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Microbiological Safety of Raw Poultry Meat from different markets in Bahry town

A Dissertation Submitted to Sudan University of Science And Technology in partial fulfillment for the degree of B.Sc. in food Science and Technology.

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الآية

قل تعالى:

"وَفَاكِهَةٍ مِمَّا يَتَخَيَّرُونَ (20) وَلَحْمِ طَيْرٍ مِمَّا يَشْتَهُونَ (21)"

صدق الله العظيم

سورة الواقعة

DIDICATION

To

Our fathers and mothers

Our brothers and sisters

And to all friends

ACKNOWLEDGEMENTS

With all due humbleness and gratitude we render ultimate thanks and special praise to Allah (Almightly) who gave us health, power and patience to accomplish and conduct this research.

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ABSTRACT

This study was conducted to determine the safety of raw poultry meat in Bahry town. Raw poultry meat samples were collected from different markets (Bahry, Hajyousif and Aldrowshab). Two types of samples were considered for microbial tests (covered and uncovered). (Total bacteria, total aerobic and anaerobic, total coliform, *E.coli*, *Salmonella* and *Staphylococcus aureus*), moisture and pH. The levels of different bacteria were higher in uncovered samples as compare with covered one. This may be due to the non-sound supply and not healthy environmental surrounding. Moreover the presence of *Salmonella* and *E. coli* that indicate external pollution should will have negative effect on health of consumers. There is no significant difference in pH between covered and uncovered samples, either in than a moisture content except for Hajyousif and Aldrowshab samples. Therefore the tested raw poultry meat samples did not comply with the safety requirement of the SSMO standard for raw meat safety.

الملخص

أجريت هذه الدراسة لتحديد سلامة لحوم الدواجن الخام في مدينة بحري.تم جمع عينات من الاسواق المختلفة (بحري، الحاج يوسف والدروشاب) حيث تم أخذ عينتين من كل منطقة (مغطاة ومكشوفة) وأجريت عليها الاختبارات الميكروبية التالية (العد الكلي للباكتيريا، العد الكلي للباكتيريا الهوائية واللاهوائية، العد الكلي لباكتيريا القولون، *E.coli*، السالمونيلا والباكتيريا العنقودية)، المحتوى الرطوبي ودرجة الحموضة. وكانت مستويات البكتريا المختلفة أعلى في العينات غير االمغطاة مقارنة مع العينات المغطاة وقد يكون هذا بسبب العرض غير السليم والبيئة المحيطة غير الصحية وعلاوة على ذلك وجود السالمونيلا وباكتيريا *E.coli* الذي يشير الى التلوث الخارجي مما يؤثر تأثيرا سلبيا على صحة المستهلكين. كذلك لايوجد إختلاف كبير في درجة الحموضة بين العينات المغطاة والمكشوفة لكل من المناطق المختلفة أما المحتوى الرطوبي لا يوجد إختلاف معنوي ما عدا في عينتي الدروشاب والحاج يوسف وقد أوضحت هذه الإختبارات أن هنالك عينات من لحوم الدواجن الخام غير مطابقة لمتطلبات السلامة للمواصفات والمقاييس السودانية للحوم الخام.

CHAPTER ONE

INTRODUCTION

1.1 Introduction:

Food is vital to life. It can be defined as any solid or liquid substance which, when taken by the body, provides it with the necessary materials to enable it to grow, to replace worn-out and damaged parts, and to function normally **(Tull, 2000).**

Food, like other substances, is composed of different chemical elements, arranged in a variety of ways to form molecules. These molecules collectively give individual foods their flavor, color, and texture, and effect their reaction to heat and their digestion **(Tull, 2000)**.

The term poultry is used collectively to designate those species of birds that have been domesticated to reproduce and grow in captivity and that render products of economic value. Chickens, turkeys, ducks, geese, some quail and pheasants, guineas, and pigeons generally meet the above criteria. They provide meat, eggs, feathers, fertilizer, animal food, and other by-products such as pharmaceuticals **(Parkhurst and Mountney, 1988)**.

Poultry comprises domestic fowls used as food. It includes the flesh, eggs or feathers obtained from these domesticated birds like chicken, geese, duck, turkey and pigeons. Out of these most widely consumed and popular ones are chicken and turkey **(Sharma, 2006)**.

On the other hand, food safety is a major global issue and, in the developed world, the claim that `our food has never been safer' is continually being challenged. Greater public awareness and concerns over food safety issues have been fuelled by various crises that have arisen, while consumer fears greatly increased following the widespread and damaging publicity given to these problems by the news media **(Mead, 2004)**. Food safety has become a phenomenon of the modern age and their consequences cannot be taken lightly. In Sudan the consumption of poultry meat has become vital nowadays. Poultry meat is widely available, relatively inexpensive and can be of central importance in helping to meet shortage in essential nutrients. The incidence of several common metabolic diseases associated with deficiencies of critical dietary minerals, vitamins and amino acids can be reduced by the contribution of poultry products rich in all essential nutrients except vitamin C **(Cherian *et al*., 2005)**.

However, extra study on safety of row poultry meat in Sudan is needed to explore the microbiological quality of poultry meat at retail areas.

1.2 The objectives of this study are:

1- To determine the microbiological quality (Total count, Total anearobe, Total earobe, Total coliform, *E.coli*, Salmonella, staph.) of raw poultry meat collected from different markets in Bahary town.

2- To examine the physicochemical (pH), and chemical (moisture) properties of raw poultry meat.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Introduction to meat and meat products:

Raw meat generally refers to any type of uncooked muscle tissue of an animal used for food. In the meat production industry, the term ‘meat’ refers specifically to mammalian flesh, while the words ‘poultry’ and ‘seafood’ are used to differentiate between the tissue of birds and aquatic creatures **(Kauffman, 2001)**.

Meat and meat products are rich and concentrated sources of nutrients including fats, proteins, vitamin B12, zinc and iron. Meat is categorised into red meat and white meat. Meat from any source is of similar nutritional value, whether it is white or red. The intensity of color in meat depends on the amount of myoglobin (Myoglobin is small, monomeric protein which serves as an intracellular oxygen storage site) it contains. It is incorrect to assume that white meat from birds is superior to red meat, or vice versa. Offal meat (i.e. internal organs such as liver and kidney), however, tends to have a higher nutritional value, and red meat is known to contain a rich source of iron **(Kauffman, 2001)**.

There are many reasons for people to eliminate meat and meat products from their diet. Some of these reasons may include health concerns,

religion, cultural background, income and accessibility of meat **(Uoda, 1984)**.

2.2 White meat:

White meat or light meat refers to the lighter-colored meat of poultry as contrasted with dark meat. In a more general sense, white meat may also refer to any lighter-colored meat (In European legislation, the term meat refers to the edible food removed from the carcass of domestic ungulates including bovine, porcine, animals as well as poultry **(European Comission, 2004)**), as contrasted with red meats like beef and some types of animals. White meat is made up of fast-twitch muscle fibers, while red, or dark meat is made up of muscles with fibers that are slow-twitch.

White meat is made up of muscles with fast fibers. Fast fiber muscles are used for quick bursts of activity, such as fleeing from danger. These muscles source of obtain their energy from glycogen, which is also stored in the muscles. White meat is a valuable source of iron and zinc, however, the mineral content per unit weight of white meat is about half the mineral content of red meat per unit weight. Similarly, vitamin B12 content is less in white meat. Myoglobin content is low in white meat. This explains why chicken breast, pork and veal are slightly pink or white. The color of fish meat is white because it lives in water and does

not need to support its own body weight, and therefore has no myoglobin in its muscles **(Kauffman, 2001)**.

2.3 Poultry meat:

Within poultry, there are two types of meats-white and dark. The different colors are based on the different locations and uses of the muscles. For ground-based birds like chicken and turkeys, dark meats occur in the legs, which are used to support the weight of the animals while they move. These muscles are designed to develop endurance for long-term use and contain a large amount of myoglobin, allowing the muscle to use oxygen more efficiently for aerobic respiration. In contrast the white meat, generally found within the breasts of the birds, are used for quick bursts of power which requires little of the meat-darkening myoglobin. Birds which use their chest muscles for sustained flight (such as geese and ducks) have dark meat throughout their bodies **(Sharma, 2006)**.

Dark meat contains 2.64 times more saturated fat than white meat, per gram of protein. One commentator wrote that dark meat contains more vitamins, while another has stated the two meats are nearly identical in nutritional value **(Sharma, 2006).**

2.4 Nutritive value of poultry meat:

Chicken meat can be considered an important component in healthy diets **(Jahan et al., 2004)** as it contains a higher proportion of polyunsaturated fatty acid compared with meat from other species **(Berzaghi *et al*., 2005)**, high nutritional value an distinct flavor **(Patsias *et al*., 2008)**. Meat continuous to supply nutrients and plays a vital role in human life because of its high biological value protein, iron, zinc, selenium and vitamin B12 contents being a crucial component of a well balanced diet. The main quality features of poultry meat are chemical composition and the ratio of muscles to fat in carcass **(Steinhauser *et al*., 2000)** claimed that proteins are the most important components of meat from nutritional and technological aspects. The content of proteins in muscles is reported to range between 18 and 22%.An important feature of poultry meat from dietetic aspects is an increased content of fatty acid particularly linoleic acid, linolenic acid and arachidonic acid. Table (1) shows the nutritional composition of chicken meat cuts **(Pereira, 2013)**.

Table (1) Nutritional composition of chicken meat cuts:

Item	Chicken breast skinless raw	Chicken breast raw	Chicken average raw
Energy value (kcal)	108	179	110
Protein (g)	24.1	24.1	22.9
Fat (g)	1.2	8.9	2
Saturated fat (g)	0.3	2.1	0.5
Vitamin B12 (mg)	0.37	0.37	0.72
Na (mg)	60	72	77
P (mg)	220	200	204
Fe(mg)	0.5	1	0.9
Zn (mg)	0.8	0.8	1

(Pereira, 2013).

2.4.1 Sensory quality attributes:

Meat quality is defined by the combination of many factors; however, consumers attach a special importance to color and texture. Inherent characteristics of animal, long and short-term environmental influences on animal and processing parameters that affect the carcass or

meat directly are all factors that influence meat color, texture and flavor **(Lyon *et al.*, 2004)**. Sensory evaluation is analysis of product attributes perceived by the human senses of smell, taste, touch, sight and hearing. People (consumers or users of the product) are used to assessing the sensory characteristics and providing a response.

2.4.2 Color:

The color of the meat is an important quality attributes which affects consumer preference. The appearance of fresh meat depends on color which is defined as the concentration of the pigment myoglobin and by the relative proportions of its three common forms, oxymyoglobin (bright red), myoglobin (purplish red) and metmyoglobin (brown). Chicken muscles can be classified by color and enervation type. Two main muscles types are distinguished: red muscles (with high myoglobin content, aerobic oxidative metabolism and abundant blood irrigation) and white muscle (low myoglobin content, anaerobic metabolism and low blood irrigation) white muscles undergo a fast contraction whereas red muscles may present fast or slow contractions. Slow contraction muscles use, in addition to glucose, fatty acids in the presence of oxygen. The slow contraction muscles have more abundant blood irrigation **(Leo and Terri, 2007)**.

Age and genetic strain are two inherent factors that affect meat color and texture. Age of the animal may be important because myoglobin, the primary muscle pigment, tends to increase with age in chicken **(Lyon *et al*., 2004)**.

2.4.3 Flavor:

Flavor analyses of poultry or poultry meat involves methods to extract compounds that are assumed to contribute to aroma. Taste is usually associated with the basic solutions of salt, sweet, sour and bitter, while aroma is associated with stimulation of receptors in the nasal cavity by volatiles released by foods **(Sams, 2001)**.

The volatile compounds responsible for meat flavors and odors develop during cooking by complex reactions between natural components in raw meat. In chicken, ribose has been shown to be of particular importance for flavor generation **(Aliani and Farmer, 2005)**.

2.4.4 Tenderness and Texture:

Tenderness or texture is a major quality concern with boneless skinless broiler breast fillets. Tenderness has been described as the most important sensory characteristic of meat. Therefore, this attribute has drawn attention from researchers. Meat texture sensation is dictated by the presence of several factors including the amount of intramuscular fat

and water holding capacity. However, it is the quality of collagen, which gives toughness to meat **(Coro *et al*., 2003)**.

Collagen is the major component of the intramuscular connective tissue and plays a key role in determining meat toughness. Nevertheless, chicken is commercially slaughtered at an age when collagen normally does not constitute a texture problem **(Coro *et al*., 2003)**.

2.4.5 Natural Preservatives:

A natural product in the meat and poultry industry is defined by united states department of Agriculture food safety and inspection service (USDA\FSIS) as a product that does not contain any artificial flavor, coloring ingredient or chemical preservative, or any other artificial or synthetic ingredient; and the product and its ingredients are not more than minimally processed **(USDA, 2005)**. Preventing microbial growth and retarding lipid and protein oxidation during storage and retail display is essential to maintain the quality and safety of meat.

Synthetic preservatives are being currently used to curtail the microbial growth and thereby extending the shelf life of meat. Synthetic preservatives used in extending shelf life of meat are less preferred over bio-preservatives by the consumer. Bio-preservatives are mainly derived from plant extracts. Majority of the plant extracts contain phenolic

compounds as secondary metabolites. Biological significance of these compounds is immense due to enormous reducing power of free hydroxyl group (antioxidant property) and protein binding capacity (causes inhibition of microbial growth – antimicrobial property) **(Adhami and Mukhtar, 2006)**. The protein binding capacity of phenolics is involved in antimicrobial and antiviral activity.

In view of certain disadvantages associated with the use of chemical preservatives, emphasis is being givekn on the use of natural preservatives for meat products. There is growing consciousness among consumer for foods with high nutritional value, free from chemical preservatives and microbiological safety. Meat is an idea medium for bacteria because of high moisture content, richness in nitrogenous compound (essential amino acids, proteins) good source of minerals, vitamins and other growth factors. Furthermore, it's PH (Hydrogen ions concentration) favorable for the growth of microorganisms. The meat preservatives restrict microbial activity, enzymatic, chemical and physical reactions that cause deterioration and spoilage of meat and meat products. Meat preservation works by lowering the amount of substances in meat that pathogens\microbes prefer to grow on **(Yadav *et al.*, 2004)**.

2.5 Chemical composition of raw poultry meat:

Poultry require almost the same nutrients-water, carbohydrates, fats, proteins, minerals, vitamins, and certain unidentified growth factors-as mammals but the proportions differ **(Parkhurst and Mountney, 2013)**.

2.5.1 Water:

Water is the largest single constituent of animal tissue, amounting to approximately 58% in chickens. It is used for temperature regulation, it serves as a medium and transportation agent for dissolved substances, and it gives animals form and shape. It is the cheapest and one of the most important of all nutrients. Lists water consumption by chickens and turkeys of different ages under average conditions. These amounts vary depending on the food consumption, temperature, humidity, activity of the chicken, and nature of the food. Fresh, clean, uncontaminated water should be easily and readily accessible, both in quantity and location **(Parkhurst and Mountney, 2013)**.

2.5.2 Carbohydrates:

Carbohydrates are organic chemical compounds structured from carbon, hydrogen, and oxygen. They are used as a source of energy by the organism because they are easily burned in the body to produce heat and energy for body movement and function. Sugars, starches, and fiber are

the most common forms in which carbohydrates are found in feeds. Glycogen is a carbohydrate synthesized in the body and stored in small amounts in the liver and muscles. It can be utilized rapidly under emergency and stress situations in the bird. Excesses of carbohydrates are converted to fats. The important forms of carbohydrates are those which can be digested by the enzyme systems in the bird. For this reason the term "nitrogen-free extract" is used to refer to the soluble and digestible portion of carbohydrates. The indigestible carbohydrates, which are the structural components of plants, are referred to as fiber. Fiber is also important as an aid to normal digestive functions and it may also affect absorption of certain minerals **(Parkhurst and Mountney, 2013)**.

2.5.3 Fats:

Fats also consist of carbon, hydrogen, and oxygen but in ratios that are different from carbohydrates. They contain about two and one quarter times the energy value of carbohydrates. Fats can be formed from carbohydrates and stored in the body. Generally, fats also include oils, the only difference being that fat is solid and oil is liquid at room temperature (65°F, 18°C). Fats are further classified as vegetable fat or oil and animal fats and oils **(Parkhurst and Mountney, 2013)**.

Fats are composed of fatty acids and glycerol. Some have a saturated chemical structure and others are unsaturated. They are also classified as

essential and nonessential fatty acids. The polyunsaturated linoleic and arachidonic acids are considered to be "essential fatty acids." They have specific functions in the body that are not related to energy production. Birds exhibit poor growth, fatty livers, reduced egg size, and poor hatchability without these essential fatty acids. Other types of fats contain phosphorus and are known as phospholipids. The type and condition of the fats consumed by poultry will also have a marked influence on the type of fat produced in the carcass. For example, if the fats are soft, the carcass fat will be soft. Poultry consuming large amounts of fish oil or rancid fats can have fishy or rancid flavors or undesirable flavors. Fat is considered to be all the material in the carcass that will dissolve in ether. It is referred to as crude fat. Small amounts of fat are generally added to rations in addition to that supplied by other feed ingredients. Processed fats added to rations may readily become rancid and form peroxides that destroy vitamins A and E. Antioxidants are usually added to delay or prevent rancidity **(Parkhurst and Mountney, 2013)**.

2.5.4 Proteins:

Proteins contain carbon, hydrogen, oxygen, nitrogen, and sometimes sulfur. They differ chemically from carbohydrates and fats in that all proteins contain nitrogen. About 16% of protein is composed of the nitrogen molecule. Because proteins are the principal constituents of

organs and soft tissues of the body they are needed for proliferation of new tissue (growth) and replacement of old or damaged tissues (injury or disease). Proteins are classified chemically according to their solubility in dilute salts and strong alkalies. From a commercial standpoint they are classified by the number of amino acids that can be used by poultry and by their digestibility, which is measured by the ability of the bird's enzyme system to break them down. Despite the amino acid qualities of this group of proteins with processing, they have high nutritive value because poultry enzyme systems cannot break them down **(Parkhurst and Mountney, 2013)**.

The quality or usefulness of a protein depends on the number and proportions of required amino acids present **(Parkhurst and Mountney, 2013)**. Although 23 chemically different amino acids have been isolated, poultry require only 11 of them. The essential amino acids for poultry are phenylalanine, isoleucine, lysine, threonine, histidine, arginine, tryptophan, methionine, valine, leucine, and glycine. Glycine is required only by the growing chick. Proline, a twelfth amino acid, may be required under some conditions.The amount of protein in a feed can be calculated by determining its nitrogen content and then multiplying by a factor of 6.25 to give an estimated crude protein content. Although a protein may show a high amino acid content, it may not be a high-quality protein for poultry. The practical usefulness of the protein can be determined only by

feeding it, though chemical tests may give some indication. The result is called the biological value **(Parkhurst and Mountney, 2013)**.

2.5.5 Minerals:

Minerals, unlike carbohydrates, fats, and protein that are organic, are inorganic. They exist primarily in the form of salts and ash. They are essential for bone and eggshell formation, and for regulatory processes in the body. The essential minerals required by poultry include calcium, phosphorus, magnesium, manganese, sodium, potassium, iron, copper, zinc, sulfur, fluorine, chlorine, iodine, selenium, and molybdenum. Macro minerals such as calcium, phosphorus, and sodium are required in the largest amounts and must be included as specific additives in the ration. Frequently manganese is deficient and supplementation is necessary **(Parkhurst and Mountney, 2013)**.

2.5.6 Vitamins:

Vitamins are complex organic structures essential in minute amounts for the growth, reproduction, and overall health of poultry. Some vitamins are dietary essential and some are metabolic essential. For poultry, vitamins are all literally dietary essential. Their principal role is body regulation rather than the development of the structural components of the body. Table 8.3 shows the vitamins needed by poultry, their sources, and deficiency symptoms the amounts of vitamins, linoleic acid,

and minerals required by poultry of various ages **(Parkhurst and Mountney, 2013)**.

2.5.7 Energy:

A source of energy is the largest single dietary need for poultry. The true energy value can be determined only by feeding it to poultry. Energy is needed by the bird for basal metabolism (cellular activity, respiration, and circulation), voluntary activity, digestion and absorption, thermal regulation, waste formation and excretion, formation of tissue and feathers, and reproduction. The first use of any feed consumed is for body maintenance functions. When this need is met energy can be used for growth followed by use for reproduction and, finally, when all these previous needs are met energy is stored in the body as fat. A number of terms have been created to describe the idealized flow of energy through an animal **(Parkhurst and Mountney, 2013)**.

2.6 Meat Handling:

Make sure to wash your hands frequently when preparing any type of meat, fish, or poultry. Prepare the meat on a separate surface from other cooking materials. Because germs spread easily. It’s particularly important to keep vegetables and other ingredients away from meat, especially if you are not cooking them together in the same dish. Use separate cutting boards. You should also clean all cooking utensils after

they come into contact with raw meat. Different utensils should be used to serve food than prepare it **(Healthline Editorial Team, 2014)**.

2.7 Poultry Storage:

As a general rule, uncured raw poultry lasts safely for around three days in the refrigerator. If you are planning to keep uncooked poultry longer, then freezing is your best choice. Meat should be sealed in an airtight package before freezing. Then it can usually be frozen for at least several months. Safe freezing and refrigeration time also depends on the storage temperature. Freezers should be kept as close to 0 degrees Fahrenheit as possible. This helps keep food fresh and retain nutrients. Refrigerators should be kept at around 34 F to effectively prolong the shelf life of foods. This temperature is just above freezing. Below is a general guide to how long basic meats can be kept safely if they are stored properly **(Healthline Editorial Team, 2014)**.

- In the Refrigerator:
  - uncooked poultry: one to two days
  - uncooked ground meat: one to two days
- In the Freezer
  - uncooked poultry: nine months (pieces) to one year (whole)
  - uncooked ground beef: three to four months

Rare and medium meats may not be cooked thoroughly enough to kill all bacteria. However, risk varies for different types of meats.

- Undercooked poultry can spread salmonella and other diseases **(Healthline Editorial Team, 2014)**.

2.8 Poultry Meat quality and consumer requirements:

Throughout the world, consumption of poultry meat continues to rise in both developed and developing countries. In 1999, global production of broiler chickens reached 40 billion for the first time and, by 2020, poultry is predicted to become the overall meat of choice **(Vanhorne, 2002)**. The continued growth and competitive nature of the industry have been attributed to a variety of factors, some of which relate to economies of scale in intensive production and processing, and extensive use of mechanization, while others include the more recent development of a wide range of convenience and ready-to-eat products that meet both direct consumer demand and the rapid expansion of fast-food outlets. Poultry products are universally popular, because they are not subject to cultural or religious constraints and the meat itself is perceived as wholesome, healthy and nutritious, being relatively low in fat and with a more desirable unsaturated fatty-acid content than other meats. Most importantly, high-quality poultry products are available to

many people at affordable prices, although production costs vary widely around the world **(Vanhorne, 2002)**, and are likely to increase as new legislation appears and retailers and consumers become more demanding in their requirements. In poorer regions, poultry are often sold live or are slaughtered at the point of sale, and 30% of all the world's poultry are said to be marketed in this way **(Holroyd, 2001)**. Processing in developing countries tends to be more labor intensive and is often confined to the production of relatively simple items, such as whole carcasses and cut portions. Nevertheless, poultry production and consumption are increasingly significant in those countries, especially where there are high population densities and sustained economic growth **(Vanhorne, 2002)**.

In parallel with these changes in lifestyle, there has been a significant uptake of labor-saving devices in the home, including the microwave oven, while traditional skills in food preparation are often forgotten. This is largely because only limited time is available to prepare meals at home. Among more discerning consumers there is, however, a greater awareness of food safety, animal welfare and environmental issues associated with food production and processing. All of the above considerations have had, and are continuing to have, a major impact on food product development and marketing, and the poultry industry has responded successfully to the market demands and opportunities that have arisen.

With regard to poultry, the most important growth area of recent times has been the development of value-added, further-processed products. In the USA, for example, less than 10% of all broilers are sold as whole carcasses **(Thornton and O'Keefe, 2002)**. The majority of carcasses are cut up, deboned or further processed and the meat is portioned, sliced, ground, flavored, marinated or cooked. There is also a large production of breaded and coated items. At one company alone, manufacture of these products increased by 16.5% per annum between 1996 and 2000. Therefore, there is growing demand for convenient, high-quality food products that provide variety and are quick and easy to prepare, while remaining attractive in price. At the same time, quality expectations are rising and products of `restaurant quality' are of increasing interest **(Thornton and O'Keefe, 2002)**. Attention will be paid to the range of chemical, microbiological and physical hazards in poultry meat production and the ways in which consumer concerns are being tackled through the development of a `farm to fork' approach to meat quality and safety control.

2.9 Diseases Caused by poultry:

2.9.1 Salmonellosis:

Salmonella poisoning, or salmonellosis, is probably the most common bacterial affliction that humans can attribute to chickens. The

infection is passed by eating chicken meat or eggs that have been contaminated with this bacterium. Salmonellosis is characterized by high fever, abdominal cramping and diarrhea, and it is especially dangerous when it affects young children, the elderly or people with compromised immune systems **(Buckley, 2015)**.

2.9.2 *E.Coli* Infection:

Though the *E.coli* bacteria is more often associated with the consumption of beef products, Escherichia coli infections can result from eating contaminated chicken as well. *E.coli* is commonly found in the intestines of humans and other mammals, but particular strains of the microorganism can cause serious illness or even death in some cases. Symptoms of infection include severe abdominal cramping, vomiting and bloody diarrhea **(Buckley, 2015)**.

2.9.3 Campylobacteriosis:

Campylobacter is another pathogenic bacterium that can be transferred to humans by eating infected chicken meat. This microorganism is one of the most common sources of food-related poisoning in people. High temperatures are very effective in killing Campylobacter, so proper cooking methods are important to follow when preparing chicken dishes. Like other chicken-related infections, this

bacterium can cause abdominal pain and diarrhea. It also can lead to Guillain-Barre syndrome, a disease that could result in paralysis. Concerns related to Campylobacter have arisen in the health community due to the identification of increased levels of antibiotic-resistant strains of the organism. According to by the Food and Drug Administration (FDA), data from industrialized countries have demonstrated that a significant source of antibiotic-resistant food borne infections in humans is the acquisition of the resistant bacteria from animals via food **(Buckley, 2015)**.

2.9.4 *Staphylococcus aureus* Infection:

Chicken-related Staph infections are usually attributed to the microorganism Staphylococcus aurous. The organism can be passed to people via ingestion of contaminated meat, and can also be transferred as a result of physical contact with live birds. Toxins released by this bacterium can cause nausea, vomiting, abdominal cramps, and severe muscular pain. Similar to Campylobacter, this bacterium has grabbed the attention of health officials due to virulent strains that are resistant to antibiotic drugs Staph aurous-commonly identified as MRSA-which has become a rising issue in hospital environments, according to the CDC (Center for disease control) **(Buckley, 2015)**.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Raw meat

Raw poultry meat.

3.1.2 Microbiological Media

- Plate Count Agar.
- Nutrient Agar.
- Potato – Dextrose Agar.
- MacConkey Broth.
- Brilliant Green 2% Bile Broth.
- EC Broth.
- Eosin Methylene Blue Agar.
- Selenite Cystine Broth.
- Bismuth Sulphite Agar.
- Triple Sugar Iron Agar / Mannitol Salt Agar (Substituted).
- Baird-Parker Agar.
- Cetrimide Fucidin Cephaloridine Agar (CFC).

3.1.3 Used Diluents

- 0.1% Peptone Solution.

3.1.4 Raw Poultry meat Samples

Twelve samples of raw poultry meat were collected from different markets in Bahry town. These samples were covered and uncovered types.

3.2. Methods

3.2.1 Sterilization of Glassware

Petri dishes, test tubes, flasks, pipettes…etc., were sterilized in hot air oven at 160 – 180º C for 2 to 3 hours before they were put in the oven they were washed dried and packed in stainless steel cans or sometimes in aluminum foil.

3.2.2 Sterilization of Media

Culture media were first adjusted to the required pH and then sterilized. Sterilization was achieved by autoclaving at 121.5º C for 15 minutes.

3.2.3 Total Viable Count of Bacteria

It was carried out by using the pour plate count method as described by **Harrigan (1998)**. Suitable medium for this purpose is Plate Count Agar.

3.2.4 Preparation of serial dilutions

Aseptically 10 grams of the sample were homogenized in 90 ml of sterile diluents (0.1% Peptone solution). It was mixed well to give dilution (10^{-1}) by using sterile pipette 1 ml was transferred aseptically from dilution (10^{-1}) to a test tube containing 9 ml of sterile diluents and it was mixed well to give dilution (10^{-2}). In the same way the preparation of serial dilution was continued until the dilution (10^{-6}). One ml of each dilution was transferred into sterile petri dish; to each plate 15 ml of sterile melted Plate Count Agar medium were added. The inoculum was mixed with medium and allowed to solidify. The plates were incubated at 37º C for 48 hours. A colony counter was used to count the viable bacterial colonies after incubation and the results were expressed as colony-forming units (CFU) per gram.

3.2.5 Determination of Coliform Bacteria

It was carried out by using the Most Probable Number (MPN) technique as following:

3.2.5.1 Presumptive Coliform test

10 and 0.1 ml prepared samples was inoculated in triplicates of MacConkey Broth test tubes containing Durham tubes. The tubes were incubated at 37º C for 48 hours. The production of acid together with sufficient gas to fill the concave of the Durham tube is recorded as positive presumptive test.

3.2.5.2 Confirmed test for Total Coliforms

From every tube showing positive result a tube of Brilliant Green 2% Bile Broth was inoculated by using a sterile loop. The tubes were inoculated at 37ºC for 48 hours, and then the tubes showing positive and negative result were recorded. The Most Probable Number (MPN) of total coliform was found out by using the Most Probable Number (MPN) tables.

3.2.5.3 Confirmed *E. coli* test

Medium used was EC Broth. From every tube showing positive result in the presumptive test used to inoculate a tube of EC Broth containing Durham tube were inoculated at 44.5º C for 24 hours. Tubes showing any amount of gas were considered positive. For further confirmation of *E.coli* tubes of EC Broth showing positive results at 44.5º for 24 hours were streaked on Eosin Methylene Blue Agar (EMB) plates.

The plates were incubated at 37º C for 48 hours. Colonies of *E.coli* are usually small with metallic green sheen on EMB Agar.

3.2.5.4 *Staphylococcus aureus* enumeration:

Medium used was Baird-Parker medium; 0.1 ml of every dilution was transferred onto the surface of each well dried Baird-Parker medium plates. The inoculums were spreader all over the plate using sterile bent glass rod. The plates were incubated at 37º C for 24 hours; after that period of incubation had been finished the plates were examined. *Staphylococcus aureus* after 24 hours appear black shiny convex and surrounded by a zone of clearing 2-5 mm in width of colony.

3.2.5.5 Detection of *Salmonella*:

Ten gram of the sample were added to a conical flask containing 100 ml of sterile Nutrient Broth and incubated at 37º C for 24 hours. A loop full of 24 hours incubated Nutrient Broth was transferred aseptically into sterilized Selenite Cystine Broth and incubated at 37º C for 24 hours. A loopful of 24 hours inoculum of Selenite Cysteine Broth was streak on Bismuth Sulphite Agar surface and incubated at 37º C for 24 – 72 hours. Black metallic sheen discrete colonies indicated the presence of *Salmonella*.

3.2.5.6 Total anaerobically:

Counting of anaerobic bacteria by using sterile pipette 1ml was transferred aseptically form every dilution to sterile petri dish, about 15ml of sterile plate count agar media were added to every plate and it was mixed well and left to be solid .the plates were incubated by using anaerobic system at 37c for 48 hours after the incubation period had been finished the number of colony counter and the result were expressed as colony forming unit {cfu/gram}.

3.3. Chemical methods

3.3.1. Moisture content

The moisture content was determined according to the standard method of the Association of Official Analytical Chemists **(AOAC, 2003).**

Principle: The moisture content in a weighed sample is removed by heating the sample in an oven (under atmospheric pressure) at 105 $^{\circ}$C. Then, the difference in weight before and after drying is calculated as a percentage from the initial weight.

Procedure: A sample of 5 g±1 mg was weighed into a pre-dried and tarred dish. Then, the sample was placed into an oven at 105 ± 1 $^{\circ}$C until a constant weight was obtained. After drying, the covered sample was

transferred to desiccators and cooled to room temperature before reweighing**.** Duplicate results were obtained for each sample and the mean value was reported to two decimal points according to the following formula:

Calculation:

$$\text{Moisture content (\%)} = \frac{(Ws - Wd)}{\text{Sample weight (g)}} \times 100\% \qquad \text{[eq.1]}$$

Where:

Ws = weight of sample before drying.

Wd = weight of sample after drying.

3.4. Physicochemical methods:

3.4.1. Hydrogen ions concentration (pH):

The different samples were determined as described by **Ranganna (2001).**

Principle: The pH value of the different samples was measured with a pH-meter. After standardization of the pH-meter electrodes with buffer solution, the reading of the sample is recorded as pH value.

Procedure: After standardization of the pH-meter with buffer solutions and the electrode of the pH-meter were rinsed with distilled water, immersed in the sample and left to stand until a staple reading was achieved. All the readings were expressed as pH to the nearest 0.01-pH units.

3.4 Statistical analysis method

The results were subjected to Statistical Analysis (Minitabsoft ware) by using two sample t test to compare significant different between means.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Microbiological quality of raw poultry meat:

The microbiological safety and quality of raw poultry meat are equally important to producers, retailers and consumers, and both involve microbial contaminants on the processed product **(Pooni and Mead, 1984, 1995).**

Contamination of raw poultry meat with foodborne pathogens remains an important public health issue because it can lead to illness if there are malpractices in handling, cooking or post- cooking and storage of the product. In developed countries foodborne illness causes human suffering and loss of productivity and adds significantly to the costs of food production and healthcare. It is also a possible cause of mortality which is even more of a problem in developing regions, where the health status of many individuals is already compromised **(Mead, 2004)**.

4.1.1 Microbiological count of raw poultry samples from Bahry:

From the table 2 the total aerobic count in covered and uncovered raw poultry meat were (1.07± 0.15 and 5.22± 0.15) respectively. **Cohen *et al*. (2007)** Reported that the total aerobic count in covered and uncovered raw poultry meat were (5.9 ±0.6 and 6.6 ±0.7) respectively.

This result was in disagreement with our findings, they reported higher level in covered sample but the level of total aerobe in the un-covered one was lower as compared with the result presented in Table 2. These different may be due to the source of the poultry or to the experimental conditions.

In the same table the *E.Coli* in covered and uncovered samples were (NF and 16.00 ± 2.58) respectively. **Cohen *et al*. (2007)** Reported that the *E.Coli* in covered and uncovered were (2.5±0.6 and 2.9±0.8) respectively. This result was in disagreement with our findings, they reported higher level in covered sample but the level of total aerobe in the un-covered one was lower as compared with the result presented in Table2. These different may be due to the source of the poultry or to the experimental conditions.

Also in table 2 the total coliform in covered and uncovered samples were (7.25 ± 3.50 and 69.50 ± 6.35) respectively. **Cohen *et al* (2007)** Reported that the total coliform in covered and uncovered were (3.6 ±0.7 and 3.8±0.6) respectively. This result was in disagreement with our findings, they reported higher level in covered sample but the level of total aerobe in the un-covered one was lower as compared with the result presented in Table2. These different may be due to the source of the poultry or to the experimental conditions.

As were as in table 2 the *staph.* In covered and uncovered samples were (2.68 ±0.06 and 3.13 ±0.56) respectively. **Cohen *et al* (2007)** Reported that the *staph.* In covered and uncovered were (2.4 ±0.7 and 2.5 ±0.6) respectively. This result was in disagreement with our findings, they reported higher level in covered sample but the level of total aerobe in the un-covered one was lower as compared with the result presented in Table2. These different may be due to the source of the poultry or to the experimental conditions.

The result in table 2 showed that the microbiological safety of cover raw poultry meat sample was better as compared with uncovering one. There was significant increase in levels of total count, total anaerobe, total coliform, *E.coli* and *staph.* in uncovered raw poultry sample. Moreover *salmonella* was detected only in uncovered raw poultry sample.

Table 2: Microbiological quality of raw poultry meat samples from Bahry market*

Source of poultry	**Total count Log cfu**	**Total anaerobe Log cfu**	**Total aerobe Log cfu**	**Total coliform (MPN)**	***E. coli*** (MPN)	***Salmonella***	***Staph.*** **Log cfu**
Bahry covered	4.72 ± 0.16[a]	3.65 ± 0.06[a]	1.07± 0.15[a]	7.25 ± 3.50[a]	NF	NDT	2.68 ±0.06[a]
Bahry un-covered	8.79 ± 0.10[b]	3.58 ± 0.08[a]	5.22± 0.15[b]	69.50 ± 6.35[b]	16.00± 2.58	**DT**	3.13 ±0.56[b]

*Values are mean ± SD of replicate in depended sample.

*Values that carry the same superscript latter in same Column are not significant different.

*NF = No Found.

*NDT = Not Detect.

*DT = Detect.

4.1.2 Microbiological quality of raw poultry meat samples from Hajyousif market:

From the table 3 the total aerobic count in covered and uncovered raw poultry meat were (3.01± 0.09 and 3.81±1.69) respectively. **Cohen *et al*. (2007)** Reported that the total aerobic count in covered and uncovered raw poultry meat were (5.9 ±0.6 and 6.6 ±0.7) respectively. This result was in disagreement with our findings, they reported higher level in covered sample but the level of total aerobe in the uncovered one was lower as compared with the result presented in Table 3. These different may be due to the source of the poultry or to the experimental conditions.

In the same table the *E.Coli* in covered and uncovered samples were (6.25±7.23 and 15.00 ± 3.16) respectively. **Cohen *et al*. (2007)** Reported that the *E.Coli* in covered and uncovered were (2.5 ±0.6 and 2.9 ±0.8) respectively. This result was in disagreement with our findings, they reported higher level in covered sample but the level of total aerobe in the un-covered one was lower as compared with the result presented in Table2. These different may be due to the source of the poultry or to the experimental conditions.

Also in table 3 the total coliform in covered and uncovered samples were (9.75 ± 2.22 and 76.3 ± 21.00) respectively. **Cohen *et al* (2007)** Reported that the total coliform in covered and uncovered were (3.6 ±0.7 and

3.8±0.6) respectively. This result was in disagreement with our findings, they reported higher level in covered sample but the level of total aerobe in the uncovered one was lower as compared with the result presented in Table3. These different may be due to the source of the poultry or to the experimental conditions.

As were as in table 3 the *staph.* In covered and uncovered samples were (2.57 ± 0.08 and 4.70 ± 20.18) respectively. **Cohen *et al* (2007)** Reported that the *staph.* In covered and uncovered were (2.4 ±0.7 and 2.5 ±0.6) respectively. This result was in disagreement with our findings, they reported higher level in covered sample but the level of total aerobe in the un-covered one was lower as compared with the result presented in Table3. These different may be due to the source of the poultry or to the experimental conditions.

Relate to result in table 3 the microbiological safety of cover raw poultry meat sample was better as compared with uncover one. There was significant increase in levels of total count, total anaerobe, total coliform, *E.coli* and *staph.* in uncover poultry sample. Moreover *salmonella* was detected only in uncover poultry sample.

Table 3: Microbiological quality of raw poultry meat samples from Hajyousif market*

Source of poultry	**Total count Log cfu**	**Total anaerobe Log cfu**	**Total aerobe Log cfu**	**Total coliform (MPN)**	***E. coli* (MPN)**	***Salmonella***	***Staph.* Log cfu**
Hajyousif covered	5.60± 0.10[a]	2.51± 0.06[a]	3.01± 0.09[a]	9.75± 2.22[a]	6.25± 7.23[a]	NDT	2.57± 0.08[a]
Hajyousif un-covered	9.34± 0.68[b]	5.53 ± 1.01[b]	3.81± 1.69[a]	76.3± 21.00[b]	15.00± 3.16[b]	**DT**	4.70± 20.18[b]

*Values are mean ± SD of replicate in depended sample.

*Values that carry the same superscript latter in same Column are not significant different.

*NF = No Found.

*NDT = Not Detect.

*DT = Detect.

4.1.3 Microbiological quality of raw poultry meat samples from Aldrowshap market:

From the table 4 the total aerobic count in covered and uncovered raw poultry meat were (3.22±0.12 and 4.21±0.07) respectively. **Cohen *et al*. (2007)** Reported that the total aerobic count in covered and uncovered raw poultry meat were (5.9 ±0.6 and 6.6 ±0.7) respectively. This result was in disagreement with our findings, they reported higher level in covered sample but the level of total aerobe in the un-covered one was lower as compared with the result presented in Table 4. These different may be due to the source of the poultry or to the experimental conditions.

In the same table the *E.Coli* in covered and uncovered samples were (NF and 14.00 ± 1.63) respectively**. Cohen *et al*. (2007)** Reported that the *E.Coli* in covered and uncovered were (2.5 ±0.6 and 2.9 ±0.8) respectively. This result was in disagreement with our findings, they reported higher level in covered sample but the level of total aerobe in the un-covered one was lower as compared with the result presented in Table 4. These different may be due to the source of the poultry or to the experimental conditions.

Also in table 4 the total coliform in covered and uncovered samples were (10.00 ± 3.16 and 39.75 ± 3.59) respectively. **Cohen *et al* (2007)** Reported that the total coliform in covered and uncovered were (3.6 ±0.7

and 3.8±0.6) respectively. This result was in disagreement with our findings, they reported higher level in covered sample but the level of total aerobe in the un-covered one was lower as compared with the result presented in Table4. These different may be due to the source of the poultry or to the experimental conditions.

As were as in table 4 the *staph.* In covered and uncovered samples were (2.81 ± 0.06 and 3.83 ± 0.11) respectively. **Cohen *et al* (2007)** Reported that the *staph.* In covered and uncovered were (2.4 ±0.7 and 2.5 ±0.6) respectively. This result was in disagreement with our findings, they reported higher level in covered sample but the level of total aerobe in the un-covered one was lower as compared with the result presented in Table 4. These different may be due to the source of the poultry or to the experimental conditions.

As were as in table 4, the microbiological safety of cover raw poultry meat sample was better as compared with uncovered one. There was significant increase in levels of total count, total anaerobe, total aerobe, total coliform, *E.coli* and *staph*. in uncover poultry sample. Moreover *salmonella* was detected only in uncover raw poultry meat sample.

Table 4: Microbiological quality of raw poultry meat samples from Aldrowshap market*

Source of poultry	**Total count Log cfu**	**Total anaerobe Log cfu**	**Total aerobe Log cfu**	**Total coliform (MPN)**	***E. coli* (MPN)**	***Salmonella***	***Staph.* Log cfu**
Aldrowshap covered	5.75± 0.14[a]	2.54 ± 0.11[a]	3.22± 0.12[a]	10.00± 3.16[a]	NF	NDT	2.81± 0.06[a]
Aldrowshap un-covered	7.87± 0.09[b]	3.67 ± 0.16[b]	4.21± 0.07[b]	39.75± 3.59[b]	14.00± 1.63	DT	3.83± 0.11[b]

*Values are mean ± SD of replicate in depended sample.

*Values that carry the same superscript latter in same Column are not significant different.

*NF = No Found.

*NDT = Not Detect.

*DT = Detect.

4.2 pH of raw poultry meat sample:

Hydrogen ions concentration (pH) is the measure of acidity and alkalinity of solution that is a number on a scale on which a value of 7 represents neutrality and lower numbers indicate increasing acidity and higher numbers increasing alkalinity.

From table 5 the pH in covered samples from different markets (Bhry, Hajyoussef and Aldrowshab) were (5.99±0.00, 6.34 ±0.00 and 6.10±0.00) respectively and in uncovered samples were (6.41±0.00, 6.40±0.00 and 6.23± 0.00) respectively. **Al-Dughaym *et al*. (2002)** Reported that the moisture was (5.84) this result was in disagreement with our findings. That may be due to different in gene type or feeding of poultry and breeding system of poultry.

The result in table 5 showed the pH of raw poultry meat sample collected from three different markets. There was no significant different between covered and uncovered samples of raw poultry meat from each markets.

Table 5: pH of raw poultry samples from different markets*

Type of sample	Bahry	Hajyousif	Aldrowshap
Covered	5.99 ± 0.00[a]	6.34 ± 0.00[a]	6.10± 0.00[a]
un-covered	6.41± 0.00[a]	6.40± 0.00[a]	6.23± 0.00[a]

*Values are mean ± SD of replicate in depended sample.

*Values that carry the same superscript latter in same Column are not significant different.

4.3 Moisture content:

Moisture is the quantity of water contained in foods, is used in wide range of scientific and technical areas, and is expressed as a ratio, which can range from 0 (completely dry) to the value of the foods.

From table 6 the moisture in covered samples from different markets (Bhry, Hajyoussef and Aldrowshab) were (74.20± 0.00, 82.20± 2.08 and 76.40± 0.46) respectively and in uncovered samples were (73.00±1.16, 76.00±2.31 and 73.00±1.15) respectively. **Al-Dughaym *et al*. (2002)** Reported that the moisture was (73.43%), this result was in agreement with our findings.

Table 6 showed the moisture content of raw poultry meat sample (cover and uncover) for each markets. There was significant different between covered and uncovered samples except in Bahry markets.

Table 6: Moisture content of raw poultry meat samples from different markets*

Type of sample	**Bahry**	**Hajyousif**	**Aldrowshap**
Covered	74.20± 0.00[a]	82.20± 2.08[a]	76.40± 0.46[a]
un-covered	73.00± 1.16[a]	76.00± 2.31[b]	73.00± 1.15[b]

*Values are mean ± SD of replicate in depended sample.

*Values that carry the same superscript latter in same Column are not significant different.

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion:

From the results obtained in this study it can be concluded that the cover raw poultry meat is more safe than the uncover, in addition the percentage of salmonella, *E.coli*, staph. In uncovered samples will have negative effect on health.

5.2 Recommendations:

1- Poultry meat should never be eaten raw. It should always be cooked thoroughly.
2- The surrounding environmental of raw poultry meat should be clean and hygiene and the person who Sells the raw poultry meat must have health card.
3- The raw poultry meat should be stored in good conditions under refrigeration or freezing.
4- Further researches are needed on safety of raw poultry meat in Sudan.

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