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Microbiological Characteristics of Shawarma from Different Restaurants in Khartoum State and Hygienic Awareness of the Workers

الخصائص الميكروبيولجية للشاورما من مطاعم مختلفة في ولاية الخرطوم ومدى معرفة العمال بالممارسات الصحية

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الآية

قال تعالى :

{ وَأَمْدَدْنَاهُمْ بِفَاكِهَةٍ وَلَحْمٍ مِمَّا يَشْتَهُونَ }

سورة الطور (22)

Dedication

This work is dedicated to my parents who raised me on love of reading and respect for education and to my friends

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Firstly, my deeply thank **ALLAH** upon completion of this work.

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Abstract

This study was conducted to evaluate the microbiological contamination of shawarma sandwiches according to the hygienic awareness of workers in Khartoum State. Twenty seven random shawarma sandwich samples were collected from three different locations (Khartoum, Khartoum North and Omdurman) in three types of restaurants (Closed, Semi-closed and Open). Also twenty seven questionnaires were distributed to assess the extent of workers knowledge of good hygienic practices. High contamination with total viable count was found in open restaurants in Omdurman $7.5x10^5$ log/cfu. Moulds and yeasts were found in all samples but the highest contamination was found in open restaurants in Omdurman $3.85x10^3$ log/cfu. *Staphylococcus aureas* were found in all samples and the highest contamination was found in open restaurants in Khartoum-North $5.05x10^3$ log/cfu. Coliform bacteria was isolated and found in all samples with a high level in open restaurants in Khartoum 32.4 MPN/g. Open restaurants in Khartoum-North had the highest value of *E.coli* (9.33 MPN/g). Detection of *Salmonella spp* was positive in semi-closed and open restaurants, while it was negative in closed restaurants. There were statistically significant relationships between type of restaurants and detection of pathogenic microorganisms. It can be concluded the type of restaurants from which the samples were collected has a major influence in the levels of contamination with different microorganisms including pathogenic bacteria. Highest contamination levels were obtained in the samples from open restaurants while lowest levels were in the samples from closed restaurants. The questionnaire proved that workers had significant weakness in knowledge of good hygienic practices.

الملخص

أجريت هذه الدراسة لتحديد الخصائص الميكروبيولوجية لساندويتشات الشاورما ومدى معرفة العمال بالممارسات الصحية الجيدة في ولاية الخرطوم، تم جمع 27 عينة من ثلاث مناطق مختلفة في ولاية الخرطوم (أمدرمان – الخرطوم بحري والخرطوم) ومن 3 أنواع مختلفة من الكافتيريات (مغلقة – شبه مغلقة – مفتوحة). أيضا تم توزيع 27 استبيان لتقييم مدى معرفة العمال بالممارسات الصحية الجيدة. تمت ملاحظة أعلى تلوث بالعدد الكلي للباكتيريا في المطاعم المفتوحة في أم درمان ($7.5x10^5$ log/cfu). أيضا تم العثور على الأعفان والخمائر في جميع العينات, لكن أعلى تلوث وجد في المطاعم المفتوحة في أمدرمان ($3.85x10^3$ log/cfu). تم العثور على باكتيريا المكورات العنقودية في جميع العينات ووجدت أعلى نسبة تلوث بهذه الباكتيريا في المطاعم المفتوحة في الخرطوم بحري ($5.05x10^3$ log/cfu). تم عزل بكتيريا القولون، حيث وجدت في جميع العينات بمستوى عالٍ خاصة في المطاعم المفتوحة بالخرطوم (32.4 MPN/g). حصلت المطاعم المفتوحة في شمال الخرطوم على أعلى قيمة للتلوث بباكتيريا الايشيريشيا كولاي (9.33 MPN/g). جميع المطاعم شبه المغلقة والمفتوحة أظهرت نتائج ايجابية لباكتيريا السالمونيلا ، في حين كانت النتيجة سلبية في جميع المطاعم المغلقة. اوضحت النتائج وجود إختلاف معنوي بين مختلف أنواع المطاعم و الكشف عن الميكروبات الممرضة. لقد تم استنتاج أن اختلاف نوع المطاعم التي جمعت منها العينات لها تأثير كبير في مستوى التلوث بالكائنات الحية الدقيقة المختلفة بما في ذلك الباكتيريا المسببة للأمراض. أعلى مستويات التلوث كانت في المطاعم المفتوحة بينما أدنى مستويات التلوث كانت في العينات التي أخذت من المطاعم المغلقة. لقد أثبت الإستبيان أن العمال لديهم ضعف كبير في معرفتهم وإلمامهم بالممارسات الصحية الجيدة.

CHAPTER ONE

INTRODUCTION

Shawarma is a type of grilled meat loafs characterized by its palatability and acceptable price. In Khartoum, shawarma sandwiches one of the famous ready to eat foods, which are prepared from beef or chicken meat, vegetables, spices and bread and sold in fast food restaurants.

Shawarma can be originally traced back to Turkey, where it was called "çevirme", which means "turning", but the dish itself is usually called döner kebab, meaning, and "turning kebab". In Greek, it is called gyros, meaning, "turned". It is becoming more popular among consumers of fast foods in Jordan, the Middle East, Europe, Canada, and other countries. It is a wrap of shredded meat (beef, lamb, or marinated chicken) prepared by alternately stacking strips of fat and pieces of seasoned meat on a rotating vertical skewer. The meat is roasted from the outside, while most of the inside remains rare. Shavings are cut off the block of meat for serving, and the remaining block of meat is kept heated on the rotating skewer, (**Okafo *et al.*, 2010).**

Shawarma sandwiches contain sliced chicken, beef, or lamb meat with fat, are seasoned with peppers and with tahini (sesame seeds paste and oil) and served in a pita bread wrap. Some restaurants use local mayonnaise to season the sandwiches, (**Okafo *et al.*, 2010).**

Microorganisms in fast and traditional fast foods are responsible for many human diseases. e.g. Salmonella bacteria is a common cause of food borne illness, particularly in-undercooked chicken and chicken eggs, **(Woodward, 1996).**

More than 250 different food borne diseases have been described; most of these diseases are caused by a variety of pathogenic bacteria, parasites, and viruses that can be food borne and can cause food poisoning, **(Center for Disease Control and Prevention, 2012).**

Campylobacter spp., Staphylococcus spp., Escherichiacoli, Salmonella spp., Yersinia Spp. and *Listeria* were found on meat, sea foods, vegetable ingredients, chicken shawarma, raw and cooked foods, raw chicken, beef burger sandwiches, ready-to eat salad vegetables, commercial mayonnaise, frozen chicken, poultry products and on the hands of food workers, **(Pelczar *et al*.,2006).**

The disease causing agents spread by sandwich not only incapacitate large groups of people, but also sometimes result in serious disability and even death. The transmission of human diseases through food is a global problem, particularly in developing countries where gastrointestinal diseases are one of the most important causes of mobility and mortality. However, food habits adopted by populations may mitigate or increase the hazards, **(WHO 1968; WHO, 1976).** The above-mentioned hazards can be minimized to a great extent simply by monitoring the microbiological quality of food e.g. sandwich and creating awareness among the people about the fundamental principles of sanitation and hygienic quality of foods.

The normal temperature of *Staphylococcus aureus* growth is 37 C° but it may grow at 6.5°C. However, it cannot grow easily under chilled storage conditions. *Salmonella* can grow on meat at temperature below 6C° in 10 hours. The minimum growth temperature for *Salmonella* is 6.7 but it can grow up to 5.3 C°. Studies showed that it was isolated from farmhouse, slaughterhouse and market meat to be 7, 50 and 20%, respectively. The importance of food as a vehicle for the

transmission of several diseases has been documented, especially in developing countries where hygienic standards are not strictly followed or enforced, **(Harakeh *et al.*, 2007).**

The knowledge of microbiology of meat and its products is very important to control the growth of undesirable microorganisms and retarding the conditions favorable for their growth and activity, **(Farooq *et al.*, 2013).**

General objective:

To study the microbiological properties of shawarma in different restaurants and assess the hygienic awareness of workers.

Specific objectives:

1- To measure the level of microbiological awareness among shawarma restaurants workers.

2- To determine the microbial load of shawarma sandwiches from the different restaurants.

3- To determine the presence of pathogenic bacteria in shawarma.

CHAPTER TWO

LITERATURE REVIEW

2.1 Definition of street food:

In many cities and towns of developing countries, street food vending is a large source of employment .The street food is prepared on the streets and ready-to-eat, or prepared at home and consumed on the streets without further preparation. Street vended food not only appreciated for their unique flavors, convenience and the role which they play in the cultural and social heritage of societies, it also become important and essential for maintaining nutritional status of populations**.** The Street foods provide a source of affordable nutrients to the majority of the people especially the low-income group in the developing countries**, (Choudhury *et al*., 2011).**

Street foods are ready-to-eat foods and beverages prepared and sold by vendors and hawkers especially in streets and other similar public places, **(FAO, 1989).** Also its known to be popular due to their accessibility, low cost, variety and nutritional value, however sometimes they are considered unsafe due to unacceptable handling practices of food servers**, (WHO, 2011).**

Besides offering business opportunities for developing entrepreneurs, the sale of street foods can make a sizeable contribution to the economies of developing countries**, (FAO, 1997).**

Street foods are perceived to be a major public health risk due to lack of basic infrastructure and services, difficulty in controlling the large numbers of street food vending operations because of their diversity, mobility and temporary nature**, (DeSousa, 2008**). Whereas, in developing countries traditional methods of

water, sewage system and interferences with the city processing and packaging, improper holding plan through congestion and littering adversely affecting temperature, poor personal hygiene of food handlers are daily life still observed during food marketing and technology FAO and several authors stipulated that street-vended, **(Barro*et al*., 2002)**.

Street foods have become one of the most common risks associated with the increase in outbreaks of food-borne diseases in developing countries in recent years. There have been several documented cases of food poisoning outbreaks associated to street foods. Street foods were responsible for 691 food poisoning outbreaks and 49 deaths from 1983 to 1992 in Shandong Province (China), **(Lianghui, 1993).** In 1988, 14 deaths were reported in Malaysia because of food-borne diseases related to street foods, **(Bryan, 1988).** In the same year 300 people became ill in Hong Kong after consumption of street vended foods, **(Bhat and waghray, 2000).** In 1981 a cholera epidemic in Pune, India was linked to consumption of street vended juice**.** An outbreak of cholera in Singapore in 1987 was attributed to the consumption of street foods, **(FAO, 1990).**

However, microbial contamination of ready-to-eat foods sold by street vendors and hawkers has become a major health problem. Street food vendors are mostly uninformed of good hygiene practices (GHP) and causes of diarrheal diseases**,** which can increase the risk of street food contamination, **(Bhaskar *et al.* 2004; Tambekar *et al.* 2009).** The vendors can be carriers of pathogens like *E. coli*, *Salmonella, Shigella*, Campylobacter and *S. aureus* who eventually transfer these food borne hazards to consumers.

According to **Rane (2011)**, the poor knowledge and improper food handling of street vendors in basic food safety measures and poor knowledge and awareness among consumers on the potential hazards associated with certain foods could

explain the health and safety issues that street foods may pose. Moreover, it is important to state that the costs of food-borne illness include the cost of medical treatment, productivity loss, pain and suffering of affected individuals, industry losses, and losses within the public health sector.

2.2 Definition of meat:

Meat is defined as the flesh of animals used as food. In practice, this definition is restricted to a few dozen of the 3000 mammalian species; but it is often widened to include, as well as musculature, organs such as liver and kidney, brains and other edible tissues, **(Lawrei and Ledward, 2006)**. In addition, Meat means the whole or part of a carcass of any buffalo, camel, cattle, deer, goat, hare, pig, poultry, rabbit or sheep that is slaughtered. Meat flesh is defined as skeletal muscle to distinguish it from other parts of a carcass of meat such as offal, bone and bone marrow. Meat flesh includes any attached fat, connective tissue rind, nerves, blood vessels and blood, and skin, **(AFSC, 2001).**Meat is a foodstuff that can be spoiled extremely quickly. Certain species of bacteria multiply on fresh meat thanks to its chemical composition, favorable water activity and pH. Their numbers soon reach levels that cause sensory deviations and lead finally to spoilage of the meat, **(Kamenik, 2013).**

2.2.1 Nutritional value of meat:

Components involved in the composition of meat are water, protein, fat, sugars, minerals, vitamins and enzymes. According to Kauffman (2001) composition is defined as the aggregate of ingredients, their arrangement, and the integrated interrelationship that forms a unified, harmonious whole. The composition of meat can be approximated to 75% of water, 19.9% of protein, 3.5% of soluble, non-protein, substances and 2.5% of fat, but an understanding of the

nature and behavior of meat, and of its variability, cannot be based on such a simplification. On contrary, it must be recognized that meat is the post-mortem aspect of a complicated biological tissue, and that the latter reflects the special features which the function of contraction requires, both in the general sense and in the relation to the type of action which each muscle has been elaborated to perform in the body. The essential unit of muscular tissue is fiber which consists of formed protein elements, the myofibrils, between which is a solution, the sarcoplasm, and a fine network of tubules, the sarcoplasmic reticulum, the fiber being bounded by a very thin membrane (the sarcolemma) to which connective tissue is attached on the outside. The spatial distribution, between these structural elements, of the 19% of protein in the muscle. Meat is an excellent source of many nutrients, especially proteins, B vitamins, iron and zinc. As a nutrient dense food, meat provides major nutritive contributions to your diet related to the amount of calories it contains. In addition, meat is a major dietary source of thiamin, riboflavin, niacin, vitamin B6 and vitamin B12**, (Lawrie and Ledward, 2006)**.

2.3 Health and hygiene:

What is good health? Different people may consider good health differently. Butto define it formally, health is a state of complete physical, mental and social well-being. We take health as being free from diseases but it is much more than just the absence of a disease. Good health may enable us to do well at work and in life. Good health involves proper functioning of all body organs. It also involves feeling well both in body and in mind. People enjoying good health are cheerful, free from stress, and enjoy life to the fullest. Only if you are in good health you can be of help to others and the community. The word hygiene comes from a Greek word hygiene that means ‘Goddess for health’ and deals with personal and

community health. Thus, health and hygiene go hand in hand or they are interrelated, **(WHO, 2009).**

Hygiene is a set of practices performed to preserve health. According to the World Health Organization (WHO), "Hygiene refers to conditions and practices that help to maintain health and prevent the spread of diseases. Personal hygiene refers to maintaining the body's cleanliness, **(WHO, 2011).**

2.3.1Types of hygiene:

A. Personal hygiene

The aim of personal hygiene is to promote standards of personal cleanliness, within the setting of the condition where people live. Personal hygiene includes bathing, clothing, washing hands and toileting, care of nails, feet, teeth, spitting, coughing, sneezing, personal appearance, and inculcation of clean habits when young.

Hand washing (hand care)**,** The cleanliness of our hands is very important in all our daily activities. In our normal activities our hands frequently get dirty. There are many situations in which microorganisms are likely to attach to our hands along with the dirt. There are many communicable diseases that follow the route of faeco-oral transmission. Hand hygiene plays a critically important role in preventing this transmission, **(WHO, 2011).**

B. Environmental hygiene

a) Domestic hygiene: domestic hygiene comprises of home, use of soap, need of fresh air, light, ventilation, hygiene in storage of food, disposal of waste, avoidance of household pests, rats, mice, insects.

b) Community Hygiene: It includes safe disposal of human excreta, control of vectors responsible for transmission of diseases, control of air and water pollution, **(WHO, 2011).**

2.4 Definition of shawarma:

Shawarma is a traditional Arab food that represents meat like lamb, chicken, turkey, beef, veal, or mixed meats grilled on a spit and cut off into small pieces for serving in sandwich with some vegetables and mayonnaise mostly, **(Mattar, 2004)**. Different terms can be used to describe ready- to- eat foods, these include convenient, ready, instant and fast foods. An example of such ready to eat food includes; pastries, meat pie, sausage rolls, burger, doughnut, Shawarma, salads or coleslaw, milk and milk products, **(Tsang, 2002).**

2.4.1 Shawarma sandwich components:

1/Meat:

It is a group of muscles, connective tissues and fats, which are taken from the carcasses of animals, either red meat (livestock) or white meat (poultry). Preparation of shawarma is by cutting meat (beef or chicken) into suitable pieces, add some spices and leave it for a period of time at a temperature between 1-4 ° C.

2/Vegetables:

Vegetables are parts of plants that are consumed by humans as food as part of a meal e.g. (cucumber, tomato, lettuce …etc).

3/Sauces:

A liquid or semi-liquid substance served with food to add moistness and flavor e.g. (tomato sauce, garlic sauce, Mayonnaise sauce and tahina sauce).

4/Bread:

It is a kind of food made of flour or meal that has been mixed with milk or water, made into a dough or batter, with or without yeast or other leavening agent, and baked.

2.4.2 Contamination of shawarma:

Consumption of contaminated ready to eat foods including red meat, eggs, cheese & vegetables has been documented to serve as vehicles for transmission of several bacterial pathogens and food-borne outbreaks **(Borch and Arinder, 2002).**

Hot foods have been the source of outbreaks of *staphylococcus aureus, clostridium perfringens* and *salmonella enteritidis,* **(Hatakka.1998).** The main sources of pathogenic bacteria in food are contaminated raw food, food handlers, dust, water, utensils and insects, **(Ray, 1996).**Ready to eat food has been implicated in cases of food poisoning or gastroenteritis in human beings, **(Eley, 1996).**Shawarma is a type of grilled meat loafs characterized by its palatability and accepted low price. In Middle East, Shawarma sandwiches are the most popular ready to eat foods, which are prepared. From chicken meat, vegetables, spices and bread and sold in fast food restaurants. The ready to eat foods must be examined at regular intervals in order to assess their microbiological quality as the microbial quality of ready to eat food reflects its sanitary condition during its production and distribution. **(Hubbert *et al.*, 1996).** Furthermore, the prevalence of *Campylobacter spp., Staphylococcus spp., Escherichia coli, Salmonella spp.,*

Yersinia Spp. and *Listeria* on meat, sea foods, vegetable ingredients, chicken shawarma, raw and cooked foods, raw chicken, beef burger sandwiches, ready-to eat salad vegetables, commercial mayonnaise, frozen chicken, poultry products and on the hands of food workers who reported by **Kaneko *et al*., 1999 and Pelczar *et al*., 2006.**

These microorganisms are carried on hands, wiping cloths and utensils, especially chopping boards. The slightest contact can transfer them to food and cause food borne disease. Examples of zoonotic pathogens that may be transmitted in this way include *Salmonella*, *Campylobacter*, *Escherichia coli* and eggs of the tape worm, **(Meng and Doyle,1998).**

Microorganisms in fast and traditional fast foods are responsible for many human diseases. e.g. Salmonella bacteria is a common cause of food borne illness, particularly in undercooked chicken and chicken eggs **(Angelillo *et al*., 2000).**In recent years, just about all the quick service restaurants have added salads fresh vegetables (Lettuce, Cabbage, Carrot, Cucumber, Onion), Ketchup and Mayonnaise). Some foods will be cooked prior to consumption others will be eaten raw. Products that might be classed with both fresh and processed vegetables are the chopped salad ingredients sold in the grocery store and to the institutional trade. Although essentially fresh produce, contamination during processing, and changes in microbial growth patterns during storage, may later to micro flora of these foods quantitatively and qualitatively. The inner tissues of healthy plants and animals are free of microorganisms. However, the surfaces of raw vegetables and meats are contaminated with a variety of microorganisms and this depends on the condition of the raw product, the method of handling, the time and conditions of storage**, (Pelczar *et al.*, 2006).**

2.5 Pathogenic bacteria

2.5.1 *Salmonella*:

Salmonella is one of the most important pathogenic genera implicated in food borne bacterial outbreaks that include nausea, vomiting, septicemia and diarrhea, each year millions of cases occur, most of these infections cause mild illness, severe infections and serious complications-including death, **(Fratamico *et al.*, 2005).** Salmonellosis continues to be a major public health problem worldwide. It also contributes to negative economic impacts due to the cost of surveillance investigation, treatment and prevention of illness. There are 16 million annual cases of typhoid fever, 1.3 billion cases of gastroenteritis and 3 million deaths worldwide due to *Salmonella,* **(Bhunia, 2008).**In brief, *Salmonella* is facultative anaerobe, gram negative flagellated rod-shaped bacterium which is about 2-3 x 0.4-0.6 μm in size, **(Montville and Matthews, 2008).**They are sensitive to heat and often killed at temperature of 70°C or above. Salmonellae grow in a pH range of 4 to 9 with the optimum between 6.5 and 7.5. They require high water activity (aw) between 0.94 and 0.99 (pure water aw=1.0) yet can survive at aw <0.2 such as in dried foods. Complete inhibition of growth occurs at temperatures <7°C, pH <3.8 or water activity <0.94, **(Bhunia, 2008).***Salmonella* has changed through the years and presently is comprised of only two species (*enteric*and *bongori*).*S. enteric* consists of six subspecies and each one contains multiple serotypes. Some *Salmonella* serotypes, *Dublin* and *typhimurium* affect cattle and some, cholerasuis and *typhimurium* affect pigs and others, *pullorum* and *gallinarum* affect poultry. *Salmonella enteric* subsp. *Enterica* serotype *typhi* and *paratyphi* A or B are human specific and can cause typhoid fever. The genus *Salmonella* is composed of more than 2300 serotypes. The main antigens used to distinguish between its serotypes are the somatic (O), flagellar (H), and capsular (K). *Salmonella* spp. has a wide

occurrence in the natural environment. Intense husbandry practices in the meat, poultry, and fish and shellfish industry, along with the recycling of offal into animal feed have favored the presence of this pathogen in the global food chain. This pathogen is a part of the microflora of many animals like chicken, cattle and reptiles. The predominance of *Salmonella* spp. in the poultry and egg industry has overshadowed its importance in meat, such as pork, beef and mutton, **(Downes and Ito, 2001).**There have been some reports on the incidence of *Salmonella* in food, in Spain **(Capita *et al.*, 2003)**, in Northern Ireland **(Madden *et al.* 2001)**, in England **(Jorgensen *et al.*, 2002)**, in USA **(Cason *et al.*, 1997)**, in Nigeria **(Adetunji and Isola ., 2011)**, in Turkey the prevalence of *Salmonella* in meat has been determined by (Aydin *et al*, 2006, Cetinkaya *et al*., 2008, Goncagul *et al.*, 2005), but their incidence in Syria has not been investigated.

2.5.2 *Staphylococcus*:

Staphylococci are spherical gram-positive cocci arranged primarily in form of irregular clusters. They are present mostly in the upper respiratory tract and on the other epithelial surfaces of warm-blooded animals. The genus Staphylococci are mainly contains 20 species amongst *S. aureus* is considered as a common pyogenic agent in humans and several animal species, and constitutes a primary cause of mastitis in dairy cattle **(Virgin *et al.*,, 2009).***S. aureus* is one of the most important amongst *Staphylococci* species. The species is found primarily on human skin, mucous membranes and can also be found in other areas of human contact including soil, water, and food products. The species is capable of causing a wide variety of diseases, including septicemia, sepsis, wound sepsis, septic arthritis, osteomyelitis, food poisoning, and toxic shock syndrome **(Boyd and Brussow, 2002).***Staphylococcus aureus* could cause food poisoning and if it grows in large numbers can leave toxins in the product, which may survive heating. It lives on the skins of humans and animals and can easily be transferred to food products

(Mousa *et al.*, 2014). *Staphylococcus aureus* produces five distinct enterotoxins (type A through E) which are single polypeptide proteins with a molecular size from 22 to 28 kDa. The growth of 50 *Staphylococcus aureus* in foods may lead to the production of sufficient Enterotoxins which may cause illness when these contaminated foods are consumed. These toxins cause disease even in the absence of the organism. In the majority of cases SFP is associated with food being contaminated by the food handler who might have a minor *Staphylococcus aureus* in faction such as a boil or cut. The contaminated food must be permitted to sit at an adequate temperature that will allow the Staphylococci to multiply and produce the toxin. Reheating the food before eating may kill the organism but does not eliminate the heat stable toxin. Some of the foods commonly linked with SFP are meat (beef, pork and poultry), meat products (sausages, hotdogs, ham), salads ham, chicken, potato), cream filled baked products and dairy products. **(Downes and Ito, 2001)**

2.5.3 *Escherichia coli*:

Escherichia coli are groups of bacteria that indicate the possible presence of organisms of concern, and may point to the origins of microbial contamination **(CSIRO, 2002).***E. coli* is a normal inhabitant of the intestinal tract of humans and warm-blooded animals. Its presence in raw foods is considered an indication of direct or indirect fecal contamination. Thus, it is used as an indicator organism for possible presence of enteric pathogens in food and water (**Cohen et al., 2007**). *Escherichia coli* are straight rod measuring 1.1 to 1.5 μm by 2.0 to 6.0 μm which occur singly or in pairs and has an optimum growth temperature of 37 ^{o}C. Capsules or microcapsules occur in many strains and some strains are motile by peritrichous flagella. *Escherichia coli* are part of the normal flora of the intestinal tract of humans and various animals. It can be classified as an overt or an opportunistic pathogen and usually constitutes about 1% of the total biomass of feces. Most

Escherichia coli do not cause gastrointestinal illnesses, but some can cause life threatening diarrhea and chronic sequelae or disability. *E. coli* is serologically classified on the basis of three major surface antigens: O (somatic), H (flagella) and K (capsule). The serogroup of the strain is identified by the O antigen and its combination with the H antigen identifies the serotype. There are more than 170 different serogroups of *E. coli* identified. Diarrhea causing *E. coli* isolates are classified into specific groups based on virulence properties, pathogenicity mechanisms, clinical syndromes, and specific O:H serotypes. These groups include enterotoxogenic *E.coli* (ETEC), enteroaggregative *E.coli* (EAEC), enteropathogenic *E.coli* (EPEC), enterohemorrhagic *E.coli* (EHEC), enteroinvasive *E.coli* (EIEC), and diffuse-adhering *E.coli* (DAEC). **(Downes and Ito, 2001).**

No recently described enteric pathogen has received as much scientific and medical examination as *Escherichia coli* O157:H7. There are many serotypes belonging to the EHEC group, but serotype O157:H7 is the predominant food pathogen. It was first recognized as a pathogen in 1982 when it was identified as the causative agent of two outbreaks involving the consumption of undercooked ground beef leading to hemorrhagic colitis. It is estimated that more than 700 individuals were affected and 4 deaths occurred between 1992 and 1993 due to this pathogen. According to CDC estimates there are more than 20,000 cases and 250 deaths annually in the U.S. due to *Escherichia coli* O157:H7 infections. Cattle are considered to be the main reservoirs of this pathogen and many outbreaks are associated with the consumption of undercooked ground beef and unpasteurized milk, **(Downes and Ito, 2001).**

2.5.4 Moulds and yeasts:

a) Yeasts

Yeasts are a subset of a large group of organisms called fungi that also includes moulds and mushrooms. They are generally single-celled organisms that

are adapted for life in specialized, usually liquid, environments and, unlike some molds and mushrooms, do not produce toxic secondary metabolites. Yeasts can grow with or without oxygen (Facultative) and are well known for their beneficial fermentations that produce bread and alcoholic drinks. They often colonize foods with a high sugar or salt content and contribute to spoilage of maple syrup, pickles, and sauerkraut. Fruits and juices with a low pH are another target, and there are some yeasts that grow on the surfaces of meat and cheese. There are four main groups of spoilage yeasts: Zygosaccharomyces and related genera tolerate high sugar and high salt concentrations and are the usual spoilage organisms in foods such as honey, dried fruit, jams and soy sauce. They usually grow slowly, producing off-odours and flavours and carbon dioxide that may cause food containers to swell and burst. *Debaryomyces hansenii* can grow at salt concentrations as high as 24%, accounting for its frequent isolation from salt brines used for cured meats, cheeses, and olives. This group also includes the most important spoilage organisms in salad dressings, **(Mandrell *et al*., 2006)**.

Saccharomyces spp. are best known for their role in production of bread and wine but some strains also spoil wines and other alcoholic beverages by producing gassiness, turbidity and off flavours associated with hydrogen sulfide and acetic acid. Some species grow on fruits, including yogurt containing fruit, and some are resistant to heat processing, **(Martinez *et al*., 2004)**.Candida and related genera are a heterogeneous group of yeasts, some of which also cause human infections. Theyare involved in spoilage of fruits, some vegetables and dairy products, **(G.D. Casey and A.D.W. Dobson., 2003)**. Dekkera/Brettanomyces are principally involved in spoilage of fermented foods, including alcoholic beverages and some dairy products. They can produce volatile phenolic compounds responsible for off-flavors, **(Couto *et al*., 2005).**

b) Moulds

Moulds are filamentous fungi that do not produce large fruiting bodies like mushrooms. Molds are very important for recycling dead plant and animal remains in nature but also attack a wide variety of foods and other materials useful to humans. They are well adapted for growth on and through solid substrates, generally produce airborne spores, and require oxygen for their metabolic processes. Most molds grow at a pH range of 3 to 8 and some can grow at very low water activity levels (0.7–0.8) on dried foods. Spores can tolerate harsh environmental conditions but most are sensitive to heat treatment. An exception is By ssochlammys, whose spores have a D value of 1–12 minutes at 90ºC. Different mold species have different optimal growth temperatures, with some able to grow in refrigerators. They have a diverse secondary metabolism producing a number of toxic and carcinogenic mycotoxins. Some spoilage molds are toxigenic while others are not, **(Pitt and Hocking, 1977).**

Spoilage moulds can be categorized into four main groups: Zygomycetes are considered relatively primitive fungi but are widespread in nature, growing rapidly on simple carbon sources in soil and plant debris, and their spores are commonly present in indoor air. Generally they require high water activities for growth and are notorious for causing rots in a variety of stored fruits and vegetables, including strawberries and sweet potatoes. Some common bread molds also are zygomycetes. Some zygomycetes are also utilized for production of fermented soy products, enzymes, and organic chemicals. The most common spoilage species are Mucor and Rhizopus. Zygomycetes are not known for producing mycotoxins but there are some reports of toxic compounds produced by a few species, **(Mandrell. *et al*., 2006)**.

Penicillium and related genera are present in soils and plant debris from both tropical and Antarctic conditions but tend to dominate spoilage in temperate

regions. They are distinguished by their reproductive structures that produce chains of conidia. Although they can be useful to humans in producing antibiotics and blue cheese, many species are important spoilage organisms, and some produce potent mycotoxins (patulin, ochratoxin, citreoviridin, penitrem). Penicillium spp. cause visible rots on citrus, pear, and apple fruits and cause enormous losses in these crops. They also spoil other fruits and vegetables, including cereals. Some species can attack refrigerated and processed food ssuch as jams and margarine. A related genus, Byssochlamys, is the most important organism causing spoilage of pasteurized juices because of the high heat resistance of its spores, **(Mandrell *et al*., 2006)**.

Aspergillus and related molds generally grow faster and are more resistant to high temperatures and low water activity than Penicillium spp. and tend to dominate spoilage in warmer climates. Many aspergilla produce mycotoxins: aflatoxins, ochratoxin, territrems, cyclopiazonic acid. Aspergilli spoil a wide variety of food and nonfood items (paper, leather, etc.) but are probably best known for spoilage of grains, dried beans, peanuts, tree nuts, and some spices, **(Couto, *et al*., 2005).**

Other molds, belonging to several genera, have been isolated from spoiled food. These generally are not major causes of spoilage but can be a problem for some foods. Fusarium spp. cause plant diseases and produce several important mycotoxins but are not important spoilage organisms. However, their mycotoxins may be present in harvested grains and pose a health risk, **(Couto, *et al*., 2005).**

CHAPTER THREE

MATERIALS and METHODS

3.1 Materials:

3.1.1 Shawarma sandwich samples:

The major materials used for the analysis were 27 samples of instant prepared ready to eat shawarma samples (chicken shawarma) which were purchased at 3 different locations (Omdurman, Khartoum and Khartoum North) and from 3 types of restaurants closed (shawarma preparation done in isolated area), semi-closed (shawarma preparation done in one side open area) and open (shawarma preparation done in street without boundaries). Then the samples was transferred in ice containing container and immediately handled to the laboratory for analysis under strict hygienic measures.

3.1.2 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 Diluents:

- Peptone solution 0.1%

3.2 Preparation of shawarma methods:

3.2.1 Sterilization:

3.2.1.1Sterilization of glassware:

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

3.2.1.2 Sterilization of media:

Culture media were first adjusted to the required pH and then sterilized.

Sterilization was achieved by autoclaving at 121 ºC for 15 minutes under pressure 15 lb/in^2.

3.2.2 Preparation of serial dilutions:

Aseptically 10 grams of the sample were homogenized by mixer for 1.5 min in 90 ml of sterile diluent (0.1% Peptone water). 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 1ml of sterile diluent (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, and then 15 ml of sterile melted

Plate Count Agar medium were added to each plate. The inculum was mixed with medium and allowed to solidify.

The plates were incubated at 37 ºC 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, **(Harrigan, 1998).**

3.2.3Total 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 Tests

3.2.4.1 Determination of coliform bacteria:

It was carried out by using the Most Probable Number (MPN) technique, **(Harrigan, 1998).**

3.2.4.2 Presumptive coliform test:

1 ml of each of the three first dilutions ($10^{-1,}$ $10^{-2,}$ $10^{-3)}$ was inoculated in triplicates of MacConkey Broth test tubes containing Durham tubes. The tubes were incubated at 37 ºC 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, **(Harrigan, 1998).**

3.2.4.3 Confirmed test for total coliform:

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, **(Harrigan, 1998).**

3.2.4.4 Confirmed *E. coli* test:

Medium used was EC Broth. From every tube showing positive result in the presumptive test inoculate a tube of EC Broth containing Durham tube were inoculated at 44.5ºC for 24 hours. Tubes showing any amount of gas were considered positive, and then the Most Probable Number (MPN) was recorded. For further confirmation of E. coli tubes of EC Broth showing positive results at 44.5ºC for 24 hours were streaked on Eosin Methylene Blue Agar (EMB) plates. The plates were incubated at 37ºC for 48 hours. Colonies of *E.coli* are usually small with metallic green sheen on EMB Agar, **(Harrigan, 1998).**

3.2.4.5 *Staphylococcus aureas* enumeration:

Medium used was Baird-Parker Agar; 0.1 ml from every dilution was transferred onto the surface of each well dried Baird-Parker Agar medium plates. The inoculum was spreaded all over the plate using sterile bent glass rod. The plates were incubated at 37º C for 24 hours, after that period of incubation the plates were examined. *Staphylococcus aureus* appear black shiny convex and surrounded by a zone clearing 2-5 mm in width of colony after 24 hours of incubation, **(Harrigan, 1998).**

3.2.4.6 Yeasts and moulds:

From suitable dilutions of sample 0.1 ml was aseptically transferred onto solidified Potato-Dextrose Agar containing 0.1 gram chloramphenicol per one litre of medium to inhibit bacterial growth. The sample was spread all over the plates using sterile bent glass rod. Plates were incubated at 28º C for 72 hours. Colonies

were counted by using a colony counter and the result were presented as CFU/gram. **(Harrigan, 1998).**

3.2.4.7 Detection of *Salmonella*:

Ten gram of the sample were added to a conical flask containing 100 ml of sterile Nutrient Broth and incubated at 37ºC for 24 hours. A loopful of 24 hours incubated nutrient broth was transferred to aseptically into sterilized selenite cystine Broth and incubated at 37ºC for 24 hours. A loopful of 24 hours inoculum of selenite cysteine Broth was streak on Bismuth Sulphite Agar surface and incubated at 37ºC for 24 – 72 hours. Black metallic sheen discrete colonies indicated the presence of *Salmonella*, **(Harrigan, 1998).**

3.3 Study questionnaire:

Information was collected to estimate the awareness of the different restaurants workers about the hygienic practices which should be followed and to assess their workers personal or hygiene cleanness, hygienic practices during preparation of shawarma and history of food poisoning cases. (Study questionnaire attached).

3.4 Statistical analysis:

Statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) Ver.23. Analysis of variance (One way ANOVA test and Fisher exact test) was performed to examine the significant effect of parameters measured. Least significant difference (LSD) was used to separate the means.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Microbial characteristics of shawarma samples:

4.1.1 Total viable count:

As shown in table 1, the mean total viable count of bacteria of Shawarma samples collected from different sources in Khartoum State recorded $2.1x10^4$, $3.8x10^4$ and $2.2x10^4$cfu/g for closed restaurants in Khartoum, Khartoum North and Omdurman, respectively. Results in semi-closed and open restaurants were $1.9x10^5$, $2.9x10^5$ and $5.6x10^4$ and $1.8x10^5$, $4.2x10^5$ and $7.5x10^5$cfu/g, respectively.

There is a significant difference between type of samples and total viable count p-value = 0.003 according to One-way ANOVA test. According to (LSD) least significant tests shown in (table 2), open restaurant was statistically significant different from semi-closed and closed restaurants p-vale = 0.023 and 0.001 respectively. The mean differences between closed and semi-closed restaurants were not statistically significant p-value = 0.171.

The total bacterial count is considered as index of sanitary and quality of foods **(Forsythe and Hayes, 1998).** Closed restaurants in Omdurman proved to be the best source of shawarma sandwich with mean value $2.0x10^4$ cfu/g due to proper display and good handling practices. Open restaurants in Omdurman showed high level of contamination with mean total viable count of bacteria $7.5x10^5$cfu/g due to bad hygienic practices. These results are similar with findings of **Abdalhamis *et al.*, (2013)** who found that total viable count in shawarma samples ranged between $2.8x10^4 – 8.4x10^5$cfu/g and **Nimri *et al.*, (2014)** found that the mean value of the total viable count were $5.91x10^4$ cfu/g which is not similar to the result obtained in

this study. This variation may be due to huge number of samples subjected to this study. Elfaki and Elhakim (2011) found a range of $5.5x10^3$ – $5.3\ x10^4$ cfu/g. TVC in gulf cooperation council standardization organization should not be over than $10x10^4$cfu/g, U.S. Food and drug administration (FDA) tvc must not over than $5x10^4$cfu/g in food ready to eat.

4.1.2 Moulds and yeasts:

Table 3 shows that mean of moulds and yeasts detected in samples from closed restaurants $1.90x10^2$, $1.30x10^2$ and $1.30x10^2$ cfu/g in Khartoum , Khartoum North and Omdurman respectively. Moulds and yeasts in semi-closed restaurants were $1.3x10^3$ in Khartoum, $2.9x10^3$ in Khartoum North and $2.4x10^2$ in Omdurman. Open restaurants samples recorded $3.9x10^3$, $3.2x10^3$ and $3.4x10^3$ cfu/g in Omdurman, Khartoum and Khartoum North, respectively.

According to One-way ANOVA there is a significant difference between type of samples and moulds and yeasts p-value = 0.00.

According to least significant test (LSD) open restaurant was statistically significant different from semi-closed and closed restaurants p-vale = 0.00 and 0.000 respectively. The mean differences between closed and semi-closed restaurants were not statistically significant (p-value = 0.514) as shown in (Table 4).

Results showed high contamination with moulds and yeasts in open restaurants in Omdurman with mean value $3.9x10^3$ cfu/g closed restaurants showed the lowest contamination. High results recorded by **Afzal. (2014)** who found the fungal count was observed in dal (Dal is an Indian dish made from pulses such as chickpeas or lentils) from street food was $5.2x10^7$, $1.30x10^2$ cfu/g, Also **Sharaf and Sabra (2012)** found that the contamination of shawarma with moulds and

yeasts was $6.2x10^4$, $5.2x10^5$ cfu/g respectively, which is not similar to the result obtained in this study.

4.1.3 *Staphylococcus aureus:*

Table 5 shows mean of *Staphylococuos aureus* count of Shawarma from closed, semi-closed and open restaurants in Omdurman, Khartoum and Khartoum North. Result of closed restaurants was $3.70x10^2$, $4.80x10^2$ and $3.01x10^2$ cfu/g respectively. Semi-closed restaurants showed $2.67x10^3$ cfu/g to Omdurman, $1.70x10^3$ cfu/g to Khartoum and $3.73x10^3$ cfu/g to Khartoum North. $3.91x10^3$, $3.71x10^3$ and $5.05x10^3$ cfu/g for Open restaurants respectively.

The mean deference between type of sample and Staphylococcus aures were statistically significant p-value = 0.00 and showed contamination with Staphylococcus aures according to One-way ANOVA test.

According to (LSD) least significant test as shown in (table 6), closed restaurant was statistically significant different from semi-closed and open restaurants p-vale= 0.07 and 0.00 respectively. The mean differences between open and semi-closed restaurants were not statistically significant p-value = 0.063.

Staphylococcus aureus were found in all samples from semi-closed and open restaurants however highest contamination were found in open restaurants in Khartoum North with mean value $5.05x10^3$ cfu/g and less contamination were found in closed restaurant in Khartoum North with mean value $0.31x10^3$ cfu/g. **Abdalhamis *etal*., (2013)** reported that mean value of *Staphylococcus aures* in shawarma samples were $8.3x10^3$ cfu/g which is not similar to the result obtained in this study. **Nimri *et al*., (2014)** found that the mean value of *Staphylococcus* count were $9.42x10^3$ cfu/g these result is not similar with the result obtained in this

study. Lower result recorded by **Zaki (2003)** who found that the mean value of staphylococci count was $1.2x10^2$ cfu/g in the examined samples of cooked shawarma. According to gulf cooperation council standardization organization *Staphylococuos aureus* should not over than $1.0x10^2$cfu/g in food ready to eat.

4.1.4 Total coliform bacteria:

Table 7 shows the mean total coliform count in shawarma samples collected from closed, semi-closed and open restaurants in Omdurman, Khartoum North and Khartoum. Samples from closed restaurants recorded 10.1, 11.6 and 8.4 MPN/g respectively. Semi-closed restaurants recorded 19.2, 19.7 and 24.3 MPN/g respectively. 19.4, 22.9 and 32.4 MPN/g open restaurants from Omdurman, Khartoum North and Khartoum respectively.

According to One-way ANOVA there is a significant difference between type of samples and presence of coliform bacteria p-value = 0.000. According to least significant test (LSD) test open restaurant was statistically significant different from closed restaurants p- value = 0.000. The mean differences between closed and semi-closed restaurants were statistically significant p-value = 0.002. The mean differences between open and semi-closed restaurants were not statistically significant p-value = 0.239 as shown in (table 8).

Presence of coliform bacteria in high levels is an indicator of unsanitary condition however, open and semi-closed restaurants proved to be the bad source of shawarma sandwich. The results of this study were in disagreement with the findings of **Nimri *et al.*, (2014)** who found that the mean values of coliform count in shawarma samples were $0.35x10^3$cfu/g. However, higher findings were obtained by **Rafaie and Moustafa (1990)** who found that the mean value of coliform was $3.4x10^6$ cfu/g. A range of zero – 2.6 $x10^3$ was found by **Elfaki and Elhakim**

(2011). According to (FDA) U.S. Food and drug administration coliform bacteria should not be over than 10 MPN/g in food ready to eat, otherwise in gulf cooperation council standardization organization (GCC) coliform bacteria should not be detected.

4.1.5 *Escherichia coli*:

As shown in table 9 *E.coli* was found in all samples from all type of restaurants. The mean of *E.coli* from closed restaurants of Omdurman, Khartoum North and Khartoum was 2.44, 1.78 and 2.44 MPN/g respectively. Mean of *E.coli* in semi-closed and open restaurants 5.78, 4.78 and 6.44and 3.78, 9.33 and 8.78 MPN/g respectively.

There is a significant difference between type of samples and presence of *E.coli* bacteria p-value = 0.050 According to One-way ANOVA test.

According to least significant test (LSD) test as shown in (table 10), open restaurant was statistically significant different from closed restaurants p-value = 0.017.The mean differences between open and semi-closed restaurants were not statistically significant p-value = 0.42. The mean differences between closed and semi-closed restaurants were not statistically significant p-value = 0.096.

E.coli plays an important role as human pathogens, which give rise to gastroenteritis outbreaks. The presence of *E.coli* is indicating to bad hygiene practices. All type of restaurants showed positive results to the presence of *E.coli* and the mean value ranged between 1.78 – 9.33 MPN/g. Sharaf and Sabra (2012) found that the contamination of shawarma with *E.coli* was 3.9×10^2 cfu/g which is not similar to the result obtained in this study. Results of **Nimri *et al*., (2014)** was 6

MPN/g, which is in agreement with the findings of this study. According to FDA and GCC *E.coli* should not be detected in food ready to eat.

4.1.6 *Salmonella*:

Results of *Salmonella* detection in Shawarma samples are presented in (Table 11). Samples from closed restaurants showed negative results. Samples from semi-closed and open restaurants showed positive results.

According to Fisher exact test there was statistically significant differences between type of sample and detection of *salmonella* p-value= 0.000.

Results of the study showed absence of *Salmonella* in all closed restaurants, semi-closed and open restaurants showed positive Results. The findings of **Abdalhamis *et al.*, (2013)** showed negative results to the presence of *Salmonella* in all samples, which is in disagreement with the results obtained in this study. Also **Alyaaqoubiet *et al.*, (2009)** did not find any *Salmonella* contamination in ready-to eat foods. The presence of *Salmonella* in cooked shawarma and hamburger from chicken or meat can be explained on the basis that during cooking the outer surface and shallow layers thickness, reached temperatures that were high enough to kill pathogenic food borne bacteria, also, in shawarma the internal layers, temperatures were not high enough to kill these organisms, **(Al-Zahraa, 2018).** One sample out of three was found contaminated with pathogenic bacteria **(Elfaki and Elhakim, 2011).** According to FDA and GCC *Salmonella* should not be detected in food ready to eat.

Presence of pathogenic bacteria in high levels is an indicator to bad conditions or poor hygiene practices during or after food production.

Table (1): Mean value of total viable count of bacteria in shawarma samples

Type of Sample	Mean (cfu/g)	±SD	Maximum	Minimum
Closed area –Omdorman	**$2.0x10^4$**	**$2.2 x10^4$**	**$4.7 x10^4$**	**$5.5 x10^3$**
Closed area -Khartoum North	**$3.2 x10^4$**	**$2.6 x10^4$**	**$5.7 x10^4$**	**$5.33 x10^3$**
Closed- Khartoum	**$2.1 x10^4$**	**$2.9 x10^4$**	**$5.4 x10^4$**	**$3.6 x10^3$**
Type of Sample	**Mean (cfu/g)**	**±SD**	**Maximum**	**Minimum**
Semi-Closed area –Omdorman	**$5.6 x10^4$**	**$1.7 x10^4$**	**$7.3 x10^4$**	**$3.9 x10^4$**
Semi-Closed area -Khartoum North	**$2.9 x10^5$**	**$4.0 x10^5$**	**$7.6 x10^5$**	**$4.4 x10^4$**
Semi-Closed area – Khartoum	**$1.9 x10^5$**	**$2.0 x10^5$**	**$4.2 x10^5$**	**$6.4 x10^4$**
Type of Sample	**Mean (cfu/g)**	**±SD**	**Maximum**	**Minimum**
Open area-Omdorman	**$7.5 x10^5$**	**$1.8 x10^5$**	**$9.3 x10^5$**	**$5.6 x10^5$**
Open area -Khartoum North	**$4.2 x10^5$**	**$3.3 x10^5$**	**$7.1 x10^5$**	**$5.4 x10^4$**
Open area – Khartoum	**$1.8 x10^5$**	**$1.5 x10^5$**	**$3.5 x10^5$**	**$9.4 x10^4$**

±SD: Standard deviation

Table (2): Statistical analysis of total viable count of bacteria in shawarma samples

Type of Sample	Type of Sample	P-value	Mean Difference (I-J)
Open area	Semi-Closed	0.023[a]	268.02778*
	Closed	0.001[a]	424.14667*
Semi-Closed area	Open	0.023[a]	-268.02778*
	Closed	0.171[b]	156.11889
Closed area	Open	0.001[a]	-424.14667*
	Semi-Closed	0.171[b]	-156.11889
a: Significant value , b: Insignificant value			

Table (3): Mean value of moulds and yeasts in shawarma samples

Type of Sample	Mean (cfu/g)	±SD	Maximum	Minimum
Closed area-Omdorman	1.30×10^2	2.30×10^2	4.0×10^2	0
Closed area -Khartoum North	1.30×10^2	2.30×10^2	4.0×10^2	0
Closed- Khartoum	1.90×10^2	3.20×10^2	5.6×10^2	0
Type of Sample	**Mean (cfu/g)**	**±SD**	**Maximum**	**Minimum**
Semi-Closed area –Omdorman	2.4×10^2	2.5×10^2	5.0×10^2	0
Semi-Closed area -Khartoum North	2.94×10^3	2.23×10^3	5.03×10^3	0.6×10^3
Semi-Closed- Khartoum	1.30×10^3	1.92×10^3	3.5×10^3	0
Type of Sample	**Mean (cfu/g)**	**±SD**	**Maximum**	**Minimum**
Open area –Omdorman	3.85×10^3	2.76×10^3	5.8×10^3	7.0×10^2
Open area -Khartoum North	3.36×10^3	2.53×10^3	5.73×10^3	7.0×10^2
Open area – Khartoum	3.24×10^3	2.21×10^3	4.63×10^3	7.0×10^2

Table (4): Statistical analysis of moulds and yeasts in shawarma samples

Type of Sample	Type of Sample	P-value	Mean Difference (I-J)
Open area	Semi-Closed	0.00[a]	2.89078*
	Closed	0.00[a]	3.33556*
Semi-Closed area	Open	0.00[a]	-2.89078*
	Closed	0.514[b]	.44478
Closed area	Open	0.000[a]	-3.33556*
	Semi-Closed	0.514[b]	-.44478
a: Significant value , b: Insignificant value			

Table (5): Mean value of total *Staphylococcus aureus* in shawarma samples

Type of Sample	Mean (cfu/g)	±SD	Maximum	Minimum
Closed area-Omdorman	$3.7x10^2$	$3.5x10^2$	$7.0x10^2$	0
Closed area -Khartoum North	$3.1x10^2$	$2.8x10^2$	$5.3x10^2$	0
Closed area – Khartoum	$4.8x10^2$	$2.9x10^2$	$8.0x10^2$	$2.3x10^2$
Type of Sample	**Mean (cfu/g)**	**±SD**	**Maximum**	**Minimum**
Semi-Closed area –Omdorman	$2.67x10^3$	$1.67x10^3$	$4.0x10^3$	$8.0x10^2$
Semi-Closed area -Khartoum North	$3.73x10^3$	$2.75x10^3$	$6.06x10^3$	$7.0x10^2$
Semi-Closed area – Khartoum	$1.70x10^3$	$2.20x10^3$	$4.23x10^3$	$3.6x10^2$
Type of Sample	**Mean (cfu/g)**	**±SD**	**Maximum**	**Minimum**
Open area –Omdorman	$3.91x10^3$	$3.26x10^3$	$7.0x10^3$	$5.0x10^2$
Open area -Khartoum North	$5.05x10^3$	$1.47x10^3$	$6.43x10^3$	$3.5x10^3$
Open area – Khartoum	$3.71x10^3$	$4.5x10^2$	$4.23x10^3$	$3.43x10^3$

Table (6): Statistical analysis of *Staphylococcus aureus* in shawarma samples

Type of Sample	Type of Sample	P-value	Mean Difference (I-J)
Open area	Semi-Closed	0.063[b]	1.52556
	Closed	0.000[a]	3.83822*
Semi-Closed area	Open	0.063[b]	-1.52556
	Closed	0.007[a]	2.31267*
Closed area	Open	0.000[a]	-3.83822*
	Semi-Closed	0.007[a]	-2.31267*
a: Significant value , b: Insignificant value			

Table (7): Mean value of total coliform bacteria in shawarma samples

Type of Sample	Mean (MPN/g)	±SD	Maximum	Minimum
Closed area –Omdorman	10.1	9.3	18.3	0
Closed area -Khartoum North	11.6	10.4	20	0
Closed area – Khartoum	8.4	7.6	14.7	0
Type of Sample	**Mean (MPN/g)**	**±SD**	**Maximum**	**Minimum**
Semi-Closed area –Omdorman	19.20	3.1	22.6	16.7
Semi-Closed area -Khartoum North	19.7	2.7	21.7	16.7
Semi-Closed area – Khartoum	24.3	3.9	27.7	20
Type of Sample	**Mean (MPN/g)**	**±SD**	**Maximum**	**Minimum**
Open area –Omdorman	19.4	6.6	27	14.7
Open area -Khartoum North	22.8	4.9	28	18.3
Open area – Khartoum	32.4	4.7	35.3	27

Table (8): Statistical analysis of total coliform bacteria in shawarma samples

Type of Sample	Type of Sample	p-value	Mean Difference (I-J)
Open area	Semi-Closed	0.239[b]	3.82444
	Closed	0.000[a]	14.85556*
Semi-Closed area	Open	0.239[b]	-3.82444
	Closed	0.002[a]	11.03111*
Closed area	Open	0.000[a]	-14.85556*
	Semi-Closed	0.002[a]	-11.03111*
a: Significant value , b: Insignificant value			

Table (9): Mean value of *E.coli* in shawarma samples

Type of Sample	Mean (MPN/g)	±SD	Maximum	Minimum
Closed area –Omdorman	2.44	4.23	7.33	0
Closed area -Khartoum North	1.78	3.08	5.33	0
Closed area – Khartoum	2.44	4.23	7.33	0
Type of Sample	Mean (MPN/g)	±SD	Maximum	Minimum
Semi-Closed area –Omdorman	5.78	6.01	12	0
Semi-Closed area -Khartoum North	4.78	4.53	9	0
Semi-Closed area – Khartoum	6.44	6.05	12	0
Type of Sample	Mean (MPN/g)	±SD	Maximum	Minimum
Open area –Omdorman	3.78	3.67	7.33	0
Open area -Khartoum North	9.33	4.37	14	5.33
Open area – Khartoum	8.78	3.34	12	5.33

Table (10): Statistical analysis of *E.coli* in shawarma samples

Type of Sample	Type of Sample	p-value	Mean Difference (I-J)
Open area	Semi-Closed	0.420[b]	1.62889
	Closed	0.017[a]	5.07333*
Semi-Closed area	Open	0.420[b]	-1.62889
	Closed	0.096[b]	3.44444
Closed area	Open	0.017[a]	-5.07333*
	Semi-Closed	0.096[b]	-3.44444
a: Significant value , b: Insignificant value			

Table (11): Detection of *Salmonella in* shawarma samples

Type of Sample	Mean
Closed area –Omdorman	Negative
Closed area -Khartoum North	Negative
Closed area – Khartoum	Negative
	Mean
Semi-Closed area –Omdorman	Positive
Semi-Closed area -Khartoum North	Positive
Semi-Closed area – Khartoum	Positive
	Mean
Open area –Omdorman	Positive
Open area -Khartoum North	Positive
Open area – Khartoum	Positive

4.2 Questionnaire results:

This section describes the analyses performed on the data and presents the result of the study. Sociodemographic characteristics of the studied participants will be presented, followed by hygienic knowledge of the participant.

4.2.1 Sociodemographic characteristics of the study participants:

Of the twenty-seven participants, 37% were with secondary education level; their mean age was 33.52 years (± 8.77) range from 21 years to 50 years. The mean duration of employment was 10.1 years (± 9.83) as shown in Figure 1and table 12.

4.2.2Knowledge of hygienic practices:

4.2.2.1 Personal hygiene:

1- Cleaning hands:

About 55.6% of the participants clean their hands before they work, while 40.7% of participants sometimes clean hands and 3.7% do not clean their hands before working (Figure 2).

1- Wearing gloves :

Figure 3, 4 show most participants did not wear gloves while working 59.3%.

2- Wearing accessories :

About 40.7% of participants were wearing accessories while working, 33.3% were not wearing it and 25.9% were sometimes wearing it (Figure 5).

3- Cover head :

Most of participants did not use head cover while working (66.7%), the rest wore head covers (Figure 6).

4.2.2.2 Hygienic practices:

1- Cleaning tools :

About 59.3% of participant-cleaned tools before and after work, while 25.9% of participants sometimes cleaned tools and 14.8% did not clean their tools before or after working (Figure 7).

2- Defrost chicken and meat:

The highest percentage of participant defrosts chicken or meat in water 55.6% as shown in (Figure 8).

3- Closing heat source :

Figure 9, 10 shows 66.7% keep heat source on, 33.3% of participants closed heat source, and participants claimed that the main reason for closing the heat source is the fear of burning the shawarma 66.67%.

4- injury:

Most of participants responded that they were sometimes keep working despite they were injured (85.2%), while 11.1% of the participants stop working if they had injury and 3.7% of the participants responded that they complete their work (Figure 11).

5- Thermostat :

About 63% of restaurants did not have thermostat in their fridges as shown in (Figure 12).

6- Temperature of storing :

Figure 13 shows most of participants didn't know the ideal storage temperature 51.85%.

7- Storing additives :

Most of participants stored shawarma additives in room temperature 74.1% as shown in (Figure 14).

4.2.3 Knowledge of hygiene:

1- Reuse yesterday Shawarma:

About 63% did not use yesterday shawarma (Figure 15).

2- Knowledge of food poisoning :

Most of participant had no idea about food poisoning 56% and 59.3% were not reading about food poisoning as shown in (Figure 16, 17).

3- Knowledge of Spoiled food:

Figure 18 shows 74.1% of participants agreed that the texture is the main sign of food spoilage.

4- Cases of poisoning:

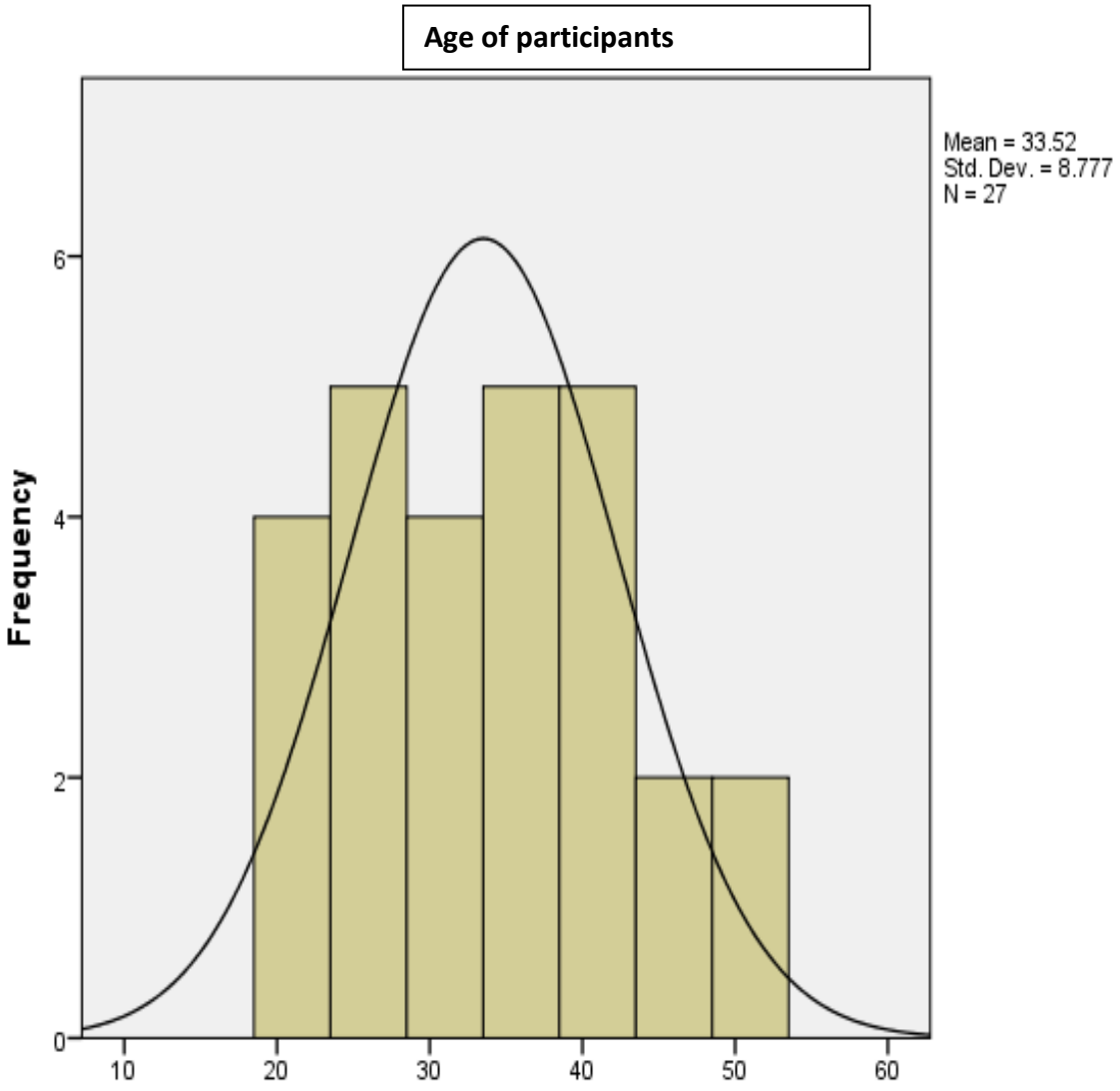
Most participants claimed that they never encountered any food poisoning case 92.6% as shown in (Figure 19).

The effect of microorganisms on human health has been reported. Food hygiene means all conditions and measures necessary to ensure the safety of the food chain. The food hygiene procedures and practices in different food establishments should be improved in order to reduce food borne illness related to poor hygiene practices. This study aimed to evaluate the total viable count of bacteria and to detect pathogenic bacteria in different hygiene conditions. Samples were collected from three types of restaurants (closed, semi-closed and open) and in three different cities (Omdurman, Khartoum North and Khartoum).

Most of participants do not clean their hands, wear gloves while working or use head cover and they ware accessories while working.

High percentage of participants cleaned their tools before and after work and most of them kept the heat source on shawarma, which indicates good hygienic practices. However, most of participants defrosted meat or chicken in water, Stored sandwich additives in room temperature, did not have thermostat in fridge, had poor knowledge of the proper storing temperature and kept working if injured, all these factors indicate poor knowledge about hygienic practices, sanitary condition, good handling practices and lack of training.

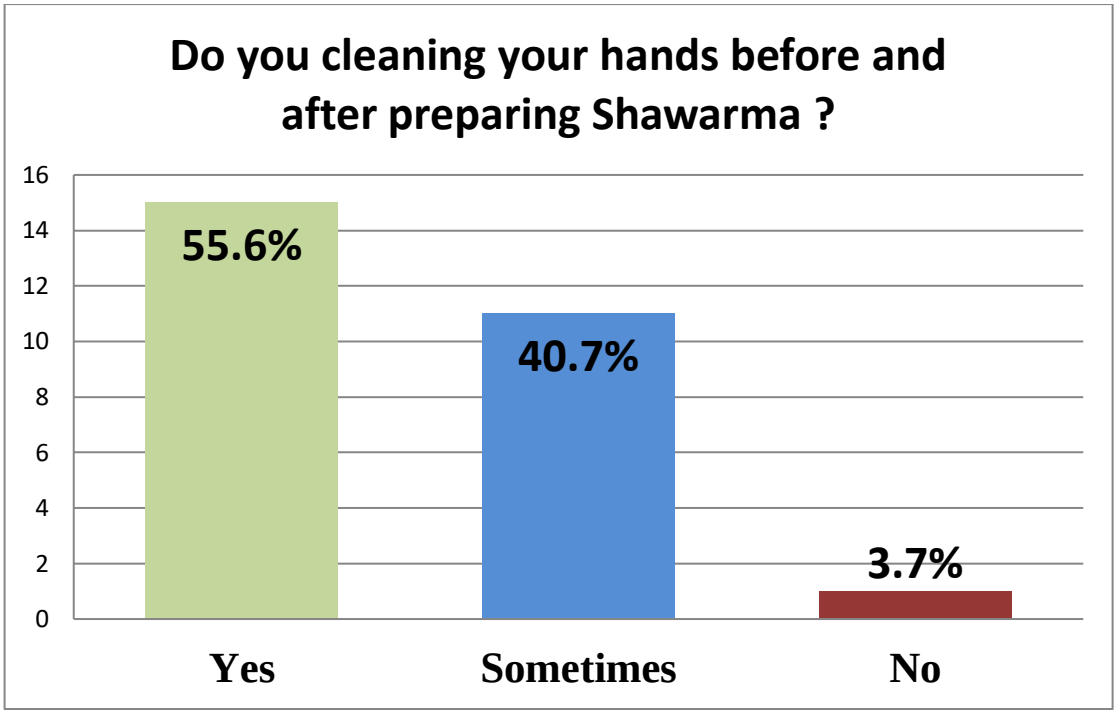
Despite of the poor hygienic knowledge most participants agreed to not reuse old shawarma and they responded that the texture was the main sign of food spoilage and there were no food poisoning cases.



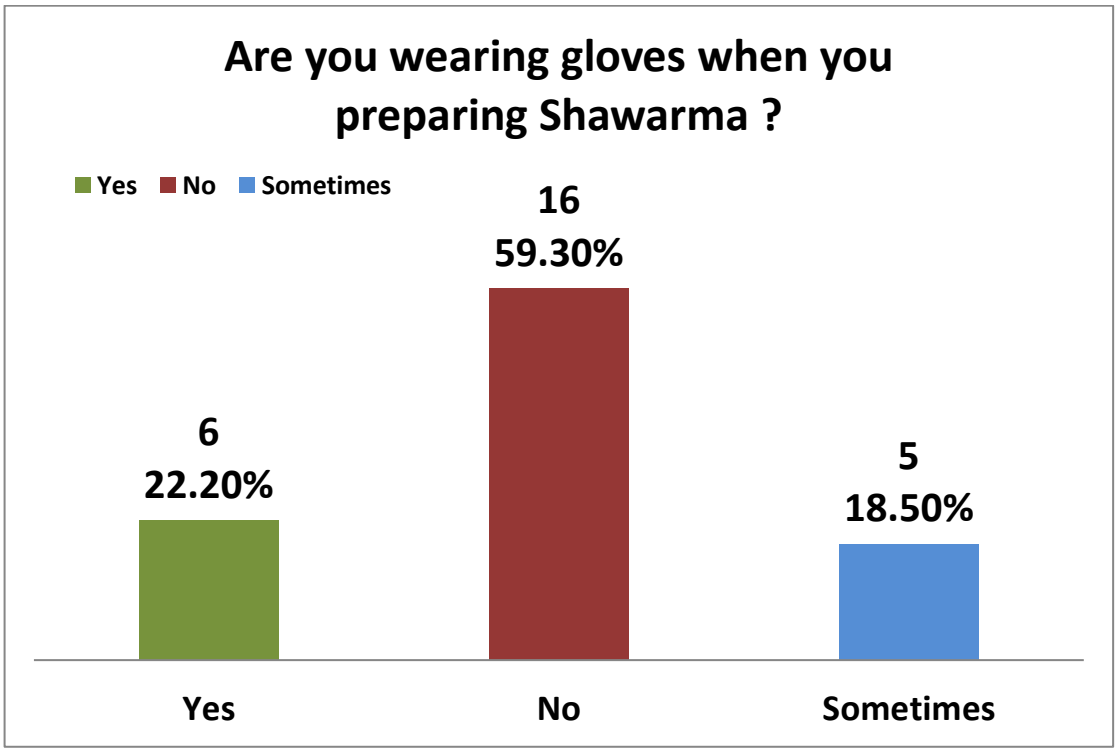
Fig(1) Age of questionnaire participants

Table (12) Sociodemographic characteristics of workers

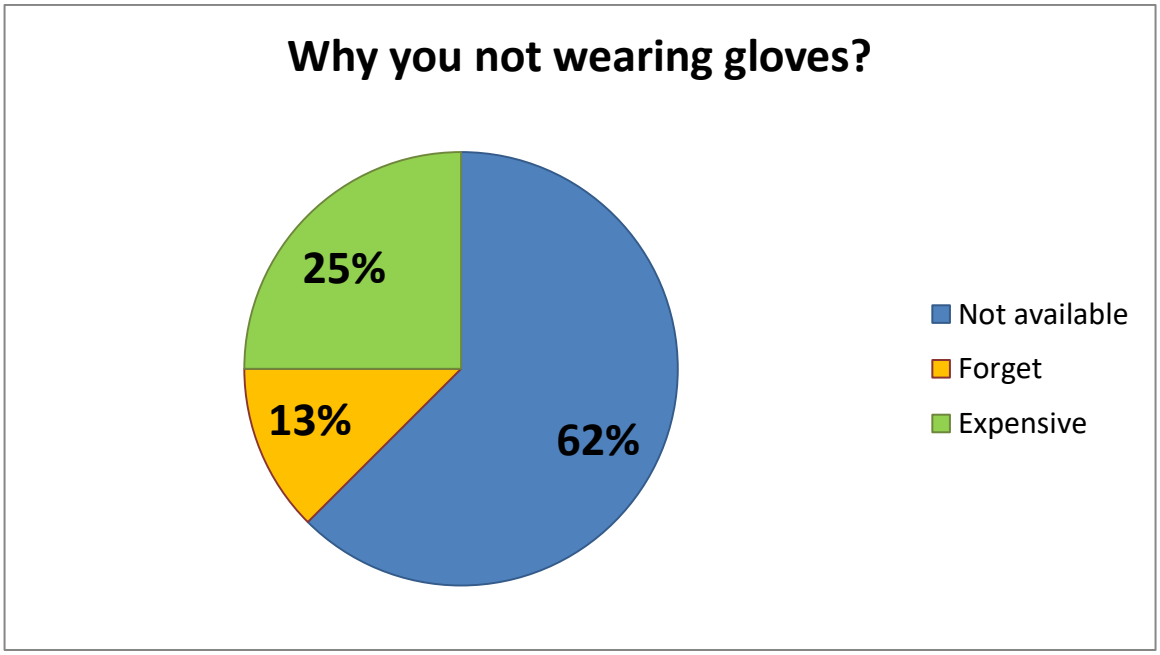
Variable	Number	Percentage
Education level		
Illiterate	3	11.1%
Primary school	6	22.2%
Secondary school	10	37.0%
University	8	29.6%
Age		
Mean age (SD±)	33.52 ±8.77	
Min and Max	21 , 50	
Duration of employment		
Min and Max	2 , 35	



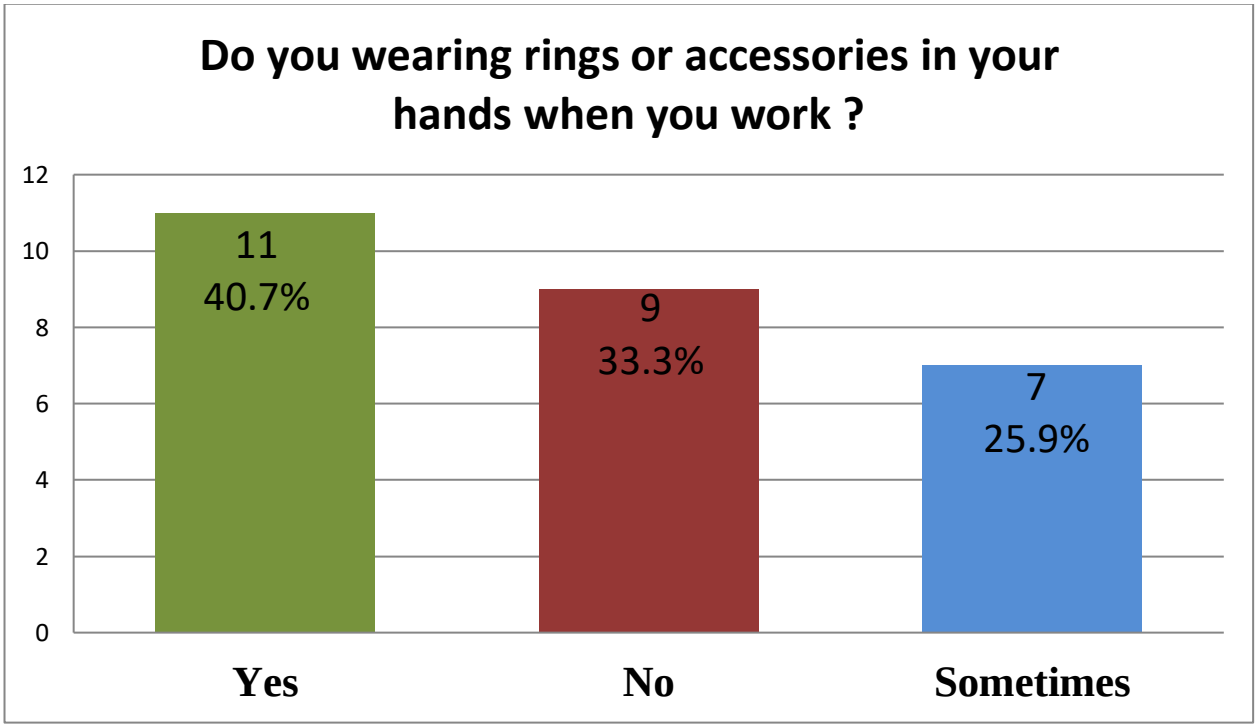
Fig(2) Cleaning hands



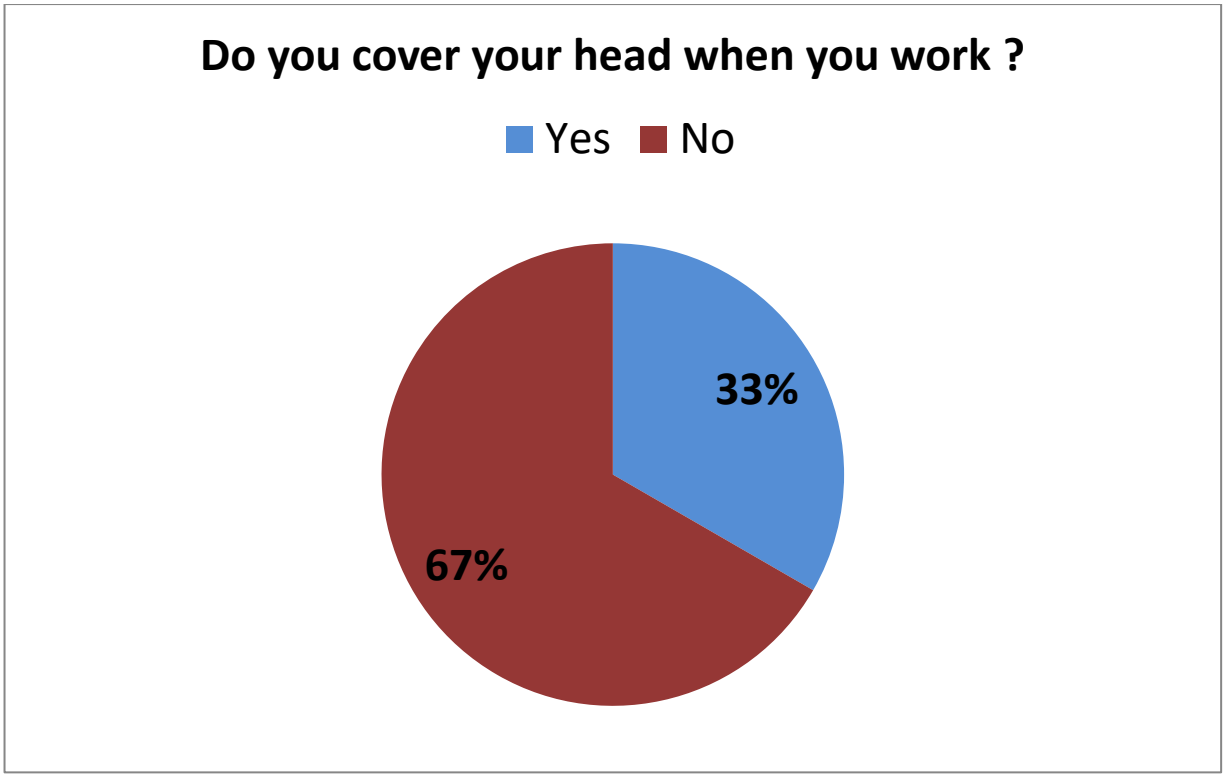
Fig(3) Wearing gloves



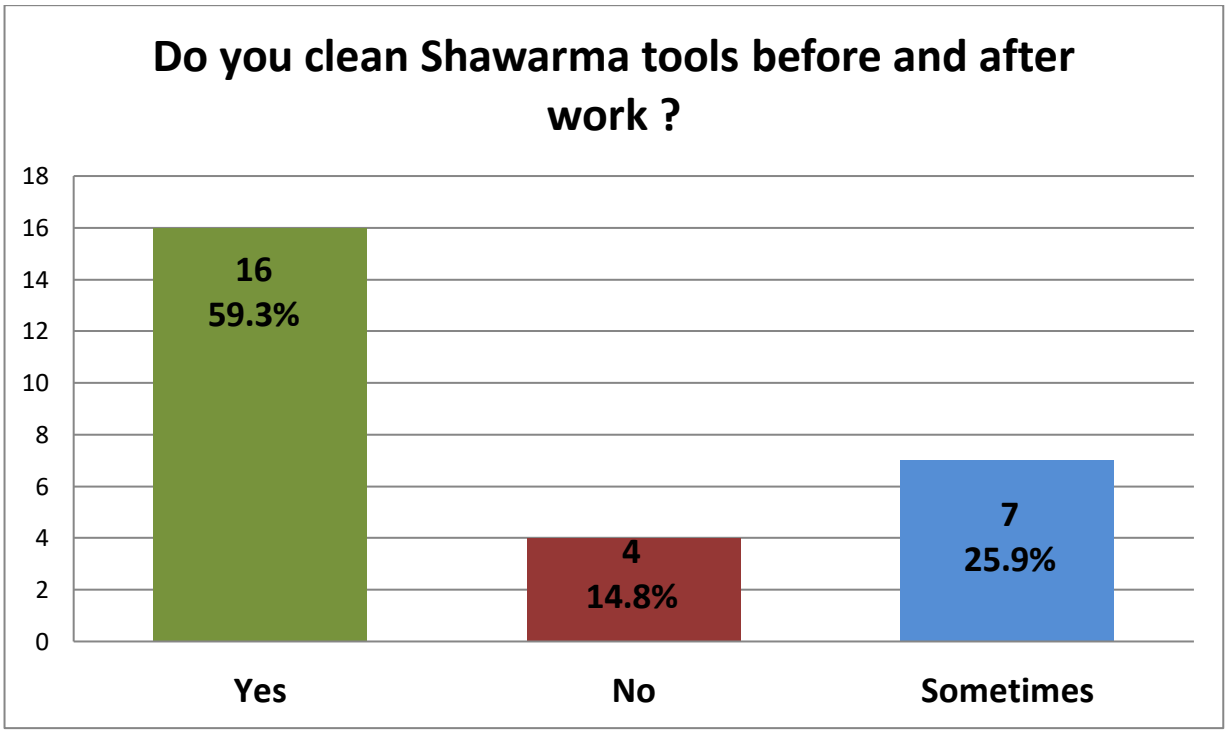
Fig(4) If answer is no wearing gloves



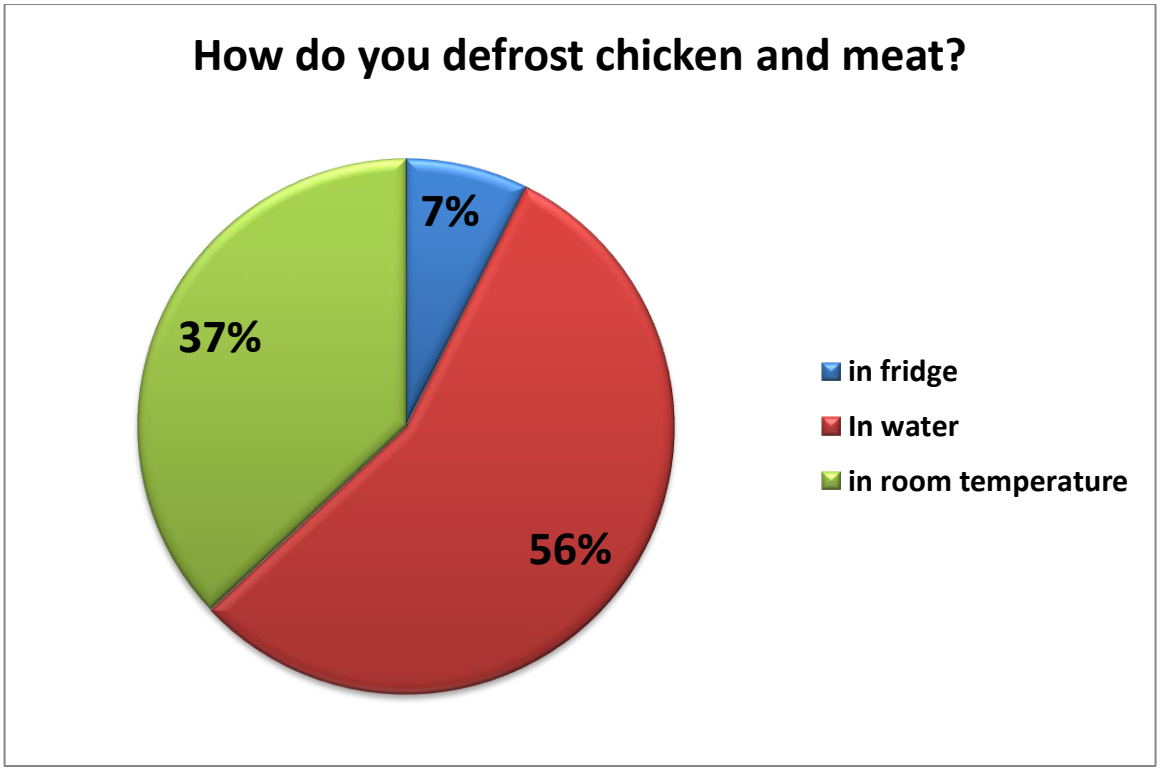
Fig(5) Wearing accessories



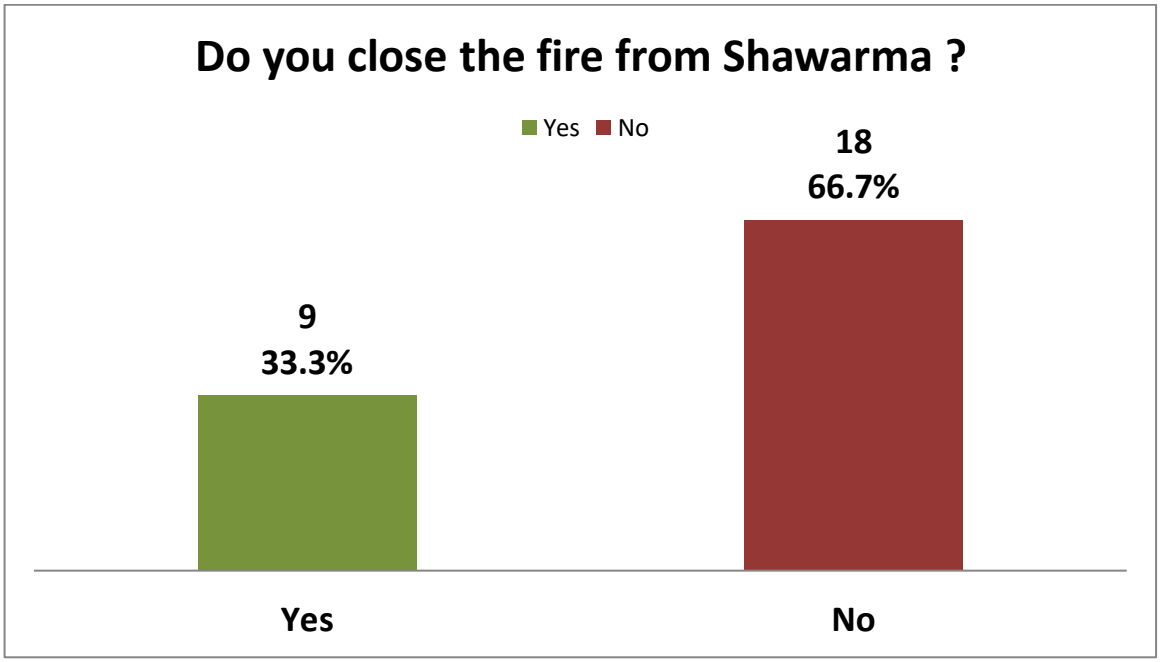
Fig(6) Cover head



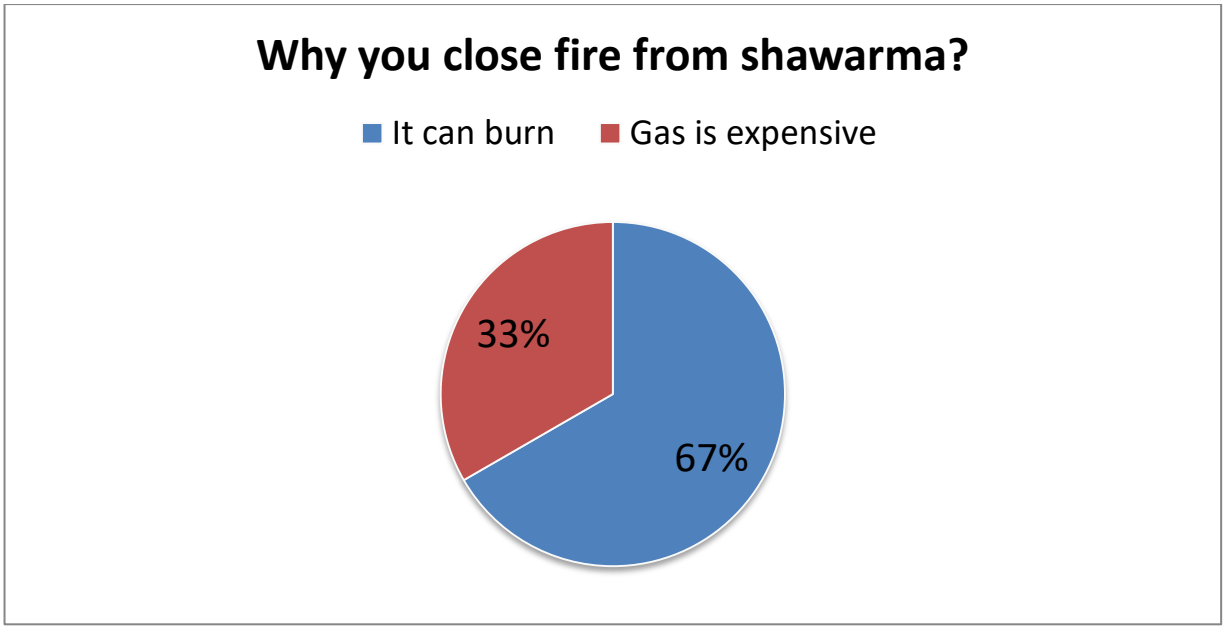
Fig(7) Cleaning tools



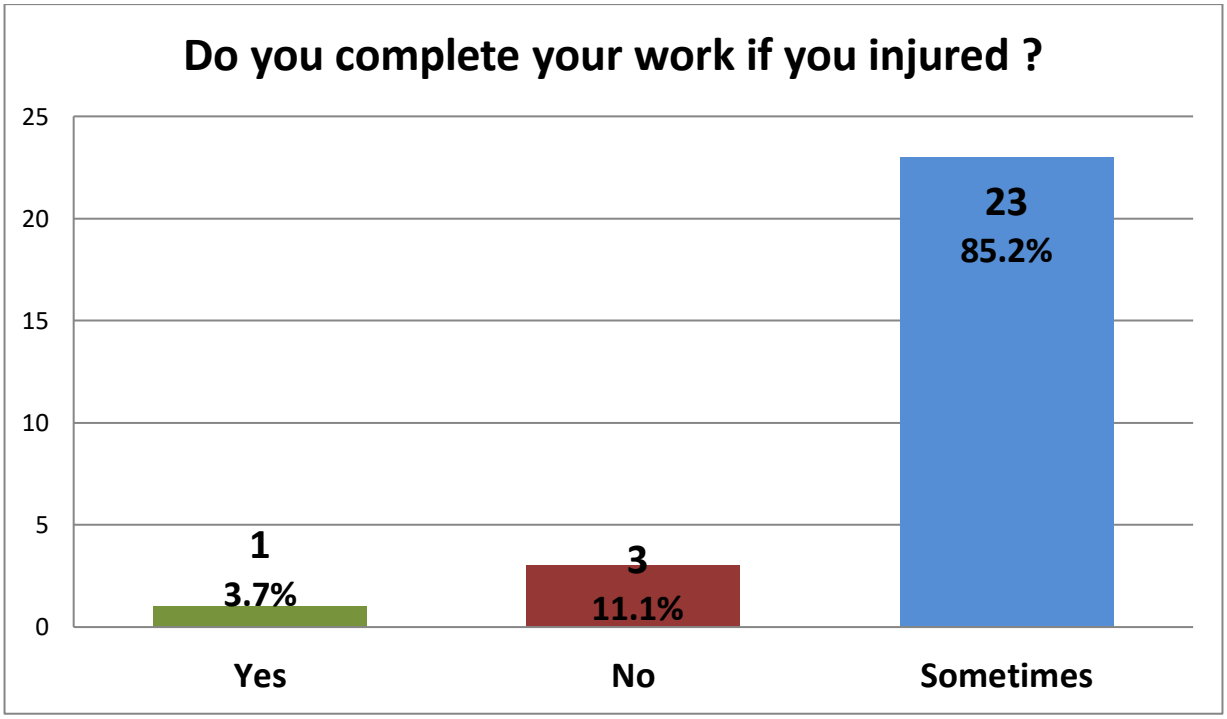
Fig(8) Defrost chicken and meat



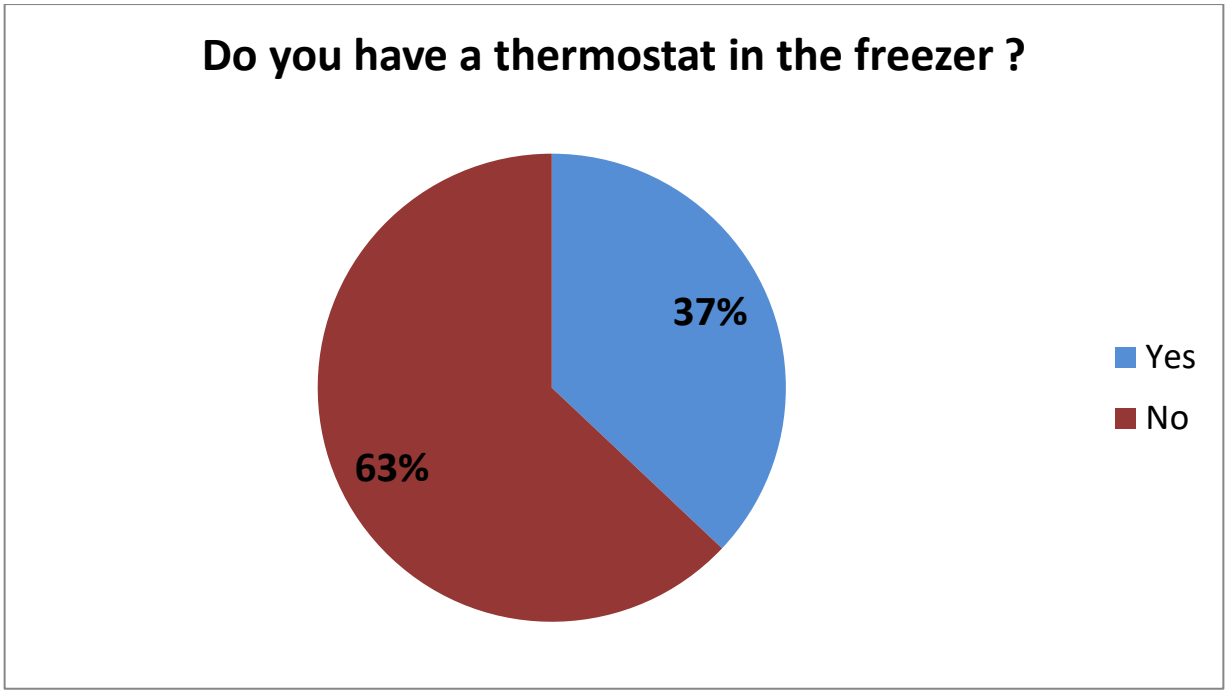
Fig(9) Close fire source from shawarma



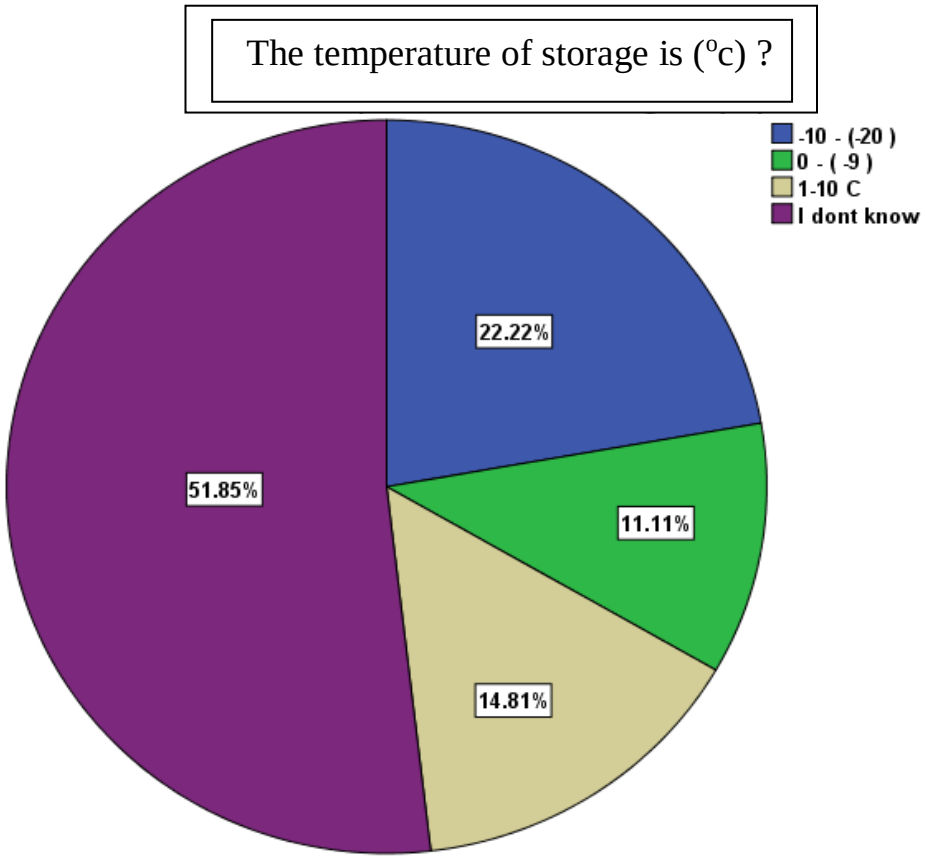
Fig(10) Why you close fire source from shawarma?



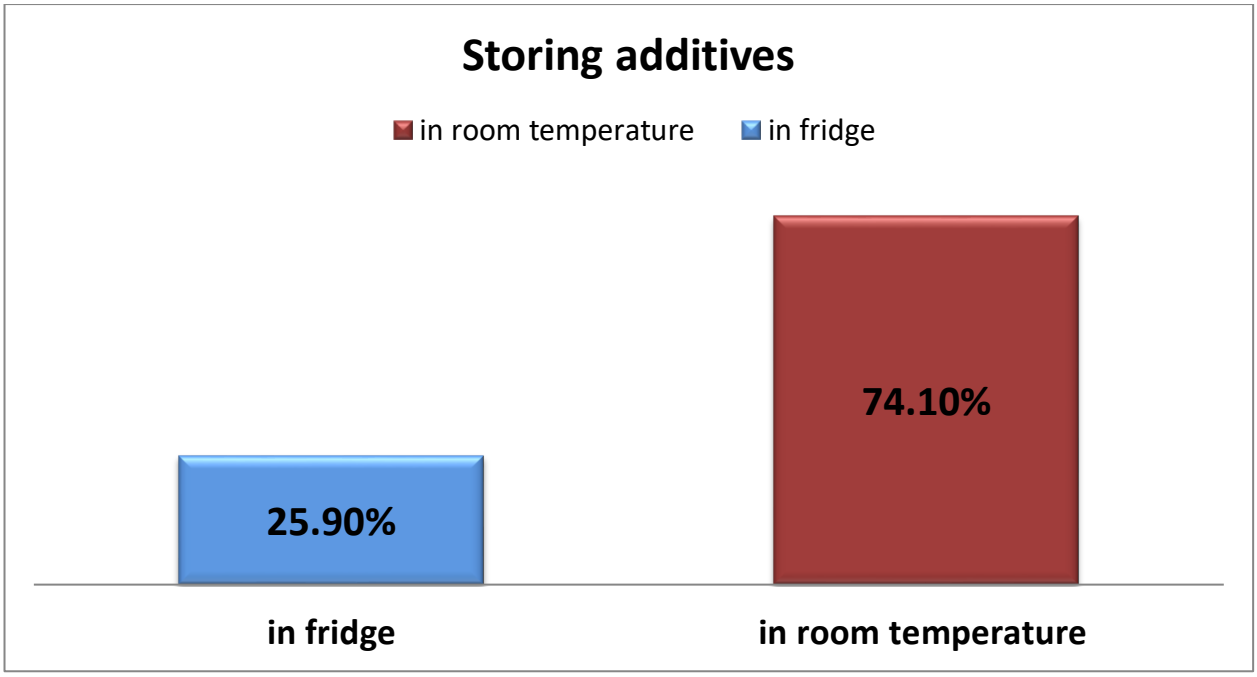
Fig(11) Complete work if you injury?



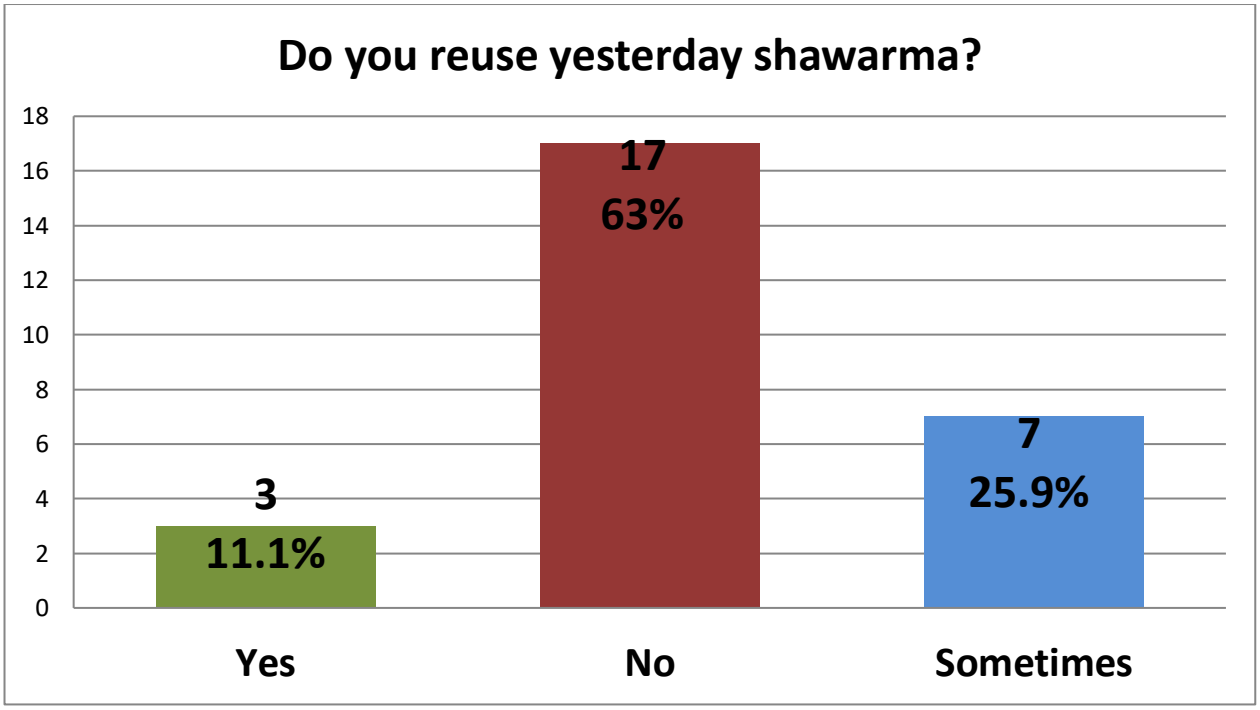
Fig(12) Thermostat



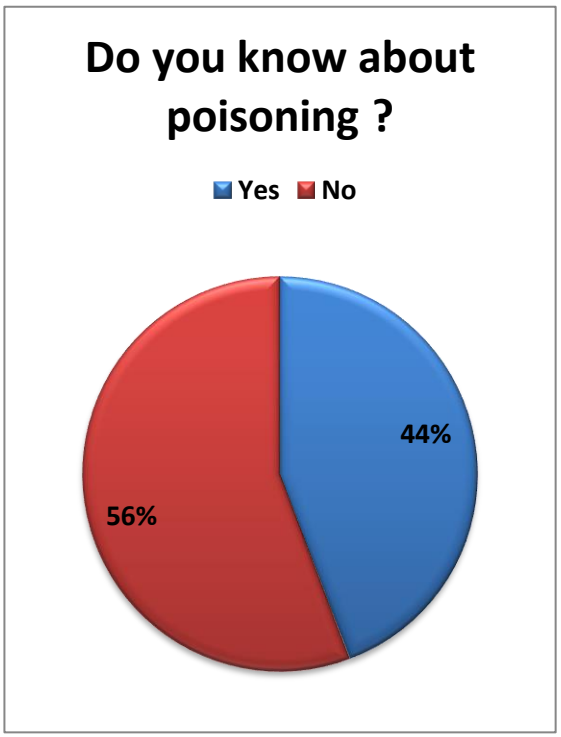
Fig(13) Temperature of storage

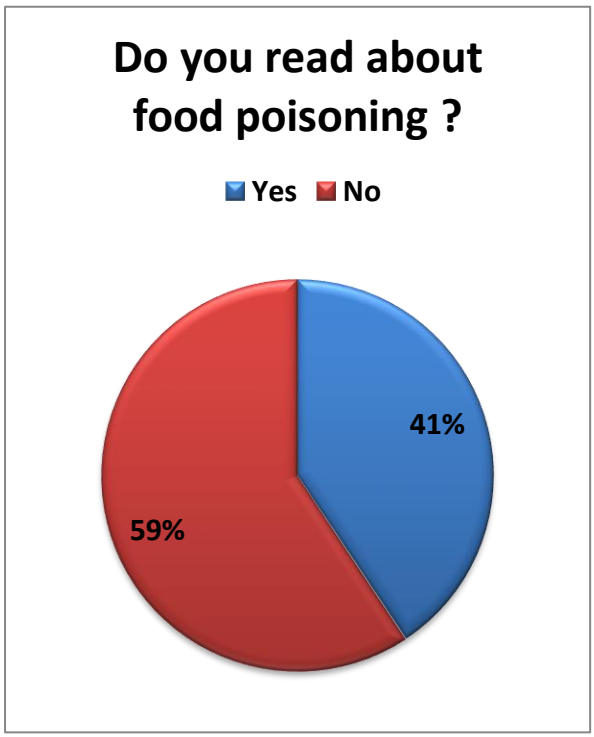


Fig(14) Where you storing shawarma additives?

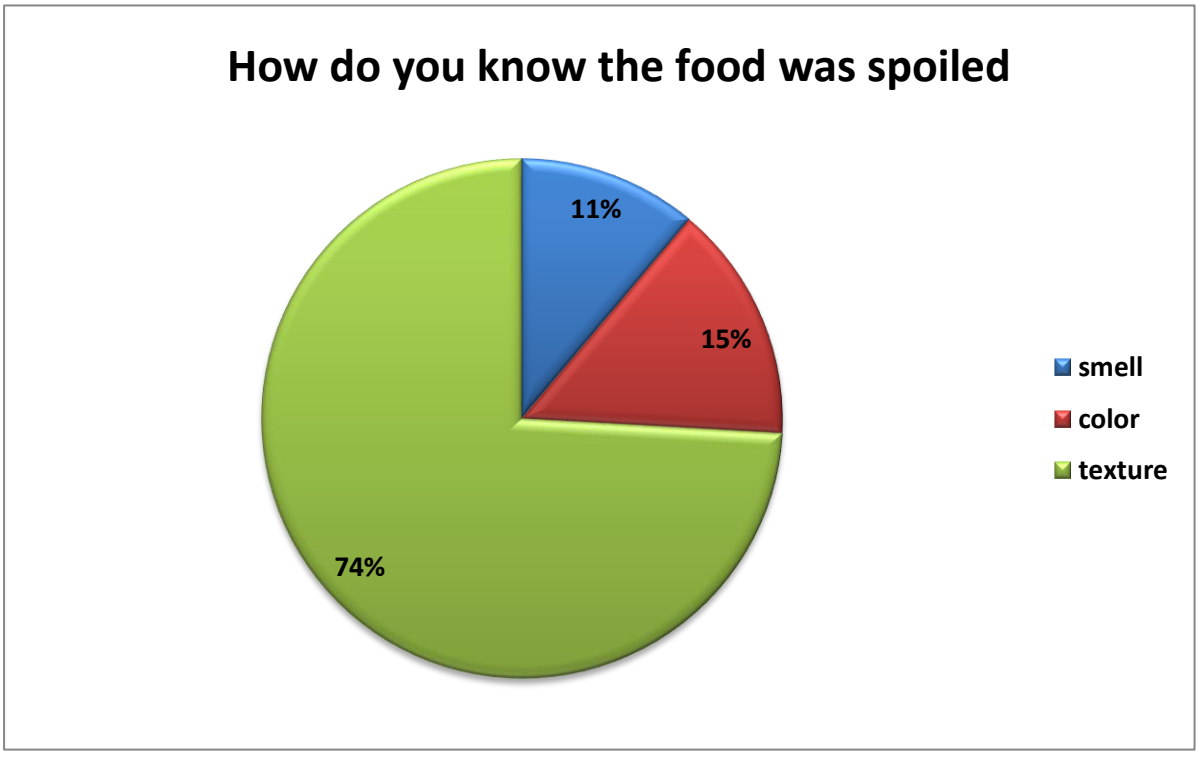


Fig(15) Do you reuse yesterday shawarma

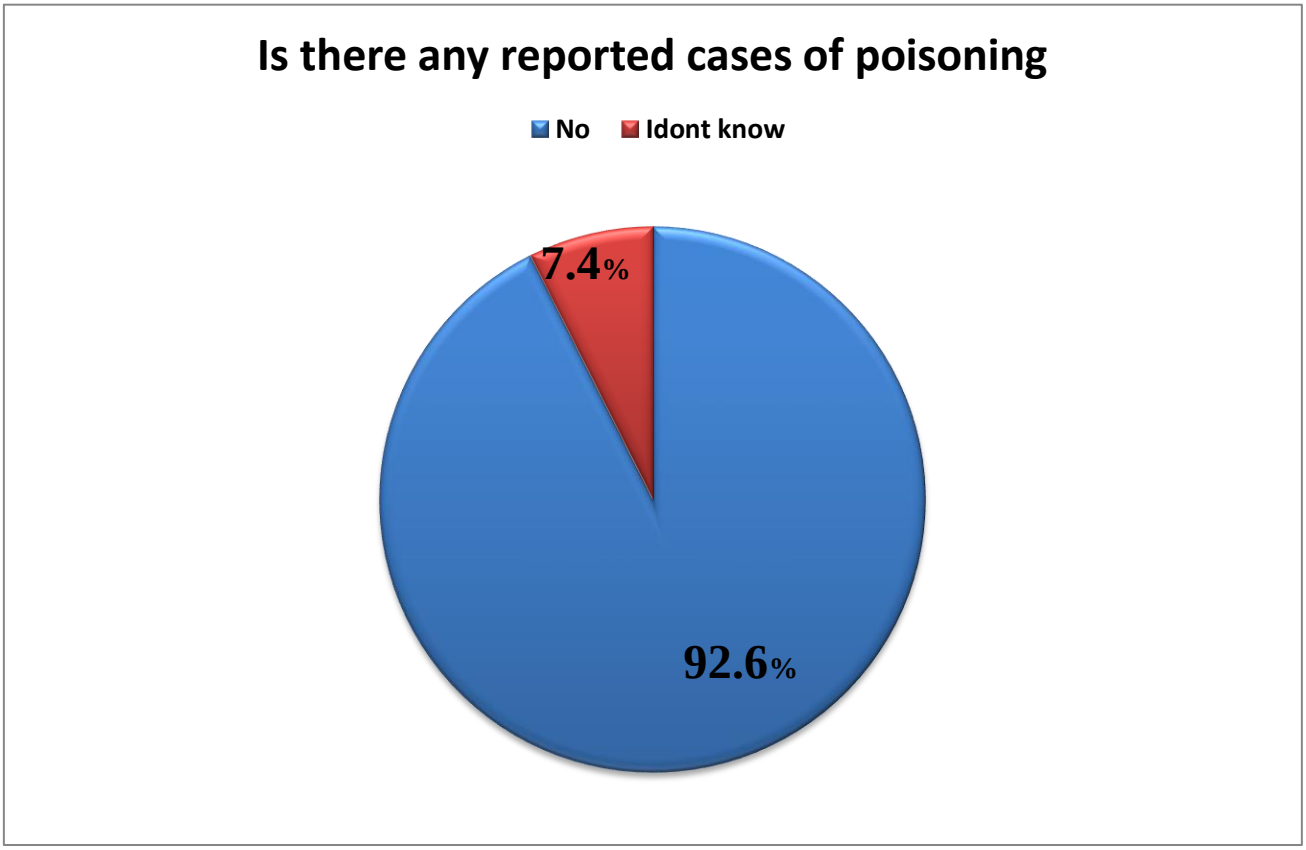




Fig(16, 17) knowledge about food poisoning



Fig(18) Spoiled food



Fig(19) Percentage of cases of poisoning during the work period

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion:

From the results obtained in this study, it can be concluded that:

1- The type of restaurants from which the samples were collected has a major influence in the levels of contamination with different microorganisms including pathogenic bacteria.
2- It was clearly obvious that this contamination was also influenced by the improper handling practices and the absence of sanitary conditions.
3- Highest contamination levels were obtained in the samples from open restaurants while lowest levels were in the samples from closed restaurants.
4- The questionnaire proved that workers had significant weakness in knowledge of good hygienic practices.
5- There is a lack of monitoring and public health education.

5.2 Recommendations:

1- Corrective measures for reducing risks associated in restaurants should be identified and addressed.
2- The relevant authorities should develop minimum guidelines on basic hygiene practices in Khartoum State and ensure enforcement.
3- Training restaurant workers on good hygiene practices.
4- Prevent workers from working until having health card and GHP certificate.
5- Securing adequate resources and the legal power for implementation.

6- Establish laws that deter any laxity in food safety.
7- Short and long action plan should be in place regarding the restructure of restaurants to enable proper hygienic practices with efficient monitoring.
8- Further studies are needed including mineral levels and chemical contaminate for shawarma and other street foods.

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APPENDIX

Study questionnaire :

1- Age ..... (years)

2- Education level

- elletry
- primary
- secondary
- high
- universal

3- Duration of employment .....

4- Wearing gloves when you preparing Shawarma ?

- yes
- no
- sometimes

if no, why?

.....

5- Are you cleaning your hands before and after preparing Shawarma ?

- yes always
- sometimes
- never

6- Are you wearing rings or accessories in your hands when you work ?

- yes
- no

- sometimes

7- Are you clean Shawarma tools before and after work ?

- yes
- no
- sometimes

8- Are you complete your work if you injured ?

- yes
- no
- sometimes

9- How do you defrost the chicken or meat ?

- in fridge
- in water
- in room temperature

10- Do you cover your head when you work ?

- yes
- no
- sometimes

11- Do you reuse yesterday Shawarma ?

- yes
- no
- sometimes

12- Do you close the fire from Shawarma ?

- yes
- no

if yes, why? .....

13- Do you have a thermostat in the freezer ?

- yes
- no

14- The temperature of meet storing is .....

15- Where you store the sandwich additives ?

- in room temperature
- in fridge

16- Do you know about poisoning :

- yes
- no

17- Do you read about food poisoning ?

- yes
- no

if yes, what is the source of information

.....

18- How do you know the food spoiled ?

- smell
- color
- texture

19- Is there any reported cases of poisoning ?

- yes
- no
- I don't know