

Sudan University of Science and Technology College of Graduate Studies

Quality and Safety of Unpacked Beef Sausage in Khartoum State

دراسة جودة وسلامة السجق البقري الغير مغلف في ولاية الخرطوم

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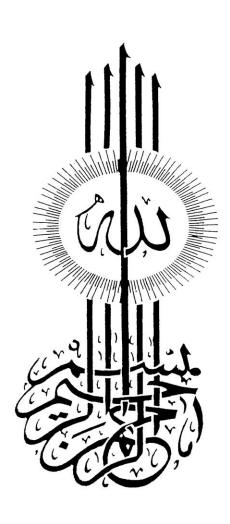
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قال تعالى :

(وَمَا أُورِيتُهُ مِنْ الْعِلْمِ إِلاَّ هِنْ الْعِلْمِ إِلاَّ

مدرة الإسراء الآية 85

الإهداء

الى معلم البشرية الأول رسولنا الأعظم , سيدنا مدمد حلى الله عليه والم وسلم.

إلى ابيه:

من أسكنني قلبه وعلمني السلام قبل النحام.

إلى أمين

من سقتيني من حنانما شمد المراء وعلمتني الوئاء قبل الفطاء.

إلى زوجيى:

الشمس التي ترسل لي كل حباج أجمل وأرق كلمة ومن ينتظرني مع مولد كل يوم جديد بشوق وحنين .

شكر وغرفان

الشكر والعند والمنة أولاً وآخراً لله وحده أن يسر هذا العمل فله الشكر عدد ما شكره الشاكرون , شكراً بكل معنى فله الشكر عدد ما شكره الشاكرون .

ثو من بعد يطيب لنا أن نتقدو بنالس الشكر وأعطره لكل من مد لي يد المساعدة والتشبيع في بدتي هذا إبتداء بالأب الفاضل والمعلو البروفيسور: داوؤد الزبير احمدالذي تشرفت بتعيينه مشرفاً على مشروعي.

ولا ننسى أن نقدم شكري إلي ا.د.محمد عبدالسلام, د.محمد الطيب حمد, ا.محمد سرالختم,ا.سمام عبدالوهاب, ا.محمد لرين الطيب حمد, ا.محمد سرالختم,ا.ربابابراهيم, ا.وحال عباس,

واخيرا اشكر د. منير ابوبكر عبدالله لكو جميعاً الشكر والتقدير

وفاء

Abstract:

This study was conducted in the college of Veterinary Medicine, Sudan University of Science and Technology. During the period from February to May 2013 to investigate the effect of storage period on chemical composition, some quality attributes and conformity of unpacked beef sausage in Khartoum state with Sudanese Standard Metrology Organization Specifications. The results of study showed that there was a high significant difference (p \le 0.01) between locations of collection. Khartoum samples recorded highest moisture % (69.13-60.03), whereas Bahri samples recorded highest content of protein (14.37- 10.15%), while highest fat content recorded by Omdurman samples (26.00- 21.65%). Khartoum samples recorded highest ash percentage (2.32 and 1.07%) at the storage periods (0 and 45 days) respectively, while Omdurman samples recorded the highest percentage in ash (1.82 and 1.38%) at the storage period (15 and 30 days) respectively. The physio-chemical parameters of unpacked beef sausage showed high significant differences (p≤0.01) between Khartoum, bahri and Omdurman samples. Bahri samples recorded the highest value of water holding capacity and cooking loss% as (4.42-2.39) and (36.10-34.05%) respectively. Omdurman samples recorded the highest pH value (6.04-6.21).

Sensory evaluation results showed high significant differences ($p \le 0.01$) between the location samples. Omdurman samples recorded the highest score of color (4.97, 5.23 and 5.64) during storage periods (0, 15 and 30 days) respectively. Whereas Bahri samples recorded the highest score (5.94) at storage period (45 days). Omdurman samples recorded highest score in the texture, juiciness and flavor, while bahri samples recorded highest score of juiciness at storage period (0,15,30 and45 days) respectively.

There was no significant differences (p \leq 0.05) between the samples collected from Khartoum State in bacterial contaminants and total bacterial count.

All beef sausage samples collected in Khartoum state were contaminated with *salmonella* and *E. coli*, which *disagreed* with the SSMO Specification.

ملخص الدراسة

أجريت هذه الدراسة في كلية الطب البيطري – جامعة السودان العلوم والتكنولوجيا. خلال الفترة من فبراير - مايو 2013 لدراسة تأثير فترة التخزين علي التركيب الكيميائي، بعض خصائص الجودة للسجك البقري غير المغلف بولاية الخرطومومطابقتها للمعابير والمواصفات السودانية. أظهرت نتائج الدراسة أن هناك فروق معنوية عالية (0.01 بين العينات المجموعة من ولاية الخرطوم . سجلت عينات الخرطوم أعلى نسبة رطوبة (0.03 69.13)، في حين سجلت عينات بحري أعلى محتوى في البروتين (0.03 69.13) بينما سجلت عينات امدرمان اعلي نسبة للدهون (0.03 69.15٪). سجلت عينات الخرطوم أعلى نسبة رماد (0.03 69.13٪) في فترتي التخزين (0.03 69.14٪) في التوالي، الخرطوم أعلى نسبة رماد (0.03 69.14٪) في فترتي التخزين (0.03 69.14٪) في فترتي التخزين (0.03 69.14٪) في فترتي التخزين (0.03 69.14٪) معنوية عالية يوما). أظهرت الخواص الفيزيوكيميائية للسجك البقري غير المغلف فروق معنوية عالية مسك الماء والفاقداثناء الطهي حيث كانت (0.03 60.14٪) و (0.03 60.15٪) على التوالي. مسك الماء والفاقداثناء الطهي حيث كانت (0.03 60.14٪) و (0.03 60.16٪) على التوالي. بينماسجلت عينات أم درمان أعلى قيمة للأس الهيدروجيني (0.03 60.16٪).

أظهرت نتائج التقييم الحسي فروق معنوية عالية ($p \le 0.01$) بين عينات المواقع الثلاث. سجلت عينات أم درمان على أعلى درجة في اللون (4.97، 5.23 و 5.64) خلال فترات التخزين (0، 15 و 30 يوما) على التوالي. بينما سجلت عينات بحرى أعلى درجة (5.94) في فترة التخزين (45 يوما). سجلت عينات أم درمان أعلى الدرجات في الملمس، والعصيرية والنكهة، بينما سجلت عينات بحري أعلى درجة من العصيرية في فترة التخزين (0, 5, 00 و 45يوم)

لا توجد فروق معنوية ($P \le 0.05$) بين العينات المجموعة من ولاية الخرطوم في الملوثات البكتيرية والعدد الكلى للبكتيريا.

كل عينات السجك البقري المجموعة من ولاية الخرطوم ملوثة بالسالمونيلا والايكولاي وغير مطابقة للمواصفات والمقائيس السودانية.

List of contents

Subject	Page
Dedication	I
Acknowledge	II
Abstract	III
Arabic abstract	V
List of content	VI
List of table	X
Chapter One	1
Introduction	1
Chapter Two	3
Literature Review	3
2.1: Meat	3
2.1.1: Chemical composition of meat	3
2.1.2: Nutritive value of meat	4
2.2: Sausage	4
2.2.1: Type of sausage	5
2.2.1.1: Cooked Sausage	5
2.2.1.2: Cooked, Smoked Sausage	5
2.2.1.3: Uncooked, Smoked Sausage	5
2.2.1.4: Dry and Semi-dry Sausage	6
2.2.1.5: Specialty Sausage	6
2.2.1.6: Fresh Sausages	6
2.2.2: The chemical composition of fresh beef sausage	7
2.2.3: Some global Standards of fresh sausage	8
2.2.4: Physico-chemical properties	8
2.2.4.1: Water holding capacity (WHC) of meat	8
2.2.4.2: Cooking loss%	10
2.2.4.3: Hydrogen ionic potential (pH)	10

2.2.5: Sausage ingredient	11
2.2.5.1: Meat	11
2.2.5.2: Fat	12
2.2.5.3: Salt	12
2.2.5.4: Spices	13
2.2.5.5: Cold water or ice (Water added)	13
2.2.5.6: Casing	14
2.2.5.7: Extra ingredient: Additive	14
2.2.5.8: Binders and Extenders	15
2.2.6: Manufacturing sausage	15
2.2.7: Quality of sausage	16
2.2.7. 1: Color	16
2.2.7. 2: Tenderness and Juiciness	16
2.2.7.3: Flavour and aroma	17
2.2.7.4: Texture	18
2.2.8: Contamination of sausage	18
2.2.9: Bacteriology Contamination	19
2.2.9.1: E. coli O157:H7	20
2.2.9.2: Salmonella	22
2.2.9.3: Listeria monocytogenes	23
2.2.9.4: Campylobacter	24
2.2.9.5: Staphylococci	24
2.3: Effect of storage on sausage	25
2.3.1: chemical composition	25
2.3.1.1: Moisture content	25
2.3.1.2: Protein content	26
2.3.1.3: Fat content	26
2.3.1.4: Ash content	26
2.3.2: Physico-chemical properties	27

2.3.2.1: Water holding capacity (WHC) of sausage	27
2.3.2.2: Cooking loss %	27
2.3.2.3: pH	27
2.3.3: Effect of storage period on sensory evaluation	28
2.3.4: Effect of storage period on total bacterial count	28
Chapter Three	29
Materials and Methods	29
3.1: Preparation of samples	29
3.2: Proximate Chemical composition	29
3.2.1: Moisture determination	29
3.2.2: Crude protein determination	30
3.2.3: Fat determination	30
3.2.4: Ash determination	31
3.3: Physico-chemical properties	31
3.3.1: Cooking loss %	31
3.3.2: Water holding capacity (WHC)	31
3.3.3: pH	32
3.4: Sensory evaluation	32
3.5: Bacterial assessment	32
3.5.1: Total bacterial count	32
3.5.2: Bacterial Isolation and Identification	33
3.6: Statistical analysis	33
Chapter Four	34
4. Results	34
4.1: Effect of storage period on the chemical composition	34
4.1.1: Moisture content	34
4.1.2: Protein content	34
4.1.3: Fat content	34
4.1.4: Ash content	35

4.2: Effect of storage period on the physico-chemical properties	35
4.2.1: Cooking loss %	35
4.2.2: Water holding capacity (WHC)	35
4.2.3: pH	35
4.3: Effect of storage period on the sensory evaluation	36
4.4: Effect of storage period on the bacterial assessment	36
4.4.1: Total bacterial count	36
4.4.2: Bacterial contaminants	36
Chapter Five	44
Discussion	44
5.1: Effect of storage period on the chemical composition	44
5.1.1: Moisture content	44
5.1.2: Protein content	44
5.1.3: Fat content	44
5.1.4: Ash content	44
5.2.: Effect of storage period on physio-chemical properties	45
5.2.1: Water Holding Capacity (WHC)	45
5.2.2: cooking loss%	45
5.2.3: pH	46
5. 3: Effect of storage period on sensory evaluation	46
5.4: Bacterial assessment	46
5. 4.1: Total bacterial count	46
5.4.2: Bacterial contaminants	47
Conclusion and Recommendation	48
Reference	49

List of tables and diagrams:

Table No	Page
Table (1): Effect of storage period on the chemical composition	37
of unpacked beef sausage	
Table (2): Effect of storage period on some physico-chemical	38
properties of unpacked beef sausage	
Table (3): Effect of storage period on sensory evaluation of	39
unpacked beef sausage	
Table (4): Effect of storage period on total bacterial count of	40
unpacked beef sausage	
Table (5) Contamination of unpacked beef sausage with	41
Salmonella and E. coli in Khartoum state:	
Diagram (1): Effect of storage period on the chemical	2نة4
composition of unpacked beef sausage	
Diagram (2): Effect of storage period on some physico-chemical	42
properties of unpacked beef sausage	
Diagram (3): Effect of storage period on sensory evaluation of	43
unpacked beef sausage	
Diagram (4): Effect of storage period on total bacterial count of	43
unpacked beef sausage	

Chapter One

Introduction:

Meat and meat products are considered as an excellent source of high quality animal protein, vitamins especially B complex, and certain minerals, especially iron (Gracey *et al.*, 1986). Meat in diet is an important source of protein which is not only of high biological value but for its amino acid contents (Shahidi, 1989).

Meat consumed by human for variety reasons including taste, nutrient, prestige, tradition and availability (Rogowski, 1980). 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 (Gunter and peter.2007). The average meat intake per person was 167 ± 84 g per day. Where beef accounted for 58% of the weekly consumption of meat (Jussara *et al.*,2006).

Meat quality is defined as the combination of physical, structural and chemical characteristics of meat which result in maximum desirability from the stand point of appearance and eatability (Pearson, 1960). Meat quality includes tenderness, palatability, aroma, flavor, color and juiciness (Kerry *et al.* 2002).

The microbiological quality of meat depends on the physiological status of the animal at the slaughter, spread of contamination during slaughter and processing, and temperature and other conditions of storage and distribution. In fact, some of microorganisms originate from the animal intestinal tract as well as from the environment with which the animal had content at some times before or during slaughtering (Adam, *et al* 2010).

Meat products may be contaminated with microorganisms from meat handlers, which carry pathogenic microorganism during the

processes of manufacturing, packing and marketing. Improper cooking, refrigeration or storage may lead to meat borne illness. Foodborne pathogens are the leading causes of illness And death in developing countries costing billions of dollars in medical care, medical and social costs (Fratmico *et al.*, 2005) In the United States, foodborne disease have been estimated to cause to 24 to 81 million of human illness (Archer and Kvenberg, 1985). In the developed world, frequent reports of food poisoning have increased public concern in relation to the potential presence of pathogenic organisms in food. Changes in eating habits, mass catering, unsafe food storage conditions and poor hygiene practices are major contributing factors to food associated illnesses (Hedberg *et al.*, 1992). Contamination of raw meat is one of the main sources of foodborne illnesses (Bhandare *et al.*, 2007; Podpecan *et al.*, 2007).

The objectives of this study are summarized as follows:-

- ❖ To study the safety of unpacked beef sausage.
- ❖ To study the effect of storage period on contamination level of unpacked beef sausage.
- ❖ To study the effect of storage period on the chemical composition of unpacked beef sausage.
- ❖ To study the effect of storage period on the sensory evaluation of unpacked beef sausage.
- ❖ To study whether the unpacked beef sausage satisfy (SSMO) specifications.

Chapter Two:

Literature Review:

2.1: Meat:

Meat is defined as those animal tissues, which are suitable for use as food. All processed or manufactured products, which might be prepared from those tissues, are included in the definition (Judge *et al*, 1990). Lawrie (1991) defined meat as the flesh of animals used as food and it is often widened to include as well as musculature organs such as liver and kidney, brain and other edible tissues. FSANZ(2002) and Williams(2007)defined meat as the whole or part of the carcass of cattle, camel, goat, sheep, buffalo, poultry, rabbit, hare and deer slaughtered. Gunter and Peter (2007) can be defined as "the muscle tissue of slaughter animals"

"Beef" is meat from full-grown cattle about 2 years old. A live steer weighs about 1,000 pounds and yields about 450 pounds of edible meat. Some major beef breeds are Angus, Hereford, Charolais, and Brahman(USDA, 2014).

2.1.1: Chemical composition of meat:

The muscle consist of 75% water, 20% protein, 3% fat and 2% soluble non –protein substance (tornberg, 2005). Biesalski (2002) mentioned that, meat and meat products are important sources of protein, some micronutrients and fat. It is mainly composed of water, protein (15-21%), fat (0.5-25%), oligonutrients and vitamins (especially rich in B group vitamins) (Hugas *et al.*, 2002). Rabaa (2009) reported the chemical composition of beef meat as 75% moisture, 19%.7% protein, 2.3% fat and 1% ash. Shawgi (2008) found the beef meat content of 71% moisture, 22.6% protein, 3.2% fat 0.98% ash and 0.3% carbohydrate. Mahassin (2008) found the chemical composition of beef meat as 71.9% moisture, 21.38% protein, 2.68% fat and 1.15% ash. Gunter and peter (2007)

reported the chemical composition of beef meat as 75% moisture, 22.3% protein, 1.8% fat and 1.2% ash. Generally the composition of meat is 75% moisture, 18% protein, 3% fat and 3.5% soluble non –protein substance (Lawire,1991).

2.1.2: Nutritive value of meat:

The nutritive value of meat is attributed to it is protein, fat, carbohydrates, vitamins and minerals content (Mahassin, 2008).

Meat is an excellent source of many nutrients, especially protein, B vitamins, iron and zinc. As a nutrient dense food, meat provides major nutritive contributions to the diet relative to the amount of calories it contains (Boyle, 1994).

Red meat contains protein of high biological value and important micronutrients that are needed for good health throughout life. It also contains a range of fats, including essential omega-3 polyunsaturated fats. Although the nutritional composition of meat vary somewhat according to breed, feeding, season and meat cut, In general lean meat has a low fat content, moderate in cholesterol and rich in protein and many essential vitamins and minerals (William, 2007). The nutritive value of 3 ounce cooked portion of lean beef containing 195 calories would provide 25 g of protein, 9g of fat, over one-third of the daily requirement for zinc and nearly 15% of the daily iron needs (Boyle, 1994; Fennema, 1996). The average amount of protein and calories for beef meat is 20% protein and 310 calories (FAO, 1989). USDA (2014) reported the nutrient composition of beef is179 Calories, 7.9(g) Fat, 3.0 Saturated Fat and 25 (g) Protein.

2.2: Sausage:

The process of preserving meat by stuffing salted, chopped meat flavored with spices into animal casings dates back thousands of years, to the ancient Greeks and Romans, and earlier(AFDO, 1999).It is

difficult to define sausage in single definition due to the variety of different type. Sausage can be defined as comminuted processed meat product made from red meat, poultry or combination of these with water, binder and seasoning (Essien, 2003). The term sausage is derived from the Latin word (salsus) meaning salt, or literally translated refers to chopped or minced meat preserved by salting and seasoned (Pearson and Tauber, 1984). Jihad *et al*(2009) define Sausage is prepared food, usually made of ground meat animal fat, salt, spices (sometimes with other ingredient such as herbs) and typically packed in a casing.

2.2.1: Type of sausage:

These are six type of sausages: fresh sausages, uncooked smoked sausages, cooked smoked sausages, and cooked sausages, dry and semi-dry sausage (Judge *et al.*, 1990; Boyle, 1994; Tronsky *et al.*, 2011).

2.2.1.1: Cooked Sausage:

Made from meat which are ground, seasoned, often cured, stuffed into casings, and cooked. No smoke is used. Cooked sausages are often served cold. Examples: braunschweiger; liverwurst; liver cheese. Cooked sausage may be cured or uncured, are heat processed and sometimes smoked. They often contain a variety meat or organ meat such as liver.

2.2.1.2: Cooked, Smoked Sausage:

Made from meat which are ground, seasoned, stuffed into casings, smoked and cooked. These can be eaten cold or reheated. Examples: bologna; berliner; cotto-salami; frankfurters.

2.2.1.3: Uncooked, Smoked Sausages:

Made from meat which is ground, seasoned, stuffed into casings, and smoked. These must be fully cooked before eating.

Examples: some kielbasas, mettwurst; teawurst; smoked country-style pork sausage.

2.2.1.4: Dry and Semi-dry Sausages:

Made from meat which are ground, seasoned, cured, stuffed into casings, fermented, often smoked, and carefully air-dried; true dry sausages are not cooked. These sausages have a distinctive tangy flavor due to the presence of lactic acid that is produced by fermentation. The meat is stuffed into casings and allowed to "ferment," the process by which bacteria metabolize sugars and produce acids and other compounds as byproducts. In meat fermentation, bacteria which produce lactic acid are utilized to produce the tangy flavor of dry sausages. They are sometimes referred to as "summer sausages" and eaten cold. Examples: pepperoni; German salami, Lebanon bologna, Genoa salami; thuringer; cervelat (Tronsky et al., 2011). Dry and semi-dry sausages are cured and sometimes smoked before the sausage is dried. These sausages have a tangy flavor due to a controlled bacterial fermentation or the addition of acids. Dry sausages are dried for a longer period of time than semi-dry sausages and are generally not heat processed. Uncut dry sausages should be stored in a cool, dry place. Semidry sausages, such as summer sausage, are usually heat processed and should be stored refrigerated

2.2.1.5: Specialty Sausage:

This is a diverse category that may contain cured, uncured, smoked, and non-smoked meats that do not readily "fit" into the other categories. They are seasoned and often formed into loaves. Examples: olive loaf; head cheese; jellied corned beef; scrapple; souse.

2.2.1.6: Fresh Sausages:

Made from ground meats which are seasoned and stuffed into casings, or left in bulk form. Fresh sausage is not cured or smoked; it

must be fully cooked before eating. Examples: pork breakfast sausage; Italian; bulk pork sausage (Tronsky *et al.*, 2011).

Fresh Sausages are made from selected cuts of fresh and or frozen meat. Fresh sausages are not allowed to contain curing agents (sodium or potassium nitrites, nitrates) or salt in sufficient quantities to preserve the product and are not cooked. Fresh sausages are usually seasoned, and have limited water content. These types of sausages require refrigerated storage, and must be thoroughly cooked before serving (AFDO, 1999).

Fresh sausages are not as widely produced as cooked sausages, and are typically consumed as breakfast meals. Typical fresh sausages include products such as pork sausages, beef sausage breakfast sausage, Italian sausage, and fresh chorizo sausages. (AFDO.1999)

2.2.2: The chemical composition of fresh beef sausage:

Mahassin (2008) reported the chemical composition of beef sausage as 66.63% moisture, 18.67% protein, 6.72% fat and 1.51% ash. Shawgi (2008) reported the chemical composition of beef sausage as 59.15% moisture, 14.09% protein, 16.49% fat, 1.7% ash and 7.39% carbohydrate. In another study, Rabaa (2009) reported the chemical composition of beef sausage as 72% moisture, 17.5% protein, 7.8% fat and 2.02% ash. Mousab (2009) reported the chemical composition of beef sausage as 64.12% moisture, 18.72% protein, 11.12% fat, 1.57% ash. Gadiyaram and Kannan(2004) reported the Chemical composition of beef sausage as 20% protein and7% fat, moisture content as 63%. The fresh sausages had overall moisture percentage of 55 % as reported by Agnihotri, (2002). Agnihotri and pal (2000) mentioned that the moisture content of sausage is (66%) and fat content did not exceed (25%). Ali (2012) reported the chemical composition of beef sausage as 68% moisture, 17% crude protein and 2 % fat. Jihad *et al* (2009) reported the

proximate analysis of beef sausage as 63.94% moisture, 12.76% protein 16.6% fat and 3% ash. Dharmaveer *et al* (2007) found the chemical composition of beef sausage as 62.5% moisture, 18.36% protein, 17.05 % fat and 2.06% ash.

2.2.3: Some global Standards of fresh sausage:

The sausage manufacturing industry must adhere to government standards for ingredients and processes. In addition, accurate labeling requirements ensure that the consumer is informed of the ingredients of a sausage product. The objective of these standards is to ensure that sausage products maintain a consistent quality and are safe to consume (AFDO, 1999). According to AFDO (1999) the fat contents up to 30% and added Water or ice up to 3%. Sudanese standards and Metrology Organization (SSMO, 2008) reported that the fresh sausage contains; 25% fat, 55% meat and binders 20%. According to SSMO (2010) in final product, the ice and water added should not exceeded 10%, 15% protein and fillers should not exceeded 1%. Draft Uganda Standard (DUS, 2012) reported the fresh sausage contains 3% added water / ice and fat as 30%. Jordanian standards of specification (JSS816,1996) reported the standard of fresh sausage as protein content up 12.75% of weight, moisture content as 63.94, fat content not exceed 25% and ash content as 2.27%. Gulf Standard Organization (GSO, 2008) reported the standard of fresh sausage as fat content should not exceed35%.

2.2.4: Physico-chemical properties:

2.2.4.1: Water holding capacity (WHC) of meat:

It is the ability of meat to hold its own or added water during the application of any force (Hamm, 1986). Judge *et al* (1989) defined the water holding capacity as the ability of meat to retain its own or added water during the application of external forces such as cutting, grinding or processing .The water holding capacity of meat or meat product can determine not only the amount of product that can be sold, but it can also influence the sensory properties of the product such as juiciness, texture and flavor (Trout, 1988).Water is present in meat as bound water (4-5% of total water) and free water. Hydrophilic groups attach bound water to protein, while free water is immobilized by physical configuration of meat protein. Changes only take place in free water and manifested as weep, drip or shrink (Aberle *et al.*, 2001).

Many of the physical properties of meat including the color, texture and firmness of raw meat, and the juiciness and tenderness of cooked meat are partially dependent on water holding capacity of muscle tissue which has a direct effect on the shrinkage of meat during storage. When the tissues have poor water holding capacity; the loss of moisture and consequently, the loss of weight during storage is great (Miller et al., 1975; Judge et al., 1990). The water holding capacity of meat is attribute of obvious importance .this particularly so in comminuted meat such as sausage where the structure of the tissue has been destroyed and longer able to prevent the release of the fluid from the protein (Lawrie, 1991). Ahmed (2012) reported the importance of WHC in quality is summarizing in three reasons, first the drip or exudates results from poor WHC affect the appearance of the meat for the myoglobin escapes from muscles in the drip. Second, loss of drip leads to weight loss in fresh meat and in the processed meat poor WHC reduces water retention and hence the weight of the product. Third, WHC is thought to influence the perceived juiciness of fresh meat after cooking for the increased cooking loss and so taste dry and lack of succulence. Fat reduction decreased emulsion stability and water holding capacity and resulted in higher cooking losses (Meltem and Meltem, 2003).

Qiao *et al.* (2001) reported the water holding capacity of sausage as ranged from 0.38-0.51. In another study the water holding capacity was 0.80 in beef sausage (Ali,2012) .Shawgi (2008) reported the water holding capacity of beef sausage as 0.62 .Mousab (2009) found the water holding capacity of beef sausage as 0.64 . Mahassin (2009) reported the water holding capacity of beef sausage as 1.39.

2.2.4.2: Cooking loss%:

It is the ability of meat to hold its own or added water during cooking. The cooking loss % defined the water lost during the cooking meat expressed as %. (Ahmed, 2012). Mahassin (2008) age of animal and wholesale cuts had a significant influence on cooking loss% and shear force values, they added that freezing and cooking methods had no significant effect on hear force value.

Cooking loss% increased as the salt level in the formulation decreased, In addition, the cooking loss also increased with use of lower quality meat. It possible to reduce cooking loss by using binder (Sofos, 2008). The loss in sausage after cooking may be affected by several factors such as make sausage by incorrect recipe, Temperature at comminution, cooking system and cooking time, water holding capacity, moisture, fat retention and the type of ingredient used in their formulation (Nurul *et al*,2010, Essien2003).

Gadiyaram and Kannan (2004) reported the cooking loss % in beef sausage as 19.88%. In another study Ali (2012) found the cooking loss% of beef sausage as 22.07 %. Mahassin (2008) reported the cooking loss % of beef sausage 18.04%. Mousab (2009) found the cooking loss % in beef sausage as 10.1%.

2.2.4.3: Hydrogen ionic potential (pH):

Ahmed (2012) defined pH as the acidy or alkalinity of media. The pH is an important determinant of microbial growth. Most of the

microorganisms grow at a neutral pH of 7.0 (Cannon et al., 1995; Romans et al., 2001; Kim, 2006). A reason for this is that the proteins are more heat stable at their isoelectric point, which is normally near neutral. The majority of bacteria function most efficiently in neutral environments and they can repair and recover easily when grown in neutral pH (Mossel et al., 1995). High pH has spoilage potential and short shelf—life (Newton and Gell, 1981). Walker and Betts (2000) reported that, ultimate pH of meat was significant for resistance to spoilage because most bacteria grow optimally at about pH below pH 7 not below pH 4. The ultimate pH of meat is result of combination between many factors including pre slaughter handling, postmortem treatment and muscle physiology (Marsh, 1977and Thomposn, 2002). The pH value of beef meat varies from 4.8-7.2 depending on the glycolytic potential at the time of slaughter but normal range of pH is 5.4-6.0 (Tarrant and Lister, 1989).

Dharmaveer et al (2007) reported the pH in beef sausage as 6.44. Shawgi (2008) found the pH in beef sausage as 6.23.and Ali (2012) reported that, the pH was 5.93 in beef sausage. Mahassin (2008) found the pH in beef sausage as 5.59. Mousab (2009) reported the pH in beef sausage as 5.61. Meltem and Meltem (2003) found the pH ranged from 5.86 to 6.04 for sausage.

2.2.5: Sausage ingredient:

Good sausage starts with good ingredients. To improve the flavor or the quality of meat by making it into sausage. Off flavors or germs in the raw meat will become part of the final product (Roxie. 2010)

2.2.5.1:Meat:

The finished product is only as good as the ingredients it contains. Meat should be fresh of high quality, have the proper lean-to-fat ratio and have good binding qualities. The meat should be clean and not

contaminated with bacteria or other microorganisms. In other words, meat used in sausage production should be safe (Martin, 2012).

Fresh and high quality meat such as lamb, beef, pork, mutton, poultry, veal and wild game may be used in sausage production. Less costly cuts such as round cuts, beef short ribs, pork shoulders or chuck cuts may be used. Cuts from the head and leftover trimmings from slaughter can also be used in sausage (Roxie 2010; Lashanda, 2011).

The characteristics of the meat ingredients used to create the sausage define the type of sausage - the overall taste, texture, aroma, along with the protein and fat content (AFDO, 1999).

2.2.5.2: Fat:

Fat content of meat used for comminuted meat products is influenced primarily by carcass grade and particular cut or type of trimming from the carcass. Variations in fat content greatly exceed those of moisture and protein. If moisture and protein are known, fat content may be approximated by difference, allowing about 0.8% for ash (Pearson & Tauber, 1984). Isidor *et al.* (1972) reported that in fresh or smoked sausage fat may reach about 20% in semidry or dry beef sausage, like frankfurters, the fat is part of an emulsion system participating in the formation of characteristic structure of product.

2.2.5.3:Salt:

Salt is an ingredient that is always used in sausage products. Technically, it is the only non-meat substance required for a product to be considered a sausage. Salt serves three functions in the meat. It lowers the amount of available water (which allows for preservation or shelf-life extension), extracts the meat myofibrillar proteins needed to make the product bind and to emulsify fat, and for flavor enhancement (Lessonson Meat, 1991).

In addition to taste, salt has the ability to extract some proteins from meat. As the sausage is heated, the protein matrix coagulates, the meat particles bind together and the texture of the sausage becomes firm. Salt also enhances flavor, reduces microbial spoilage, and increases water absorption and retention. (Judge*et al* .,2001;Kerry *et al* .,2002). In general, salt is added at a concentration of 1% to 2% (w/w) of the total sausage batter weight (Tronsky *et al.*, 2011). Salt is added for flavoring function—at concentration between 2-3%. Fresh sausages generally have a lower salt level due to detrimental color effects, 1.5% salt in finished sausage works out satisfactorily for color and flavor (Baumgartner, 1985).

2.2.5.4: Spices:

Depending on the variety of sausage, various types and amounts of spices and herbs may be used to add flavor to the product. Black pepper, white pepper, paprika, sage, garlic, cumin, fennel, oregano and many other seasonings are used to improve appearance and add flavor to sausages. (All about sausage, 2004)

Seasonings influence the flavor, appearance or shelf life of the product; Seasonings are classified further as spices, herbs, aromatic vegetables, flavoring enhancers and stimulated meat flavors. Certain spices such as black pepper .ginger and mace have antioxidant properties and will help extend the shelf life of sausage (Pearson and Gillett, 1996). The characteristic flavor of given type sausage depends in large extent on the spices used in its formulation (Toldra, 2002; Roxie, 2010).

2.2.5.5: Cold water or ice (Water added):

Water and ice are added to provide moisture and keep the sausage cold. Cold temperature delays microbial growth and also ensures a better final product texture. Ice and water can also be added to increase the yield of sausage, but there are upper limits for wholesale or retail

marketing. Water also aids in dissolving salt to facilitate its distribution within the meat. Texture and tenderness of the finished sausages are markedly affected by added water content (Pearson and Gillet, 1996). SSMO (2008) reported the level of added water in fresh sausage should not exceed 10%. Water is added to many products for several reasons. Some products would be dry and unpalatable without adding water. Using water improves tenderness and juiciness and it serves as a processing aid when the product is made. The amount of water added to the product is regulated by the Jordanian Standards of Specification (JSS816, 1996). The water added in fresh sausage up to 3% of total product weight (AFDO, 1999). SSMO (2010) the limits of ice or water added should not exceeded 10% of final product.

2.2.5.6: Casing:

These natural casings are largely made up of collagen which has the unique characteristic of variable permeability. Moisture and heat make casings more porous and tend to soften them. Natural casings readily permit smoke penetration and do not contribute any undesirable flavors. Sausage made from natural casings have a "snap" when bitten into that is considered a desirable sensory characteristic (A Brief History of Natural Casings, 2003).

Sausages may be stuffed into natural or artificial casings. Natural casings are hog, cattle and sheep gastrointestinal tracts. These edible casings are permeable to moisture and smoke. They have an innate curved shaped and are used in fresh bratwurst and other fresh sausages. Use of natural casings presents a challenge due to splitting of the casings, handling problems, and difficulty in standardizing dimensions and weights of the sausages (All about sausage, 2004).

2.2.5.7: Extra ingredient: Additive:

Food additives are used to accomplish certain functions such as coloring, antimicrobial, antioxidant, preservation, improved nutrition, increased emulsification and altered flavor (Okerman,1986;Jihad, 2009). The use of food additives has become more prominent in recent years due to the increased production of prepared, processed and convenient foods (USDA, 2008; Directive No 95/2/EC, 2006).

Additives can be included in sausage products but under strict conditions and legal limits. They are used to impact the color, minimize rancidity or to inhibit microbial growth. Examples of these are sodium nitrite, phosphates, sodium ascorbate, and sodium erythorbate (Tronsky *et al.*,2011; Knipe, 2003; Lampila and Godber, 2002; Molins, 1991).

2.2.5.8: Binders and Extenders.

Binders used in meat processing technology divided into two groups: first plant proteins such as soy isolates, soy concentrates and flours second protein of animal origin such as milk protein (Meltem and Meltem, 2003). Soy products have been used in meat processing to improve functional properties such as water binding and textural properties, they are hydrophilic (absorb and retain water) and have adhesive properties (Dexter *et al*, 1993; Mittal and Barbut, 1993; Meltem and Meltem,2003). Milk proteins can be act both as emulsifier and water fat binders in foods (Sebranek, 1996). Milk-protein derived extenders are used widely in processed meat products. These include nonfat dry milk, dried whey, and buttermilk solids and are added to improve binding qualities, flavor, cooking yields and slicing characteristics. They also help to stabilize meat emulsion products such as bologna and frankfurters (Tronsky *et al.*, 2011). A sausage formulation can include up to 3.5 of binder and extender (AFDO, 1999).

2.2.6: Manufacturing sausage:

Sausage making and manufacturing is a continuous sequence of events. Each step in proper sequence is important to successful operation in studying sausage processing; it is convenient to separate the process into four basic processing: selecting ingredient, grinding, mixing and thermal processing (Pearson and Gillett, 1996).

2.2.7: Quality of sausage:

2.2.7.1: Color:-

Color is an important criterion of raw or cooked meat products. It reflects the proper composition of the products, particularly relation of meat to other compounds, freshness of the raw materials, texture, taste and proper conditions of storage (Klak *et al.*, 2001; Alberti *et al.*, 2002).

In general sausages; whatever their formulation and composition should have a uniform basically red color. To obtain the desirable color of finished sausages, it's necessary to add an optimum amount of nitrite or nitrate. Good color in sausage can be destroyed by improper storage or by prolonged contact with air (Isidor *et al.*, 1972; Maha, 2009). Colour is an important quality property which contributes to its sensory acceptability (Garcia- Esteban *et al.*, 2003and Bekhit and Faustman, 2005).

Color is an important quality attribute that influences consumer acceptance of many food products, including meat. Consumers will often reject products in which the color varies from the expected normal appearance. Pointed out that color is everywhere and that psychological responses to color, as they relate to appetite, are considered important to processors and consumers. Consequently, color is often used to determine economic value of food. (Qiao *et al.*, 2001).

2.2.7.2: Tenderness and Juiciness

Meat tenderness is defined by the ease of mastication, which involves initial penetration by the teeth, the breakdown of meat into fragments and the amount of residue remaining after chewing (Lawrie, 1998; Fanbin *et al.*, 2007). Juiciness; it difficult to give a definition of juiciness. The sensation of juiciness is composed of two organoleptic components. First is impression of wetness during the first few chews produced by rapid release of meat fluid; the second is a sustained juiciness largely due to the stimulatory effect of on salivation. Tenderness and juiciness are closely related and, in general, the more tender the meat, the more readily juices appear to be liberated during eating.(Lawrie, 1991). Juiciness varies inversely with cooking loss (Lawrie, 1991; Judge et al., 2001). McMillan and Brock (2005) reported that, age, breed, and diet influence tenderness, juiciness and flavor.

2.2.7.3: Flavour and aroma:-

Meat aroma develops from the interactions of the non-volatile precursors, including free amino acid, peptides, reducing sugars, vitamins, nucleotides and unsaturated fatty acids, during cooking (Mottram and Nobrega, 2002). Flavor, it involves odor and taste of cooking meat. It originates from water or fat soluble precursors and from the release of volatile substances pre-existent in meat (Howard & Lawrie, 1956). Aberle *et al.* (2001) reported that, components of meat responsible for flavor and aroma have not been completely identified. It is likely that many constituents of muscle connective and adipose tissues become flavor compounds upon being heated. Some evidence shows that inosine monophosphate (IMP) and hypoxanthine enhance flavor or aroma. Because IMP and hypoxanthine are break down products of ATP, it is obvious that muscles with large energy stores would have more pronounced flavor. Sausage should have a pleasing flavor, typical for

each product .The flavor of sausage depends upon meat, spice blend and method of processing. Fresh sausage and some of the smoked and cooked sausages show their optimum flavor characteristic when heated, while sausage eaten cold should be already fully flavored at room temperature (Isidor *et al.*, 1972; maha, 2009).

2.2.7.4: Texture:

Food texture has been defined as the composite food of those properties which arise from the structural element, and the manner in which it registers with the physiological sense (Szezeriak, 1963). The texture of meat can be defined as the sensory manifestation of the structure of meat and manner, which the structure reacts the force applied during biting (Mahassin,2008). The texture of sausage depends upon the selection of meats, proper chopping and curing, and through smoking and cooking (Maha, 2009).

Meat texture is influences not only quality of collagen but also its solubility on heating .As a consequence, fat reduction can significantly affect the acceptability of a product and increase the toughness of meat product. In some cases, it is of such importance that several studies have attempted to maintain sensory and texture attributes through the use of fat-replaces (Sandrou and Arvanitoyannis, 2000).

2.2.8: Contamination of sausage:

Meat and meat product considered as an ideal culture medium for growth of many microorganisms because of the high moisture, high percentages of nitrogenous compounds of various degrees of complexity, plentiful supply of minerals, accessory growth factors and some fermentable carbohydrates (glycogen) and favourable pH for most of the enteric microorganisms (*Salmonella* and *Escherichia coli* O157:H7). Meat products may be contaminated with microorganisms from meat handlers, which carry pathogenic microorganism during the process of

manufacturing, packing and marketing. (AFDO1999; Fratmico *et al.*, 2005; peter, 2009; Mohamed, 2011). The micro organisms found on or in meat and poultry may consist of viruses, molds, yeasts, and bacteria. Viruses are very small organisms that do not usually contribute to meat spoilage but may be infectious for meat plant workers or consumers. Yeasts and molds spores, can be spread through the air or by other means, and will contaminate meat and equipment surfaces wherever they settle. Bacteria also are unicellular and vary in morphology from elongated and short rods to spherical or spiral forms (Aberle *et al.*, 2001). These bacteria require three conditions for growth: first Low acidity (near natural pH) level within the meat; second an available supply of water or other moisture such as meat juices; third warm temperature generally between 45° and 127°F (Loveday and Sheri, 2004).

2.2.9: Microbiology Contamination

Bacteria grow best between 40°F to 140° F. When cooking or cooling meat (for cooked sausages), make sure the product temperature passes through this range quickly. During meat processing, cooked sausages should have a final internal temperature of 160° F as this effectively kills pathogenic bacteria. Pans of water can be placed near the sausages to provide humidity and prevent over drying (Meat Science and Meat Sense, 2004). Once cooked, the sausages must be cooled quickly or pathogenic bacteria that "land" on the product during subsequent handling will have the opportunity to grow. Once a sausage is finished, its shelf-life is limited. It should be stored under refrigerated or frozen conditions to minimize bacterial growth. When refrigerated, fresh and uncooked sausages can be kept for a few days. Hard/dry and summer sausages can be kept up to three weeks. Cooked sausages can be kept for approximately one week (Safe Handling of Sausage and Hot Dogs, 1999). Fresh sausage microbial profiles have been characterized by the presence

of aerobes, facultative anaerobes and mesophiles, which are responsible for spoilage and potentially pathogenic bacteria (Cocolin et al, 2004). Aerobic colony counts range from $1.5 \times 10^3 - 2.1 \times 10^8$ cfu/g for fresh sausage and for frozen sausage from $1.4 \times 10^3 - 3.1 \times 10^7$ cfu/g (Farber et al 1988). In deboned meat the aerobic counts have been shown to range from $1.4 \times 10^5 - 1.5 \times 10^7$ cfu/g (Nel et al 2004).SSMO (2010) reported that for fresh sausage the total aerobic plate count should not exceed 5.25×10^{-5} CFU\ml, yeast 4.61×10 , mould 6.03×10 , Coliform 13.8×10 and Salmonella was zero. DUS (2012) reported the microbial limit for fresh sausage as TPC 106 per g and E.coli 10CFUg. According to the United States Department of Agriculture (USDA, 1999), sausage makers should ensure that their products are not contaminated by pathogens such as Listeria, E. coli O157, Salmonella, Trichinae and Staphylococcus enterotoxin. The British processer Association (2011) reported the microbial standard of raw sausage and sausage stuffing as $<5\times10^5$ CFU/g).

2.2.9.1: E. coliO157:H7:

Is often found on undercooked minced beef and unpasteurized milk (peter, 2007). The presence of E. coli in high numbers indicates the presence of organisms originating from faecal source. This is due to improper slaughtering techniques, contaminated surfaces and/or handling of the meat by infected food handlers (Nel *et al.*, 2004). *Escherichia coli* O157:H7 is a bacterial contaminant of sausage and other meat products that can cause serious diarrheal illness, sometimes resulting in complications that can lead to death. The presence of *E. coli* in cooked sausages can be controlled by proper cooking temperatures and times. *E. coli* contamination of dry sausages can be reduced by closely controlling the fermentation heating process, the acid content, and via post-fermentation heating to 145°F or above. And with all sausage

products, proper hygiene, handling, and storage procedures are essential to control contamination. One of the five options described by the *BlueRibbon Task Force* in their *Dry Fermented Sausage and E. coli*O157:H7 report can be used to eliminate *E. coli* O157:H7 in thefinished product(AFDO1999).

Escherichia coli are considered the most commensally living microorganism in the alimentary tract of nearly all domestic and wild animals as well as human. Enteropathogenic E. coli organisms usually lead to severe diarrhea in infants and it may also be the causal organisms In appendicular abscess, peritonitis and cholecystitis (Frazier, 1988; Mackie and Mecartney, 1989). The Enterobacteriaceae group of bacteria is the most challenging bacterial contaminant to raw and processed meat products worldwide. Salmonella, E. coli, Proteus, and Klebsiella species are the most predominant species in all food poisoning cases associated with some meat products. Due to the rising incidence of food borne infections, there is an urgent need for control and/or prophylaxis for food poisoning outbreaks associated with meat products. It depends greatly on investigating the causative agents in food (meat products), eliminating Them to ensure food safety and to protect public health from microbial contamination of food (Mohammed, 2011). Nel et al (2004) stated that the maximum limit of E. coliin meat and meat products should not be more than 10 cfu/g.According to Department of Health (DOH) of South Africa (2001), E. coli and other pathogens should not be present in any of the perishable meat products. Farber et al. (1988) showed that there is no correlation between the presence of Salmonella spp. and other organisms such as E. coli and Staph. aureus on fresh sausage and frozen sausage. Although Arthur et al (2004) stated that there is no correlation between the level of pathogens and the counts of aerobic bacteria and Enterobacteriaceae, and that these indicator organisms cannot be used for

direct indications for the presence or absence of E. coli O157:H7, the indicator organisms can be useful as a guideline for reduction of E. coli contamination.

2.2.9.2: Salmonella

Main sources are poultry, and red meat, unpasteurized milk and raw egg products. Food can be contaminated by improper handling and poor hygiene. *Salmonella* causes two types of food-borne human disease. First, *Salmonellosis* is most commonly caused by *S. enterica* subsp. *typhimurium* or *S. enterica* subsp. *enteritidis* (World Health Organization, 1995; D'Aoust, 2000). Secondly, *S. enterica* subsp. *typhi* and *S. enterica* subsp. *paratyphi* are the causes of typhoid fever or paratyphoid fever, respectively (Anon., 1992c).

Salmonella can replicate both inside the vacuoles of host cells (Garcia-del Portillo and Finlay, 1994) and in the external environment. Salmonella are the second most common pathogens isolated from humans with gastro enteric disease in developed countries ,Salmonella are non-sporing, motile rods, and are facultatively anaerobic (Le Minor, 1984). Salmonella; growth occurs between 5 and 47°C. Grow best at pH 7, but can grow in relatively acidic conditions, pH 4.0 to 5.4. Nitrite and high salt concentrations are inhibitory at low pH. Salmonella survive very well in dried foods, particularly those with protective fats and proteins. Salmonella are not heat-tolerant, so will be destroyed by thorough cooking. Contamination raw meat is one of the main sources of foodborne illnesses(Podpecan et al., 2007).

Salmonella species remains a leading cause of food poisoning in the developed world, resulting in multiple cases of absenteeism, illness, hospitalization and death each year(CDC, 2006). Salmonella is one of the microorganisms most frequently associated with food-born outbreaks of illness. Meat products in general and poultry, in particular,

are the most common sources of food poisoning by Salmonella (Antunes et al., 2003). Nontyphoidal Salmonellosis is a leading cause of foodborne illness in the U.S. As with E. coli, salmonella organisms can be eliminated from cooked sausages by proper cooking processes. In dry sausages, the producer must follow a combination of processes to control the pathogen, including use of a fermentation starter culture, increased product temperatures during fermentation, and careful control of the product pH, cure, and salt content. In addition, product handling procedures must be designed and monitored to ensure that cross contamination of the finished product with contaminants present in raw materials does not occur (AFDO.1999). Studies conducted by Mreme et al (2006) about the prevalence of Salmonella in raw minced meat, raw fresh sausage and raw burger patties from retail outlets in Gaborone, Botswana, showed that the prevalence of Salmonella was the highest in fresh sausages (26%) followed by minced meat (20%).

2.2.9.3: Listeria monocytogenes:

Listeria monocytogenes is recognized as a human pathogen, and the occurrence of *L. monocytogenes* results in listeriosis, which is a gastrointestinal food infection that leads to bacteremia and meningitis in humans (Gombas, Chen, Clavero & Scott, 2003; Madigan, Martinko & Parker, 2003). This organism has been detected in a variety of ready-to-eat food products such as deli-style salad, processed meat, smoked fish, ice cream and cheese(Gombas et al., 2003; Hoffman, Gall, Norton & Wiedmann, 2003; Madigan et al., 2003). Listeria monocytogenes is a bacterium found in soil and water that can contaminate meat, and can cause a serious infection in humans, called listeriosis. The organism can be found in many food processing environments, and has been isolated from floor drains and refrigeration drip pans. Cross contamination between raw and cooked product can also result in the presence of the

bacteria on ready to eat product. Detection of post processing product contamination by Listeria monocytogenes can include sampling the processing lines and environment (AFDO1999). The level of this organism that has been detected in food is not clear, but it has been suggested that levels of $> 10^3$ cfu/g L. monocytogenes may result in listeriosis (Gombas et al., 2003).

2.2.9.4: Campylobacter:

Is found in raw and undercooked poultry; other sources include red meat, unpasteurized milk and untreated water. Food can be contaminated by improper handling and poor hygiene (Peter ,2009).

Campylobacteriosis is transmitted through consumption of food contaminated with Campylobacter species (Hussain, Mahmood, Akhtar & Khan, 2007; Little, Richardson, Owen, de Pinna & Threlfall, 2008). Campylobacter jejuni is known to cause diarrhea/dysentery in children, and undercooked food such as poultry or other meats, raw milk and surface water. Studies conducted in the United Kingdom (Little et al., 2008) and Pakistan (Hussain et al., 2007) on the prevalence of Campylobacter in raw red meat, showed that the meat was frequently contaminated with Campylobacter jejuni, followed by Campylobacter coli. The incidence of Campylobacter has been suggested to be due to cross-contamination during slaughtering processing in abattoirs, manual skinning and evisceration (Hussain et al., 2007).

Campylobacter jejuni is the most commonly reported bacterial cause of foodborne infection in the U.S., with an estimated 2.1 to 2.4 million cases each year. Campylobacter jejuni is found in many foods of animal origin, including poultry and meats. Methods of controlling and reducing Campylobacter jejuni in processing facilities include forced air chilling of carcasses, and implementation of standard hygienic practices (AFDO.1999).

2.2.9.5: Staphylococci:

Staphylococci-contaminated food products that include red meat have been implicated in food-poisoning outbreaks (Shale, Lues, Venter & Buys, 2005). The presence of Staph.aureus can be used as indicator of personal hygiene and also is known to produce harmful enterotoxins. According to Shale et al. (2005) the South African legislation stipulates that a maximum count of 102 cfu/g in meat is acceptable. The amount of Staph. aureus required for production of toxin is 105 - 108 cfu/g (Farber et al., 1988; Nel et al., 2004; Shale et al., 2005). In deboned meat cuts the counts of Staph. aureus has been shown to range from $3.8 \times 103 - 2.42 \times 105$ cfu/g (Nel et al., 2004). The prevalence of Staph.aureus in the meat and meat products is due to the fact that it is part of the micro biota of animals and humans (Voster et al., 1994; Nel et al., 2004). High counts of E. coli and Staph. aureus have been found in the intestine of cattle and broiler chickens. This may result in contamination of the meat during the slaughtering process due to the negligence of good manufacturing practice (GMP) and/or Hazard Analysis Critical Control Point (HACCP) systems (Voster et al., 1994; USDA, 1999).

2.3: Effect of storage on sausage:

2.3.1: chemical composition:

2.3.1.1: Moisture content:

Mousab(2009) reported the moisture content as (64.43%, 64.22% and 63.61% at 0, 7 and 14 days) which decreased with storage period (0, 7 and 14 days) respectively in beef sausage .Ali (2012) found the moisture in beef sausage was decreased(70.14%, 68.65% and 66.34%) with storage period (0, 15 and 30 days) respectively. Shawgi (2008) reported the moisture (59.15%, 57.56% and 57.22%) in beef sausage which decreased with storage period (0, 3and 7days)

respectively). Rabaa (2009) found the moisture (68.35% and 68.19%) decreased with storage period (0 and 6 days) respectively in beef sausage.

2.3.1.2: Protein content:

Rabaa (2009) found the protein content as (18.8% and 19.04%) was increased with storage period (0 and 6 days) respectively in beef sausage. Shawgi (2008) reported the protein (14.09%, 14.47% and 15.05%) in beef sausage which increased with storage period (0, 3and 7days) respectively. Ali (2012) found the protein (18.70%, 17.21% and 16.7%) in beef sausage was decreased with storage period (0, 15 and 30 days) respectively. Mousab (2009) found the protein (18.66%, 18.49% and 18.37%) at (0, 7 and 14 period) which increased with storage period (0, 7 and 14 days) respectively in beef sausage

2.3.1.3: Fat content:

Shawgi (2008) reported the fat (16.49%, 16.53% and 16.19%) in beef sausage which decreased with storage period (0, 3and 7days) respectively. Mousab (2009) found the fat (10.44%, 10.24% and 10.18%) at (0, 7 and 14 period) was decreased with storage period (0, 7 and 14 days) respectively of beef sausage. Ali (2012) reported the fat (3.36%, 2.32% and 1.19%) in beef sausage which decreased with storage period (0, 15 and 30 days) respectively. Rabaa (2009) found the fat (9.3% and 7.9%) was decreased with storage period (0 and 6 days) respectively of beef sausage.

2.3.1.4: Ash content:

Ali (2012) found the ash (1.64%, 1.41% and 1.13%) in beef sausage was decreased with storage period (0, 15 and 30 days) respectively. Rabaa (2009) reported the fat (2.3% and 1.9%) which decreased with storage period (0 and 6 days) respectively in beef sausage. Mousab (2009) found the ash (1.54%, 1.55% and 1.67% at 0, 7 and 14 period) was increased with storage period (0, 7 and 14 days) respectively

in beef sausage. Shawgi (2008) reported the fat (1.79%, 1.76% and 1.71%) in beef sausage which decreased with storage period (0, 3and 7days) respectively.

2.3.2: Physico-chemical properties:

2.3.2.1: Water holding capacity (WHC) of sausage:

Ali (2012) reported that, water holding capacity improved with storage period as (0.65, 0.46 and 0.34) at (0, 15, and 30 days) respectively of storage. Similarly to Mahassin (2008) reported that, water holding capacity improved with storage period (1.39, 1.23 and 1, 12) at (0, 7 and 14 days) respectively. Shawgi (2008) reported that, water holding capacity improved with storage period (0.62, 0.59 and 0.58)at(0, 7 and 14 days)respectively. Mousab (2009) found the water holding capacity decreased with storage period in beef sausage as (0.64, 0.58 and 0.53)at(0, 7 and 14 days) respectively.

2.3.2.2: Cooking loss %:

Mousab (2009) reported that, the cooking loss of beef sausage was decreased with storage period as (10.1, 9.0 and 8.6) at (0, 7 and 14 days) respectively. Mahassin (2008) found the cooking loss% of beef sausage was decreased with storage period as (18.04, 16.66 and 14.30) at (0, 7 and 14 days) respectively. Ali (2012) reported the cooking loss was decreased as storage period (21.75, 18.82 and 16.62) at (0, 15 and 30 days) respectively. Lawrie (1991) stated that higher W.H.C of meat decreased cooking loss% in final products.

2.3.2.3: pH:

Van den et al (1961) reported that, changes in pH during freezing stored might be caused by the increase in concentration of soluble materials, by the subsequent precipitation of salt, and probably by the interaction of protein with ionic substances. Ali (2012) reported that, pH was' decreased (5.93, 5.83 and 5.80)at (0, 15 and 30 days)

respectively. Dharmaveer *et al* (2007) reported that, pH was decreased with storage period (0, 7 and 14 days)at(6.44, 6.40 and 6.28days) respectively. Shawgi (2008) found the pH was decreased with storage period of beef sausage as (6.22, 6.18 and 6.10) at (0, 3 and 7 days) respectively. Mahassin (2008) found pH was increased with storage period (5.59, 5.63 and 5.7) at (0, 7 and 14 days) respectively. Mousab (2009) found pH was increasing in beef sausage with storage period as (5.61, 5.62 and 5.64) at (0, 7 and 14 days) respectively.

2.3.3: Effect of storage period on sensory evaluation:

Mousab (2009) found the color (6.26, 6.13 and 5.79), flavor (6.62, 6.17 and 5.75) juiciness (6.09, 5.92 and 5.75) tenderness (6.4, 6.04 and 5.67) and Overall acceptance at storage period (0, 7 and 14days). Ali (2012) who reports the scores of sensory evaluation increased with during storage period.

2.3.4: Effect of storage period on total bacterial count:

Ali (2012) found the total bacterial count decreased with storage period (14log 10, 9 log10 and 2.22log 10 CFUg⁻¹ at 0, 15 and 30days respectively). Mousab (2009) reported the total bacteria count was increase with storage period.

Chapter three:

Materials and methods:-

The study was conducted at laboratory of Meat Science and Technology, Collage of Veterinary Medicine, Sudan University of Science and Technology. For determination of the chemical composition, some physico-chemical properties, sensory evaluation and assessment of bacterial contamination. In the period February- May 2013.

3.1: Preparation of samples:

Samples were collected from butcher shops from the three towns of Khartoum state (Khartoum, Bahri and Omdurman). Thirty samples of unpacked beef sausage were taken (ten samples from each town); the size of samples was about 3 kg of unpacked beef sausage. The samples were marked according to the location of collection (Khartoum, Bahri and Omdurman). The samples were stored at 18° c for determination of chemical composition, quality attributes, sensory evaluation and bacterial assessment at the storage period (0, 15, 30 and 45 days).

3.2: Proximate Chemical composition:

Determination of moisture, crude protein, fat (ether extract) and ash of the beef sausage samples was done according to (A.O.A.C., 2002)

3.2.1: Moisture determination:-

Five grams from each sample were put in an oven at 100c for overnight, and then the samples were taken out the oven, cooled in desiccators. The loss of weight was considered as the moisture content.

The moisture percentage was calculated as follows:-

Moisture%=

Weight of the sample before drying –weight of dried sample × 100

Weight of the sample before drying

3.2.2: Crude protein determination

Kjeldahl method was used to determine nitrogen percentage .Crude protein was determined by multiplying the amount of nitrogen times 6.25. One grams of each sample was digested in Kjeldahl flask by adding 10 gm of catalysts (mercury) and 25ml conc. H₂SO₄ .The mixture was heated for3 hours. The digested samples were cooled and then 100ml of distilled water was added to each flask .50ml of boric acid containing methyl blue were placed under condenser of each distilled unit. The mixture was then titrated against 0.1 N Hcl. The formula used for calculation of cured nitrogen was as follows:-

Nitrogen content% = $\underline{\text{Tv} \times \text{N} \times 14} \times 100$

1000×wt.of sample

Where:

Tv: Actual volume of Hcl used for titration.

N: Normality of Hcl.

14: Each ml of Hcl is equivalent to 14 mg nitrogen.

1000: To convert from mg to g.

Crude protein %: Nitrogen %×6.25

3.2.3: Fat determination:-

Fat was determined by the ether extraction .Two grams from the minced samples were taken into Soxhlet apparatus. The sample was subjected to continuous extraction with ether for 6hrs. The samples were then removed from the extractor and allowed to dry for 4hrs at 80°c in drying oven till no traces of ether remained. The sample was cooled and weighted for ether extraction percentage. The calculation was as following:-

Fat
$$\%$$
= fat weight $\times 100$
Sample weight

3.2.4: Ash determination:

Two grams of fat free sample were placed into dried crucible of known weight. The crucible was placed inside a muffle furnace at 105°c. The temperature was increased gradually till it reached 600°c for 3hrs, and then the crucible was taken out, cooled into desiccators and weighed. The ash percentage was calculated by the following formula:

$$Ash\% = wt. of ash x 100$$

$$\overline{Wt. of sample}$$

3.3: Physico-chemical properties:

3.3.1: Cooking loss %:

Cooking loss percentage was determined according to (Honikel, 1998) method, by weighing differences in sausage samples before and after cooking. Individual samples are placed in thin walled polyethylene bags in the water bath at 80 °C for 90 minutes, then it was removed from the water bath and cooled in running tap water. Then, sausage samples were taken from the bags, mopped dry and weighed. The cooking loss percentage was expressed as the following equation:

Weight of the sample before cooking – Weight of the sample aftercooking X100

Weight of the sample before cooking

3.3.2: Water holding capacity (WHC):-

About one gm from each sample was used for WHC determination. Each sample was placed on humidified filter paper and pressed between two Plexiglas plates for 1 minute at 25kg/cm² load. The meat filter area was traced with a ball pen and the filter paper was allowed to dry. Meat and moisture areas covered by meat (meat film area) was subtracted from the moisture area and then divided by meat film area to give the ratio expressed as water holding capacity of the meat. A large

ratio indicates an increase in the watery condition of the flesh or a decreased in the water holding capacity (Babiker and Lawrie, 1983).

Water Holding Capacity =

Loose water area – Meat film area

Meat film area

3.3.3: pH:-

10 gm of each sample was blended with 100ml distilled water in a blender jar at high speed for one minute pH measurement was done in the laboratory by pH meter (Okerman, 1981).

3.4: Sensory evaluation:

Sensory evaluation was conducted in the meat laboratory. Samples were offered to 10 semi-trained sensory panelists who evaluated color, texture, juiciness, flavor, and overall acceptability using an 8-point (hedonic scale) card (cross *et al.*, 1978), in which the highest score of 8 being extremely desirable and score 1 being extremely undesirable.

3.4: Bacterial assessment:

3.5.1: Total bacterial count:

Standard plate count agar media was used to determine the total viable bacterial count. Samples were prepared according to the technique described by ICMSF (1987). One gram from each sample was transferred under aseptic condition to glayuss tube containing nine ml of sterile normal saline. The content of the tube was homogenized by dipping and shaking the sample to have a dilution of 10-1. Such homogenate was used for all bacterial investigation. Further, 5 fold serial dilutions were prepared up to 10-5. About 10-15 ml of plate count agar media poured aseptically into sterile Petri- dishes. One ml from the dilutions was added to each Petri- dish, and then they were incubated at 37°c for 48 hours. A colony counter was used for counting colonies grown in the incubated Petri – dishes

3.5.2: Bacterial Isolation and Identification:

For isolation of *Salmonella* spp. The samples were incubated in salmonella agar plate and incubated at 37°c for 24 hours. Well isolate individual colony of different type were sub-culture on fresh agar for purification. For isolation of E. coli form bacteria, the samples were incubated on Mac-Conkey agar and incubated at 37°c for 24 hours and colonies of different morphology were sub-cultured and purified, purified and identified (ICMSF, 1987).

3.6: Statistical analysis:

Complete Randomised Design was used to analyses the results obtained from this study and subjected to General Linear Model (GLM) followed by least significant difference test (LSD) using the SPSS 17.0 (2007) computer program, as 3X4 factorial design.

Chapter four

4. Results

The results obtained from this study were shown in table 1, 2, 3, 4 and 5

4.1: Effect of storage period on the chemical composition:

There was high significant differences (p <0.01) between samples collected from Khartoum state as showed in table (1). All the chemical composition components (moisture, protein, fat and ash) values decreased with storage period.

4.1.1: Moisture content:

The moisture content of samples collected from Khartoum markets recorded the highest values (69.13, 66.95, 64.29 and 60.03), Bahri markets (68.36, 65.90, 61.07and57.72%) and Omdurman markets (60.04, 56.65, 53.83 and 50.37%) at (0, 15, 30 and 45 days) of storage period respectively.

4.1.2: Crude protein:

The crude protein of the samples collected from Bahri markets recorded the highest values (14.37, 12.57, 11.50 and 10.15), followed by Omdurman markets (13.26, 11.52, 10.49 and 8.10) and Khartoum markets (12.61, 11.40, 10.14 and 8.83%) at (0, 15, 30 and 45 days) at storage period respectively.

4.1.3: Fat content:

The fat content of the samples collected from Omdurman markets reported the highest fat content (26.00, 25.48, 23.62, and 21.65), while Bahri markets (15.82, 14.64, 13.29 and 12.41) and Khartoum samples (15.23, 14.23, 12.33 and 11.09) at (0, 15, 30 and 45 days) of storage period respectively.

4.1.4: Ash content:

The ash content of the samples collected from Khartoum markets was recorded the highest content (2.38, 1.44, 1.19 and 1.07%) while Omdurman markets (2.36, 1.82, 1.38 and 1.2%) and Bahri markets (2.21, 1.70, 1.29 and 1.5%) at (0, 15, 30 and 45 days) of storage period respectively.

4.2: Effect of storage period on physiochemical analysis:

There was high significant differences (p <0.01) between the samples collected from Khartoum state. The water holding capacity (WHC) and cooking loss% was decreased with storage period, while the pH increased with storage period.

4.2.1: Water Holding Capacity (WHC):

Table (2) showed that, Bahri sausage samples recorded the highest WHC value (4.42, 3.82, 3.43 and 2.39) compared with Khartoum markets (3.94, 3.19, 2.75 and 2.01) and Omdurman markets (3.75, 3.41, 2.27 and 1.35) at (0, 15, 30 and 45 days) of storage period respectively.

4.2.2: cooking loss%:

Table (2) showed that, Bahri sausage sample recorded the highest cooking loss% (40.26, 39.51, 38.93 and 34.05) compared with Khartoum and Omdurman samples at (0, 15, 30 and 45 days) of storage period respectively.

4.2.3: pH:

Table (2) showed that, Omdurman sausage samples recorded the highest pH value (6.04, 6.14, 6.16 and 6.21) compared with Khartoum (5.73, 5.93, 6.13 and 6.16) and Bahri samples (5.75, 5.75, 6.13 and 6.15) at (0, 15, 30 and 45 days) respectively.

4.3: Effect of storage period on sensory evaluation:

Table (3) showed significant difference (p<0.01) for all sensory parameters (color, texture, juiciness, tenderness, flavor and

overall acceptability). The results of all sensory parameters increased with storage period. The sample collected from Omdurman markets recorded the highest scores in the all parameters compared to other locations.

4.4: Bacterial assessment:

There was no significant difference (p<0.01) between the locations. The result showed in table (4 and 5).

4.4.1: Total bacterial count:

Table (4) showed that, Bahri sausage sample recorded the highest total bacterial count value (7.33, 8.00, 7.33 and 13.67) $\times 10^6$, Khartoum (4, 5.33, 5.33 and 13.67) $\times 10^6$ and Omdurman samples (6, 4.67, 6.67 and 13) $\times 10^6$ at (0, 15, 30 and 45 days) of storage period respectively.

4.4.2: Bacterial contaminants:

Table (5) showed that, all the samples collected in Khartoum state (Khartoum, Bahri and Omdurman) were contaminated with *salmonella* and *E. coli*.

Table (1) Effect of storage period the chemical composition of unpacked beef sausage in Khartoum state

Treatment	Parameter	Moisture	Crude	Ether	Ash
			protein	extract	
Storage	Collection sites				
period					
(days)					
0	Khartoum	69.13±0.29	12.61±0.55	15.23±0.10	2.38±0.31
	Bahri	68.36±0.83	14.37±0.34	15.82±0.53	2.21±0.03
	Omdurman	60.04±0.70	13.26±0.91	26.00±0.64	2.36±0.31
15	Khartoum	66.95±0.57	11.40±0.39	14.23±0.10	1.44±0.46
	Bahri	65.90±0.48	12.57±0.25	14.64±0.30	1.70±0.29
	Omdurman	56.65±0.48	11.52±0.52	25.48±0.14	1.82±0.18
30	Khartoum	64.29±0.87	10.14±0.23	12.33±0.08	1.19±0.19
	Bahri	61.07±0.35	11.50±0.36	13.29±0.16	1.29±0.17
	Omdurman	53.83±0.10	10.49±0.63	23.62±0.15	1.38±0.05
45	Khartoum	60.03±0.81	8.83±0.35	11.09±0.18	1.07±0.06
	Bahri	57.72±0.35	10.15±0.26	12.41±0.10	1.05±0.06
	Omdurman	50.37±0.21	8.10±0.13	21.65±0.26	1.02±0.04
Stander		0.54	0.37	0.23	0.18
Error					
Siginificant	**	**	**	**	**
deferent					

 a,b,c mean with different superscript in the same column are significantly different at (P \leq 0.05)

^{**:} Significance different P≤ 0.01

Table (2) Effect of storage period on some physico -chemical unpacked beef sausage in Khartoum state:

Treatment	Parameter	WHC	Cooking loss%	pH
Storage	Collection sites			
period (days)				
0	Khartoum	3.94±0.25	36.10±0.38	5.73±0.09
	Bahri	4.42±0.08	40.26±0.22	5.75±0.15
	Omdurman	3.75±0.08	34.61±0.20	6.04±0.09
15	Khartoum	3.19±0.14	35.28±0.35	5.93±0.17
	Bahri	3.82±0.09	39.51±0.05	5.75±0.14
	Omdurman	3.41±0.04	33.19±0.35	6.14±0.02
30	Khartoum	2.75±0.06	34.00±0.25	6.03±0.12
	Bahri	3.43±0.01	38.93±0.44	6.13±0.01
	Omdurman	2.27±0.09	32.35±0.25	6.16±0.04
45	Khartoum	2.01±0.24	30.91±0.89	6.15±0.04
	Bahri	2.39±0.08	34.05±0.10	6.15±0.04
	Omdurman	1.35±0.09	28.25.0.20	6.21±0.03
Stander		0.04	0.21	0.10
Error				
Siginificant	**	**	**	**
deferent				

a,b,c mean with different superscript in the same column are significantly different at $(P \le 0.05)$

^{**:} Significance different P≤ 0.01

Table (3) Effect of storage period on sensory evaluation of unpacked beef sausage in Khartoum state:

Treatment	Parameter	Color	Texture	Tenderness	Juiciness	Flavor	Overall
							acceptance
Storage							
period	Collection						
(days)	sites						
0	Khartoum	3.37±1.69	3.67±1.49	3.43±1.61	3.60±1.84	3.60±1.42	3.77±1.31
	Bahri	3.97±1.42	4.47±100	4.06±1.26	4.20±1.39	4.17±1.42	4.47±0.99
	Omdurman	4.97±1.40	4.57±1.13	4.03±1.27	4.33±1.59	4.23±1.30	4.68±1.11
15	Khartoum	4.16±1.80	4.30±1.82	4.20±1.66	4.13±1.45	4.46±.43	4.25±1.87
	Bahri	5.10±1.20	4.83±1.44	4. 73±1.51	4.70±1.17	4.79±1.31	4.85±1.22
	Omdurman	5.23±1.27	4.95±1.70	4. 85±1.34	4.96±1.32	4.83±1.19	4.87±1.14
30	Khartoum	4.35±1.52	4.40±1.08	4.64±1.31	4.89±1.00	4.75±1.00	4.60±1.27
	Bahri	5.24±1.10	4.99±1.45	5.02±1.46	5.7±1.13	4.98±1.13	5.10±1.09
	Omdurman	5.64±1.14	5.11±1.76	5.94±1.01	5.15±1.51	5.03±1.37	5.35±1.57
45	Khartoum	5.07±1.07	4.88±1.23	5.00±1.20	5.09±1.03	5.13±1.27	5.03±1.26
	Bahri	5.94±1.54	5.26±1.11	5.31±1.90	5.33±1.28	5.21±1.93	5.41±1.04
	Omdurman	5.13±1.05	5.78±1.20	6.37±1.37	5.41±1.52	5.33±1.45	5.76±1.38
Stander		0.02	0.06	0.01	0. 83	0.04	0.01
Error							
Siginificant	**	**	**	**	**	**	**
deferent							

a,b,c mean with different superscript in the same column are significantly

different at $(P \le 0.05)$

**: Significance different P≤ 0.01

NS: No significant.

Table (4) Effect of storage period on total bacterial count of unpacked beef sausage in Khartoum state:

Treatment	Parameter	Total bacterial count(10 ⁻		
		⁶ CFU)		
Storage period	Collection sites			
(days)				
0	Khartoum	4.00±2.00		
	Bahri	7.33±3.06		
	Omdurman	6.00±2.00		
15	Khartoum	5.33±1.15		
	Bahri	8.00±2.00		
	Omdurman	4.67±2.31		
30	Khartoum	5.33±1.15		
	Bahri	7.33±3.01		
	Omdurman	6.67±3.01		
45	Khartoum	13.67±2.08		
	Bahri	13.67±2.52		
	Omdurman	13.00±2.00		
Stander Error		0.70		
Siginificant deferent	NS	NS		

NS: No significant.

Table (5) Contamination of unpacked beef sausage with Salmonella and E.coli in Khartoum state:

Treatment	Parameter	Salmonella	E.coli
Storage			
period	Collection sites		
(days)			
0	Khartoum	+ve	+ve
	Bahri	+ve	+ve
	Omdurman	+ve	+ve
15	Khartoum	+ve	+ve
	Bahri	+ve	+ve
	Omdurman	+ve	+ve
30	Khartoum	+ve	+ve
	Bahri	+ve	+ve
	Omdurman	+ve	+ve
45	Khartoum	+ve	+ve
	Bahri	+ve	+ve
	Omdurman	+ve	+ve
Stander		0.17	0.23
Error			
Time X	NS	NS	NS
Type			

NS: No significant.

Diagram (1): Effect of storage period on the chemical composition of unpacked beef sausage:

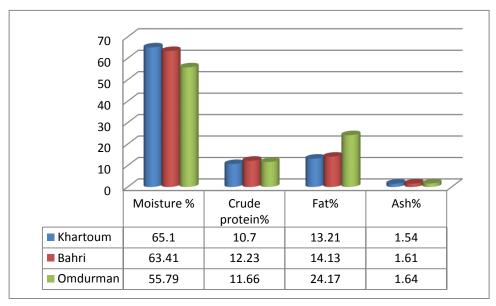


Diagram (2): Effect of storage period on some physico-chemical properties of unpacked beef sausage

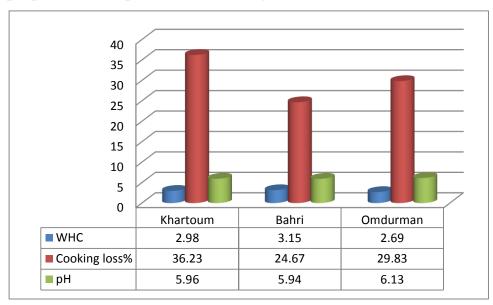


Diagram (3): Effect of storage period on sensory evaluation of unpacked beef sausage

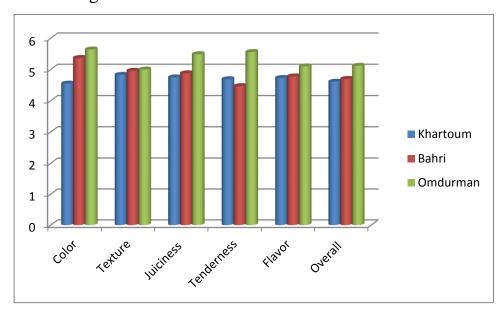
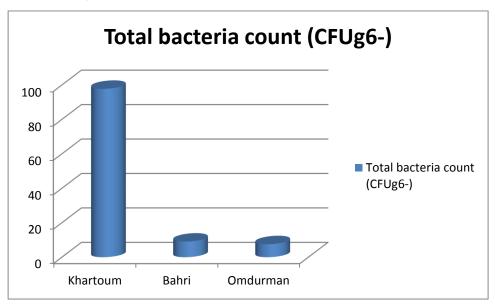


Diagram (4): Effect of storage period on total bacterial count of unpacked beef sausage



Chapter Five

Discussion:

5.1: Effect storage period on the chemical composition of unpacked beef sausage in Khartoum state:

5.1.1: Moisture content:

The result of this study was similar to Mousab (2009) who reported moisture % in beef sausage as (64.43%, 64.22% and 63.61%) at (0, 7 and 14 days) respectively. This result agreed with SSMO (2008) limits who reported that, the ice and water added should not exceeded 10% of the final products. The calculated moisture content from standard not be less than 52% in fresh sausage (2008).

5.1.2: Crude protein:

In the present study the results agreed with Rabaa (2009) who reported the crude protein in beef sausage as (18.8% and 19.04%) at (0 and 6 days) respectively. The result of this study disagreed with SSMO (2008) the limits of protein as not to be less than 15%. Also disagreed with Ali (2012) who reported the protein% as (18.70%, 17.21% and 16.7%) in beef sausage at (0, 15 and 30 days) respectively.

5.1.3: Fat content:

The result of this study was similar to Shawgi (2008) who reported the fat content in beef sausage as (16.49%, 16.53% and 16.19%) at (0, 3and 7days) respectively, This result was agreed with AFDO(1990) who reported that, the fat content in beef sausage as up 30%, and Draft Uganda Standard (DUS,2012) reported as 30%, Gulf Standard Organization(GSO,2008) reported as not exceed 35%, whereas disagreed with SSMO(2008) who reported the fresh sausage content 25% fat.

5.1.4: Ash content:

In the present study the results agreed with Ali (2012) who reported the ash% as (1.64%, 1.41% and 1.13%) at (0, 15 and 30 days)

respectively. The result was disagreed with Mousab (2009) who reported the ash content was (1.54%, 1.55% and 1.67%) at (0, 7 and 14) respectively.

5.2.: Effect of storage period on physio-chemical properties:

5.2.1: Water Holding Capacity (WHC):

In the present study the results agreed with Ali (2012) who found the water holding capacity increasing with storage period (0.65, 0.46 and 0.34) at (0, 15, and 30 days) respectively, and agreed with Mousab (2009) who found water holding capacity as (0.64, 0.58 and 0.53 at 0, 7 and 14 days) respectively.

5.2.2: cooking loss%:

The result of this study agreed with Mousab (2009) reported the cooking loss in beef sausage as (10.1, 9.0 and 8.6) at (0, 7 and 14 days) respectively. And agreed with Mahassin (2008) who reported the cooking loss% of beef sausage was decreased with storage period as (18.04, 16.66 and 14.30) at (0, 7 and 14 days) respectively.

5.2.3: pH:

The result of this study agreed with Ali (2012) who reported the pH value in beef sausage as (5.93, 5.83 and 5.80) at (0, 15 and 30 days) respectively. The result of study disagreed with Dharmaveer et al (2007) who reported the pH in beef sausage as (0, 7 and 14 days) for (6.44, 6.40 and 6.28days) respectively.

5. 3: Effect of storage period on sensory evaluation:

This result of this study disagreed with Mousab (2009) who reported the color as (6.26, 6.13 and 5.79), flavor (6.62, 6.17 and 5.75) juiciness (6.09, 5.92 and 5.75) and tenderness (6.4, 6.04 and 5.67) at storage period (0, 7 and 14days) respectively.

5.4: Bacterial assessment:

5. 4.1: Total bacterial count:

The result disagreed with SSMO (2010) limits who state that, the total bacterial count not exceeded 2.25×10⁵). Also disagreed with Ali (2012) who reported the total bacterial count decreased with storage period (14 log 10, 9 log10 and 2.22log 10 CFUg-1) at (0, 15 and 30days) respectively. The results agreed with Mousab (2009) who state that, the total bacterial count increased with storage period.

5.4.2: Bacterial contaminants:

The result of this study disagreed with SSMO (2010) who reported that, fresh sausage should be free of *salmonella* and *E. coli* not exceed 13×10 .

Conclusion and Recommendation

This study was concluded to:-

- All samples of unpacked beef sausage in Khartoum state were contaminated with *Salmonella* and *E.coli*.
- All samples of unpacked beef sausage were in conformance with SSMO Standard in chemical composition.
- The public health authorities should take necessary actions to safeguard the public from such contaminated products.
- Further studies should continue in this field to produced meat and meat products that matches with SSMO Standard.

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