . أظهرت نتائج العد البكتيري تأثير ملحوظ لحمض الستريك في التعداد الحيوي للبكتيريا ، إذ أدى إلى نقصان

التعداد الحيوي للبكتيريا من (7.03 لوغريثم 10) عند المستوى (صفر %) من حمض الستريك إلى (6.9 لوغريثم 10) عند المستوى (1%) من حمض الستريك. أعلى مستوى منشود يمكن إضافته من حمض الستريك إلى وصفة البرجر هو نسبة 0.8 والذي يكون مقبولا للمستهلك. مزيد من الدراسات المستقبلية لإستخدام حمض الستريك في صناعة اللحوم .

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CHAPTER ONE

Introduction

Sudan has a huge amount of animal resources which has qualified it to compete in meat industry Marfr,(2012).

In the past Sudanese preferred fresh meat to processed meat, but now the profitability and technological advances, and the change in the life style (the working women have a little time for food preparation), will probably affect the rate and ultimate extent to which meat is processed Maha and Abogroun ,(2014).

In recent years there has been an increased consumer demand in meat and meat products requiring minimal home preparation Stubbs *et al.*,(2002).

The demand for convenient food will grow continuously Richards and Padilla ,(2009).Beef and meat products, particularly beef patties, exhibited considerable increases in production and consumption throughout the world in recent years. This is due to the rapid growth in the fast food market beside the exceptional nutritional quality of meat products as it contains appreciable amounts of protein, vitamins and trace minerals with significant health benefits National Health and Medical Research Council,(2006). Meat consumption in developing countries has been continuously increasing from a modest average annual per capita consumption of 10 kg in the 1960s to 26 kg in 2000 and will reach 37 kg around the year 2030 according to FAO projections. This forecast suggests that in a few decades, developing countries' consumption of meat will move towards that of developed countries where meat consumption remains stagnant at a high level. The only possible alternatives are making better use of the meat resources available and reducing waste of edible livestock parts to a minimum. This is where meat processing plays a prominent role. It is fully utilizes meat resources, including nearly all edible livestock parts for human food consumption Heinz and Hautzinger, (2007).

Meat is an excellent source of many important nutrients; however, it is subject to high rate of deterioration. It is also an ideal environment for bacterial to thrive due to its high protein and moisture contents Bhaisare *et al.*, (2014).

Meat is a perishable animal product; microbial spoilage of meat has always been of great concern to the food industry. Meat can be a source for food borne pathogenic microorganisms Paulsen *et al.*, (2006).

Meat is also very much susceptible to spoilage due to chemical and enzymatic activities. The breakdown of fat, protein and carbohydrates of meat results in the development of off-odors, off-flavor and slim formation which make the meat objectionable for human consumption. It is, therefore, necessary to control meat spoilage in order to increase its shelf life and maintain its nutritional value, texture and flavor Dave and Ghaly,(2011).

Many efforts have been made to improve the quality and stability of burgers because consumer demand for fast food has been increasing rapidly in the recent years Papadina and Bloukas, (1999).

Acidifier additives are used for flavoring, health benefits and other functions besides food preservation. Food acidulates can be used in most foods where acidity is desired or necessary for the keeping quality. Citric acid is the prominent general Purpose acidulates. It is used widely in consumed food Products, accounts more than 80% of general purpose acidulants used (Frederick, 1999) and inhibits bacteria such as salmonella and E.Coli when added to sodium chloride Ransom *et al.*, (2003).

Objectives

- 1/ To assess the effect of adding different levels of citric acid on Burger shelf life.
- 2/ To evaluate physicochemical and sensory characteristics of beef burger.

CHAPTER TWO

LITERATURE REVIEW

2.1 Meat definition

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).

Meat can be define as the whole or part of the carcass of any buffalo, camel, cattle, deer, goat, hare, sheep, poultry, or rabbit, slaughtered, but does not include eggs, or fetuses Williams, (2007).

Meat is animal flesh that is eaten as food, it is mainly composed of water, protein, and fat. It is edible raw, but is normally eaten after it has been cooked and seasoned or processed in a variety of ways Lawrie and Ledward, (2006).

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 Olusolatet and Mojola, (2010).

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).

Meat can be broadly classified as "red" or "white" depending on the concentration of myoglobin in muscle fibre when myoglobin is exposed to oxygen, reddish oxymyoglobin develops, making myoglobin-rich meat appear red. The redness of meat depends on species, animal age, and fibre type: Red meat contains more narrow muscle fibres that tend to operate over long periods without rest, while

white meat contains more broad fibres that tend to work in short fast bursts Lawrie and Ledward, (2006).

2.2 Nutritive value of meat

Rich nutrient matrix meat is the first-choice source of animal protein for many people all over the world Heinz and Hautzinger, (2007).

Fresh beef, as a high-energy type of food is considered to be the food of choice due largely to its nutritional value Rule *et al.*, (1997).

Meat and meat products are important sources for protein, fat, essential amino acids, minerals and vitamin and other nutrients Biesalski, (2005).

Quantitatively and qualitatively, meat and other animal foods are better sources of protein than plant foods (except soy bean products). In meat, the essential amino acids – the organic acids that are integral components of proteins and which cannot be synthesized in the human organism – are made available in well balanced proportions and concentrations. As well, plant food has no Vitamin B12; thus animal food is indispensable for children to establish B12 deposits. Animal food, in particular meat, is rich in iron, which is of utmost importance to prevent anemia, especially in children and pregnant women Heinz and Hautzinger, (2007).

Meat is well known as an excellent protein and energy source for our daily diets and after digestion, provides excellent nutrition Chang and Huang, (1991); Jihad *et al.*, (2009).

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, Percentages of some minerals in processed meat products are higher than fresh meat because of added salt and seasoning, it 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).

The level of vitamin in meat is reduced by cooking, the amount depends on temperature and time employed Gracey, (1986).

On nutritional basis alone, meat is vital to the diet, it is one of the few foods which is provides complete protein, as well as being a rich source of such essential nutrients as iron, Niacin and vitamin B12 National livestock and meat board, (1983).

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).

Meat is the most important single item in the diet; primarily because of its palatability and high nutritive value. 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 Physical 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 Copper and Willis, (1984).

2.3 1 Meat color

Color defines as “that aspect of things that is caused by differing qualities of the light reflected or emitted by them, definable in terms of the observer or the light American Heritage College Dictionary, (2000).

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 4(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).

Of the several quality attributes of fresh meat, color is the most important one influencing purchase decisions Mancini and Hunt, (2005).

The presence of muscle pigments, myoglobin and hemoglobin is the main limiting factor of meat color. At the point of sale, consumers, in general, cannot evaluate the odor or feel the texture of meat without opening the packages. Thus, a bright cherry-red color is commonly utilized as an indicator of wholesomeness in fresh meat. Surface-discolored whole muscle cuts are ground to low-value products, such as ground beef, to salvage the cuts’ interiors, which might still be red or are discarded often well before microbial safety is compromised; both practices lead to sales loss and wastage of valuable food. Various live animal–related factors, such as management, diet, and genetics, are also known to influence meat color Faustman and Cassens, (1990). In addition, several endogenous factors contribute to meat color, and the most prominent among them are pH, muscle source, presence of antioxidants, lipid oxidation, and mitochondrial activity Mancini and Hunt (2005).

A wide variety of exogenous factors commonly encountered in meat processing are known to influence the meat color. Presence of ligands, antioxidants, and pro oxidants governs meat color stability, and several extensive reviews may be consulted for the details Faustman and Cassens (1990); Mancini and Hunt (2005); McMillin,(2008).

2.3. 2 Tenderness and texture

Tenderness has been identified as the most important factor affecting consumer satisfaction and perception of taste Robbins *et al.*, (2003).

Tenderness is considered to be the most important trait of meat acceptability by the consumers Miller *et al.*, (1995).

It is known that the meat tenderness is one of the most important factors affecting the meat consumer's satisfaction Shackelford *et al.*, (2001).

Tenderness of meat is related to a combination of breakdown within muscle fibers, predominantly due to the activity of enzymes, and loosening of connective tissue, specifically collagen. Collagen in raw meat is usually loosened by the enzyme collagenase over a prolonged period of time but this process takes place only to a small degree as the action of collagenase is very slow and meat would be microbiologically spoiled before collagen would be significantly softened. Another method of softening collagen in raw meat is to place meat in a sour (acidic) soaking solution Gerhard Feiner, (2006).

Meat tenderness is related principally to the two structural elements: the connective tissue and the myofibrillar protein components of muscle Lawrie, (1998).

Basically, the mechanism responsible for increased meat tenderness is connected with the weakening of connective tissue and the weakening of myofibrillar protein component Takahashi, (1996).

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 myofibril protein Zaglul and Cassens (,1987).

When the meat is heated in the water, the connective tissue is changed to assort of tender gelation and it became more tender Inekoronye and Ngoddy , (1992) .

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).

Pre-slaughter and post-slaughter factor effecting meat texture includes species, sex, age, feed, pre-rigor factors and processing Kumar *et al.*, (1974).

Meat tenderness is one of the main attributes of meat quality. Currently, Being a mechanical property, tenderness is related to final pH, postmortem temperature, sarcomere length, connective tissue content and enzymatic proteolysis of myofibrillar proteins Yu *and* Lee, (1986); Perez *et al.*, (1998).

Maintaining the sensory and textural properties of meat products is a matter of challenge, which necessitates more effort to protect the product integrity, taste, flavor, and textural sensory attributes Gehan and Emara, (2010).

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).

Evaluation of taste and odor still depends mainly on taste panel Lawrie, (1979).

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. Some undesirable flavor changes occur during storage could be due to metabolic products. Judge *et al.*, (1990).

Generally, meat from an older animal of the same species exhibits stronger flavor than meat from a young animal. The flavor of raw meat is also influenced to some

degree from sulphuric components present in meat. Other flavor components in meat are amino acids, peptides and carbonic acids. During ageing (ripening and maturing) of meat, lipolysis (breakdown of fats) as well as proteolysis (breakdown of proteins) causes proteins and fat to become a much more significant part of meat flavor Gerhard Feiner, (2006).

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 Judge *et al.*, (1990).

The majority of water in muscle is held either within the myofibrils, between the myofibrils and between the myofibrils and the cell membrane (sarcolemma), between muscle cells and between muscles bundles (groups of muscle cells). Once muscle is harvested the amount of water and location of that water in meat can change depending on numerous factors related to the tissue itself and how the product is handled Honikel and Kim, (1986); Honikel, (2004).

Many of the technological and sensory properties of meat and meat products depend on the capacity of muscle tissue to bind and hold water. All these properties are associated with changes that take place in meat after slaughter and the application of substances added to meat in the course of technological processes Medynski *et al.*, (2000).

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.*, (1989).

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

Juiciness is important to meat texture and palatability .it has two major components; the first is the impression of wetness produced by the release of fluid

from the meat during chewing, the second is the more sustained juiciness that apparently results from the stimulating effect of fat on the production of the saliva Lawrie, (1991).

The degree of shrinkage on cooking is directly correlated with loss of juiciness to the palate. Juiciness in cooked meat has two organoleptic components; the first is impression of wetness during the first chew produced by rapid release of meat fluids, and the second is sustained juiciness, largely due to stimulatory effect of fat on salivation Weir, (1990).

The principal source of juiciness in meat, as detected by the consumer are intramuscular lipids and 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 barrier to moisture lost during 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 at this pH level Mohammed, (2005).

2. 4 Chemical composition of meat

Adult mammalian muscle flesh consists of roughly 75 percent water, 19 percent protein, 2.5 percent intramuscular fat, 1.2 percent carbohydrates and 2.3 percent other soluble non-protein substances. These include nitrogenous compounds, such as amino acids, and inorganic substances such as minerals Lawrie and Ledward, (2006).

Meat is composed of water, fat, protein, mineral (ash) and a small proportion of carbohydrates. Meat and other animal products make valuable contributions to diets of developing countries due to its high nutritional qualities Olusola and Omojola , (2010).

Numerous aspects of the biochemical composition of meat vary in complex ways depending on the species, breed, sex, age, plane of nutrition, training and exercise

of the animal, as well as on the anatomical location of the musculature involved .Even between animals of the same litter and sex there are considerable differences in such parameters as the percentage of intramuscular fat Lawrie and Ledward, (2006).

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

Meat and meat products are concentrated sources of high quality protein and they supply easily absorbed iron Snijders and Collins, (2004); Lawrie and Ledward, (2006).

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).

Muscle proteins are often classified into three groups based on their solubility: sarcoplasmic protein, myofibrillar proteins and stroma proteins. The sarcoplasmic protein which includes 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 is soluble protein (1% salt concentration) mainly consists 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).

2.4.2 Meat fat

Fat in meat can be either adipose tissue, used by the animal to store energy and consisting of "true fats" (esters of glycerol with fatty acids or intramuscular fat,

which contains considerable quantities of phospholipids and of unsaponifiable constituents such as cholesterol Lawrie and Ledward, (2006).

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 factors, including genetic predisposition, age, gender and sex status, level of nutrition and exercise Alan and Jane, (1995).

The percentage of fat in the meat depends on the type of animal, how it is raised, and the type of food, but in general it is possible to know how much fat in the meat by looking at the color of meat, the meat is dark reddish fat is less than meat, which is open color Sherifa, (2013).

Fat has considerable importance in meat products, since it affects technological properties and sensory aspects, mainly tenderness and juiciness. However, diets high in fat provide large amounts of saturated fatty acids and cholesterol, which are associated with the occurrence of obesity, hypertension, cardiovascular disease and coronary heart disease Ozvural and Vural, (2008).

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

The pH value of beef varies from 4.8-7.2 depending on the glycolytic potential at the time of slaughter but the normal variation is of pH 5.4-6.0. Fresh beef quality is highly determined by the muscle pH and its change in time, which in turn is strongly dependent on pre-slaughter conditions (stress), imposed on the animals Tarrant and Lister, (1989).

Stress experienced by animal before slaughter cause glycogen reserves to be depletion resulting in higher ultimate pH. The pH is also an important determinant of microbial growth and the high pH beef has a high spoilage potential and a short shelf life Newton and Gill, (1981).

The pH value has a significant impact on color, shelf life, taste, microbiological stability, yield and texture of meat and meat products and is therefore one of the most important parameters within the production of meat products and meat itself. Quite often, the pH value is referred to as the 'acidity of meat', which is only partly correct as the pH scale ranges from 0 to 14 and covers not only the sour range. The pH values of meat and meat products lie generally between 4.6 and 6.4. At a pH value of around 6.4, meat is spoiled owing to enzyme activity, which produces a large amount of metabolic byproducts as well as ammonia. Sliminess, bad smell and discoloration can be seen at this point as well Gerhard Feiner, (2006).

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 6.8 to 5.7. The pH usually determines fresh rather than cooked meat sample Leo and Nollet, (2012).

the low pH affects beef colour by promoting oxidation of the haem pigments from the purple or red myoglobin (Mb) and oxymyoglobin (MbO₂) to the brown met myoglobin (met Mb) Abril *et al.* , (2002) .

Ultimate pH of meat was significant for its resistance to spoilage because most bacteria grow optimally at about pH 7 and not well below pH 4 or above pH9 Walker and Betts, (2000).

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. 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 an 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 Meat Quality Characteristics

Quality was defined as the consumer acceptance or preference of food or food product by consumers . Traditionally meat quality is either eating quality or processing quality; therefore quality is directly associated usage and is a multifaceted concept Webb *et al.*, (2005).

Meat eating qualities including tenderness, juiciness and flavor are regarded the major essential meat palatability traits by consumers Lawrie and Ledward, (2006); Smith *et al.*, (2008).

Meat eating quality involves five attributes namely, colour, water holding capacity, tenderness, juiciness and flavor .All attributes are influenced by breed, sex, age, anatomical location, exercise, nutritional and internal variability Lawrie, (1991).

There is currently no consensus on what the term ‘quality’ really stands for, given that ‘quality’ is generally seen as a combination of two major elements. On the one hand, ‘total quality’ of meat and meat products includes characteristics which can be measured, such as microbiological status, tenderness, colour, juiciness, shelf life, pH value and pesticide levels. On the other hand, total quality also includes an aspect which is less easy to measure: the consumer’s personal perception of the value of meat and meat products. This perception is different for every individual human being as external factors such as television advertising for example have an influence on perceptions of total quality. The term ‘quality’, from the consumer’s point of view, could be simply said to mean whether the consumer thinks a product is good value for money and this judgment will vary from person to person and from product to product Gerhard Feiner, (2006).

Meat quality is normally defined by the compositional quality (lean to fat ratio) and the palatability factors such as visual appearance, smell, firmness, juiciness, tenderness, and flavor. The nutritional quality of meat is objective yet "eating" quality, as perceived by the consumer, is highly subjective FAO, (2019):

2.5.1 Visual Identification

The visual identification of quality meat is based on colour, marbling and water holding capacity. Marbling is small streaks of fat that are found within the muscle and can be seen in the meat cut. Marbling has a beneficial effect on juiciness and flavor of meat. Meat should have a normal colour that is uniform throughout the entire cut. Beef, lamb, and pork should also have marbling throughout the meat FAO, (2019).

2.5.2 Smell

Another quality factor is smell. The product should have a normal smell.

This will be different for each of the species (i.e. beef, pork, chicken), but should vary only slightly within the species. Any rancid or strange smelling meat should be avoided FAO, (2019).

2.5.3 Firmness

Meat should appear firm rather than soft. When handling the retail package, it should be firm, but not tough. It should give under pressure, but not actually be soft FAO, (2019).

2.5.4 Juiciness

Juiciness depends on the amount of water retained in a cooked meat product. Juiciness increases flavor, helps soften meat - making it easier to chew, and stimulates saliva production in the mouth. Water retention and lipid content determine juiciness. Marbling and fat around edges helps hold in water. Water losses are from evaporation and drip losses. Meat aging can increase water retention and therefore increases juiciness FAO, (2019).

2.5.5 Tenderness

Has been linked to several factors, such as the animal's age, sex or the muscle location. One important way to tenderize meat is by aging. Carcasses are aged by holding them at refrigeration temperatures for extended periods of time after slaughter and initial chilling. Several studies have suggested tenderness is directly influenced by water holding capacity. Water holding capacity is the amount of

water that can be held within a muscle during some form of mechanical forces such as cutting, tumbling, etc FAO, (2019).

2.5.6 Flavor

Flavor and aroma are intertwined to create the sensation the consumer has during eating. These perceptions rely on the smell through the nose and on the sensations of salty, sweet, sour and bitter on the tongue. Meat flavor is affected by type of species, diet, cooking method and method of preservation (e.g. smoked or cured) FAO, (2019).

2.6 Deterioration of meat quality

Fresh beef has unique biological and chemical properties and its nutrients composition represents an optimum medium for microbial growth. It undergoes deterioration progressively from slaughter until consumption. The shortened shelf-life is due to microbial growth and/or rancidity development which is strongly influenced by initial beef quality, package parameters and storage conditions Zhao *et al.*, (1994).

Meat and meat products are susceptible to deterioration in view of their rich nutritional composition Devatkal *et al.*, (2012).

Microbial growth is the most important factor in spoilage of fresh beef and this is followed by colour deterioration. Different types of spoilage and pathogenic microorganisms may be introduced into and on the surface of fresh beef during slaughtering and processing, which causes rapid spoilage, great loss of valuable protein and also affect human health. The shelf life represents the useful storage time of food product. Beyond this period, changes in smell, colour, taste, texture or appearance make the product unacceptable. Several factors influence the shelf life of fresh beef such as temperature, pH, oxygen, pressure and light. Oxidation of beef to metmyoglobin is essentially affected by myoglobin oxidation rate, oxygen availability and reducing capacity of the muscle Catherine *et al.*, (1989).

The rate of beef discolouration is more influence by factors such as autoxidation of myoglobin and oxygen consumption Renerre and Labas, (1987).

Quality characteristics of meat products deteriorate due to microbial growth and rancidity of lipids in the course of storage Aguirrezabal *et al.*, (2000).

Lipid oxidation is an important factor limiting both the quality of meat products and their acceptability to the customer. Food become unacceptable to the consumer once a certain degree of rancidity is attained Sammet *et al.*, (2006).

During the course of lipid oxidation, a number of compounds are formed that have an influence on the quality of meat and meat products expressed in the form of sensory changes (colour, texture and taste) and a deterioration of nutritional quality Min and Ahn,(2005); Karre *et al.*, (2013).

Problems in meat quality are primarily caused by changes in the biochemistry and morphology of the muscles themselves Sosnicki and Feb, (1993).

During the deboning process, meat undergoes extensive handling and may susceptible to bacterial contamination resulting in pigment decomposition, discoloration and development of off odors Nel *et al.*, (2004).

During slaughtering process, meat is exposed to many sources of bacterial contamination Jo *et al.*, (2004).

During chilled storage of burger, growth of spoilage microorganisms which enhanced by an increase in A_w , cause alternations in flavor, texture and disagreeable – smelling volatile compounds as a result of breaking down of complex organic components into simpler compounds. Lipid oxidation products and free radicals can also cause oxidation of oxymyoglobin to metmyoglobin indicating discoloration of meats Lee *et al.*, (1999).

There is a gradual loss of flavor during storage of beef and this may occur even in the frozen condition due to the slow loss of highly volatile substances Lawrie, (1991).

Undesirable odor and taste may arise during storage of beef because of microbial growth and chemical deterioration on the surface. The lipases of microorganisms which attack fat, splitting of fatty acids with more or less unpleasant consequences according to their nature. The nature of the off-odor depends on the types of microorganisms growing and those will be determined by such factors as the

temperature of storage and the nature of product (fresh, cured and comminuted). It is generally accepted that autoxidation of membrane of phospholipids is largely responsible for the development of off-odor Angelo *et al.*, (1987).

Control of microorganisms in meat products is the major concern in the preparation of high quality foods. The hygienic status of animals prior, during and after slaughter can be critical to the finished product quality Satin, (2002).

2.7 Preservation of meat

Meat and food preservation was an important tool that assisted ancient farmers to increase their produce and prolong food supply by retarding spoilage. Also meat preservation became imperative for conveying meat over extended distances without deterioration of sensory attributes and nutritional properties after the establishment and fast expansion of grocery stores and shopping malls. Meat preservation became necessary for transporting meat for long distances without spoiling of texture, color and nutritional value after the development and rapid growth of super markets Nychas *et al.*, (2008).

In meat processing, the preferable preservation methods are application of good slaughter, meat handling and processing hygiene and submission of semi- and fully-fabricated products to an uninterrupted cold chain. In complying with these requirements, bacterial counts in meat can be kept low and chemical preservatives are actually not needed FAO, (2007).

Natural methods of preservation usually aim to exclude air, moisture, and microorganisms, or to provide environments in which organisms that might cause spoilage cannot survive Daniel, (2007).

Natural way of Food Preservation can be done by Boiling, freezing, pasteurizing, dehydrating, smoking and pickling Heldman, (1994).

The principle of preservation is to create unfavorable conditions for the growth of microorganisms, which result spoilage of food. Due to spoilage, the texture, flavor and nutritive value of meat are altered and thereby, rendering it inedible for human use. Unless proper preservation methods are adopted, deterioration, microbial activity, enzymatic and chemical reactions along with physical changes is bound to occur. However, once meat is contaminated with microorganisms, their removal is

difficult. Hence, preservation of meat is done by various preserving techniques such as chilling/refrigeration, freezing, curing, smoking, thermal processing, canning, dehydration, irradiation, chemicals and pressure processing Cassens, (1994); Jay *et al.*, (2005); Zhou *et al.*, (2010); Pal, (2014).

The aims of preservation methods are: (a) to inhibit the microbial spoilage and (b) to minimize the oxidation and enzymatic spoilage. Traditional methods 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).

The preservation of food has several objectives Gracy *et al.*, (2009).

1. To control foodborne infections and intoxications.
2. To ensure the safety of food from microbes.
3. To prevent the spoilage of food.
4. To extend the shelf life of food.
5. To enhance the keeping quality of food.
6. To reduce financial losses.

The food has limited shelf life, in order to increase the shelf life and maintain the quality certain preservatives are used Sanjay Sharma, (2015).

The maximum shelf life of meat products depends on several factors such as pH, water activity, microbial growth and temperature Marth, (1998).

Growth of bacteria and spoilage of meat is depending on the species of bacteria, nutrients availability, pH, temperature, moisture and gaseous atmosphere Cerveny *et al.*, (2009).

Current meat 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 preservation 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 .At 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 and Heijnen, (2004).

2.7.1.1 Chilling

This is the most widely used method of preservation for short term storage of meat as chilling/refrigeration slows or limit the spoilage rate at temperature below the optimal range can inhibit the microbial growth,(Cassen,1994) enzymatic as well as chemical reactions Cassen, (1994); Pal , (2014).

Storage of fresh meat is done at a refrigeration temperature of 2 to 5°C. Chilling is critical for meat hygiene, safety, shelf life, appearance and nutritional quality Cassen, (1994); Zhou *et al.*, (2010).

Carcasses are first hanged in chilled coolers (15°C) to remove their body heat, and are then passed on to holding coolers (5°C). It is essential to maintain proper spacing between carcasses so as to allow throughout air circulation. It is employed by two methods: (a) immersion chilling, in which the product is immersed in chilled (4°C) water and (b) air chilling, in which the carcasses are misted with water in a room with circulating chilled air Carroll and Alvarado , (2008).

Chilling is employed at slaughtering plants immediately after slaughtering and during transport and storage .Chilling is credited for meat hygiene, safety, shelf, appearance and nutritional quality it is necessary to reduce the temperature of carcass immediately after evisceration to 40°C within 4h or slaughtering USDC, (1995).

Generally, fresh meat remains in good condition for a period of 5-7 days if kept at refrigerated temperature of $4 \pm 1^{\circ}\text{C}$. Cold-shortening and toughening may result from ultra-rapid chilling of pre-rigor meat Ockerman and Basu, (2004).

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 quality of frozen meat is also influenced by its freezing rate. In slow freezing, there is formation of large ice crystals, which may cause physical damage to muscular tissue, giving it distorted appearance in the frozen state. In fast freezing, numerous small ice crystals are formed uniformly throughout the meat tissue. The freezing rate is increased with decreases in temperature, almost 98% of water freezes at -20°C and complete crystal formation occurs at 65°C Rosmini *et al.*, (2004).

Thus, problem of muscle fiber shrinkage and distorted appearance is not there in meat tissue. The drip losses during thawing are considerably low as water freezes within the muscle fiber itself. Numerous small ice crystals on the surface of the fast frozen meat are also important as they give a desirable light color as compared to slow frozen meat. Microbial growth stops at -12°C and total inhibition of the cellular metabolism in animal tissues occurs below -18°C Perez and Mateo, (2004).

However, enzymatic reactions, oxidative rancidity and ice crystallization will still play an important part in spoilage Zhou *et al.*, (2010).

During freezing, about 60% of the viable microbial population dies but the remaining population gradually increases during frozen storage Rahman, (1999b).

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).

Energy intensive freezing operations are the greatest way to preserve carcass, meat and meat products for a longer time which inhibits bacterial growth, but not the

psychrophiles and the spores. Most of these survive freezing and grow during thawing Neumeyer *et al.*, (1997).

2.7.2 Controlled water activity

Water activity (A_w) is an important factor in the preservation and quality of meat. It is defined as a measure of how much of the water in a product is free referring to the water that is not chemically or physically bound Young *et al.*, (2001).

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 and Budge, (2010).

Water activity in meat products is equivalent to the relative humidity of air in equilibrium with the product Comaposda and Gouand, (2000).

The main variables that affect microbial growth are the surface temperature and surface water activity. Microbial growth on the surface of foods is mainly controlled by water activity, temperature and pH Ross, (1999).

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 pickling 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).

In the United States, the additive must be GRAS-listed (Generally Recognized as Safe) according to the American Food and Drug Administration USFDA, (2009).

In Canada, it must fall under GMP (Good Manufacturing Practice) in accordance with the Canadian Food and Drug Act HC, (2006).

Chemical preservation methods are quite beneficial in combination with refrigeration in order to optimize stability, product quality while maintain freshness and nutritional value Cassens, (1994).

Chemical preservatives are a sensitive issue, but can play an important and valuable role when properly applied during meat handling and processing in order to extend the shelf life of meat and meat products and reduce losses. Other chemical preservatives are also officially authorized in most countries and applied in specific hygienically sensitive situations in the meat sector. Amongst these specific chemical preservatives, organic acids such as lactic, citric or acetic acids are the most common. They are natural food components and therefore permitted in any type of food processing. They can reduce microbial growth on fresh meat surfaces, when sprayed on. For processed meat products they are less suitable as they will have a negative impact on water binding (produce low ph.) and taste (sour) FAO, (2007).

Antimicrobial preservatives are substances which are used to extend the shelf life of meat by reducing microbial proliferation during slaughtering, transportation, processing and storage Rahman, (1999a).

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 Archer, (2002); Ray, (2004); Chipley, (2005).

Food additives used to acidified foods are normally studied and used in food production. Organic acids used in the preparation of acidic marinades include acetic acid, lactic acid, citric acid and fruit juices Stanton and Light, (1990); Lewis and Purslow, (1991); Burke and Monahan, (2003).

Soy sauce, acetic, citric, ascorbic, and tartaric acids are examples of food additives that can be used to acidify meat proteins Calhoun *et al.*, (1996)

Organic acids have a long history of being used by the food industry as either additives and/or preservatives for preventing food deterioration and extending the shelf life of perishable food and food ingredients. Organic acids are also used to control microbial contamination and dissemination of foodborne pathogens in pre-harvest and postharvest food production and processing. Ricke, (2003).

Several studies have been carried out to investigate the effects of organic acids in beef products in order to prolong the storage life Glass *et al.*, (2002); Gill *et al.*, (2000) ; Calicioglu *et al.*, (2003); Stopforth *et al.*, (2003) ; Nunez de Gonzalez *et al.*, (2004).

The effectiveness of organic acids as antimicrobials differ widely based on concentration, pH, molarity and the concentration of the nondissociated form Beth *et al.*, (2004a).

Currently, organic acids are allowed to be used at < 2.5% of solution for pre-chilled carcass washing. Lactic acid is also allowed to be used at 5% of solution as a pre and post-chilled wash for beef carcasses. The application of food acids such as citric, lactic and acetic (organic acids rinses) as a single or in combination have been shown to be effective in reducing both spoilage and pathogenic microorganisms Dorsa *et al.*, (1997).

Organic acids have bacteriostatic and bactericidal properties; however, its ability to manifest these attributes depends on the physical state of the organism and the characteristics of the external environment Davidson, (2001).

Organic acids have a long history of being utilized as decontamination of meat from several bacteria including Salmonella Mani-Lopez *et al.*, (2012).

They are generally recognized as safe (GRAS) antimicrobial agents. Various researchers have proven the antibacterial effect of organic acids on different types of pathogenic bacteria which is directly proportional to the concentration of organic acid used Samelis *et al.*, (2001); Raftari *et al.*, (2009).

Although the antibacterial mechanism(s) for organic acids (including citric acid) are not fully understood, they are capable of exhibiting both bacteriocidal and bacteriostatic depending on the physiological status of the organism and the physicochemical characteristics of the external environment Ricke, (2003).

Recently, the interest in lactic, citric and acetic acids for the decontamination of carcasses by the meat industry has escalated. Numerous research studies have shown that organic acid reduces bacterial counts on carcasses if the organic acid is applied prior to microbial attachment to the carcass or meat surface Acuff, (1991).

2.8 Meat technology:

2.8.1 Meat processing technologies

Meat processing technologies were developed particularly in Europe and Asia. The European technologies obviously were more successful, as they were disseminated and adopted to a considerable extent in other regions of the world – by way of their main creations of burger patties, frankfurter-type sausages and cooked ham. Meat processing technologies include Heinz and Hautzinger, (2007):

- Cutting/chopping/comminuting (size reduction)
- Mixing/tumbling
- Salting/curing
- Utilization of spices/non-meat additives
- Stuffing/filling into casings or other containers
- Fermentation and drying.

Generally foods are processed commercially for one of the following reasons Lund, (1979):

- (1) Extend the shelf life of the processed food form.
- (2) Alter the characteristics of the product.
- (3) Separate components from the complex mixture of bio-chemicals.
- (4) Improve the nutritional characteristics of the processed food.

2.8.2 Classification of meat products

Meat products are products in which properties of fresh meat have been modified using one or more procedures such as alteration of color, grinding or chopping,

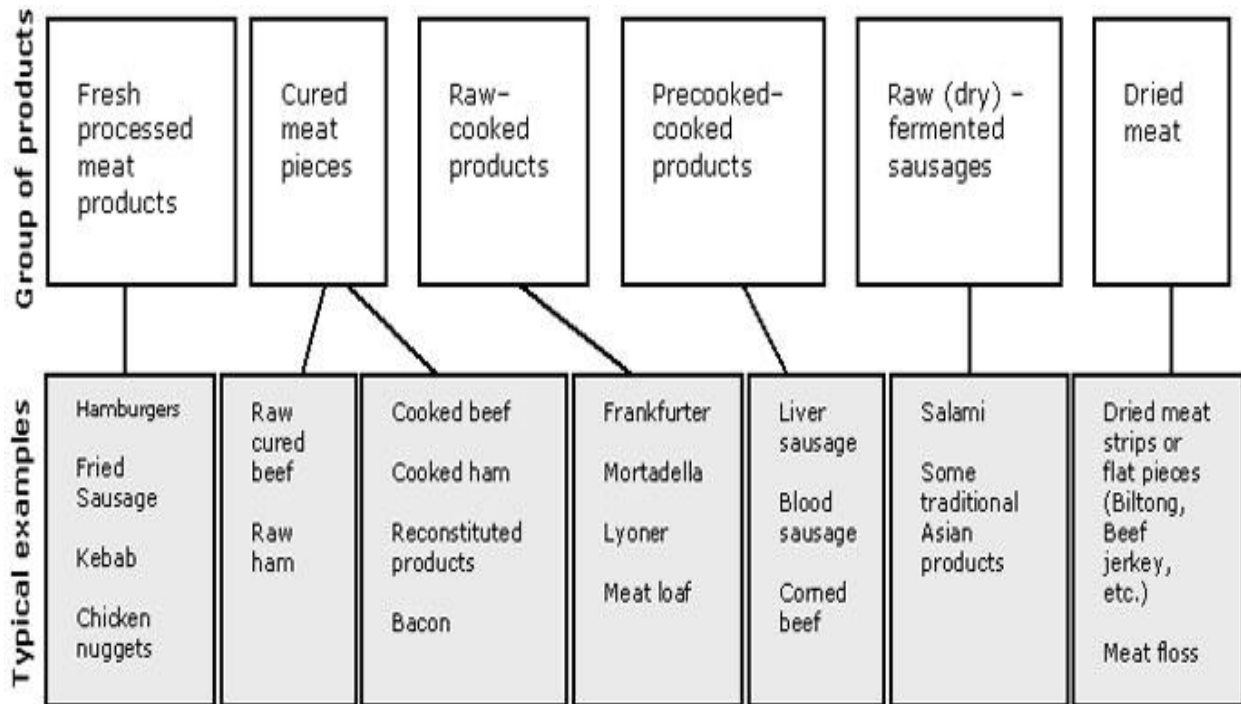
addition of seasoning or heat treatment. The original purpose of meat processing was preservation by inhibiting microbial decomposition as well as processing that result in flavorful and nutritious products. Increased price for lean meat has also altered processing practices and has encouraged the incorporation of increased percentage of less expensive fat Judge *et al.*, (1990).

The processed products should be uniform in color, texture and fat distribution and suitable to be conveniently and accurately cut into portion size with the minimum of waste to consumer. Also reduced cooking loss and improved tenderness and texture and increased shelf life are some of the most important characteristics of processed meat Price and Schweigert, (1987).

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 types of meat products and processes used to manufacture Products Borchel and cassens, (1998).

Meat products grouped according to the processing technology applied



(Heinz and Hautzinger, 2007)

2.8.3 Burger as meat product

Burger is a minced meat product. The minced meat is mixed with condiments and spices, shaped and then cooked by frying or baking Gujral *et al.*, (2002).

The word is derived from hamburger, a large beef sausage which is cut into slices before cooking but the term is now applied more commonly to product of similar organoleptic properties made as flat slices. It was common practice in Germany to name food products especially sausage, after the town where they are manufacture. In UK the same products are referred to beef burger possibly due to the misapprehension that burger are named according to their meat constituent. Such as bacon burger, lamb burger and hamburger or according to basic ingredient for example cheese burger, microwave burger which is usually intended to be fully cooked in microwave oven varanam and Sutherland, (1995).

Hamburger production is one of the way of utilization of less valuable meat cuts, fats and edible by-products obtained during trimming the basic elements of the animal's carcasses, including poultry and rabbits (Feiner, 2006 ; Souza Tavares *et al.*,2007; Prokopp *et al.*, 2008). Usually, this kind of raw material contains high amount of connective tissue, thus the crucial quality properties of its final products are sensory characteristics (Ozkan *et al.*, 2004). Those quality traits are also important due to hamburgers production is based mainly on beef meat, which quality is generally characterized tenderness (Tornberg, 1996; Hildrum *et al.*, 2002; Hwang *et al.*, 2002; Palka, 2003; Pospiech *et al.*, 2003; Purslow, 2004). Therefore, in the quality evaluation of hamburgers texture and sensory traits are the most crucial Park *et al.*, (2004); Erdogdu *et al.*, (2005).

Burger normally contains some amount of extender like powder milk dried for binding or reduces cooking loss of the product Rust, (1976).

Bread crumbs contribute to the mouth feel and texture of burger and absorbs any free moisture present. Bread crumb is made from wheat dough which is backed and ground to specified particle size Varanam and Sutherland, (1995).

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 .Ice water added to keep product temperature down during

emulsification. It is added to the burger formulations 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).

2.8.4. The history of burger:

The hamburger (also commonly called “burger”) most likely first appeared in the 19th or early 20th century McDonald, (1997).

Hamburgers are without any doubt the most popular meat products belong to convenient group. Despite the fact that they are produced for more than 250 years, due to the development of new technologies, facilities, as well as implementation of new raw materials, semi products and some changes in the idea of food and nutrition sciences, technology of hamburgers has to be still improved Komatsu, (2001); Earle *et al.*, (2007); Mater, (2002).

At the end of the Victorian period, the first genuine hamburgers appeared. Before then, the Victorians ate a version of the burger called minced collops. Minced beef patties became popular in America, in restaurants and at home. In 1890, the word ‘hamburger’ first appeared in print – it almost certainly comes from the ‘Hamburg steak’, which was an American dish of flattened fried meat balls served in the 1870s and 1880s. It was named for the German port and the high quality beef it was associated with, and initially appeared in German restaurants. It was cheap to make, using offcuts of meat otherwise unusable, easy to cook and, after the invention of mincing machines in the mid-19th century, quick and simple to prepare. By the 1890s it was being served in a bread roll to hungry workers from lunch wagons. The burger as we know it had finally arrived Annie Gray, (2015).

2.8.5 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. 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 usually grind before adding to meat Isidor and Sedky, (1972).

Several studies have been carried out for many years to explore the benefit of using herbs and spices to enhance sensory attributes and prolong shelf life of foods Fernandez *et al.*, (2005).

The chief purpose of using spices and herbs are the flavor they generate, however, some have demonstrated antimicrobial characteristics Tiwari *et al.*, (2009); Tajkarimi, *et al.*, (2010).

Salt play an important technological role in solubilization of proteins and increasing water holding capacity. Beside their role as flavor enhance and serves as preservative. Spices are used to impart unique flavor and also has preservative role Rust, (1976).

The primary purpose of a processing aid is to facilitate the manufacturing of a food product. Processing aids are used for variety of reasons Praveen and Tim, (2017):

1. Improve product quality and consistency.
2. Enhance nutrition.
3. Help maintain product wholesomeness.
4. Enhance shelf life.
5. Help packing and transportation.

An example of a processing aid is the use of organic acid(s) (e.g., lactic, acetic, or citric acid) as part of a livestock carcass wash applied pre-chill Praveen and Tim, (2017).

2.9 Citric acid:

Citric acid is a white powder extracted from the juice of citrus and other acidic fruits such as lemons, limes, pineapples and gooseberries. It is also produced by the fermentation of glucose. Citric acid is highly soluble in water and primarily insoluble in fat. Citric acid was investigated for its effect on inhibition of bacteria, yeast and molds and was shown to be inhibitorier than lactic acid and acetic acid Sorrells, (1989).

Anhydrous citric the chemical formula is $C_6H_8O_7$. This food acid is occasionally utilized for acidification purposes. Citric acid also acts as a chelating agent by binding heavy-metal ions such as copper as well as iron and therefore acts as a secondary antioxidant but its contribution as an antioxidant is marginal. Citric acid is utilized in marinades applied to portioned meats. The material can be bought in a monohydrate (some moisture within the molecule) or anhydrous form (no water within the molecule). Citric acid is a naturally occurring fruit acid and commercially produced by the fermentation of a carbohydrate material. It is a white, odorless and crystal material with a strong acid taste and is freely soluble in water and ethanol. Coated (mostly fat-coated) citric acid is used in products where acidification should be delayed Gerhard Feiner, (2006).

It was used in acidified beef, to reduce the risk of bacterial contamination and to improve the texture Anonymous, (1990).

This acid exhibits both bactericidal and antistatic effects against *L. monocytogenes* Buchanan and Golden, (1994).

The antibacterial activity of citric acid is dependent on pH, concentration and anion effects Young and Foegeding, (1993).

It is widely distributed in nature in both plants and animals. It can be used as an additive to protect the fresh color of meat cuts during storage. Citric acid also helps protect flavor and increases the effectiveness of antioxidants USDA, (2014).

Citric acid also has strong metal chelating properties and has the widest buffer range of the food acids (2.5 –6.5), it is also the most popular and commonly added acid in beverages, especially juice based ones, is citric acid Dzlezak, (1990).

Citric acid has been indicated as a standard for evaluating the effects of other acidulants in food products Gardner, (1972).

Citric acid is highly soluble in water and can deliver a “burst” or rapid built up of tartness, which makes it suitable for use in flavor modification or enhancement. Citric acid is used to increase tartness levels and enhance fruit flavors in soft drinks and confectionery Hansson *et al.*, (2001).

Citric acid has been reported to enhance flavor, storage stability and reduce microbial counts of meat products Leo *et al.*, (2012).

Citric acid, a food acidulant is often used in meat marination to improve the water holding capacity (WHC) and tenderness of beef muscle Ke *et al.*, (2009).

Acetic, lactic, and citric acid solutions at 1.5-2.5% are approved by USDAFSIS as acceptable interventions for reducing carcass contamination USDAFSIS, (1996a). Citric acid, a food acidulante, is not only often used in acid marinating to improve the water-holding capacity and tenderness of beef muscle but is also commonly used as a chelator to control the activity of pro-oxidant metals Ke, (2006).

The concentration of citric acid (CA), treatment time, temperature and the type of organism plays an important role in reducing the number of bacteria Virto *et al.*, (2005).

Citric acid and its salt form, sodium citrate, are the most widely used organic acids in the food industry. They are commonly added to food substances to chelate metal ions, control pH, and these compounds have also been shown to have effects on food color Bouchard and Merritt, (1979).

Citric acid solution may be applied to the surfaces of meat in concentrations up to 10% immediately prior to packaging as antibacterial agent Code of Federal Regulations, (1998).

Acidic marinade solutions decrease pH and suppress microbial growth. The reason for this inhibition is the presence of weak organic acids Yusop *et al.*, (2010).

The antibacterial activity of citric acid is dependent on pH, concentration and anion effects, the use of any antimicrobial depends on several factors, such as desired effect, legal limits of use and effect on food Young and Foegeding, (1993).

2.10 The usages of citric acid in meat and meat products:

The burger formulated from citric acid treated beef had low microbial count and microbial growth was significantly ($P < 0.05$) decreased at the end of the storage. Citric acid treatment significantly ($P < 0.05$) decreased the pH value of the burger, delayed the rapid increase in PH and could also delay the growth of spoilage microorganism Abd Elgadir *et al.*, (2015).

Citric acid added to intact turkey breasts at either 0.2% or 0.3% reduced pH compared with the control regardless of pinking agent Sammel or Claus, (2003).

Citric acid provides antioxidant and antimicrobial benefits to fresh beef sausage and is recommended as an additive to extend the shelf-life of meat products at levels not more than 0.2% during storage at -18°C Ikhlas and Mousab, (2011).

Meat acid marination is an applicable technique to tenderize beef burgers with high percentage of meat Hosseini and Esfahani Mehr, (2015).

Findings indicated positive effects in the physicochemical properties and storage ability of sous vide chicken breast at 2% and 5% citric acid concentrations ,also increasing citric acid concentration had an antibacterial effect on the growth of microorganisms Ji-Han Kim *et al.*, (2015).

Citric acid was investigated for its effect on inhibition of bacteria, yeast and molds and was shown to be inhibitorier than lactic acid and acetic acid Sorrells, (1989).

This acid exhibits both bactericidal and antistatic effects against *L.monocytogenes* Buchanan *et al.*, (1994).

One of the ways to extend the shelf life of fresh beef is through using of organic acids such as citric acid, acetic, lactic and tartaric acids in individually or in combination which can result in effective shelf life extension of fresh beef Mohamed *et al.*, (2008).

Citric acid had effect in reducing microbial load, because of the decrease in pH. Eduzor *et al.*, (2016).

The effect of 1% citric acid caused reducing in total bacterial count of the raw and cooked sausages Maha and Abogroun, (2014).

Ginger Extract combined with citric acid had a significant tenderization effect on duck breast muscle Hyun *et al.*, (2015)

The best acid marination technique for beef would be citric acid since it is effective at both improving texture and inhibiting lipid oxidation. Shuming ke *et al.*, (2009).

When using 1, 3, 5% acetic, lactic or citric acids applied on the carcass at 24°C then incubated for 24 hrs. At 4°C the organic acids reduced the bacterial load, but did not completely inactivate the organism Cutter and Siragusa, (1994).

A food acidulant such as citric acid is used in marinade to enhance water-holding capacity and tenderness of muscle. It controls the activity of pro-oxidant metals by acting as a chelator Ke *et al.*, (2009)

Meat treated with acid improved in tenderness within the first few days of marinating. In addition there is a slight increase in tenderness when the treated meat is marinated for a longer duration (over 21 days); this suggests that meat marinated in an acid solution for an extended period will not result in any distortion of sensory attributes Wenham and Locker, (1976); Berge *et al.*, (2001).

Citric acid at 0.2% and 0.3% and sodium citrate consistently reduced natural or induced pink color in ground turkey rolls Sammel and Claus, (2006).

Samples treated with citric acid had higher fat content when compared with control samples Desmond and Troy, (2001).

With increasing citric acid concentration, there was a clear decrease in muscle pH Meltem *et al.*, (2007).

There is no difference in the cooking loss of chicken breast marinated in solutions with various pH containing citric acid Yusop *et al.*, (2010).

The acid concentration demonstrated a positive correlation with the moisture content of meat products marinated in weak acid Aktas and Kaya, (2001); Aktas *et al.*, (2003).

Acid treatments increased moisture content, water holding capacity and decreased cooking loss Oreskovich *et al.*, (1992).

Immersion meat with citric acid solution failed to improve cooking yield Klinhom *et al.*, (2015).

The increase in the concentration of citric acid resulted in increasing the antibacterial effect, Zahran and Hendy, (2013).

Natural antimicrobials such as buffered citric acid, acetic acid, or a mixture of the two can control the growth of pathogens like *L. monocytogenes* and *C. perfringens* Glass *et al.* , (2010).

Researchers have evaluated the efficacies of ascorbic, propionic, citric, lactic, and acetic acids, ranging from 0.1 to 24%, to reduce populations of bacteria on red meat Dickson and Anderson, (1992).

CHAPTER THREE

MATERIALS AND METHODS

3.1 location

The study was conducted in the meat processing laboratory, in the the Department of Meat Production, Faculty of science and technology of Animal Production (kuku), Sudan University of science and technology, during the period from 7/10/2018 to 28/10/2018.

3. 2 Samples collection: The meat was obtained from mature beef animals purchased from Animal Production Research Centre (kuku). A total of 6kgs of fresh beef. The samples were transported hygienically to the meat processing laboratory, in the Department of Meat Production, Faculty of science and technology of Animal Production (kuku), Sudan University of science and technology. Then the samples were kept in a refrigerator at 4°C for overnight.

3.3 samples preparation : The meat ,potatoes and garlic were minced in a grinder and put into vessel of mixing ,then non meat products were added to the mixture as the showing in table(1).

Table 1: Burger Recipe

Material	Amount per gram
Beef meat	6.000
P0tatoes	1200
Skimmed milk	240
Coriander	18
Black pepper	12
Cinnamon	12
Nut meg	12
Pipper cubeb(mace)	12
Salt	108
Garlic	18
Bread crumbs	600
Chilled water	1200 ml

The mixture was divided into three parts and the citric acid was added to two parts with levels of 0.8% and 1% respectively and the third part without adding citric acid (control). Each part was processed separately by using the piston of burger. Then the processed samples were labeled to (A) control, (B) level 0.8% and (C) level 1% then put in to deep freezing at (-18°C), for doing the following tests:

3.4 Chemical analysis:

3.4.1 Determination of moisture content:

Five grams sample weighed into dried weighed crucible. the samples dried overnight in a drying oven at 100°C for 18 hours. The dried sample put into desiccators, allowed to cool and reweighed. the moisture content percentage calculated from the equation :

$$\text{Moisture (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where:

W1= initial weight of empty crucible.

W2= weight of crucible +sample before drying.

W3 =final weight of crucible +sample after drying AOAC, (1990).

3.4.2 Determination of fat content:

Crude fat was determined based on sohxlet extraction method of AOAC (1990). Two grams of sample weighed into a muslin thimble. This inserted into extraction column with condenser connected. 200ml of extracting solvent (petroleum ether, boiling point 40-60°C) was poured into the cleaned, dried and weighed round bottom flask and fitted into the extraction unit. Then, the sample subjected to continuous extrication with ether for 6hrs. After extraction, the thimble was removed and the solvent salvaged by distillation. The flask containing the fat and residual solvent allowed drying in an oven at 100°C for 30 minutes to complete evaporate the solvent. The flask containing the fat cooled in a desiccator and weighed. The fat obtained expressed a percentage of the initial weight of the sample. The fat content percentage calculated from the equation:

$$\text{Fat (\%)} = \frac{W2-w1 \times 100}{\text{Weight of sample}}$$

Weight of sample

Where:

W1=weight of empty extraction flask.

W2=weight of flask and oil extract AOAC, (1990).

3.4.3 Crude protein content:

Crude protein determined by the method of the association of official analytical chemists (AOAC, 1990). One gram of the sample weighed into kjeldahl digestion flask and one gram catalyst added. 25 ml of concentrated H₂SO₄ added and the flask shaken to mix the contents. The flask then placed on a digestion burner for 3 hours and heated until the solution turned green and clear. The sample solution then transferred into a 100 volumetric flask and made up to mark with distilled water. 25 ml of 2% boric acid pipetted into a 250ml conical flask and two drops of methyl red indicator solution added; and into the decomposition chamber of the distillation apparatus was added 12 l of 40% NaOH solution. 5 ml of digested sample solution then introduced into a kjeldahl Flask. The condenser tip of the distillation apparatus then dipped into the boric acid contained in the conical flask. The ammonia in the sample solution then distilled into the boric acid until it changed completely to blue. The distillate was then titrated with 0.1 NHCL solution until it became colourless. the percent total nitrogen and crude protein calculated using a conversion factor of 6.25 AOAC, (1990).

$$\text{Crude protein \%} = \frac{T (N \times 14 \times VF) \times 6.25 \times 100}{\text{Sample weight}}$$

Sample weight

Where:

N= normality of titrate (0.1N).

VF= total volume of the digest.

T= titrate value.

3.4.4 Determination of ash:

Five grams sample weighed into previously dried and weighed porcelain crucible, transferred into a muffle furnace at 150 °C the temperature increased gradually until reach 600° c for 3hours. The crucible with its content removed from the furnace, cooled in a desiccator to a room temperature and the crucible with its content reweighed. The percentage of ash content calculated as AOAC, (1990):

$$\text{Ash \%} = \frac{\text{weight of ash} \times 100}{\text{Weight of original sample}}$$

3.4.5 pH measurement

PH was determined for raw and processed meat samples of various treatments .10gms of sample were placed in a blinder jar, and 100gms of distilled water were added, the mixture was blended at high speed for one minute .The PH of mixture measured using a PH- meter (model l. Pustl Munchen15), which had calibrated with two standard buffers (7and 4).

3.5 Physical analysis

3.5.1 Water Holding Capacity (WHC):

Approximately 0.5gm of product placed on humidified filter paper and pressed between two plexiglass for two min at 25kg/cm². Meat and Moisture areas measured using a compensating Plano meter. The result expressed as ratio (Grau and Hamm, 1953). The water holding capacity calculated from the equation:

$$\text{Water holding capacity (WHC)} = \frac{\text{loose water area} - \text{Meat film area}}{\text{Meat film area}}$$

3.5.2 Cooking Loss:

Cooking loss determined by using thermostatically controlled water bath 90°C for 90 min, samples weighed before and after cooking. Adam and Abugroun, (2015).

$$\text{Cooking loss} = \frac{\text{weight before} - \text{weight after cooking}}{\text{Weight before cooking}}$$

3.6 Sensory Evaluation Experiment:

The prepared burger pieces were cooked on hot plate set at 180°C for 30minutes. Every piece was cut into three parts and provided to the sensory panelists to familiarize them with the properties. The panel consisted of twenty members from postgraduate students and staff of the faculty of animal production science & Technology. Cooked beef burgers were served to evaluate sensory attributes of tenderness, juiciness, flavor, color, and over all acceptability .The panelists were asked to indicate to evaluate the processed burgers using 8- point (hedonic scale) card (Cross *et al.*, 1978) in which the highest score of 8 being extremely desirable and 1 being extremely un desirable .

3.7 Bacteriological Assessment of burger

Total viable bacterial counts of fresh and frozen burger were done after variable periods of storage) 0, 7, 15, and 21 days).

3.7.1 Preparation of sample:

25 grams of burger samples were weighed , The samples were then grinded with martum in hand , then blended with 225 ml normal saline and shake well by hand. Three Duplicate samples were taken Monica, (1991).

3.7.2 Culture Media: Plate count agar. The medium was in form of dehydrated powder. It was composed of casein enzymic hydrolysate -yeast extract, Dextrose and agar. It was prepared by dissolving 1.8 gm. of medium in 100 ml of distilled water, then put it on water bath to dissolve the medium completely. Sterilize by autoclaving at 15Ibspressure (121 °C) for 15 minutes

3.7.3Total viable counts:

The best method for viable count is called Miles and Misra. The Miles and Misra Method (or surface viable count) is a technique used in Microbiology to determine the number of colony forming units in a bacterial suspension or homogenate. The technique was first described in 1938 by Miles, Misra and Irwin Miles and Misra, (1938).

3.7.3.1 Miles and Misra (dilution method):

Using sterile pipette 1.0 ml of the culture was transferred to a test tube containing 9.0 ml sterile normal solution. The contents were mixed by another sterile pipette and 1.0 ml of the mixture was transferred to a second tube until the fifth tube thus decimal serial dilutions up to 10^{-5} were prepared. Using sterile pipettes 1.0 ml of the dilutions 10^{-5} was transferred into duplicate sterile Petri dishes.

3.7.3.2 Plates preparation:

- Three plates are needed for each dilution series, for statistical reasons an average of at least 3 counts are needed.
- Plates are divided into equal sectors (divided in to 4 quarter). The sectors are labeled with the dilutions.
- Use 1/50ml dropper (Pasteur pipette) remove about 1 ml from the highest dilution (last dilution).
- Drop one drop in one quarter.
- Using the same pipette remove 1 ml of previous and go on each time from the previous dilution.
- Using a bent glass rod spread your drop starting by higher dilution.
- The plates are left upright on the bench to dry before inversion and incubation at 37 °C for 18 – 24 hours (or appropriate incubation conditions considering the organism and agar used).
- Next day remove petri- dishes from incubator.
- Each sector is observed for growth, high concentrations will give a confluent growth over the area of the drop, or a large number of small/merged colonies. Colonies are counted in the sector where the highest number of full-size discrete colonies can be seen (usually sectors containing between 2-20 colonies are counted).

Take the median of dilution 10^{-5} and calculate the colonies in one ml cfu/ml according to the equation Martin *et al.*, (2007):

The following equation is used to calculate the number of colony forming units (CFU) per ml from the original aliquot / sample:

CFU per ml = Average number of colonies for a dilution x 50 x dilution factor.

3.8 Statistical Analysis

All the data presented as Mean \pm standard deviation (std) was subjected to one way analysis of variance (ANOVA)($p < 0.05$). All statistical calculations were performed with SPSS (version 16.0) computer program Gomez and Gomez (1984).

CHAPTER FOUR

RESULTS

Table (2): Chemical analysis of beef burger treated with citric acid

Citric acid levels (%)	Chemical analysis				
	Moisture%	Fat%	CP%	Ash%	pH
0	70.43^a±0.32	1.38^b ±0.07	17.6^b ±0.27	2.36^a ±0.04	5.56^a ±0.02
0.8	68.38^b ±0.32	1.99^a ±0.11	18.33^a ±0.12	2.15^b ±0.07	4.03^b ±0.04
1	69.95^a ±1.1	1.58^b ±0.07	17.47^b ±0.42	2.07^b±0.096	4.00^b ±0.02
Sig	*	**	*	**	**

N=3/replicate

*=significant differences at P<0.05

**=significant differences at P<0.01

Different superscript letters with in the same column means significant differences at P<0.05

4.1 Changes in chemical composition of beef burger:

4.1.1 Moisture content

As shown in Table (2) moisture content of burger samples slightly decreased ,it decreased from(70.43 \pm 0.32)in level 0% citric acid to (69.95 \pm 1.1)in level 1% citric acid at (P<0.05).

4.1.2 Fat content:

The fat content increased Table (2),it increased from(1.38 \pm 0.07) in level 0% citric acid to(1.58 \pm 0.07) in level 1% citric acid at (P<0.01).

4.1.3 Protein content

The protein of beef burger Table (2) decreased from (17.6 \pm 0.27) in level 0% citric acid to(17.47 \pm 0.42) in level 1% citric acid at (P<0.05).

4.1.4 Ash content

In Table (2) the ash percentage decreased with increasing the level of added citric acid, it decreased from(2.36 \pm 0.04)) in level 0% citric acid to(2.07 \pm 0.096) in level 1% citric acid at (P<0.01).

4.1.5 pH values

As shown in Table (2) increasing the level of added citric acid resulted in decreasing of pH value, it decreased from(5.56 \pm 0.02) in level 0% citric acid to(4.00 \pm 0.02) in level 1% citric acid at (P<0.01).

Table (3)

Physical analysis of beef burger treated with citric acid

Citric acid levels%	WHC	Cooking loss	Shrinkage
0	0.73^c ±0.17	19.90^c ±0.59	15.28^c ± 0.28
.8	1.65^b±0.04	22.39^b ± 0.03	16.17^b± 0.04
1	3.01^a ±0.32	23.75^a ±0.49	17.33^a± 0.54
Sig	**	**	*

N=3/replicate

*=significant differences at P<0.05

**=significant differences at P<0.01

Different superscript letters with in the same column means significant differences at P<0.05

4.2 Changes in physical properties of beef burger

4.2.1 Water holding capacity (WHC)

As shown in table (3) Water holding capacity (WHC) decreased with increasing citric acid levels. Water holding capacity (WHC) significantly decreased from (0.73 ± 0.17) in level 0% citric acid to (3.01 ± 0.32) in level 1% citric acid at ($P < 0.01$).

4.2.2 cooking loss

Cooking loss percentages increased ($P > 0.05$) with increasing citric acid as result of increased acidity (lower pH). , cooking loss increased from (19.90 ± 0.59) in level 0% citric acid to (23.75 ± 0.49) in level 1% citric acid at ($P < 0.01$).

4.2. Shrinkage

Shrinkage percentages increased ($P > 0.05$) with increasing citric acid as result of increased acidity (lower pH). shrinkage significantly increased from (15.28 ± 0.28) in level 0% citric acid to (17.33 ± 0.54) in level 1% citric acid at ($P < 0.05$).

Table (4): Sensory Evaluation of beef burger treated with citric acid

Citric acid levels (%)	Color	Texture	flavor	Juiciness
0	2.05^b ±0.76	2.1^b ±0.64	1.85^b ±0.99	2.2^b ±0.89
.8	2.45^b ±01.32	2.5^b ±1.15	2.7^b ±1.46	2.4^b ±1.14
1	3.85^a ±2.16	3.7^a ±1.99	4.4^a ±2.35	3.3^a ±2.03
Sig	**	**	**	*

N=3/replicate

*=significant differences at P<0.05

**=significant differences at P<0.01

Different superscript letters with in the same column means significant differences at P<0.05

4.3 Changes in sensorial properties of beef burger

4.3.1 Effect of citric acid on color

Table (4) shows high significant differences at ($P < 0.01$). The colour as evaluated by panellists showed significant increased ($P < 0.01$) with the increased level of citric acid, 0% level showed the lowest colour score (2.05), while 1% level showed the highest score (3.85).

4.3.2 Effect of citric acid on texture

Table (4) shows high significant differences at ($P < 0.01$). The texture as evaluated by panelists showed significant increased ($P < 0.01$) with the increased level of citric acid, 0% level showed the lowest texture score (2.1), while 1% level showed the highest score (3.7).

4.3.3 Effect of citric acid on flavor

Table (4) shows high significant differences at ($P < 0.01$). The flavor as evaluated by panelists showed significant increased ($P < 0.01$) with the increased level of citric acid, 0% level showed the lowest flavor score (1.85), while 1% level showed the highest score (4.4).

4.3.4 Effect of citric acid on juiciness

Table (4) shows significant differences at ($P < 0.05$). The juiciness as evaluated by panelists showed significant increased ($P < 0.05$) with the increased level of citric acid, 0% level showed the lowest juiciness score (2.2), while 1% level showed the highest score (3.3).

(Table 5): Total viable bacterial count of beef burger treated with citric acid

Factors	Total viable bacterial count (log CFU/g⁻¹)
Citric acid levels (%)	
0	7.03 ^a
0.8	7.09 ^a
1	6.90 ^b
±SE	0.046
Sig	*
Storage period (day)	
0	7.04 ^a
7	7.14 ^a
14	7.11 ^a
21	6.75 ^b
±SE	0.053
Sig	**
Citric acid ×Storage period	
±SE	0.092
Sig	*
Over all	7.01
±SE	0.027

N=3/replicate

*=significant differences at P<0.05

**=significant differences at P<0.01

Different superscript letters with in the same column means significant differences at P<0.05

4.4. Effect of citric acid on total viable count of beef burger

As shown in table (5), citric Acid leads to insignificant decrease in total bacterial count. Total bacterial count in level (0%) was (7.03 log 10) it decreased to (6.90 log 10) in level 1%).

CHAPTER FIVE

DISCUSSION

Moisture content of burger samples slightly decreased, because the acidity (lower pH) reduces the ability of burger to bind water. Similar results were found by Carroll, (2005), Ikhlas and Mousab, (2011). These findings disagree with Ji-Han Kim *et al.*, (2015) who reported that, The sample marinated with citric acid solution resulted in higher moisture content due to immersion in acidic treatments, which influences the uptake of water compared to non-treated groups. Other studies also reported that the acid concentration demonstrated a positive correlation with the moisture content of meat products marinated in weak acid (Aktas and Kaya, 2001; Aktas *et al.*, 2003). Also disagrees with Oreskovich *et al.*, (1992) who found that acid treatments increased moisture content.

The fat% in this study increased significantly as citric acid levels increased because citric acid prevents oxidation of fat. This result is in line with those obtained by Desmond and Troy, (2001) who found that samples treated with citric acid had higher fat content when compared with control samples. Similar result obtained by Ikhlas and Mousab, (2011). It is also agrees with the result obtained by Eduzor *et al.*, (2016).

The protein of beef burger in this study (Table2) decreased with increasing the level of added citric acid. Protein content in this study decrease, because lowering pH causes protein degradation. Similar results were found by Carroll, (2005). It also agrees with Desmond and Troy, (2001) who found that samples treated with citric acid had low protein content when compared with control samples. Ikhlas and Mousab, (2011) obtained similar result.

Ash percentage in this study (Table2) decreased with increasing citric acid level. This result dis agrees with the result obtained by Ikhlas and Mousab, (2011) who reported that ash increased with increasing of citric acid.

As shown in (Table2) increasing the level of added citric acid resulted in decreasing of pH value.

These results show that the overall means of pH of beef burger samples were reduced significantly ($P < 0.01$) with increasing the level of added citric acid and this could be attributed to its acidic nature. This result agrees with Ikhlas and Mousab, (2011) who reported that pH of the fresh beef sausage samples were reduced significantly ($P < 0.05$) with increasing the level of added citric acid. This result agrees with Sammel and Claus, (2003, 2006) who reported that increased acidity will result in lower pH and thus a decrease in the ability of meat to bind water. Meltem *et al.*, (2007) stated that with increasing citric acid concentration, there was a clear decrease in muscle pH. Also agrees with Abd Elgadir *et al.*, (2015) and Ji-Han Kim, *et al.*, (2015) who stated that immersion of chicken meat in citric acid significantly decreased the pH. Similar result obtained by Hyun, *et al.*, (2015). Similar result obtained by Yusop *et al.*, (2010) who found that ,acidic marinade solutions decrease pH and suppress microbial growth. The reason for this inhibition is the presence of weak organic acids.

Water holding capacity (WHC) in this study decreased with increasing citric acid levels (table3). When using organic acids one can usually expect for there to be a reduction in the product pH. With a reduction in pH and becoming closer to the meats isoelectric point there is a loss of WHC due to the space between the myofibrils. Such results are in harmony with Sammel and Claus, (2003, 2006) who reported that increased acidity will result in lower pH and thus a decrease in the ability of meat to bind water. Similar result obtained by Ikhlas and Mousab, (2011) who reported that the decrease in pH which can cause the pH to approach the isoelectric point of myofibrillar proteins and affect the swelling of proteins that reduces the ability of meat to bind water. This result confirms the findings of Desmond and Troy, (2001) who stated that increase the acidity will lower the water holding capacity. Eduzor *et al.*, (2016) obtained similar result. This finding disagrees with Oreskovich *et al.*, (1992) who reported that acid treatments increased Water holding capacity. Also disagrees with the result obtained by Shuming ke *et al.*, (2009) who reported that Water-holding capacity of beef semitendinosus muscle increased significantly.

Cooking loss and shrinkage percentages increased ($P > 0.05$) with increasing citric acid as result of increased acidity (lower pH), which decreased the ability of meat to bind water, (table3). Sammel and Claus, (2003, 2006), Ikhlas and Mousab, (2011) obtained similar results. These findings disagree with Oreskovich *et al.*,

(1992) who reported that acid treatments decreased cooking loss of beef cores. Also disagrees with Hosseini and Esfahani Mehr, (2015) who reported that the lowest cooking loss was observed in samples marinated with the highest concentration of citric acid. Previous research has reported the acid treatment decreased cooking loss of beef. Yusop *et al.*, (2010) has indicated that there is no difference in the cooking loss of chicken breast marinated in solutions with various pH containing citric acid. These results might be due to the lack of change in core pH of meats.

The effect of citric acid levels on sensory evaluation is presented in Table (4). The colour as evaluated by panellists showed significant increased($P < 0.01$) with the increased level of citric acid, 0 level showed the lowest colour score (2.05), while 1% level showed the highest score (3.85).

The lightness of fresh beef burger increased by addition of citric acid because at lower pH and ionic strength, muscle proteins swell and light reflection alters and this results in lighter colour. Similar result was obtained by Aktas and Kaya, (2001). This finding dis agrees with Ikhlas and Mousab, (2011).

The texture as evaluated by panelists showed significant increased($P < 0.01$) with the increased level of citric acid(table 4), 0 level showed the lowest texture score (2.1), while 1% level showed the highest score (3.7). That because, the effects of organic acids on meat texture depend on pH drop after treatment that resulting in solubilization of the collagenous tissue and increased tenderness similar result obtained by Burke and Monahan, (2003) who found that acid marination increased meat tenderness. And in line with shuming ke *et al.*, (2009) who reported that citric acid is effective at improving texture. Similar result obtained by Anonymous, (1990).

The flavor as evaluated by panelists showed significant increased($P < 0.05$) with the increased level of citric acid(table 4). Similar result obtained by Leo (2012), who reported that Citric acid enhanced flavor, storage stability and reduce microbial counts of meat products .

The juiciness as evaluated by panelists showed significant increased ($P < 0.05$) with the increased level of citric acid, (table4), 0 level showed the lowest juiciness score (2.2), while 1% level showed the highest score (3.3), this might be due to as the

juiciness affected by the lipid content and here citric acid prevent lipid oxidation, there for lipid content increased and this led to increase of juiciness.

The effect of citric acid level on total bacterial count is presented in table (5). Citric Acid in this study leads to significant decrease in total bacterial count. Total bacterial count in level (0%) was (7.03 log 10) it decreased to (6.90 log 10) in level 1%) This might be due to the increased acidity which limits microbial growth, because the lower pH disturbs the homeostasis of bacterial cells thus decreasing the biological activity as a result of pH changes of the cell's environments. This agrees with result obtained by Ji-Han Kim, *et al.*, (2015) who reported that increasing citric acid concentration had an antibacterial effect on the growth of microorganisms. Similarly, Cutter and Siragusa ,(1994) found when using 1, 3, 5% acetic, lactic or citric acids applied on the carcass at 24°C then incubated for 24 hrs at 4°C the organic acids reduced the bacterial load. Also agrees with the result obtained by Eduzor *et al.*, (2016) who demonstrated that citric acid had effect in reducing microbial load because of the decrease in PH. This result confirms the findings of Abd Elgadir *et al.*, (2015) who found that the microbial growth was significantly ($P < 0.05$) decreased at the end of the storage in the fresh burger. Similarly, Ikhlas and Mousab, (2011) who stated that Citric Acid leads to insignificant decrease in total bacterial count. Similar result obtained by Mohamed *et al.* ,(2008), who found that One of the ways to extend the shelf life of fresh beef is through using of organic acids such as citric acid, acetic, lactic and tartaric acids in individually or in combination which can result in effective shelf life extension of fresh beef. Similarly, Zahran and Hendy, (2013) who found that the increase in the concentration of citric acid resulted in increasing the antibacterial effect.

CHAPTER SIX

Conclusions and recommendations

Conclusions:

- Citric acid provides antimicrobial benefits to beef burger.
- The results of the study showed that treating fresh beef burger with citric acid in concentration of 0.8% is effective practice in keeping quality of fresh beef products during storage period.
- The burger prepared using citric acid treatment during sensory evaluation according to the sensory attributes used gave high values.

Recommendations:

- Citric acid is recommended as an additive to extend the shelf-life of meat products at levels not more than 0.8% during storage at (-18°C).
- Future experiments are needed to explain the effect of citric acid on beef burger quality, for future application of citric acid treatments in the meat industry.

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Appendix 1

Grading chart for meat and burger

Evaluate these samples for color, texture, flavor and juiciness- for each sample, use appropriate scale to show your attitude by checking at the point that best desirable the feeling about the sample. If you have any question please ask, thanks for your cooperation.

Name:.....

Date:

Type of product:.....

Panelist number:.....

sample code	Color	Flavor	tenderness	juiciness	comments
A					
B					
C					

Key:

Color		Flavor		tenderness		juiciness	
8	extremely desirable	8	extremely desirable	8	extremely desirable	8	extremely desirable
7	very desirable	7	very desirable	7	very desirable	7	very desirable
6	moderately desirable	6	moderately desirable	6	moderately desirable	6	moderately desirable
5	less desirable	5	less desirable	5	less desirable	5	less desirable
4	less undesirable	4	less undesirable	4	less undesirable	4	less undesirable
3	moderately undesirable	3	moderately undesirable	3	moderately undesirable	3	moderately undesirable
2	undesirable	2	undesirable	2	undesirable	2	undesirable
1	extremely undesirable	1	extremely undesirable	1	extremely undesirable	1	extremely undesirable

