بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Cold Shocked *Escherichia coli* Isolated from Sausage and Recovered using Different Substrates

عزل الاشيريشيا البرازية من السجوك المعرضة للصدمة الباردة وإستراجعها بإستخدام أوساط مختلفة

A Thesis Submitted to the Sudan University of Science and Technology in Fulfillment of the requirement for the Degree of M. Sc. in Food Science and Technology (FoodMicrobiology)

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الآية

قَالَ تَعَالَىٰ:

﴿ وَلَقَدْ ءَاتَيْنَا دَاوُۥدَ وَسُلَيْمَٰنَ عِلْمًا ۖ وَقَالَا ٱلْحَمْدُ لِلَّهِ ٱلَّذِى فَضَّلَنَا عَلَىٰ كَثِيرٍ مِّنْ عِبَادِهِ ٱلْمُؤْمِنِينَ ﴿١٥﴾ ﴾

صدق الله العظيم

(سورة النمل الأية: ١٥)

DEDICATION

To the souls of my father and Husband

To My Mother

To My Family Members

To My Dear Friends

With Love

Leila

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I thank Almighty **Allah** for giving me patience and help to finish this work. I wish to express my deepest appreciation to all those who participated in this research particularly, Prof: Sulieman Mohamed ELsanousi, Who supervised the present work for his close supervision and patient leadership throughout this study.Thanks are extended to co supervisor prof. Ahmed El - Awad El- Faki for patient help, supervision,and constructive eriticism. Also Thanks are extended to Dr .Omer Abdalla and AwadSograb.As wellthanks are extended to the staff of the lab of microbiology (Sudanese Standards and Metrology Organization) for their assistance in preparing and monitoring the experiment.I thank all my friends and to everybody who helped me during this study.

ABSTRACT

Meat and meat products such as sausages and minced meat, are perishable foods and much easily consist of various sources of pollution before, during and after the manufacturing process and then become unsuitable for consumption and a source of danger to health. The objective of this reach was to isolate bacteria in frozen sausage and to improve the recovery media to escape the phenomenon of cold shock that leads to number less than the true number. *Escherichia coli* was selected as an organism for this study, it was subjected to cold-shock at -20°C, -30°C and -40°C for one hour at each temperature. The recovery medium used was EMB. For improvement recovery of shock bacteria the following treatments were followed :vitaminB_6 and B_{12}were incorporated in the medium concentrations 1ml, 2ml and 3ml and sodium pyruvate concentrations 0.5%,1% and 1.5% the results revealed that when vitaminB_6 (ml)was incorporated in the recovery medium improving of recovering shock cells was observed,(98.3%). at -20 °C while at -30 °C and-40 °C no improvementwas observed .

When vitaminB_{12} (3ml)was incorporated in the recovery medium improving of recovering shocked cells was observed (96.6%) at -20°C, (95.2%) at -30°Cand (99%) at -40°C.

When sodium pyruvate(1%g)was incorporated in the recovery medium, improving of recovering shocked cells was observed (99%), at -20°C and (99.7%) at -30 °C while at -40 °C noimprovementwas observed.

When sodium pyruvate and B6(3ml+1.5%g)was incorporated in the recovery media, improving of recovering shocked cells was observed (98.3%), at -20°C, at-30 °C and -40 °C improvementwas observed .

When sodium pyruvate and B12(3ml+1.5%g)was incorporated in the recovery media, improving of recovering shocked cells was observed (97.2%), at -20°C while at-30 °C and -40 °Cno improvement was observed.

الملخص

اللحوم ومنتجات اللحوم مثل السجق واللحم المفروم، من الأطعمةالقابلة للتلفوكثيرا ما تتعرضبسهولةلمختلف مصادر التلوثقبل وأثناء وبعد عملية التصنيعومن ثمتصبحغير صالحة للإستهلاك ومصدرا خطراً للصحة .الهدف من هذا البحث دراسة البكتيريا في السجق المجمدة وتحسين عملية الإسترجاع ودراسة ظاهرة الصدمة الباردة التى تؤدي إلى إنخفاض واضح فى عدد بكتريا الدراسة من العدد الحقيقي. لقد تم استخدام بكتريا الإشرشيا كولاي لتطبيق هذه الدراسة، حيث تم تعريض البكتريا للصدمة الباردةعند درجات حرارة مختلفة وهى- 20و-30 و-40 درجة مئوية ولمدة ساعة واحدة لكلدرجة حرارة .و الوسط المستخدم لإسترجاع عدد البكتيريا التى تعرضت للصدمة الباردة هو EMB ومن ثم تم معالجتها بإضافة فيتامين(B_6)و(B_{12}) للوسط بتركيز (1مل , 2 مل و3 مل) وصوديوم بيروفيت بتراكيز (0.5%,1%and1.5%) وكانتالنتائج كالآتى: عند إضافة فيتامين B_6 بتركيز 1 مل كانت نسبة الإسترجاع .98.8% عند درجة حرارة -20 درجة مئوية لم يحدث تحسن فى الإسترجاع عند درجة حرارة -30 و-40 درجة مئوية.

عند إضافة فيتامينB_{12} بتركيز (3 مل) كانت نسبة الإسترجاع 96.6% عند درجة حرارة -20 درجة مئوية و 95.2 % عند درجة حرارة -30 درجة مئويةو99%عند درجة حرارة - 40درجة مئوية

عند إضافةصوديوم بيروفيت بتركيز (1. %) كانت نسبة الإسترجاع99% عند درجة حرارة -20 درجة مئوية 99.7%عند درجة حرارة -30 درجة مئوية لم يحدث تحسن فى الإسترجاع عند درجة حرارة -40 درجة مئوية.

عند إضافةصوديوم بيروفيت وفيتامين(B_6) بتركيز (1.5% + 3مل)كانت نسبة الإسترجاع .98.3%عند درجة حرارة -20 درجة مئويةحدث تحسن فى الإسترجاع عند درجة حرارة -30 و-40 درجة مئوية.

عند إضافةصوديوم بيروفيت وفيتامينB_{12} بتركيز (1.5% + 3مل)كانت نسبة الإسترجاع .97.2% عند درجة حرارة -20 درجة مئويا لم يحدث تحسن فى الإسترجاع عند درجة حرارة -30 و-40 درجة مئوية.

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CHAPTER ONE

INTRODUCTION

Meat is the most important source of food proteins to the people of the Sudan. Because of its high consumption rate, every effort should be made to provide clean and safe meat to the consumers. Fresh and processed beef is consumed by most Sudanese, and it is subjected to contamination at various stages of production.Food safety is important for consumers, food producers and inspection authorities for number of reasons, including consumer protection and producers, risk and international trade. (Van Gerwen *et al*. 2000).

Quality control include the suitability of facilities, control of suppliers safety and maintenance of production equipment, cleaning and sanitation of equipment and facilities, personal hygiene of employees, control of chemicals, pest control, and the like (Jay,2000). Food manufacturers apply microbiological criteria to products at point of manufacture asconsistent reference and as check on the success of all the process control procedures, that a particular product at point of manufacture will remain safe and wholesome to eat at any time during its shelf- life(PFMG,1995). In producing good quality market food, it is important to keep microorganisms at a low level for reasons of aesthetics, public health, and product shelf- life. Other than those foods that have been made sterile, all foods should be expected to contain a certain number of microorganisms of one type or another. Ideally, the number of organisms should be as low as it is possible under good conditions of production. Excessively high number of microorganisms in fresh foods causes harm. It should be kept in mind that the inner parts of healthy animal tissue are generally sterile and that it is theoretically possible to produce many foods free of microorganisms.

Therefore the number of microorganisms in fresh food products may be taken to reflect the overall conditions of raw products quality, processing, handling, storage and so forth. Raw meat is a sensitive ingredient and the product is subjected to recontamination after processing and during distribution (Jay, 2000). In the Sudan, where environmental factors favoring the growth of microorganisms and rapid spoilage of products, it is vital to follow rapid, dependable methods in protecting the consumers. Preserving food by cooling remains one of the major methods of providing food with minimal risk of health problems and decreases substantially the economic loss of food. On the other hand, cooling also can affect the food microbiological quality control, which results in minimizing the real view of danger due to the injury of cells which in subsequent times repair themselves andconsistute health hazards (Mustafa, 2005).Fresh processed and frozen food constitutes a majority in human consumption. In the Sudan, variety of fresh and processed frozen foods are being consumed. These include: meat, fish, minced meats, beef burger, pastrami, martidella, sausages, poultry, as well as ice creams. The bacterial load of such foods is governed by local and international standards. For example: Five gram of "X" foods must not contain more than "Y"organisms. Also food must be free of *Salmonella* andother hazardous organisms. The problem in the sense that usually the estimated bacterial count is less than true value. This is due to the bacterial being injured by cold-shock. Such injured cells require special care in their isolation and counting and precautions are not usually practiced in such field. Freezing of such foods will expose the bacterial load to a minimized false number that might urge itself to pass food as suitable for human consumption while it is not.The effect of cold diluents on viability was first reported by Sherman and Albus (1923) who reported that ca 95% of cells were killed within 1 h when the temperature of a4 –h culture of *E.coli* was reduced suddenly from 45°Cto a medium at 10C°.

The phenomenon was later observed by several workers (Strange and Dark,(1962), Williams, (1956) and Duncan *et al*.(1972).Colleese*t al* (1961) termed the phenomenon as "phoenix" phenomenon due to disappearance of colonies at the recovery media and attributed this to the temperature of the inoculums: a finding which was disqualified by El Sanosui, (1975) who explained the phenomenon to be mere a cold- shock one. This is due to cells being shocked when transferred from high temperature to low one.When microorganisms are stressed a change in the genome, the proteome or the environment take place. In this study by "stress" we mean subjection to low temperatures: cooled, frozen, or deep frozen. This is in term will produce a decrease in the growth rate or survival (Vorobeva, 2004).

1.3 Rationale

The spoilage of products may lead to many health problems among different population categories (children's,elders,patients… etc) and also has negative effect on the economy of the country.

General Objective

To assess the quality of fresh and frozen sausages, from microbiological point of view.

Specific Objectives

1. To determine the total number of microorganisms per gram of food sample and compare it with the standards.
2. To isolate and identify the dominant microorganisms.
3. To improve recovery of injured cells to show visible colonies.

CHAPTER TWO

LITERATURE REVIEW

2.1 Meat nutritive value

Red meat and poultry contribute about a sixth of all protein consumed by humans. Not only is meat a very concentrated source of protein, but this has a high biological value because its composition matches closely that of our own protein. It contains all the amino acids essential for human health. Meat is also an important source of the B vitamins, and Avitamins. It is a major source of iron, copper, zinc and selenium.Lawrie, (1998).

2.1.1 Sausage:

From antiquity to the present, man has searched for methods of preserving and extending his food supply. The oldest method of preservation is salting. In 830 B.C., Homer wrote about smoking and salting meats in The Odyssey. Different types of sausage were created all over the world, and each region developed their own distinctive style of sausage influenced by the availability of local ingredients, spices, and casings. Climate was another important factor for the development of region-specific fresh and dry sausages.

Regions with distinct seasons used different techniques to preserve meat. In the cold seasons, fresh sausage was able to keep for short periods of time without refrigeration. The smoking process was developed to preserve sausages during the warmer seasons.

Dry sausage, which does not require any refrigeration, was created in warmer regions. Some sausages became associated with their country or city of origin. A good example is Bologna, which originated in the town of Bologna in Northern Italy. Almost every culture has created its own

characteristic type of sausage. Even the Native Americans created sausages made from a wide variety of meats and berries Basic sausage – Making (2004). Any meat which has been changed from its original form (e.g. minced) and seasoned is considered sausage.

Of all the various processed meats, sausage is the most appetizing and widely utilized. The word "sausage" is derived from the Latin word salsas', which means salted. Sausage is any salted, ground meat, and there are many different types of sausages produced in the United States.

There are six basic categories of sausage:

1. Fresh Sausages - made from ground meats which are seasoned and stuffed into casings, or left in bulk form. Fresh sausage is not cured or smoked; it must be fully cooked before eating. Examples: pork breakfast sausage; Italian; bulk pork sausage.
2. Cooked Sausages - made from meats which are ground, seasoned, often cured, stuffed into casings, and cooked. No smoke is used. Cooked sausages are often served cold. Examples: braunsch weiger; liverwurst; liver cheese.
3. Cooked, Smoked Sausages - made from meats which are ground, seasoned, stuffed into casings, smoked and cooked. These can be eaten cold or reheated. Examples: bologna; berliner; cotto-salami; frankfurters.
4. Uncooked, Smoked Sausages - made from meats which are ground, seasoned, stuffed into casings, and smoked. These must be fully cooked before eating. Examples: some kielbasas, mettwurst; teawurst; smoked country-style pork sausage.
5. Dry and Semi-dry Sausages - made from meats which are ground, seasoned, cured, stuffed into casings, fermented, often smoked, and carefully air-dried; true dry sausages are not cooked. These sausages have a distinctive tangy flavor due to the presence of lactic acid that

is produced by fermentation. The meat is stuffed into casings and allowed to "ferment," the process by which bacteria metabolize sugars and produce acids and other compounds as by-products. In meat fermentation, bacteria which produce lactic acid are utilized to produce the tangy flavor of dry sausages. They are sometimes referred to as "summer sausages" and eaten cold. Examples: pepperoni; German salami, Lebanon bologna, Genoa salami; thuringer; cervelat.

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

2.1.2 Meat contamination:

Sudan is one of the few countries of the word that could contribute greatly towards alleviation of at least part of the present world deficit in animal protein. This position is acquired due to its great potential resources of both livestock numbers and animal feed material (L M M C, 1985).

Raw materials, as well as final meat products are exposed to a high risk of microbial contamination at the time of their production, processing, storage and distribution .Chemical composition of food, properties of the outside environment and specific growth requirements determine the type of microorganisms and the course of physical and chemical reactions in the contaminated food (Lcia kova al, 2004).

Meat is an ideal environment for the growth of microorganisms particularly bacteria (Frazier, 1967; Forsythe and Hayes, 1998). Microorganisms can multiply readily on the cut surfaces and exposed surfaces which become easily contaminated after slaughter and during and

after dressing and butchering, although the microbial count of the interior of the meat usually remains much lower (Harrigan, 1998).

Center for disease control and prevention (2000) reported that food of animal origin such as poultry eggs and beef are the main sources of food borne illnesses. Raw beef, salami and sausages have all been associated with *Salmonella* outbreaks in United States of America.

Ban wart (1981) reported that so as to control contamination, microbial load on the food must be kept as low as possible it is important to know the main sources of contamination. Hence contamination of meat product was extensively studied. in general it can be concluded that meat can become contaminated if the meat is eaten from an animal that had septicemia if infectious lesions remain on sites used for meat if edible organs are infected or inedible infected organs leaked bacteria on surfaces or if cross-contamination occurs from animal faces.The microbiology of meat is highly dependent on the condition under which animals are reared, slaughtered and processed (Brown, 1982). (Vanderzant and Neckelaon 1969) and Frazier and Westhoff (1978,1989),it was found that the main sources of contamination of meat were the soil and drinking water. (Frazier, 1967) The level of microbial contamination on meat was influenced by the level of carcass contamination at boning and by boning process itself .Carcass contamination was the major determinant of microbiological quality , a some of more than 70% of carcass had microbial counts greater than $10^3/cm^2$.Cutting boards were a major source for microbial dissemination during boning (Widders,1994). During transport form farm to the slaughter house livestock are herded together and frightened so that cross-contamination occurs more readily (Forsythe and Hayes, 1998).

Mohamed (2000) found that meat spices, additives and machines used during processing were sources of contamination to the meat during processing.

Wagner and Jer(2000) reported that meat would be contaminated by contact with hides, feet, skin, stomach and intestinal contents and clothing of personnel, water used for washing carcass, equipments, and even air in the processing and storage areas meat when put in utensils , some or all microorganisms on the surface of these meats will contaminate the contact surface of the utensils . fresh meats put in the same utensils will inevitably pick up some of these microorganisms then become contaminated Jay,(2000).

2.1.3Contamination of minced meat by some pathogenic bacteria:

Narsimha and Ramesh (1988) proved that comminuted meat provided an ideal environment for the growth of bacteria. Federal register US A (1999) reported that, raw ground beef products presented a significant public health risk, because they were frequently consumed after preparation that did not destroy the organisms that had been introduced below the product's surface by chopping. Although beef in general may be contaminated, ground meat is a special concern because grinding combines meat from different animals and transfers bacteria from the meat's surface to its interiorMFMER ,(2006).

Minced beef is a product with large and varied microbial profile which frequently included a number of potentially pathogenic bacteria .More handling especially grinding and mixing , means a greater like hood of contamination by bacteria such as *Salmonella*, *Campylobacter* , *Listeria*, and *E.coli O157:H7* (Mary and Scottie, 1998).

2.1.4 Contamination of Sausage by some pathogenic bacteria:

Sausage is a processed meat that might be contaminated during preparation Wilson, (1981).

Herschdoerfer (1968) explained that fresh sausage was largely retailed in non-frozen condition and consequently highly vulnerable to bacteria spoilage and that the rate of deterioration depends primary upon the initial contamination of the raw material sanitation during processing, temperature history and addition of permitted preservatives. Jay (1986) reported that sausage is usually contaminated with more varied flora than most other processed meat due to different seasoning agents employed.

2.1.4.1*Salmonella:*

Salmonella is the genus name for a large number (over2500) of type ofbacteria. Each type is distinctly identifiable by its specific protein coating. The types are otherwise closely related.

Salmonella bacteria are rod – shaped, flagellated. Gram stain- negative, and are known to cause diseases in humans, animals, and bird (especially poultry)

Worldwide ,the two major diseases caused by *Salmonellaspp*- are typhoid and paratyphoid fever in humans. The terminology that identifies the particular protein coats, or samovars, is not well settled, and what previously were thought to be various species of the genus salmonella are now thought to be servers of only two species, *S.enterica* and *S.bongori*, by many researchers, however, these designations are not always accepted in the scientific literature. And so common samovars that have been named in the past are still used (for example S.*typhi*, *S.typhimurium*, *S.paratyphi*, *S.enteritidis*, *S.cholerasuis*, *S.saint-paul*). The bacteria were first isolated by Theo bald Smith in 1885 from pigs. The genus name *Salmonella* was derived from the last name of D.E.Salmon, who was Smiths director

in1896, diagnosis of *Salmonella*SPP, infection in human, was accomplished.(http://salmonella)

Salmonella genus contains over 2200 strains (termed serovars or serotypes) according to their O-and H-antigens, bacteria related to each other both phenotypically and genotypically by DNA sequence.Many serotypes are named after the place where they were first isolated e.g.*S.Dubin*,*S.london*,other names according to the disease and affected animal ,e.g.*S.typhi ,S.cholerae-suis*,*S.abortus-ovis*.*Serotypes* are further sub-divided by their resistance to bacteriaphages (phage types or lystotypes),antibiotics or heavy metals, their biochemical characteristics(biovars or biotypes)or their sensitivity to or production of bacterocins ICMSF,(1996).

Most cases of *salmonella* food poisoning outbreaks are caused by two serotypes*:* *S.enteritidis* and *S.typhimurim* (SaudiEpidemiology Bulletin,2001).

*S.typhi*and*S.paratyphi* in meat products are indicate of human origin and their presence therefore indicate poor personal hygiene occurred during handling of meat products(Neema,*et.al*(2004)*Salmonellae* induce the illness by their death following multiplication in the hosted's gut and their subsequent lyses with the release of potentend toxin D'Aoust, (1991). The principle symptoms of *Salmonella*loss are nausea, vomiting, abdominal pain and diarrhea which may be preceded by headache, fever and chills with low mortality rate. The condition needed for an outbreak is ingestion of live cells present in a raw foods or processed food via cross-contamination that leads to human infection (Wallace *et al,* 1998).

2.1.4.2Staphylococcus aurous:

Staphylococcus is a group of bacteria that can cause a number of diseases as result of infection of various tissuesof the body.*Staphylococcusit* ismore

familiarly known as staph (pronouced‘staff.).Staph-related illness can range from mild and requiring no treatment to servere and potentially fatal.

The name *Staphylococcus* comes from the Greek staph lye, meaning a bunch of grapes, and kokakos, meaning berry, and that is what Staph bacteria look like under the microscope , like a bunch of grapes or little round berries (In technical terms, these are Gram-positve, facultative , usually unencapsulated cocci.

Over 30 different type of *Staphylococci* can infect humans, but most infections caused by *Staphylococcus aureus.Staphylococci* can be found normally in the nose and on the skinand less commonly in other locations) of 25%-30% of healthy adults. In the majority of cases the bacteria do not cause disease. However, damage to the skin or other injury my allow the bacteria to overcome the natural protective mechanisms of body leading to infections. http://www Staphinfections.

Staphylococcusaureus is the type species of the genus *Staphylococcus* which occurs as Gram-positive small, non-sporeforming ,non-motile organism, forming irregular clusters of cells like bunches of grape. These organism are facultative anaerobes catalase-positive and oxidase-negative. Their optimum growth temperature is around 37°C. *Staph.aureus* is unusual in being able to tolerate low water activity levels and thus it grows in fairly high salt concentration levels (Forsythe and Hayes, 1998). The most important source of *S. aurous* is probably the human body , the principal reservoir being the nose . Between 30 and 40% of healthy individuals carry *S.aureus* and many of these nasal carriers inevitably also harbor the organism on their hands and other parts of their body. Many lesions such as boils, carbuncles, septic cuts are abounds with *S.aureus* whilst another source of the organism is hair. It is also transmitted to food from human source by cross-contamination and from another source such as utensils. Animals are important sources of organism like milk and

carcass meat (Eley,1996 ,Forsytheand Hayes,1998). Cosagulase-positive *Staphylococci* are characterized by the production of an extra cellular enzyme, coagulasethat convert fibrinogen in citrated human blood or rabbit plasma into fibrin, by aiding an activator present in plasma (Wood,*et al*,1992). All *S.aureus* strain arecoagulase positive (i.e.possess an enzyme coagulating blood plasma) but only about 30% of strains are able to produce the enteroxins associated with food poisoning, six endotoxins,A,B,C1,C2,D,and E have been identified. Types A and D being the most commonly involved with food poisoning and are produced during storage. Each enter atoxinproduced has the property of heat resistance (resist boiling for 30 minutes). They are produced when *Staph.aureus* grows in carbohydrates and protein food. Enter toxin Ais the most important cause of food poisoning.(Forsythe and Hayes,1998,Jawez and Adel,1990). The diseases caused by *Staphylococci* include acute infections, such as septicemia and acute toxemias, such as *Staphylococcal* food poisoning (ICMSF,1996).After ingestion of the contaminated food the symptoms appear within 1-6hrs.Food poisoning due to S.*aureus* is characterized by nausea, vomiting, abdominal pain and prostration . often with diarrhea but without fever, and the mortality rate is very low or nil. Recovery is rapid, usually within 2 days (Eley, 1996, Jay, 2000).Sauer's competes poorly with other bacteria and thus seldom causes food poisoning in raw product. The organism is resistant to drying and having allow water activity also it is very resistant to salt .The toxins also appear able to survive sterilization and are not destroyed by cooking (Bergdoll,1989). *Staphylococcal*toxic shock syndrome (RSS) is an acute multi system illness due to infection with *S.aureus,* it was first described by Todd *etal* .(1978), this syndrome is clinically characterized by sudden onset of fever , hypotension , vomiting ,diarrhea, rash and subsequent desquamation of skin. Case-fatality rate for TSS is in the range of 1to 2% (Wallace *et*

*al;*1998). This disease is ordinary not fatal, and it is considered that death from this disease occurs only when the patient is already weakend or in sickly condition when the poisoning occurs (John *et al*;1974).

Meat is an excellent medium for *Staphylococci* proliferation and if the temperature is warm enough only few hours are needed for the production of effective amounts of enterotoxin. In abacteriological study on meat products in the Sudan which included ground meat, pastrami, beef burger, kofta and sharmoot, Sanosui and Al mahi,(1986) isolated several pathogenic bacteria among which the pathogenic *Staphylococci* were found.The primary selective isolation of *Staphylococci* from specimens of a mixed flora is the beginning of aseries of confirmatory tests. Baird Parker selective and diagnostic medium is mainly used for coagulate-positive pathogenic species Baird-parker,(1962).

2.1.4.3*Escherichia coli*:

Since 1885, when it was first isolated from children's feaces and described by the German bacteriologist Theodor*Escherichia*, scientific attention has been lavished on *Escherichia coli* to such an extent that it is today probably the best understood free-living organisms. Strains of *E.coli* were first recognized as cause of gastroenteritis by workers in England investigating summer diarrhoea in infants in the early 1940s.Until 1982, strains producing diarrhoea were classified in to three type based on their virulence properties, enteropathoggenic *E.coli* (EPEC),enter invasive*E.coli* (EIEC), enterotoxigenic *E.coli*(ETEC).They are not very common causes of food borne illness in developed countries, but an important cause of childhood diarrhoea in less developed countries .(ETEC) is also frequently associated with so called traveller's diarrhoea . However since 1982 enteroheamorrhagic *E.coli*(EHEC) particulary associated with serotypes 0157:H7 has been recognized as the cause of number of out breaks of

heamorrhagic colitis and heamolytic uremic syndrome, particularly in north America where food such as under-cooked ground meat, raw milk and fresh product have been implicated.(Adam and Moss,2008).

2.1.5 Injury

Bacterial Injury may be defined simply as the effect of one or more sub lethal treatment on a microorganism (Hurst,1984.). By extension, sub lethal injury is a consequence of exposure to a chemical or physical process that damages but does not kill a microorganism (Russell,1984).

Yousef and Caurtney (2003) include damage to cellular components in their description of injury, and Gilbert (1984) wrote, "Sub lethal injury of microorganisms implies damage to structures within the cells, the expression of which entails some loss of cell function that may be transient or permanent." Most intervention strategies used for the control of pathogenic and spoilage microorganisms frequently produce a continuum of sub lethal effects, and a considerable proportion of microorganisms in foods likely incur some degree of sub lethal injury during food processing (Zhao,Dolye.2001).

2.1.6 Stress:

The term stress has been used to describe the effect of sub lethal treatments. However, (Hurs.1984) considers injury to the preferred term because, by analogy with higher organisms, its description evokes an image of "temporary and repairable physical damage." By similar analogy, the term stress carries a more subtle meaning, not necessarily causing physical damage but altering organism behavior. Current literature pertaining to microbial injury typically does not maintain this distinction, and the terms are often used interchangeably. The term stress, however, is universally used in reference to the agents or treatments causing injury. Although there

is a tendency to perceive food matrices as metabolically supportive environments, food is frequently bacteriostatic or bactericidal due to intrinsic factors such as water activity (a), pH, oxidation-reduction potential, competitive exclusion by protective cultures, and other environmental and processing stresses (Archer 1996). Other types of stress encountered in food environments may include exposure to acids, bases, bioactive antimicrobial peptides, oxidants, osmotic pressure differences, starvation, heating, freezing, thawing, and the presence of other innate and supplemented antimicrobial compounds (Miller *et al.*, 2000). Some emerging technologies (e.g.. high hydrostatic pressure) cause sub lethal injury, although some have argued that other technologies (e.g., pulsed electric field) do not induce injury (Wuytack *et al.*,2003, Yousef and Caurtney 2003). Bacterial stresses, which generally fit into three categories physical, chemical, or nutritional—can occur throughout the farm-to-fork continuum and lead to different types of bacterial cell damage.

2.1.7Cold Stress

Salmonella can reportedly survive during cold storage at 5°Cfor up to 8 months (Jeffrey's*et al*, 1998). This is likely due to cold shock and subsequent adaptation. The cold shock phenomenon occurs when growing bacteria are exposed to a sudden temperature drop of at least 10°C, leading to cold shock in susceptible microorganisms (Jones *et a1,*996). The associated cold shock response is divided into stages of initial cessation of growth, resumption of growth after an adaptive period, and changes in protein synthesis (Miller*et al* 2000). Microorganisms inhabiting foods that must be refrigerated. For pre- and/or post processing storage are subject to cold shock. Additionally, injury due to cold shock may occur if serial dilutions of microorganisms are held in the refrigerator when laboratory tests cannot be immediately completed (Van Schothorst and Duke 1984).

Sensitivity of bacteria to low temperatures varies widely and is based on population density, growth temperature, cooling rate, and the temperature range over which cooling occurs. The effect of food components on the degree of injury and survival of cold-stressed microorganisms has not been studied in great detail. Preliminary work has shown that water or low-nutrient diluents present more stressful environments than do nutrient-rich broths or food. The time required to reduce *Salmonella* by 90% in water at 0 to 5°C was between 2 and 16 days, with populations in vacuum-packaged beef decreasing about 50% in the same temperature range after 28 days of storage (Mackey , 1984).

However vireo vulniji.us decreased nearly 7 logs in oyster homogenate after 24 h of storage at 4°C, due to lethal cold stress, with no reduction seen in a salt-based culture medium during the same time period (Oliver, 1981).

2.1.8 Freeze injury:

Although in bacteria, freezing is generally recognized as an ineffective microbial inactivation strategy. Freeze injury results from continued exposure to concentrated solutes and physical damage caused by ice crystal formation. Many constituents of food and culture media are protective against freeze damage. These cryoprotectants include glycerol, sodium glutamate, certain sugars, peptides, and proteins (Mackey ,1984). Utilization of cryoprotectants in one study may have contributed to minimizing the impact of freeze injury on *Escherichia coli 0157:H7* (Semanchek andGolden ,1998).

2.1.9 Heat shock

In contrast to other bacterial stresses, heat shock is perhaps the most studied and best understood. Heat shock occurs when organisms are exposed to temperatures above their normal growth range (Bunning *et al*,

1990 and Farber *et al,* 1990 and Mackey 1986,and Pagan 1997). Temperatures at which heat injury is induced may be lethal to a fraction of the bacterial population, based on the growth phase and heat sensitivity of the microorganism (Murano and Piersom 1993). When processing animal carcasses, hot acid sprays may elevate the superficial temperature of the carcass, altering the growth and resistance profile of indigenous flora (Castillo*etal*,2001). Conditions within both preprocessing and processing environs may cause heat shock or stimulate a heat shock response in the target microorganism. Guidelines for the use of acid washes to decontaminate animal carcasses include spray temperatures that vary from 20 to 60°C (Gerris 2000). The heat shock response has been reported to occur at a temperature as low as 42°C for *E. coli 0157:H7* (Murano andPierson 1993) , 46°C for Cainpviobacterjejuni (Palumbo 1984), 48°C for *SalmonellaTyphimurium* (Bunning, *et al*,1990, Mackey, and Derrick. 1986), and 45 to 48°C for *Listeria monocytogenes* within a fermented beef—pork sausage homogenate (Farber, and. Brown. 1990).Thermal processes that include extended come-up phases, such as low-temperature pasteurization of eggs, slow roasting of certain meat products, or certain sous-vide processes, might generate conditions that can induce sub- lethal thermal injury to microorganisms (Bunning, *et al*,1990 Mackey and Derrick (1987), Murano and Pierson. (1993)., Pagan *et al*,(1997). The behavior of microorganisms in foods that are heated gradually may mimic the response of microorganism to isothermal heat shock, resulting in a concomitant genetic and physiological heat shock response(Farberand Brown,1990, Mackey and Derrick, 1987). Likewise, microorganisms present in meat products*E.coli* on warming trays before receiving a final reheating could also experience heat shock (Farber, andBrown, 1990).

2.1.10Cold shock (Phoenix Phenomenon):

The effect of cold diluents on viability was first reported by Sherman and Albus (1923) who reported that 95% of cells were killed within 1 hr. when the temperature of a 4 hr. culture of *E.coli* was reduced suddenly from 45°Cto 10°C,while no similar loss in viability was observed in 12 day old cultures.

Aloss in viability also was observed by Sherman and Cameron (1934) upon transferring *E.coli* in the log phase from a medium at 45°Cto a medium at 10°C. *Pseudomonas* was shown to be sensitive to cold shock in the log phase by Gorril and McNeil (1960) who found that stationary phase cultures of *Pseudomonasaeruginosa* were susceptible to cold shock during harvesting but log-phase cells were not. The same phenomenon i.e cold – shock of log phase cells was observed in *Aerobacteraero genes* Strange and Dark, (1962); Strange and Ness, (1963) but not in *staphylococcus aurous* Gorril and MeNeil,(1960). Williams (1956) observed a similar fall in viable count with log phase cells of thermo uric bacterium (Micrococcus 9, Group III B) in glucose broth at 37.5°C , but since it never occurred when culture were incubated at 15°C or 25°C, he concluded that the effect was due solely to the temperature of incubation. The more probable reason for failure to observe the phenomenon was the small difference between the incubation temperature (i.e. 15°Cand 25°C) and the temperature of the diluents, assuming the latter was at room temperature. The term (cold-shock) or(the effect of sudden freezing on bacterial cells) was first used by El. Sanousi(1975) instead of(Phoenix phenomenon) and other investigators such as Traci and Duncan, (1974); Shoemarker and Pieron,(1976) also used this term.

Despite the fact that Gram- negative organisms are more susceptible to freezing than Gram positive, El Sanousi (1975) reported that cold –shock

phenomenon which results in loss of viability in cultures of Gram-negative bacteria, also occurred in cultures of *Clostridium perfringens*cells at different stages of growth where subjected to cold diluents. For 1 hr cells at all stages of growth when inactivated by cold diluents. Those from the early logarithmic phase were most sensitive, viability falling by 60% over 1 hr. most of which (ca 90%) occurred in the 15 minutes. Also the ″phoenix phenomenon″ was observed with *Clostridium perfringens*, serological type 9 (HT9)in a cooked meat medium at 81.7°C by a decrease in phase count (phase I), followed by an increase in count to the initial level (phase II) and a continued increase above the initial count (phase II) . This phenomenon was reproduced in experiments with speculation-negative mutants derived from HT9 inoculate of various cell ages, and different assay media (sulfite-iron agar, tryptose –soy tone yeast extract agar, prereduced peptone –yeast extract agar, prereduced veal agar, and veal agar). When strict anaerobic conditions were employed it was necessary to increase the heating temperature to 52.3°Cto observe the phenomenon .The phenomenon was eliminated at 52.3°Cwhen a combination of strict anaerobic condition, prereduced media, and prereduced veal diluents was employed. The addition of nalidixic acid at the minimum point of the growth curve (end of phase I) had no effect on the appearance of phase II; However, phase III was completely inhibited. This indicated that phase I and II were an injury-recovery process (Shoemaker and Pieron, 1976).

2.1.11 Recent Developments in Bacterial Cold- Shock Response:

Apart from the production of antifreeze proteins (AFPs)(5) or ice-nucleation agents (JNAs)(6)after cold shock several bacterial species accumulate low molecular weight compounds known as compatible

solutes.These substances comprise polios, polyamines, sugars, amino acid derivativesetc and have been shown to protect cells of many different types from damage caused by exposure to salt coldheatand free radicals. Although their precise mode of action is still unclear.Temperature is one of the major stresses that all the living organisms have to face. Heat-shock response from bacteria to human has been extensively studied, while cold-shock response has caught attention of researchers relatively recently.A major reason why heat shock is extensively studied is because it causes well-defined damage to the cells, i.e. unfolding or denaturation of proteins. Heat shock- induced proteins, chaperones, assist in protein folding. In contrast, cold shock does not cause such well-defined cellular damage. Cold-shock response is classically exhibited when an exponentially growing culture is shifted from its optimum growth temperature to a lower temperature. In case of majority of bacteria such as *Escherichia coli*, upon temperature downshift, there is a transient arrest of cell growth, during which general protein synthesis is severely inhibited. However, synthesis of a number of proteins, called cold-shock proteins, is induced under these conditions. Eventually, the synthesis of these proteins decreases, cells become acclimated to low temperature and growth resumes (Jones *et al*., 1987). The effect of cold shock is seen at multiple levels such as:

-Decrease in the membrane fluidity affecting the membrane associated functions such as active transport and protein secretion.

-Stabilization of the secondary structures of RNA and DNA, leading to reduced efficiency of mRNA translation and transcription.

-Slow or inefficient folding of some proteins.

-Ribosome's need to be cold-adapted to function properly at low temperature. The study of cold-shock response is now in limelight because of its commercial and health implications. bacterial cold-shock response are useful in avoiding potentially disastrous situations in various

industries. Understanding cold-shock response of food-borne pathogens such as Wisterias is imperative as refrigeration is a commonly used method of food storage. Cells, which are cold-shocked prior to freezing, exhibit better cry tolerance. Therefore, food-spoilage bacteria can be sensitized to damage caused by cold temperatures through direct freezing of the food articles (Willimsky *et al*,1992).

2.1.11.1 General principles of cold-shock response:

Bacteria sense the change in temperature mainly at level of cell membrane, nucleic acid and ribosome's (Phadtare*et al*, 2000). The changes in cell membrane are discussed in detail later in this article. Various environmental factors such as changes in temperature or osmolarity, exposure to chemicals can change the extent of DNA super coiling, which in turn affects the expression of various genes. The super coiling of DNA is presumed to act as a thermo sensor and its regulation is important in order to maintain the DNA related functions, such as replication, transcription and recombination (Drlica, 1992; Higgins *et al*., 1988). The usual Negative super coiling state of DNA transiently increases after the temperature downshift (Krispin and Allmansberger, 1995; Mizushima *et al*., 1997). The arrangement between the –10 and –35 region of many promoters is affected due to this change, which in turn affects recognition of some σ^{70}promoters, for example, the cold-shock-inducible *E.coli* reach promoter is one such twist-sensitive promoter (Wang and Syvanen, 1992). It is hypothesized that organisms Sense the changes in temperature also at the level of ribosome's (VanBogelen and Neidhardt, 1990). It was shown that artificially induced high levels of the guano sine 5' triphosphate-3'diphosphate (PPGPP) and guano sine 5' diphosphate-3'diphosphate (PPGPP)collectively abbreviated as (PPGPP)diminish the expression of cold-shock proteins, while low concentration increases their production. Thus,(PPGPP)affect the

magnitude of the cold-shock response (Jones *et al*., 1992). In summary ,bacteraial cold shock responses involve the typical regulatory elements founds in other bacterial stress responses as well,but are differently organized . In contrast to the latter where alternative sigma factors play acentral regulatory role especially during intial adaptation stages,cold shock-induced synthesis of RpoS in *E.coli* and increased regulatory activity of SigB in *L.monocytogenes*, although important, rather appear to represent a long-term necessity that is only part of a complex regulatory interplay dominated by the requirement for adaptation of the translation machinery. Importantly,even though individual bacterial species display subtle differences in their adaptational needs as is conceivably the case for photosynthetic cyanobacteria,large parts of function but also regulation appear to be conserved as for example indicated by the fact that heterologous expression of CspB from *B.Subtilis* in *E.coli* results in activation of hns (Geramann, and Marahiel, 1997).

2.1.11.2 Cold shock proteins:

One of the most prominent responses of the microorganisms to cold shock is induction of cold shock proteins. All the three groups of microorganisms, i. e. psychrophiles, pedophiles and thermopiles, synthesize cold shock proteins to counteract the effect of temperature downshift. The cold-shock response and cold shock proteins have been studied in detail using *E. coli* and *Bacillus subtitles* as model systems (Ermolenko and Makhatadze, 2002; Phadtare and Inouye 1999; Phadtare*et al*, 2000; Weber and Marshier, 2003; Yamanaka *et al*., 1998).The cold shock proteins of E. coli include; group I: CSPA (Goldstein *et al*., 1990), CSPB (Lee *et al*., 1994), CSPG (Nakashima *et al*., 1996), CSPI (Wang ***et*** *al.,* 1999), CSPA (Toone *et al*., 1991), RMFA (Dammel and Noller, 1995), NusA (Friedman et al., 1984), PNP (Donovan and Kushner, 1986), and group II: RecA (Walker, 1984),

IF-2 (Gualerzi and Pon, 1990), H-NS (Dersch *et al*., 1994), GyrA (Sugino *et al*., 1977), Hsc66, HSCB (Lelivelt and Kawula, 1995), dihydrolipoamide transferees and pyruvate dehydrogenises (Jones and Inouye, 1994). The induction levels of these proteins vary, proteins belonging to group I being more dramatically induced than those from group II. CSPA, CSPB, CSPG and CSPI have been proposed to function as RNA chaperones; CSPA is a ribosomal associated protein with RNA unwinding activity. RBFA is a ribosomal binding factor, recently shown to be involved in maturation of ribosomes at low temperatures (Xia *et al*., 2001b). NusA is involved in termination and ant termination of transcription and PNP is a rib nuclease. PNP selectively degrades CSPAMRNA at 15 °Cand represses production of CspA homologues at the end of the lag phase (Neuhaus *et al*., 2000; Yamanaka and Inouye, 2001a).

2.1.11.3 CSPA family of cold shock proteins:

The first cold-shock protein, CspA, was reported from *E.coli* and its homologues have been reported from a number of gram positive and gram-negative bacteria, but not from archaea and cyan bacteria. The CspA family of *E. coli* consists of nine homologous proteins, CspA to CspI, but among them only CspA, CspB, CspG and CspIis cold-shock inducible. The functions of the CspA family members overlap since they are able to substitute for each other during cold acclimation. *E. coli* cells harboring double or triple deletions of the CSP genes (CspA, CspB, CspA, CspG, CspB, CspG, CspA, CspI or CspA.CspB.CspG) are not cold sensitive, and in the triple deletion strain, CspE is overproduced at low temperatures. On the other hand, a quadruple deletion strain (CspA.CspB.CspG.CspE) is cold-sensitive, and this defect can be complemented by overproduction of any one of CspA homologues except CspD (Xia *et al.,* 2001a). In contrast, in the case of *B. subtilis*, however, CspB/CspC/ CspD triple deletion

mutation is lethal, indicating that at least one CspA homologue is essential for the survival of the organism, CspB being the most important of the three proteins (Grummane*t al*., 1997). Regulation of expression of cold-shock induction of CspA and its homologues occurs at levels of transcription, mRNA stability and translation and has been a topic of extensive studies. The cold-shock induction of CspA does not need any additional transcription factors in contrast to heat-shock induction of proteins. One of the unique features of CspA, CspB,CspG and CspI is the unusually long 5' untranslated region (5'-UTR). The 5'-UTR contains a highly conserved unique 11-base sequence called the cold box (Jiang *et al*., 1996; Xia *et al*., 2001b). It is a presumed transcriptional pausing site and is involved in the repression of CspA expression. Based on deletion analysis, CspA 5'-UTR is presumed to be responsible for the extreme instability of CspA,MRNA at 37°C, and has positive effect on mRNA stabilization at low temperature (Mitta *et al*., 1997). CspA mRNA is dramatically but transiently stabilized (half-life more than 20 min at 15 °C as compared to half life of 12 s at 37°C) immediately following cold shock. Its promoter is active at $37C^{\circ}$, but due to instability of its mRNA, CspA is hardly detected at 37°C. Interestingly, CspAis also produced at 37 °C during early exponential growth phase and its mRNA becomes unstable by mid- to late-exponential growth phase (Brandi *et al*., 1999). This expression is attributed to the position of CspA near Orica resulting in higher gene dosage effect and high concentration of its transcription activator Fis and higher stability of its MRNA due to lower RNare activity. With increasing cell density, Fis diluted out, while, a transcriptional repressor, H- NS, accumulates leading to decline in level of CspA transcript. This is followed by rapid disappearance of the caps mRNA due to increased rate of its degradation at 37°C) (Brandi *et al*., 1999). Later it was shown that production of CspA at 37°C) during early exponential growth phase was

due to nutritional up shift and the induction level of CSPA at 37°C) was one-sixth of its cold shock induction level (Yamanaka and Inouye, 2001b). The CspA MRNA contains a unique sequence located 14-bases downstream of the initiation cordon. This element is also present in CspB, CspG, CspI, CspA and RBFA and presumed to enhance translation initiation in cold shock mRNAs. It was originally termed as the downstream box (DB). It is complementary to a region in the penultimate stem of 16SRNA and was initially thought to enhance translation initiation by facilitating the formation of translation preinitiation complex through binding to 16S rRNA, however this view is disputed and the exact mechanism of the enhancing effect on translation initiation by DB is unknown at presnt(Mitta *et al*,1997 ;Moll*et al*;2001).

2.1.12Effect of cold shock in the release of toxin:

Survival and tolerance at cold temperatures, the differentially expressed cellular proteins, and cholera toxin (CTX) production were evaluated in *Vibrio cholera* O1. Rapid lossof cultivability and change to distinct coccids morphology occurred when cultures of *V. cholera* O1were exposed to 5°Cdirectly from 35°C.Also cultures of *V. cholera* first exposed to 15°C for 2 hours and then maintained at 5C° failed to exhibit an adaptive response, instead a rapid loss of viable plate count was noticed. Results from western blot experiments revealed the absence of a major cold shock protein, CS7.4Also a decreased level of CTX was noticed in*V. cholera O1* cultures exposed to 5°C or 15 °Cafter first being exposed to 15°C for 2 hours, followed by transfer to 5°C. Reduced expression of CTX at cold temperatures, compared to the cultures maintained at 35°C, may be a result of decreased cellular metabolic activity. (Carroll, *et al*; 2001).

2.1.13 Effect of cold shock in the release of U.V absorbing aterials:

Cross damage is indicated by leakage of UV absorbing materials from the cell or increased access of molecules to be cytoplasm compartment leaked materials include K+,inorganic phosphate,phosphorylated sugars,fatty acid, esters and amino compounds. An impression of the extent of injury was obtained from the size of molecules able to pass across the membrane. The cytoplasm enzyme glucose 6-phosphate dehydrogenize was unable to escape from freeze-injured *E.coli* where as molecules of molecular mass 12KD (ribonuclease) but not 16KD (dextran) where able to enter the cell from outside (Lund *et al*; 2000) Freezing and storage of *E.coli* at -20°C in phosphate buffer resulted in loss of cell viability and a pronounced leakage of cellular material which had maximal absorption at 260 mμ.Greater loss in cell viability occurred when cells were , but only small amounts of 260mμ absorbing material were detected. Unfrozen cells stored at 2°Cand 22 °C in each men strum showed little loss in viability, but cells in phosphate buffer released significant amounts of material during storage leakage material. During storageleakage material from cells in phosphate buffer contained greater amounts of ribonucleic acid and amino acids than did material from cells in distilled water . Leakage material from frozen cells contained protein in the form of peptides of relatively small molecular weight; this was not observed unfrozen cells. These compounds protected a dilute cell suspension from the lethal effects of freezing , and also possessed biological activity for the recovery of cells which had been “injured” by freezing. Direct cell counts indicated that the material released was not a result of cell lyses (Moss and Speck, 1966).

2.1.14Repair of injured cells:

Hideharu(2006)define repair as ability of injured cells to return to normal physiological function. (Janssen and Busta 1973) reported that fast freezing and slow thawing of *Salmonella anatum* cells in nonfat milk solids resulted in about 20% death and 50% injury of the cells surviving the treatment. Death was defined as the inability to from colonies on a non-selective plating medium xylose-lysine-peptone agar (XLP) after freezing and thawing. Injury was defined as the inability to form colonies on selective plating medium (XLP with 0.2% sodium desoxycholate added). The injured cells repaired rapidly and within 2 hr at 25°C, in the presence of 0.1% milk solids, all the injured cells regained the ability to form colonies on the selective medium. The treated cells showed a 1- hr extended lag phase of growth as compared to the unfrozen cells. Milk solids concentration in freezing and repair menstrual influenced injury, repair of injury and death. The same above investigators concluded that, the repair process was affected by the pH and temperature of environment in which the injured cells were incubated. Maximum repair occurred at pH values between 6.0 and 7.0 and temperatures from 25°C, to 42°C.The data suggested repair did not require the synthesis of protein, ribonucleic acid, or cell-wall mucopeptide but did require energy synthesis.

After *Escherichia coli* was injured by freezing, the repair process was studied during incubation of the cells for 2 hours at 25°C in 0.5%K2HPO4at PH 7.0 in the presence of specific metabolic inhibitors. Data indicated that cells synthesized energy in the form of ATP and probably utilized it for the repair process. Addition of ATP also facilitated the repair of injury. The freeze-injured cells showed extreme susceptibility to surface-active agents and lysozyme. The repaired cells like the uninjured

cells became relatively resistant to these compounds. (Ray and Speck,1962).

2.1.15 The effect of selective media on recovery of injured bacterial cells:

When Morichi and lrie (2004) investigated condition facilitating recovery of sublethally injured cells present in frozen or freeze-dried preparations of bacteria, they noted that some cells of *Streptococcus facials* injured by freezing required RNA resynthesis for recovery, whereas the rest did not. Addition of 6%NaCl to control recovery medium was found to inhibit RNA synthesis completely in frozen-injured cells of *Streptococcus facials*, but not in intact ones. This seems to be a principal reason why 6% NaCl inhibits the growth of the injured cells. The same investigators reported that the recovery of viable cells of freeze-dried and stored *Streptococcus thermophlus* was greatly increased by the addition of cytosine to the planting medium. The favorable effect of cytosine was mainly due to its reducing activity. On the contrary, the addition to the plating medium of certain types of peptone, such as lactalbumin hydrolysis, resulted in a considerable decrease of viability in frozen preparations of *Vibrio metschnikovii*.

Ray and Speck (1962) recorded that freezing an aqueous suspension of*Escherichia coli* NCSM at -78°C for 10 min, followed by thawing in water at 8°C for 30 minute, resulted in the death of approximately 50% of the cells, as determined by their inability to form colonies on Trypticase soy agar containing 0.3% yeast extract (TSYA).Among the survivors, more than 90% of the cells were injured, as they failed to form colonies on TSYA containing 0.1% desoxycholate.

They also concluded that injury was reversible as the injured cells repaired in many suitable media. The rate of repair is maximum in a

complex nutrient medium such as Trypticase soy broth supplement with yeast extract.

2.1.16Pathogencity of Injured cells:

In (1990) MacFadden stated that "pathogenic bacteria probably do not lose their virulence by freezing''. Freezing -75°Cand storage -20°Cof a cell suspension of *Salmonella gallinarum* and not a stable mutation. Penicillin was used to increase the ratio of injured to uninjured cells from a frozen and thawed cell suspension. Pathogen city was evaluated by observing per cent mortality after injecting injured or uninjured cells into separate sets of chicks. Mortality differences between wholly uninjured and resulted in a heterogeneous population of dead, metabolically injured, and unharmed cells. Injured cells constituted as much as 40% of those surviving freezing and storage for 1 day. Replica plating of frozen and thawed cells indicated metabolic injury was repairable predominantly injured populations were small and consistent (5% level) with a hypothesis of no difference.(Sorrels *et al* ; 1970). As in many bacteria, including such pathogens as *Salmonella enteritidis*, enterotoxic *Escherichia coli*, *Vibrio vulnifcus*, *Vibrio cholere*, the occurrence of viable but not-n-culturable (VBNC) state has been described for *Campylobacter jejuni.*This bacterium has been recognized as a leading food borne pathogen.VBNC cells from three *Compylobacterjejuni* human isolates were suspended in microcosm water at 4°C and entered the VBNC state.

The metabolic activity of these VBNC cells was monitored by CTC reduction. Once in VBNC state, the strains were inoculated per so into newborn mice and 1-day old chicks so as evaluate and compare these animal models of VBNC cell recovery. The three strains used were revived with chick model. The results showed that the marine model permits better recovery than the 1-day chick model. All three strains revived exhibited an

associative index very similar to that measured in cultural state. This study indicates that the VBNC state should be considered asplaying a role in the epidemiology of *Campylobacte*r infection, Cappelier *et al*(1999) Raw milk and fresh product have been implicated, Adam and Moss,(2008),Nagla(2010).

2.1.17 Vitamin B_{12}

Vitamin B12, also called cobalomine, is a water soluble vitamin with a key role in the normal functioning of the brain and nervous system, and for the formation of blood. It is one of the eight B vitamins. It is normally involved in the metabolism of every cell of the human body, especially affecting DNA synthesis and regulation, but also fatty acid synthesis and energy production. It is the largest and most structurally complicated vitamin and can be produced industrially only through bacterial fermentation-synthesis.

Vitamin B12 consists of a class of chemically-related compounds (vitamins), all of which have vitamin activity. It contains the biochemically rare element cobalt. Biosynthesis of the basic structure of the vitamin in nature is only accomplished by simple organisms such as some bacteria and algae, but conversion between different forms of the vitamin can be accomplished in the human body. A common synthetic form of the vitamin, cyanocobalamin, does not occur in nature, but is used in many pharmaceuticals and supplements, and as a food additive, because of its stability and lower cost. In the body it is converted to the physiological forms, methylcobalamin and adenosylcobalamin, leaving behind the cyanide, albeit in minimal concentration. More recently, hydroxocobalamin (a form produced by bacteria), methylcobalamin, and adenosylcobalamin can also be found in more expensive pharmacological products and food supplements. The extra utility of these is currently debated.

Vitamin B_{12} was discovered from its relationship to the disease pernicious anemia, which is an autoimmune disease in which parietal cells of the stomach responsible for secreting intrinsic factor are destroyed. Intrinsic factor is crucial for the normal absorption of B_{12}, so a lack of intrinsic factor, as seen in pernicious anemia, causes a vitamin B12 deficiency. Many other subtler kinds of vitamin B_{12} deficiency and their biochemical effects have since been elucidated.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Methodology:

3.1.1 *SalmonellaSSP.*

Twenty-five of the sausage was added to 225ml of the buffer peptone water and mixed with stomacher bag for 2 min and incubated at 37°Cfor 18 hr.After incubation, 10ml of culture was transferred to tube containing 90ml (MSRV)andincubated at 41.5°C. For 24 hr. After incubation in MSRV samples of culture were placed on XLD Agar to obtain isolated colonies. Petri dishes were incubated at37°C.for24h. The plates were examined for the presence of typical colonies of *Salmonella* which have a black center and lightly transparent zone of reddish color due to the color change of the indicator.

3.1.2 BiochemicalTests :

The selected colonies were streaked on to the surface of pre,dried nutrient agar plates and incubated at37°Cfor 24.Pure cultures were used for confirmation.

3.1.2.1 TSI Agar:

The agar slant surface and the buttstabbed streakedwith sterile loop Incubated at 37°C.for 24h. Interpret the changes the medium as follows. Slant (red) butt (yellow) with gas formation of hydrogen sulfide (blackening of the agar).

3.1.2.2 Urea agar

Agar slant surfacewasstreaked andincubated at 37°Cfor24hand examined at intervals (ISO 6579_2007).

3.1.2.*E .coli*:

Twenty -five gram of the sausage was added to 225ml of peptone water and mixed with stomacher bag in 2min,the final suspension is 1:10 dilution of the sample (john) 1ml of the initial suspension was added to 9ml of single strength lauryl sulfate broth. 1ml of initial suspension was transferred in to each of three tubes of single strength liquid selection enrichment medium (lauryl sulfate broth) i.e.: (10^{-1}, 10^{-2}, 10^{-3}). The set of the test tube was incubated at 37°C...after 48h incubation period for each dilution 10^{-1}, 10^{-2},10^{-3}. The number of positive result tube of single-strength medium was counted.Full loop of suspension from each tube was transferred to a new sterile tube containing 10ml of a liquid selective medium (E .C broth) Incubated at 44°C.for 24h.Full loop of suspension from each tubewastransferred to new sterile tube contain 5ml of peptone water. Incubated at 44°C.h for 84h after the incubation period checks the result.

3.1.2.1indole production:

0.5ml of indole reagent was added to tubes of peptone water which was mixed well and examined after 1minacolor in alcoholic phase the presence of Indole (ISO7251:2005).

3.1.3*Staphylococcusaureus*:

Twenty -five grams of the sausage was added to 225ml of the peptone water and was mixed with stomacher bagfor 2min, the final suspension is

1:10 dilution of the sample. Prepared 1:100, 1:1000, dilution of the sample wastransferred 0.1ml of each dilution was transferred on Petri dishes containing Baird-Parker agar and speeded with sterile bend glass rod. and incubated at 37°C for48h. After that the numbers of coagulase-positive *Staphylococcus*.was counted .

3.1.3.1 Brain heart infusion broth:

From the surface of each selected colony,inoculumswas removed with sterile wire and transferred it to tube of brain-heart infusion broth, (ISO 6888-1).

3.1.3.2 Incubated at 37°C for 24h:

0.1ml of each culturewasaseptically added to 0.3ml of to rabbit plasmain sterile haemolysis tubes and incubated at 37°C.Clotting of the plasma was examinedby tilting the tube after 4 h to 6 h of incubationand ,if the test was negative,re-examination at 24 h of incubation, ore examine at the incubation times as specified by manufacturer.

3.1.4 Recovery media:

3.1.4.1 Sampling:

Samples were collected from the final product of sausage, from different factories in Khartoum State.

3.1.4.2 Organisms:

E .coli was isolated from fresh sausages before frozen and the isolate was stored in nutrient agar slant.

3 .1. 4.3 Media

The following media were used for growth and recovery of injured cells .

1. EMB Agar: Consisted of different concentrates (0.5% 1% 1.5%) sodium pyruvate .
2. EMB Agar: Consisted of different concentrates (1000 mg/L،2000mg/L 3000mg/L) Vitamin B_{12}.
3. EMB Agar: Consisted of different concentrates (50mg/L ،100mg/L،150mg/L) Vitamin B_6 .

3.1.4.4 Method:

For recovery mediatwo steps were followed:-

3.1.4.4.1 Before shock:

Different concentrates of sodium pyruvate, vitamins B_{12} and B_6were added to sterilized EMB agar medium andcooled at 45 – 50 °C.

About 15-25 ml were poured in each sterilize Petridishes and allowed to solidity.A full loop of *E. coli* suspensionwas prepared in test tube containing peptone water, and incubated at 37°C for 24h.

1 ml of the suspension was added to 9 ml normal saline and serial dilutionswereprepared 10^{-1} , 10^{-2}, 10^{-3}….ext .From above serial dilution 0.1 ml was inoculated in EMB agar prepared with different concentration additives before the shock and incubated at 37 °Cfor 24 hrs.

3.1.4.4.2 Aftershock:

Serial dilutions as mensioned in above were shocked for I h at -20 °C.

From above serial dilution0.1 ml inculcated in EMB agar prepared with different concentration additives after the shock and incubated at 37 °C. for 24 hrs.

The same procedures was followed for shocked cells at -30 °C. and -40 °C

3.1.5 Calculation of the bacterial:

Calculation of the bacterial count after and before shock.

Calculation of the bacterial loss.

Calculation of the bacterial recovery.

3.1.6 Statistical analysis

Statistical analysis was carried out usinganova .

CHAPTER FOUR

4. RESULTS

4.1 The recovery of *E.coli* using vitamin B_6 in EMB at -20°C,-30°C and -40°C:

In this study , the effect of freezing on the loss of *E . coli* at -20 °C(*table* 2)in EMB medium without addition B6 losswas 2.7% and the recovery was97.3% . Loss and recovery with addition B6 ,(concentration 1 ml)the loss was 1.2% and recovery was 98.8%.In 2 ml the loss was1.7% and recovery was98.3% ,In 3 ml the loss was 1.7% and recovery was 98.3% .

At-30°C(table 3)in EMB medium without addition B_6 the loss was 13.4% and the recovery was 86.6% . Loss and recovery with addition of B6(concentration 1 ml) the loss was 16.8% and recovery was 83.2% . In 2 ml the loss was 16.9% and recovery was 83.1% . In 3 ml the loss was 17.9% and recovery was 82.1% .

At -40 °C (table 4) in EMB medium without addition B_6 the loss was18.3% and the recovery was 81.7%. Loss and recovery with addition of B6 (concentration 1 ml) loss was 20.7 % and recovery was 79.3%. In 2 ml the loss was22.9% and recovery was 77.1% .In 3 ml the loss was 21.6% and recovery was78.4% .

(4.2)The recovery of *E.coli* using vitamin B_{12} in theEMB at -20°C ,-30°C and -40 °C.

In this study, the effect of freezing on the loss of *E. coli* at -20 °C(table 5)in EMB medium without addition B12 the loss was 6.5% and the recovery was93.5. % . Loss and recovery with addition B12, (concentration

1 ml)the loss was 4.7% and recovery was 95.3%.In 2 ml the loss was4.6% and recovery

Table (4.1): Count and presence of bacteria from sausage before freezing**:**

Factores	Bacterial count	Coliforms	E.coli	Staph	Salmonella
A	$3.08x10^9$	$2.4x10^4$	+ve	Nil	-ve
B	$Unconut \times 10^7$	$2.4x10^4$	+ve	$3x10^3$	+ve
C	$6.2x10^9$	$2.4x10^4$	+ve	Nil	-ve
D	$3.7x10^5$	$4.6x10^3$	-ve	Nil	-ve

Table(4.2): Effect of addition of vitamin B_6 on growth of *E .coli* in EMB medium, before and after cold shock at-20°C .

Conditions	EMB Medium (cfu/g)	EMBwithvitaminB_6(ml/l) concentrations		
		1ml	2ml	3ml
Before	7.515	7.465	7.43	7.395
After	7.32	7.365	7.3	7.265
Bacterial loss	2.7%	1.2%	1.7%	1.7%
Recovery	97.3%	98.8%	98.3%	98.8%

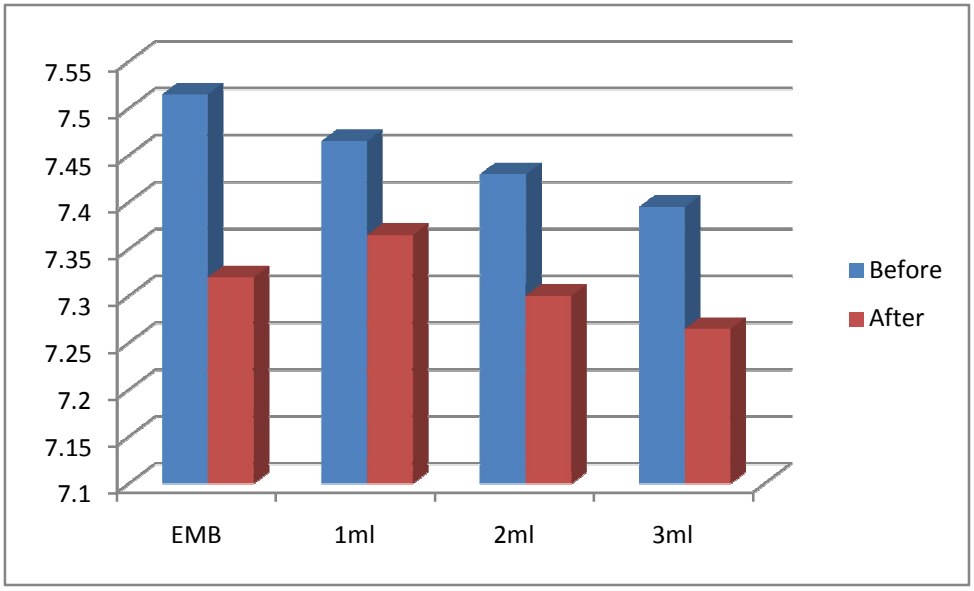


Table (4.3): Effect of addition of vitamin B_6 on growth of *E.coli* in EMB medium, before and after cold shock at -30°C

Conditions	EMB Medium (cfu/g)	EMBwithvitaminB_6(ml/l concentrations		
		1ml	2ml	3ml
Before	7.445	7.49	7.5	7.45
After	6.45	6.23	6.215	6.115
Bacterial loss	13.4%	16.8%	16.9%	17.9%
Recovery	86.6%	83.2%	83.1%	82.1%

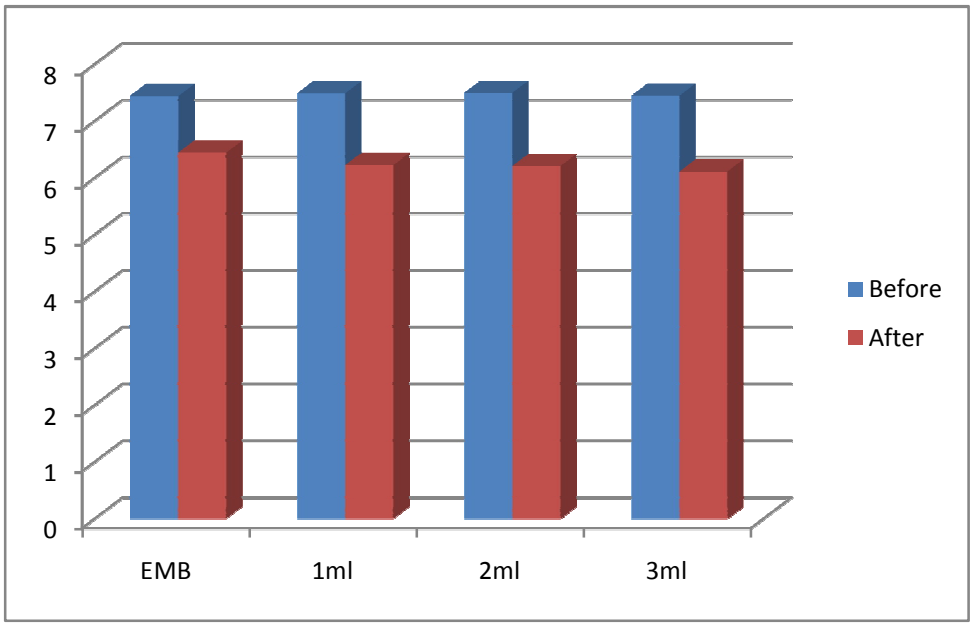


Table (4.4): Effect of addition of vitamin B6 on growth of *E.coli* in EMB medium ,before and after cold shock at-40°C .

Conditions	EMB Medium (cfu/g)	EMBwithvitaminB6(ml/l) concentrations		
		1ml	2ml	3ml
Before	7.485	7.44	7.49	7.455
After	6.1	5.89	5.775	5.845
Bacterial loss	18.3%	20.7. %	22.9%	21.6%
Recovery	81.7%	79.3%	77.1%	78.4%

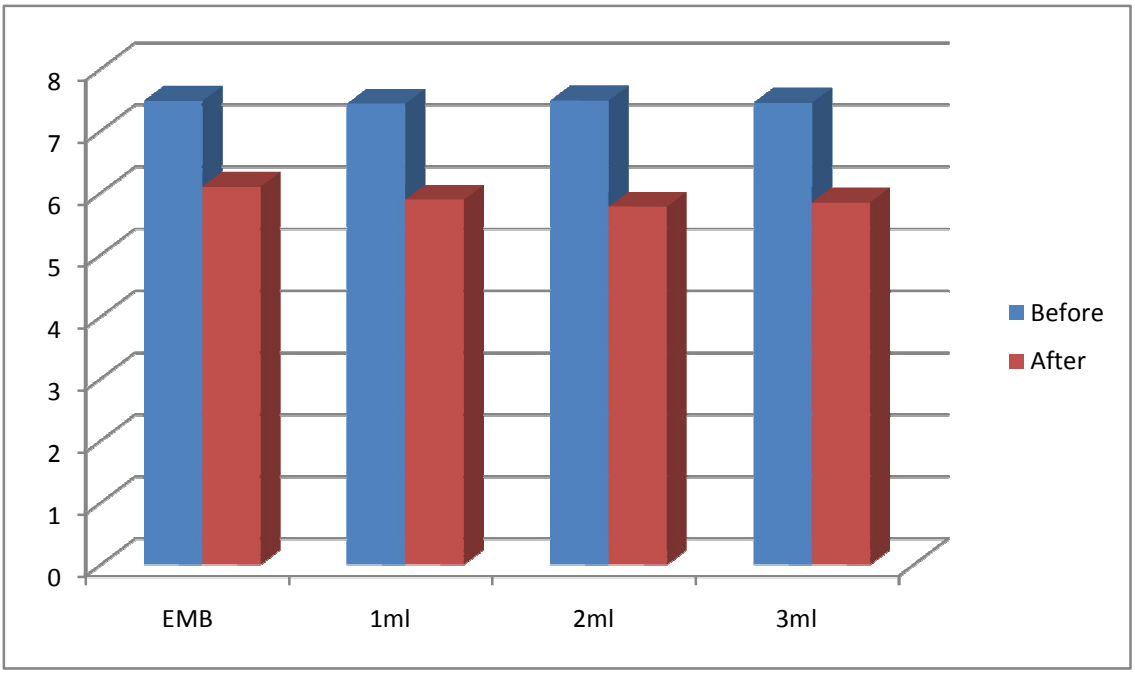
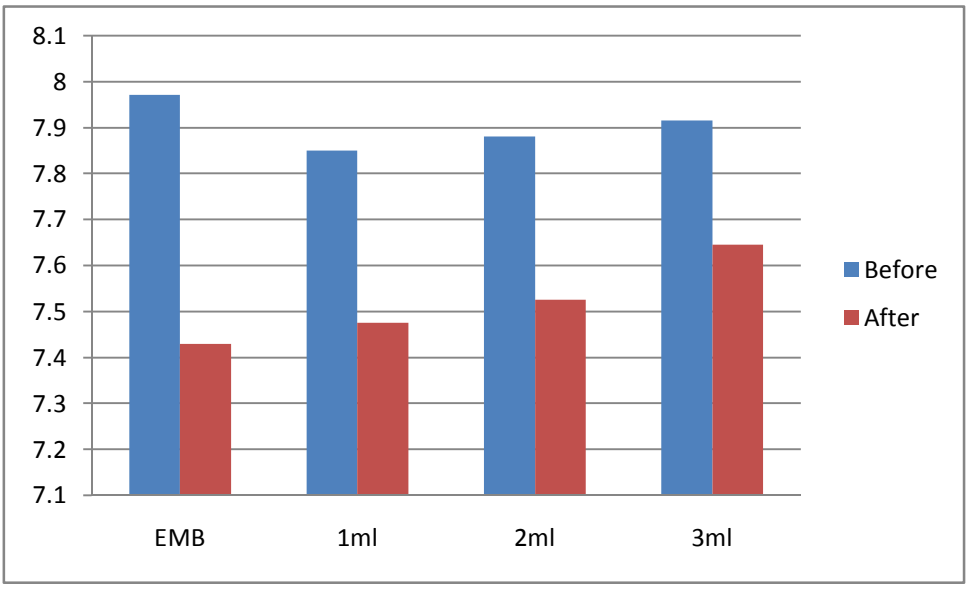


Table (4.5): Effect of addition of vitamin B_{12} on growth of *E.coli* in EMB medium ,before and after cold shock at-20℃.

Conditions	EMB Medium (cfu/g)	EMBwithvitaminB$_{12}$(ml/l) concentrations		
		1ml	2ml	3ml
Before	7.97	7.85	7.88	7.915
After	7.43	7.475	7.525	7.645
Bacterial loss	6.5%	4.7%	4.6%	3.4%
Recovery	93.5%	95.3	95.4%	96.6%



was95.4%, In 3 ml the loss was 3.4% and recovery was 96.6%. At -30 °C (table 6) in EMB medium without addition B_{12} loss was 15% and the recovery was 85% . Loss and recovery with addition of B_{12} (concentration 1 ml) the loss was 12.6% and recovery was 87.4%. In 2 ml the loss was 13.2% and recovery was 86.8%. In 3 ml the loss was 4.8% and recovery was 95.2%. At -40 °C (table 7) in EMB medium without addition B12 the loss was 2.7% and the recovery was 97.3%. Loss and recovery with addition of B12 (concentration 1 ml)the loss was 1.8 % and recovery was 98.2%. In 2 mlthe loss was 1.5% and recovery was 98.5% .In 3 ml the loss was 1% and recovery was 99% .

4 .3 The recovery of E.coli using sodium pyruvate in EMB at -20℃,-30℃ and-40℃.

In this study , the effect of freezing on the loss of E. coli at -20℃ (table 8)in EMB medium without addition of sodium pyruvate was 2.2% and the recovery was 97.8% .Loss and recovery with addition,(concentration 0.5%) the loss was 5.2% and recovery was 94.8% .In1% the loss was 1% and recovery was 99% .In the 1.5% the loss was 1.1% and recovery was 98.9%.

At -30℃ (table 9)in EMB medium without addition of sodium pyruvate was 3.7% and the recovery was 96.3%. Loss and recovery with addition(concentration0.5%) the loss was1.5% and recovery was 98.5%.In 1% the loss was 0.3% and recovery was 99.7% and concentration 1.5% the loss was 1.2% and recovery was 98.8% .

At-40℃ (*table* 10) in EMB medium without addition of sodium pyruvate the loss was 5.1% and the recovery was 94.9%.Lossand recovery with addition(concentration 0 .5%)the loss11.3% and recovery was 88.7%.In

1% theloss was16.2%and recovery was 83.8% .In 1.5%the loss was 14.3%and recovery was85.7% .

Table (4.6): The effect of addition of vitB_{12} on growth of *E.coli* in EMB medium, before and after cold shock at -30℃.

Conditions	EMB Medium (cfu/g)	EMBwithvitaminsB12(ml/l) concentrations		
		1ml	2ml	3ml
Before	7.815	7.755	7.805	7.04
After	6.95	6.75	6.775	6.7
Bacterial loss	15%	12.6%	13.2%	4.8%
Recovery	85%	87.4%	86.8%	95.2%

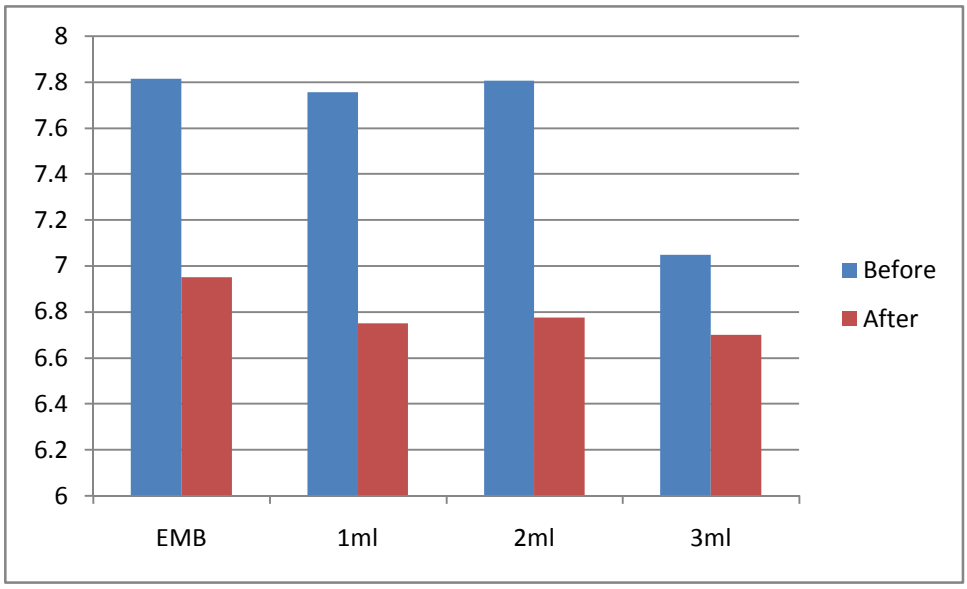


Table (4.7): Effect of addition of vitB_{12} on growth of *E.coli* in EMB medium,

Before and after cold shock at-40℃.

Conditions	EMB Medium (cfu/g)	EMBwithvitaminB_{12}(ml/l) concretions		
		1ml	2ml	3ml
Before	7.39	7.515	7.47	7.54
After	7.205	7.375	7.37	7.52
Bacterial loss	2.7%	1.8%	1.5%	1%
Recovery	97.3%	98.2%	98.5%	99%g

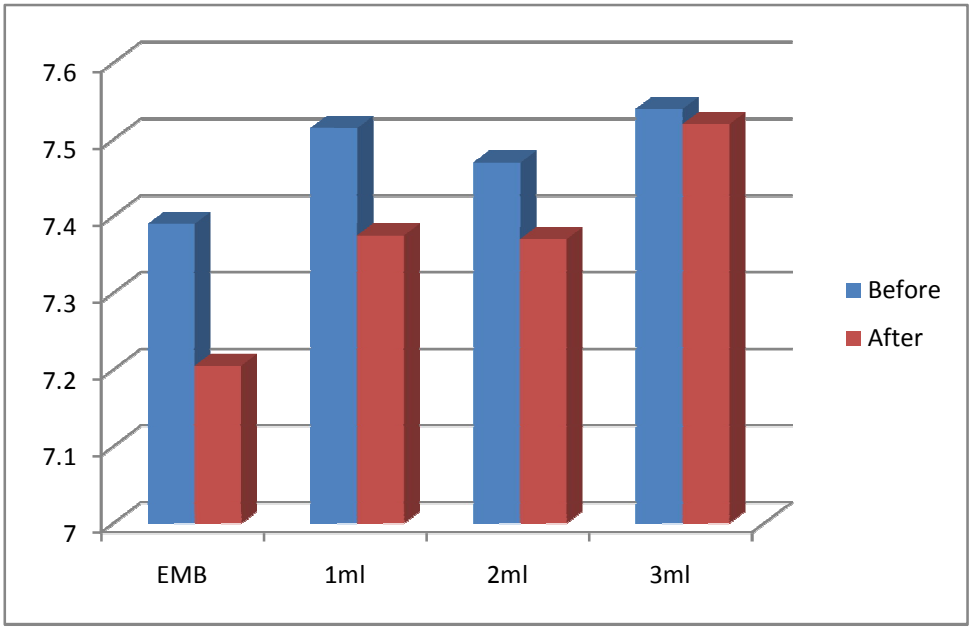


Table (4.8): Effect of addition of sodium pyruvate on growth of *E.coli*in EMB medium ,before and after cold shock at-20℃.

Conditions	EMB Medium (cfu/g)	EMB medium with Sodium pyruvate(g%) concentration		
		0.5%	1.0%	1.5%
Before	7.34	7.43	7.27	7.56
After	7.17	7.04	7.2	7.49
Bacterial loss	2.2%	5.2%	1%	1.1%
Recovery	97.8%	94.8%	99%	98.8%

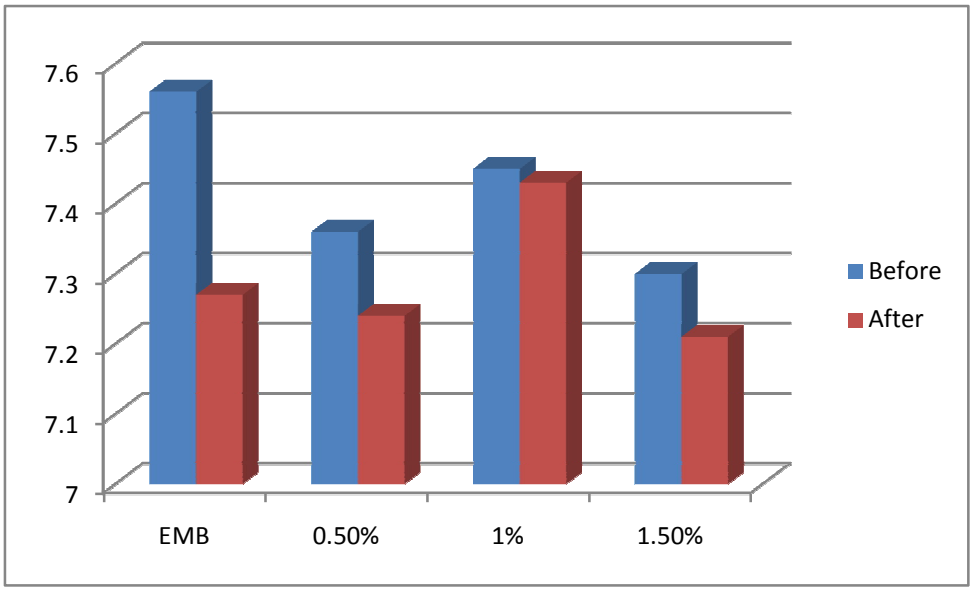


Table (4.9): Effect of addition of sodium pyruvate on growth of *E.coli* in EMB medium, before and after cold shock at -30℃

Conditions	EMB Medium (cfu/g)	EMB medium with Sodium pyruvate (g%) concentration		
		0.5%	1.0%	1.5%
Before	7.56	7.36	7.45	7.3
After	7.28	7.25	7.43	7.21
Bacterial loss	3.7%	1.5%	0.3%	1.2%
Recovery	96.3%	98.5%	99.7%	98.8%

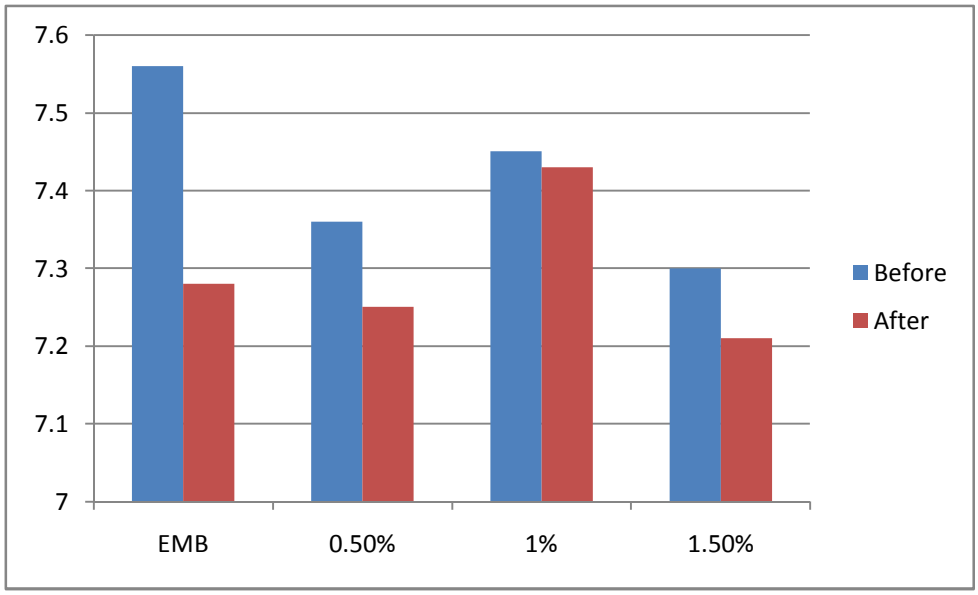
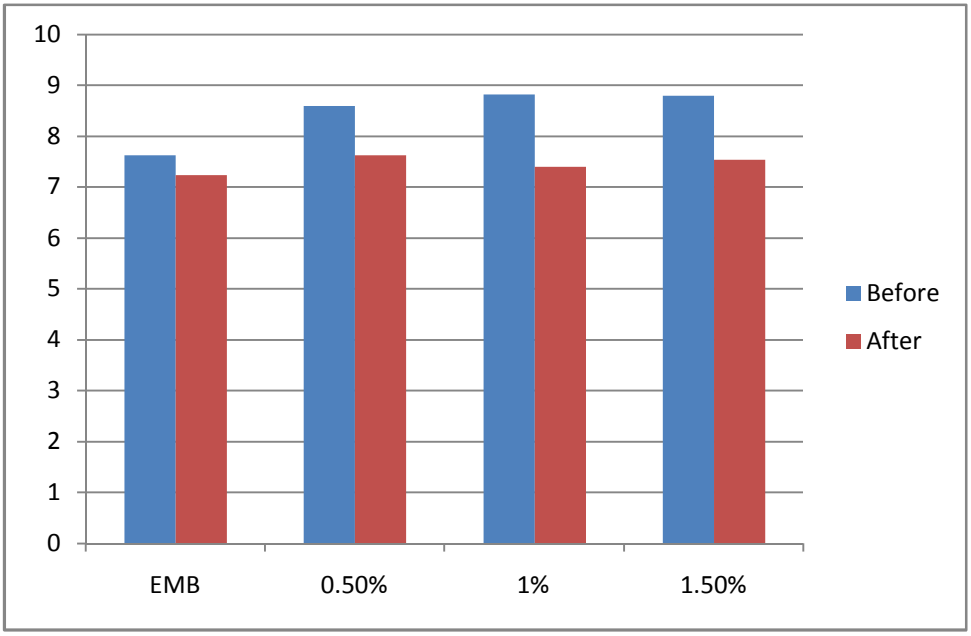


Table (4.10): Effect of addition of sodium pyruvate on growth of *E.coli* in EMB medium, before and after cold shock at-40℃.

Conditions	EMB Medium (cfu/g)	EMB medium with Sodium pyruvate (g%) concentration		
		0.5%	1.0%	1.5%
Before	7.62	8.59	8.82	8.79
After	7.23	7.62	7.39	7.53
Bacterial loss	5.1%	11.3%	16.2%	14.3%
Recovery	94.9%	88.7%	83.8%	85.7%



4.4 The recovery of *E.coli* using sodium pyruvate and B_6in EMB at -20℃, − 30℃and- 40℃.

In this study the effect of freezingon the loss of *E. coli* at -20 ℃ (*table* 11) in EMB medium without addition sodium pyruvate and vit B_6 the loss was12% and the recovery was88%.Loss and recovery with addition(concentration.5% +1ml)the loss was 5% and the recovery was95%.In (1% +2 ml)the loss was 3.4%and the recovery was 96.6% and at the concentration (1.5%+ 3ml)the loss was1.7% recovery was98.3%.

At -30 ℃ (*table* 12)in EMB mediumwithout additionon sodium pyruvate and vit B6 the loss was 8.9% and the recovery was 91.1%.Loss and the recovery with addition (concentration 0.5% +1ml) the loss was 7% and the recovery was 93%.In (concentration 1% +2 ml) the loss was7'1% and the recovery was 92.9%.In(concentration 1.5%+ 3ml) the loss was 8%and the recovery was92%.

At -40 ℃ (*table* 13) in EMB medium without addition on sodium pyruvate and vit B6 the loss was 25.5% and the recovery was 74.5%.Loss and the recovery with addition(concentration0.5% +1ml) the loss was 19.9% and the recovery was79.1% .In(concentration 1% +2 ml) the loss was 22%and the recovery was 78% .In(concentration 1.5%+ 3ml) the loss was 19.1% and the recovery was79.8% .

4.5 The recovery of *E.coli* using sodium pyruvate and B_{12} in EMB at -20℃,-30℃and-40℃.

In this study the effect of freezing on the loss of *E.coli* at -20℃ (*table* 14) in E.MB medium without addition ofsodium pyruvate and vit B_{12} the loss was 5% and the recovery was 95%.Loss and recovery with addition(concentration0.5% +1ml,) the loss was 6%and the recovery was94%.In (concentration 1% +2 ml) the loss was 3.6% and the recovery

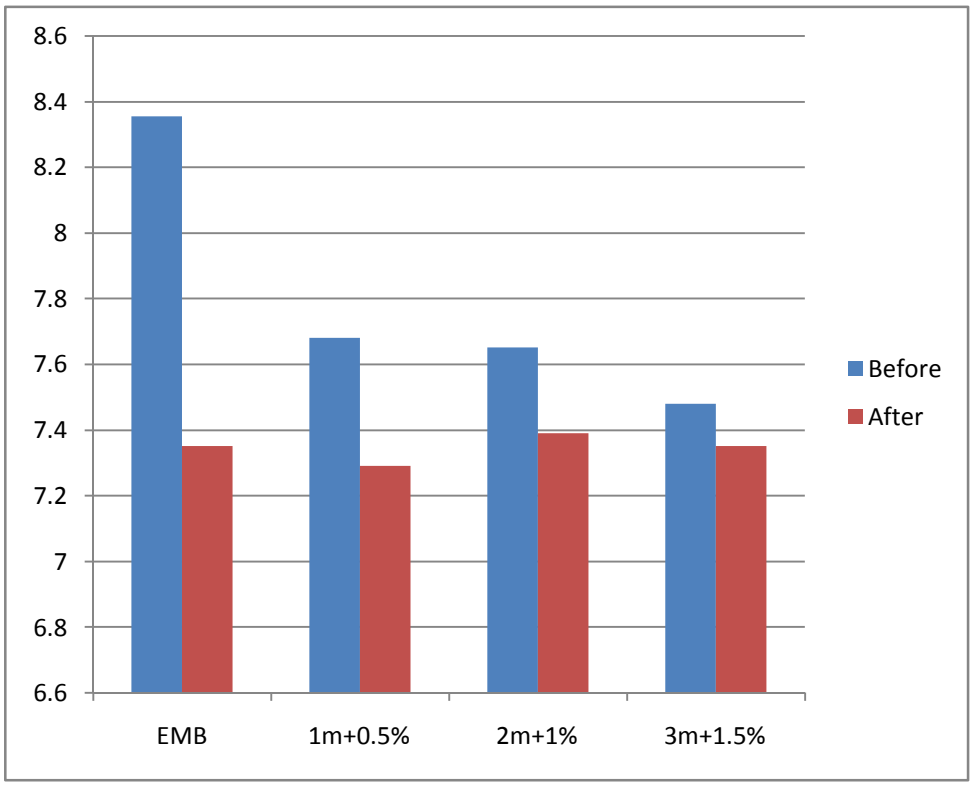
was 96.4%.In (concentration 1.5%+ 3ml)the losswas 2.8%and the recovery was 97.2%.

At -30℃ ($table$ 15) in EMBwithout additionsodium pyruvate and vit$B_{12}$the loss was 6.2% and the recovery was 93.8%.Loss and recovery with addition(concentration0.5% +1ml,) the loss was6.3%and the recovery was 93.7%.In (concentration 1% +2 ml) the loss was 10.1%and the recovery was89.9% .In(concentration 1.5%+ 3ml) the loss was 6.2% and the recovery was 93.8% .

At -40℃ ($table$16) inEMB without addition sodium pyruvate and vit $B_{12}$the loss was 8.4% and the recovery was 91.6% .Loss and the recovery with addition(concentration0.5% +1ml,)the loss was24.6% and the recovery was75.4%.In(concentration 1% +2 ml) the loss was22.2% and the recovery was77.8% and (concentration 1.5%+ 3ml) the losswas 23.5%and the recovery was .76.5% .

Table (4.11): Effect of addition of sodium pyruvate and vitB_6 (gm%+ml/L) on growth of *E.coli* in EMB medium, before and after cold shock at -20℃**.**

Conditions	EMB Medium (cfu/g)	EMB medium with Sodium pyruvate and vitB_6(g%+m/l) concentration		
		1ml+0.5%	2ml+1.0%	3ml+1.5%
Before	8.355	7.68	7.65	7.48
After	7.35	7.29	7.39	7.35
Bacterial loss	12%	5%	3.4%	1.7%
Recovery	88%	95%	96.6%	98.3%



Table(4.12):Effect of addition of sodium pyruvate and vitB$_6$ (g%+ml/L) on growth of *E.coli* in EMB medium, before and after cold shock at -30℃.

Conditions	EMB Medium (cfu/g)	EMB medium with Sodium pyruvate and vitB$_6$(g%+ml/l) concentration		
		1ml+0.5%	2ml+1.0%	3ml+1.5 %
Before	7.76	7.74	7.71	7.87
After	7.07	7.2	7.16	7.24
Bacterial loss	8.9%	7%	7.1%	8%
Recovery	91.1%	93%	92.9%	92%

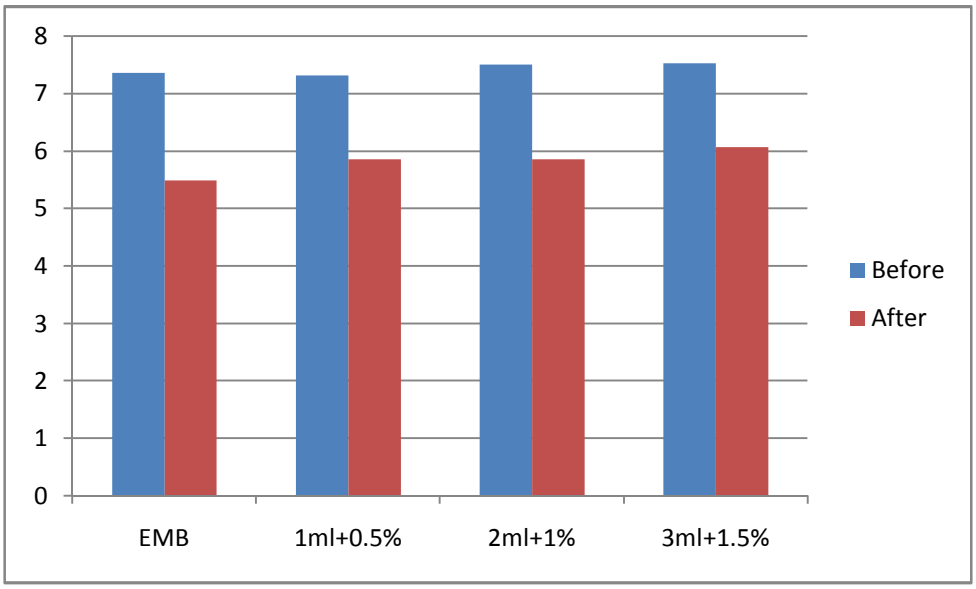


Table (4.13): Effect of addition of sodium pyruvate and vitB_6 (g%+ml/L) on growth of *E.coli* in EMB medium, before and after cold shock at -40°C

Conditions	EMB Medium (cfu/g)	EMB medium with sodium pyruvate and B_6(g%+ml/L) concentration		
		2ml+1.0%	1ml+0.5%	3ml+1.5%
Before	7.36	7.31	7.5	7.52
After	5.48	5.85	5.85	6.06
Bacterial loss	25.5%	19.9%	22%	19.1%
Recovery	74.5%	80.1	78%	80.9%

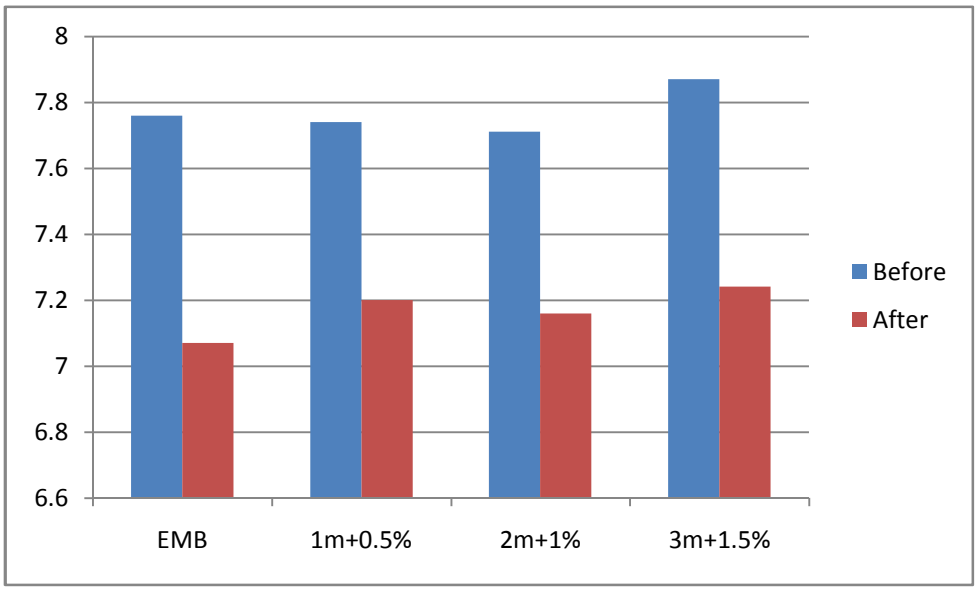
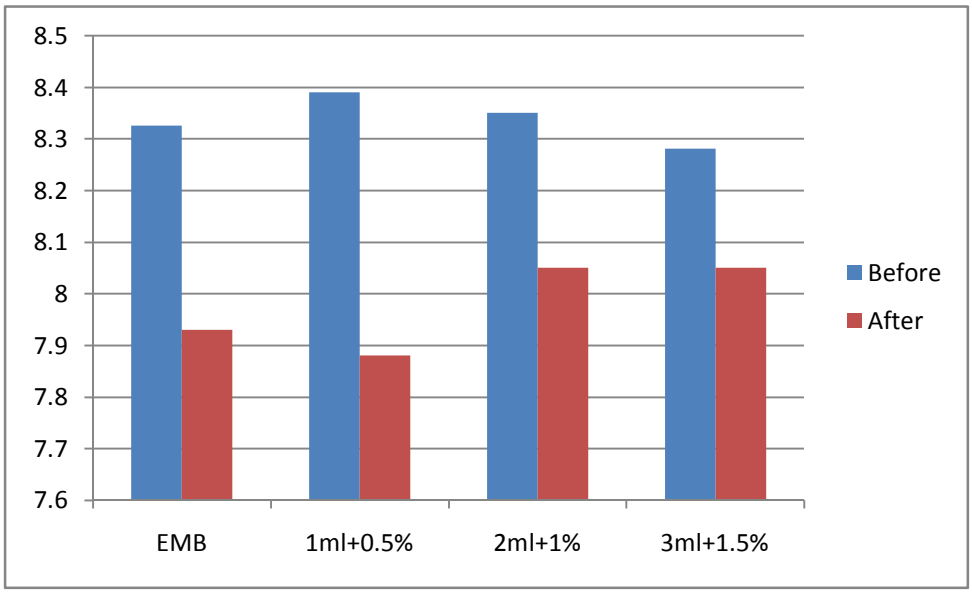


Table (4.14): Effect of addition of sodium pyruvate and vitaminB_{12} (gm%+ml/L) on growth of *E.coli* in EMB medium, before and after cold shock at -20°C.

Conditions	EMB Medium (cfu/g)	EMB medium with sodium pyruvate and B_{12}(g%+ml/L) concentration		
		1ml+0.5%	2ml+1%	3ml+1.5%
Before	8.325	8.39	8.35	8.28
After	7.93	7.88	8.05	8.05
Bacterial loss	5%	6%	3.6%	2.8%
Recovery	95%	94%	96.4%	97.2%



Table(4.15): Effect of addition of sodium pyruvate and vitB_{12} (gm%+ml/L) on growth of *E.coli* in EMB medium, before and after cold shock at -30°C.

Conditions	EMB Medium (cfu/g)	EMB medium with sodium pyruvate and B_{12}(g%+ml/L) concentration		
		1ml+0.5%	2ml+1%	3ml+1.5%
Before	8.38	8.21	8.45	8.08
After	7.85	7.69	7.59	7.58
Bacterial loss	6.2%	6.3%	10.1%	6.2%
Recovery	93.8%	93.7%	89.9%	93.8%

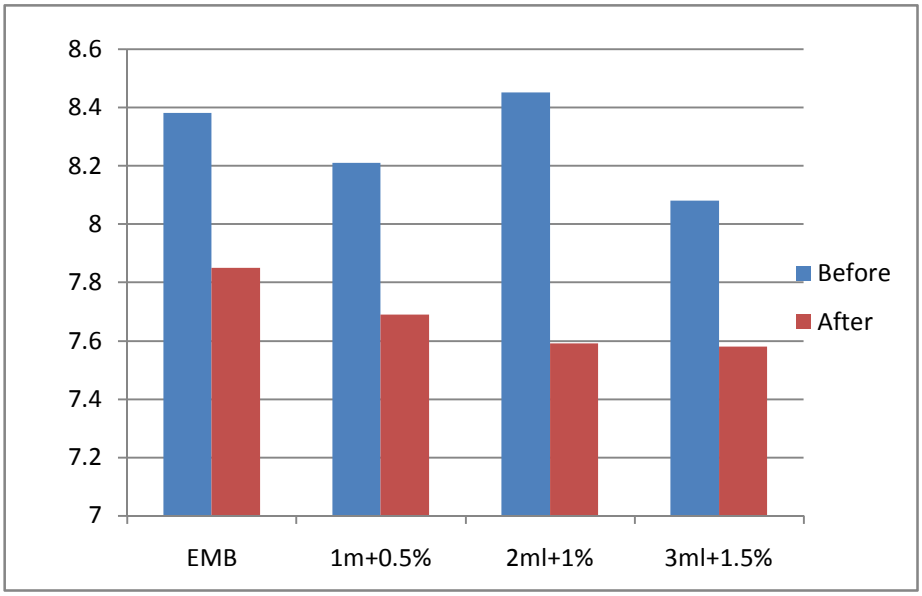
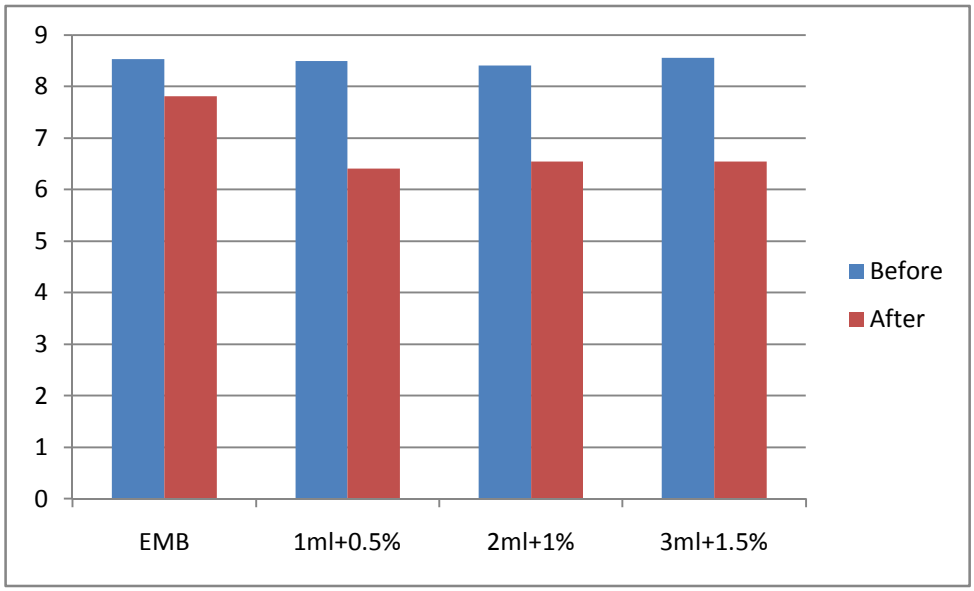


Table (4.16): Effect of addition of sodium pyruvate and vitB_{12} (g%+ml/L) on growth of *E.coli* in EMB medium, before and after cold shock at -40°C

Conditions	EMB Medium (cfu/g)	EMB medium with sodium pyruvate and B_{12}(g%+ml/L) concentration		
		1ml+0.5%	2ml+1%	3ml+1.5%
Before	8.53	8.49	8.41	8.55
After	7.81	6.4	6.54	6.54
Bacterial loss	8.4%	24.6%	22.2%	23.5%
Recovery	91.6%	75.4%	77.8%	76.5%



CHAPTER FIVE

5 .Discussion ,Conclusionand Recommendations

5 -1. Discussion

Meat surface is usually heavily contaminated with wide range of microorganisms and due to its high nutritional value (water, proteins , peptides , nucleotides , sugars , minerals and vitamin) it is a suitable medium for the development of most bacteria (Steinhauser ,1995) . One of the most important methods for quality control is using Sudanese microbiological standards for foods. These examinations include bacterial count, *E.coli*, *salmonella* , coaqulase +veStaph. (SDS 335 :2007) In this study *E. coli*, *salmonella* and *S.aureus* , were isolated from sausages collected from the different factories in the Khartoum state .Infactories A, B and C, the bacterial count and *E.coli* were found to be above the acceptable range of Sudanese microbiological limits for fresh sausage.*Salmonella* and*S.aurues*were isolated from factory B.Bacteria count was found acceptable in factory D.(table 1).*E.coli ,salmonella* and *S.aurues*isolatedfrom processed beef samples (minced meat , burger and sausage) the bacterial counts were found to be above the acceptable range of Sudanese microbiological limits for processed meat.Amanie 2000reported that spices and other additives were found to be a high source of microbial contamination.Cold shock also affects cell division . The temperature downshift results in a growth lag . During the lag phase the organism changes the composition of the cytoplasmic membrane and synthesis sets of specific proteins called cold shock proteins or cold induced proteins (Nuray and Ferhan , 2001) .Ray and Speck (1972) stated that freezing an aqueous suspension of *E.coli* in NCSM at -78°Cfor 10 min, followed by thawing in water at 8°C for 30 min , resulted in the death of

approximately 50% of the cells . There is some work of cold shock was carried out for gram positive bacteria , like the bacteria *Staph.aureus* Cells of *Staph. aureus* were almost resistant when subjected to freezing at -02°C .The results are agreement with those of Egziabber *et al* .(1982). That means cold shock affects both gram negative and gram positive bacteria . As far as vitamin B6 is concerned we could not trace similar work .

Fatima (1990) found that freezing at -20 °C injured bacterial cells. Lyla (1987) found that at -20 °C the death of *E.coli* and *S.aureus* was proportional to the storage time. Nagla (2010) reported that death of *E.coli* cells from freezing at -20°C was 55.4%, 14.8% of the cells were injured and 29.8% were uninjured cells. Ray and Speck (1972) stated that freezing an aqueous suspension of *E.coli in*NCSM at -78°C for 10 min, followed by thawing in water at 8°C for 30 resulted in the death of approximately 50% of the cells min.The results are agreement with those of Egziabber et al .(1982). That means cold shock affects both Gram negative and Gram positive bacteria . As far as vitamin B_6 is concerned we could not trace similar work.There is some work of cold shock was carried out for Gram positive bacteria , like the bacteria *Staph . aureus* . Cells of *Staph. aureus* were almost resistant when subjected to freezing at -20°C .The results are agreement with those of Egziabber *et al* .(1982). That means cold shock affects both Gram negative and Gram positive bacteria . As far as is concerned we could not trace similar work .Lyla 1987repoted that studies on effect of the curing agents on the injured cells of*E.coli,Staphylococcus aureus*, *C.perfringens* and *Ps.aeruginosa* have shown that these agents act in similar manner as the selective agents and they have an adverse effect on the injured cells i.e . freeze –injured cells were found to be more selective to the action of sodium chloride and sodium nitrate as compared with the uninjured cells.These two curing salts retarded the rate of growth of injured

cells as compared with non injured cells and also inhibited the growth of the injured cells concentrations lower than that needed for control cells.Brewer *et al*. 1977 found at addition of pyruvate and catalase to the selective media has equal effect. In this studySodium pyruvategave recoverywith concentration 1.0% at 20℃ and -30℃.

5-2 Conclusion

The effect of freezing on*Escherichia coli* varied in different temperatures, and some are very sensitive while others are less sensitive.The injury which resulted from freezing was repairable by adding some additive e.g. B_6, $B_{12}$and sodium pyruvate. B_6(ml)at -20℃ recovery of98.8%, while medium without additive gave97.3%, and no improvement was noticed at -30℃, -40℃.B_{12}(3ml) at-20℃, $-30℃$ and $-40℃$,gaverecovery of96.6.% at-20℃while medium without additive gave 93.5%, gave recovery of95.2% at-30℃ while medium without additive gave 89%and gave recovery of 99% at-40℃ while medium without additive gave 97.3%Sodium pyruvate at -20℃and -30℃.with concentration 1.0% recovery of99%,99.7% respectively .Medium without additive gave97.8% ,96.3% respectively and no improvement was noticed at -40℃.B_6+ sodium pyruvate (3ml+1.5%) at-20℃ recorded recovery of 98.3% while medium without additive recorded 88% and improvement was noticed at -30℃ and -40℃.B_{12}+ sodium pyruvate (3ml+1.5) at-20℃ gave recovery of 97.2% and medium without additive gave recovery of 95% and no improvement was noticed at -30℃-40

5.3Recommendations

1- Meat after process should be kept at-40℃.

2 - Sodium pyruvate and vitamin B_{12} can be used for recovery media.

3 -Farther studies are recommendedconcerning the present or other additives for recovering cold shocked *E.coli.*

6- REFERENCES

1. **Adams. M. R and Moss M .O(2008)** Food microbiology. 3rd edition Royal Society of Chemistry .London-Piccadilly-WIJ OBA pp: 216-252.
2. **Agafonov,D.E.;Kolb,V.A . and Spirin , A.S .(2001)** . Ribsome-associated protein that inhibits translation at the arininoacyl-tRNA binding stage. EMBO Rep. 2:399-402.
3. **Archer, D.L.(1996).**Preservation microbiology and. Sal.ety : evidence that stress enhances virulence and triggers mutations . Trends Food Sc! Te chnol. **(7):** 91-95.
4. **Baird- Parker , A. C.(1962) .** An improve diagnostic medium for isolating coagulase- positive staphylococci . J .*Appl. Bacteriol* , 25: 12 .
5. **Banwart , G . I . (1981) .**Basic Food Microbiology. The Avi publishing company, Inc West port , Connecticut .
6. **Basic Sausage – Making .(2004) .** Angharad of the coppery shields .Oct .17 , 2004 < http:// www meridies. Org /as /dmir / cooking & feasts / 0818 .html > .
7. **Bergdoll , M . S.(1989)** . *Staphylococcus aureus* . In. Food borne Bacterial pathogens (Doyle , M . P, ed) . Marced Dekker , Inc., New York,NY , U.S.A, PP .463-523.
8. **Brandi, A.; Spurio, R.; Gualerzi, C. O. and Pon, C. L.(1999).** Massive presence of the *Escherichia coli´* majar cold chock protein´ CspA under non stress conditions. Embo J. (**18)**:1653-1659.
9. **Brown , M .H .(1982) . Meat Microbiology** . Ed published LTD Essax , England cited .
10. **Bunning , V . K.; R. G. Crawford; J. F Tierney. and J.T Peeler,(1990)** Thermotolcratue at Li . vteria ; nonocvto cites and *Salmonella*

tvp/uoiiirii,n after sublethal head shock. App!. Eat/mn . Micro/do! **(56)**: 3216-3219.

11. **Cappelier, J.M.; Magras,C.;Jouve, J.L and Federighi ,M.(1999).** Recovery of viable but non- culturable *campylobacter jejuni* cells in two animal models *FoodMicrobiology*. **(16)**: 375-383.
12. **Carroll , W. Jeffrey, madalina C. Mateescu ,kiran chava, Rita R.colwell and Asimk. Bej (2001).**Response and tolerance of toxigenic *Vibro cholerae OI* to cold temperature .*Antonie van leeuwenhoek***(79)**: 377-384 .
13. **Castillo, A. L. M . Lucia, 1. Mercado. And C. R. Acuff. (2001)** . Inplant evaluation of a lactic acid tereatment for reduction of bacteria on chilled beef carcasses, .1. *Food prof* . (**64)**: 738-740 .
14. **Collees , J . G ., knowlden J .A .and Hobbs, B.C.(1961).** studies on growth, sporulation and carriage of *C. Perfringes* with special reference to food poisoning strains .*J . appl . Bact*. 24 **(3),** 236.
15. **D´Aouts,J.Y. (1991).** Pathogenicty of food borne *Salmonella International Journal of food microbiology* .**(2)**: 17-40.
16. **Dammel , C .S . and Noller , H .F .(1995).**Suppression of a cold – sensitive mutation in 16s r RNA by overexpression of a novel ribosome binding factor , Rbf A. *Genes Dev***.(9)**: 626-637 .
17. **Dersch, p.; kneip ,S . and Bremer , E.(1994)** .The nucleoidassociated DNA – binding protein H-Ns is required for the efficient adaptation of *Esherichia coli* K- 12 to a cold environment . *Mol .Gen . Genet* (**245)**: 255 -259 .
18. **Donovan , W. P . and Kushner , S. R . (1986).** polynucleotide phosphorylase and ribonuclease II are reguired for cell viability and m RNA tunover in *Echerichia coli* K-12. *porc .Natl. Acad . Sci* USA.(**83)** : 120 -124

19. **Drlica , K. (1992).**Control of bacterial DNA supercoiling *Mol.Microbiol***(6)** : 425-433 .
20. **Duncan, C.L.; Storong , D.H. and Sebald, M. (1972).** Sporulation and enteroxin production by mutants of *C . Perfringes J.Bact 110* (1), 378.
21. **EL Sanousi, S. M . (1975) .** Oxidation Reduction of*C .perfringes* ph. D. thesis University of Bristol , England.
22. **Eley , R.A. (1996) .** Microbial Food poisoning . Second edition .university of Sheffield Medical School , chapman and hall publishing company. UK. London, pp 1-176.
23. **Ermolenko , D.N. and Makhatadze, G.I. (2002).** Bacterial cold-shock proteins. *Cell. Mol. Life .Sci.* (**59)** : 1902- 1913 .
24. **Farber, J.M. and B,F .Brown .(1990).** Effect of prior heat shock on the heat resistance of *listeria genes* in meat *Appi . Eni,j-, Microbial .* **(56)**: 1584 – 1587.
25. **Fatima Alamin Mohammed(1990).** The effect of freezing on bacteria in processed meat. PhD thesis. Faculty of Veterinary Sciences, University of Khartoum.
26. **Favaro, R. and Deho ,G. (2003).**Folynucleotide phosphorylase-deficient murtants of *Pseudomonas putida .J Bacteriol* (**185)**: 5279-5286 .
27. Federal Register ,U.S.A. (1999). 'Guidance for Beef Grinders to Better protect Health Volume 64, number 11.
28. **Forsythe , S.J and Hayes, P. R. (1998).** Food Hygiene , Microbiology and. HACCP. 3rd ed Aspen publishers , Inc .Gaithersburg , Maryland 20878.2: 21- 58.
29. **Frazier , W.C. and Westhoff, D.C. (1989).** food microbiology 3rd ed McCraw – Hillbook company, New York .

30. **Frazier , W.C.(1967).** Cotamination, preservation and spoilage of cereals and products . In food microbiology. pp. 180.191, McCraw – Hillbook company, New Yor.
31. **Friedman , D. I . ; Olson , E.R.; Georgopoulos, C.; Tilly ,K.; Herskowitz , I. and Banuett, F. (1984) .** Interactions of bacteriophage and host macromolecules in the growth of bacteriophase lambda .*Microbial. Rev*.**(48)** : 299- **325.**
32. **Gerris ,C. V. I (2000) .** Use of lactic acid for carcasses decontamination meat pou /t.
33. **Gilbert, p. (1984).** The revival of microorganisms sublethally injured by chemical inhibitors,p.175-197. In M.H.E Andrew and A.D. Russell russen (ed.), the revival of injured microbes. Academic Press ,London.
34. **Goldstein ,J.Pollitt, N.S.and Inouye ,M. (1990).**Major cold shock protein of *Escherichia coli*. *Proc. Natl. Acad. Sci* . U.S.A. (87): 283-287 .
35. **Gorril , R. H. and McNeil, E .Ms. (1960).** The effect of cold diluents on the viable count of *pseudomonas pyocyanea .J. gen . Microbiol* .22:437.
36. **Graumann , P. L. and Marahiel , M. A. (1999).** Cold shock response in Bacillus subtilis .*J.Mol Microbiol Biotechnol* . (**1**): 203 - 209.
37. **Graumann , P.; Wendrich ,T.M.; Weber, M.H.; Schroder, K. and Marahiel , M. A. (1997).** A family of cold shock proteins in*Bacillus subtilis* is essential for cellular growth and for efficient protein synthesis at optimal and low temperatures *Mol. Microbiol*.**(25)**:741-756 .
38. **Graumann ,P. and Marahiel,M.A(1997).** Effects of heterologous expression of CspB, the major cold shock protein of *Bacillus*

subtillis,on protein synthesis in *Escherichia coli. Mol Gen Genet*,253,745-752.

39. **Gualerzi , C.O. and Pon ,C.L. (1990)** . Initiation of mRNA translation in prokaryotes .*Biochemistry*. (29): 5881-5889.
40. **Harrigan.W.F.**,(1998)**Laboratory Methods in Food Microbiology 3rd edition .**
41. **Hiba ,S, O (2010)**.Assessment of effect of gum arabic on recovery of cold shocked bacteria.M.Sc.Khartoum:University of Khartoum.
42. **Hideharu Shintani.(2006)** . Importance of Considering Injured Microorganisms in Sterilization Validation . *Biocontrol Sci* .**(11)** : 91- 106
43. **Higgins , C.F. ;Dorman , C. J. ;Stirling , D .A . ;Waddell, L .; Booth, I . R .;May , G. and Bremer , E. (1988) .** Aphysiological role for DNA supercoiling in the osmotic regulation of gene expression in *S.typhimuruim* and *E. coli Cell* . **(52):** 569- 584.
44. Hurst ,A . (1984).Revival of vegetative bacteria after sub lethal heating .P.77-103 .In M. H. E. Andrew and A .D. Russell (ed). The revival of injured microbes . Academic press London.
45. **ICMSF(1996). nternational Commission. On Microbiological Specifications for foods.** Salmonellae. 217 -264. In micro – organisms in foods 5 . Characteristics of Microbial Pathogens .Roberts , T.A. , Baird Parker , A. C. and Tompkin , R. B.eds . Puplished by Blackie Academic & Professional , London .
46. **ISO 4831: 2006** Microbiology of food and animal feeding stuffs-Horizontal method for the detection and emumeration of coliforms – Most probable number technique
47. **ISO 6888-1:** (1999)Microbiology of food and animal feeding stuffs-Horizontal method for the detection and emumeration of coaqulase –

positive staphylococci (Technique using Baird – Parker agar medium).

48. **ISO 7218: 2007** Microbiology of food and animal feeding stuffs- Genral requirement and quidance for microbiological examinations.

49. **ISO 7251: 2005**Microbiology of food and animal feeding stuffs- Horizontal method for the detection and enumeration of probable *Escherichia coli* – Most probable number technique .

50. **Janssen, D . W. And Busta , F. F . (1973).** Repair of injured in *Salmonella* anatomy cells after freezing and thawing in milkCryobiology. **(10)**: 386- 392.

51. **Jawez , Melnick and Adel , Berg,s (1990).** Medical microbiology . 19 th ed United state of America .

52. **Jay ,M.(2000) .** Modern Food Microbiology , 6 th Edition Aspen puplishers, Inc . Gaithersburg Maryland , vol (3) and (4) .

53. **Jay, M . (1986).** Modern Food microbiology .3rd .ed .New York : Van Nostrand Reinhold Company Inc .

54. **Jeffreys , A . G .; K . M . Hak, ; R . J . Steffan, ; J . W Foster .and A. K . Bej , (1998)** . Growth, survival and characterization of esPA in Salinane/ *La enteritidis* following cold shock . *Curr .Microbiol* ,**(36)**: 29 -35 .

55. **Jiang, W .; Fang , L . and Inouye , M .(1996)** . The role of the 5-end untranslated region of the mRNA for CspA, the major cold – shock protein of *Escherichia coli*, in cold shock adaptation . *J. Bacteriol* .**(178)** : 4919- 4925 .

56. **John , N . and Anthony, J. Sinskey (1974) .** Microbiology of foods and food processing .2 nd. ed New York .

57. **Jones , P . G .;Van Bogelen , RA . and Neidhardt , F . C .(1987) .**Induction of proteins in response to low temperature in *Escherichia coli* . J. Bacteriol .(**169)** : 2092- 2095 .

58. **Jones , P. G . and Inouye, M.(1994) .** The cold – shock response - ahot topic .*Mol .Microbiol* . (**11)**: 811- 818 .

59. **Jones , P. G .; Cashel, M . ; Glaser , G . and Neidhardt , F .C .(1992)** . Function of arelaxed – like state flowing temperature downshifts in Escherichia coli . J .Bacteriol . (**174**): 3903 – 3914 .

60. **Jones, P G.; M . Milta .Y , Kim , W. Jiang and M . Iuouyi (1996) .**Cold shock induces a major ribosomal- associated protein that un winds double –stranded RNA in E . sc/ ie pjc /, ja co/ i. Proc. Nail, . Acad . Sc- I, USA 93 : 76- 80 .

61. **Kandror ,O. ;Deleon, A. and Goldberg, AL.(2002).** Trehalose synthesis is induced upon exposure of Escherichia coli to cold and is essential for viability at low temperatures. Proc. Natl. Acad. Sci.USA**.(99)**; 9727-9732.

62. **Kandror ,O. and Gold berg , AL . (1997)** . Trigger foctor is induced upon cold shock and enhances viability of Escherichia coli at low temperatures . proc .Natl .Acad . Sci. USA**. (94)** : 4978 – 4981 .

63. **Krispin , O. and Allmansberger , R .(1995) .** Changes in DNA supertwist as response of *Bacillus Subtilis* towards different kinds of stress. FEMS Microbiol. Lett. (**134)**: 129- 135 .

64. **Lawrie, R. A. (1998)** Meat Science. 6th edition Cambridge , England: Wood-head publishing limited.

65. **Lee , S .J .; Xie , A .;Jiang , W . ; Etchegaray , J. P . ; Jones, P . G. and Inouye , M .(1994)** . Family of the major cold – shock protein , CsPA(CS7.4), of *Escherichia coli* whose members show a high sequence similarity with the eukaryotic Y – box binding proteins *Mol. Microbiol* . (**11)**: 833- 839 .

66. **Lelivelt , M.J. and Kawula , T . H . (1995) .** Hsc66, an Hsp70 c homolog in *Escherichia coli* .is induced by cold –shock but not by heat shock .*J .Bacteriol***. (177)**: 4900- 4907 .

67. **Lund , Barbara ,M ., Tony C. Baird- Parker, Grahame W . Gould , Grahame warwick Gould (2000).** The microbiological Safety and quality of food. Aspen publishers , ar chaidridg, drive Gaitherburg , Maryland 20878.1 ,pp: 317 .

68. **Lyla,I.M.(1987).Study on recovery and properties of cold injured** bacterial isolated from processed meat ,M.V.Sc. khartoum:University of Khartoum.

69. **Macfadyen(1990) .** The influences of the temperature of liquid air on bacteria .pro . R. soc**. (66)**: 78- 180 .

70. **Mackey , B . M .and Derrick, C . M . (1986)** .Elevation of the heat APP/. Bacterial .(61) : 389 -393 .

71. **Mackey , B .M .(1984)** . Lethal and sublethal effects of refrigeration , freezing and freeze drying on microorganism . P . 45- 75.in M . H .E .Andrew and A. D. Russell (ed.) , The revival of injured microbes . Academic press .London .

72. **Mary , A .and Scottie, M . (1998) .**Ground meat and poultry food safety, Part of food safety Tips , college of A agriculture , The university of A rizonea , http : // www . ag , arizona . edul pubs/ health / food safety /az 1090- html .

73. **MFMER) (2006). Mayo, Foundation for Medical Education and Research** *E.coli*: Dangers of eating raw under cooked foods .

74. **Miller , A .J .; Bayles, D. O . and Ehlen, B . S . 2000)** .Cold shock induction of thermal sensitivity in *Listeria rnonocytogenes* . APP/ .Environ .Microbiol .(**66)**: 4345 – 4350 .

75. **Mitta , M .; Fang, L . and Inouye , M .(1997)** . Deletion analysis of CsPA of *Escherichia coli* . requirement of the ATrich up element

for CspA transcription and the downstream box in the coding region for its cold shock induction *Mol . Microbiol* . (**26)**: 321 – 335 .

76. **Mizushima ,T.; Kataoka , k. ; Ogata, Y .; Inoue , R. and sekimizu, . K.(1997) .** Increase in negative supercoiling of plasmid DNA in *Escherichia coli* exposed to cold shock .*Mol .Microbiol* .(**23**): 381- 386 .
77. **Mohamed , A . E . (2000).** Arabic bacteria isolated from meat at different stages of processing . M .SC . Thesis university of Khartoum .
78. **Moll , I.; Huber, M .; Grill, S.; Sairafi , P .; Mueller, F. ;Brimacombe , R.; Londei , P. and Blasi ,U.(2001) .** Evidence against an Interaction between the mRNA downstream box and 16S rRNA in translation initiation . *J. Bacteriol* (**183)** : 3499 – 3505 .
79. **Morichi , Toskiki and Ryozaburo , lrie (2004)** . Factors affecting repair of sublethal injury in frozen or freeze –dried bacteria . Cryobiology (**10)**: 393-399 .
80. **Moss , Wayne , C. and speck ,M. L .(1966).** Identification of Nutritional components in Trypticase Responsible for Recovery of *Escherichia coli* Injured by freezing .*J .Bacteriol* . (**91)**: 1098- 1104 .
81. **Murano , E . A .and M . D .pierson .(1993)** . Effect of heat shock and incubation atmosphere on injury and recovery of *Escherichua coli*0157:H7 . J .*Food Prot* . (**56)**: 568 -572
82. **Musttafa,N.E.(2005).**. Isolation of *Bacillus* and *Clostridium spp* . From food of animal origin with special reference to their spores resistance and injury by heat.
83. **Nagala,M. (2010).**physiological attributs of cold-shocked *E.coliSamonalla sp, Staphylococcus aureus* and *Pasteurella multocida* type B,M.V.sc.Khartoum :Univesity of Khartoum.

84. **Nakashima , K .; Kanamar UK .; Mizuno , T. and Horikkoshi , K.(1996).**A , novel member of the CsPA family of genes that is induced by cold shock in *Escherichia coli*. *J .Bacteriol* . (**178)** : 2994 – 2997 .

85. **Narasimha , Rao . D and Ramesh , B . S .(I988) .**Microbial profiles of minced meat . *J .of Meat Science* (**23)**: 279- 291 .

86. **Neema Mrema , Sisai Mpuchane and Ber hanu , A .Gashe .(2004) .** prevalence of *Salmonella* in raw minced meat, raw fresh sausages and raw burger patties from retail outlets in Grabovone , Botswana . *J .of science Direct food control* ., Volume (**17)** : 2007-212 .

87. **Neuhaus,K.; Rapposch, S.; Francis ,K.P. and Scherer, S.(2000)** Restart of exponential growth of cold shocked *yersinia enterocolitica* occurs after down – regulation of CsPA1/ A2. mRNA. *J. Bacteriol.* (**182)**:3285-3288.

88. **Oliver, J.D.(1981).** Lethal cold stress of vibria i´ulnijicu.s in oysters. APP!.Enui´ on. *Microbiol*.(**41)**:710-717.

89. **Pagan, R.; Condon, S. and Sala, F. J. (1997).**Effects of several factors on the heat- shock- induced thermotolerance of *Listeria mnonacytogenes* . APP*? Environ . Microbial.* (**63)**:3225-3232.

90. **PFMG) (1995).Professional Food Microbiology Group**. Microbiological criteria for retail foods.J.I of let in Apple .Microbiol ,.(20) : 331- 332 .

91. **Phadtare, S. and Inouye, M. (1999).** Sequence-selective interactions with RNA by CspB, CspC and CspE,members of the CspA family of *Escherichia coli*. Mol.*Microbio*l. (33): 1004-1014**.**

92. **Phadtare, S., Yamanaka, K, and Inouye, M (2000).** The ColdShock Response. In: The Bacterial Stress Responses. G. Storz, and R. Hengge-Aronis, ed. ASM Press, Washington DC. P. 33-45.

93. **Phadtare, S.; Hwang, J.; Severinov, K., and Inouye, M.(2003).** CspB and CspL, thermostable cold-shock proteins from Thermotoga maritima. Genes Cells. (**8)**: 801-810.

94. **Polissi, A.; De Laurentis, W.; Zangrossi, S.; Briani, F. ;Longhi, V.; Pesole, G. and Deho, G.(2003).** Changes in Escherichia coli transcriptome during acclimatization at low temperature. Res. Microbiol. (154): 573-580.

95. **Ray, B. and Speck, M.L.(1972).** Metabolic Process during the Repair of Freeze – injury in. *Apple Environ microbiol***.(24):**585-590.

96. **Russell, A.D.(1984).** Potential sites of damage in microorganisms exposed tochemical or physical agents .p.1-18.In M. H.F.A ndress and A.D. Russell led, The revival of injured microbes Academic Press. London. Sal.ety : evidence that stress enhances virulence and triggers mutations . Trends Food Sc! Te chnol. **(7):** 91-95.

97. **Saudia Epidemiology Bulletin.(2001).***Salmonella* Food poisoning Outbreak in Riyodh, Saudi Arabia department Escherchia coli of prevention Medicin and Field Epidemiology training program volume (**9):**1-3 .

98. **Semanehek, J.J. and I.A.Golden. (1998).**Influence growth temperature oil option and injury of Esclieric / tiu coli 0157 H7 by heat, Acid, and freezing. *J. Food prof,* (**61)**:395-401.

99. **Sherman,J.M and Cameron ,M.G.(1934).**Lethal environment and factors within the natural range of growth.J.Bact. 27;341.

100. **Sherman,J.M, and Allbus , W.R.(1923).**Physiological youth in bacteria. *J.Bacteriol* . 8:127.

101. **Shoemaker , S. p . andPierson , M . D . (1976) .**Phoenix phenomenon , in the growth of *Clostridium perfringens* . APP/. *Environ microbiol* .(**32)**: 803- 807 .

102. **Sorrells, K.M.;Speck ,M.L. and Warren, J.A.(1970)**. pathogenicity of *Salmonella gallinarum* After Metabolic Injury by Freezing. *Appl environ microbial* .(**19**):39-43.

103. **Steinhauser, L.(1995).** Meat Hygiene Technology (In Gezech).LAST. Brrno,15-108.

104. **Strange, R.E.and Ness, A.G.(1963).** Effect of chilling on bacteria in aqueous suspension. Nature. 197:819.

105. **Strange,R.E.and Dark, F.A.(1962).**Effect of chilling on aerobatic aerogenes in aqueous suspension. J. gen. Microbiol.29:719.

106. Sudanese Microbiological Standars for foods-(2007). Meat and meat products . Fresh sausages (SDS 335).

107. **Sugino, A.; Peebles, C.L.; Kreuzer, K.N., and Cozzarelli,N.R. (1977)**. Mechanism of action of nalidixic acid: purification of *Escherichia coli* nalA gene product and its relationship to DNA gyrase and a novel nicking-closingenzyme. Proc. Natl. Acad. Sci. USA. (**74):** 4767-4771.

108. **Todd , J . R . Fish out , M . Kapral , F and Weth , T . (1978)** . Toxic shock syndrome associated with phage group 1 *Staphylococci* .lancet , 25: 1116 .

109. **Toone, W.M.; Rudd, K.E. and Friesen, J.D. (1991).**deaD,a new *Escherichia coli* gene encoding a presumed ATP-dependent RNA helicase, can suppress a mutation in rpsB, the gene encoding ribosomal protein S2. *J.Bacteriol.* (**173)**: 3291-3302.

110. **Traci, P.A, Duncan C.L.(1974).** Cold shock lethality and injury in *Clostridium perfringens .Appl. Microbiol***. (28)**:815-821.

111. **Van Gerwen, S.J.c ;Giffel, M.C. ;van,t. ;Riet, k.; Beumer, R.R and Zwietering, M.H, M.H. (2000).**Stepwise quantitative risk assessment a stool for characterization of microbiological Food Safety. J. OF appL.Microbiol. (88):938-951.

112. **Van Schothorst, M., and. A.M. Duke. (1984)**. Effect of sample handling oil limits lail down in standard ,. P.309-328.

113. **VanBogelen, R.A., and Neidhardt, F.C. (1990).** Ribosomes as sensors of heat and cold shock in Escherichia coli.Proc. Natl. Acad. Sci. USA. (**87)**: 5589-5593.

114. **Vanderzant, C. and Neckelson ,R.(1969).** Microbiological examination of muscle tissue of beef pork , and comb carcasses. J. Milk and Fd teach;(**32)**: 257-361.

115. **Vorobeva, L.I. (2004).** Stressors, stress reaction and survival of bacteria *; A review Appl. Biochem . and Microbial* 40 (3) :217 .

116. **Wagner, A.I.B. and Jer…,(2000).** Bacterial food poisoning. Texas Agricultural Extension Service.

117. **Walker, G.C. (1984).** Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli. Microbiol. Rev*. (**48)**: 60-93

118. **Wallace , Robert .B. Dobbling , Bradley N . and last , John ,M .(1998)** .Puplic Health and Preventive medicine . 14 th ed .M Cc R A W – Hill New York USA . 263-735 .

119. **Wang, J.Y., and Syvanen, M.(1992).** DNA twist as a transcriptional sensor for environmental changes. *Mol .microbiol* . (**6)**: 1861-1866.

120. **Wang, N.; Yamanaka, K., and Inouye, M. (1999).** CspI, the ninth member of the CspA family of *Escherichia coli*, is induced upon cold shock. J. Bacteriol. (**181)**: 1603-1609.

121. Wassenberg, D.; Welker, C., and Jaenicke, R.(1999). Thermodynamics of the unfolding of the cold-shockprotein from Thermotoga maritima. J. *Mol. Biol.***(289):**187-193.

122. **Weber, M.H. and Marahiel, M.A.(2003)**. Bacterial cold shockresponses. *Sci. Prog*. (**86):** 9-75.

123. **Widders, P.R., Coates, K.J., Warener ,S.W.,Hickey , M.W.(1994)**.Controlling microbial contamination on beef and lambmeat during processing. journal of Australian Veterinary., 72: 208- 211.

124. **Williams, D.J (1956).** The rates of growth of some thermoduric bacteria in pure culture and their effects on tests for keeping quality of milk. J. *Appl. Bacteriol* . 19; 80.

125. **Willimsky, G.; Bang, H.; Fischer, G.; and Marahiel, M.A.(1992).** Characterization of cspB, a *Bacillus subtilis* inducible cold shock gene affecting cell viability at low temperatures. *J. Bacteriol*. (**174)**: 6326-6335.

126. **Wilson, N.R.P.(1981).** Meat and Meat Products Factors affecting quality control. London and New Jersey.

127. **Wood Green Daivid, Richard, C.B. Slack and John forest peuthere (1992) .**Medical Microbiology. A guide to microbial infection. Pathogenesis. Immunity laboratory diagnosis and control, 14thed.

128. **Wuytack, F. Y.; L.D.T.Phuong ,A.Aertsen, K.M.F Regns. D.Marquenie. B. Dc ketelaere . B .Mass chalck ,I. Van Opstal , A.M.J.Diels**.And C. W. Michiels**(2003)** sublethal injung induced in *Salmonella enteric* server. Typhirnuium by heat and by different non thermal treatment . J. Food prof. (**66)**:31-37.

129. **Xia, B., Ke, H., Jiang, W., and Inouye, M.(2001b).** The Cold Box stem-loop proximal to the 5'-end of the *Escherichia coli* cspA gene stabilizes its mRNA at low temperature.J. Biol. Chem. **(277)**: 6005-6011.

130. **Yamanaka , K. and Inouye, M. (2001a)**. Selective mRNA degradation by polynucleotide phosphorylase in cold shock adaptation in *Escherichia coli* ;. J .*Bacteriol*. (**183**): 2808-2816.
131. **Yamanaka , K. and Inouye, M. (2001b).** Induction of CsPA, an *E.coli* magor cold- shock protein, upon nutritional up shift at 37 degrees C. GenesCells (**6)**:279-290.
132. **Yamanaka , K., Fang, L., and Inouye, M. (1998).** The CsPA family in *Escherichia coli* ; multiple gene duplication for stress adaptation. Mol. Microbial .(**27):**247-255.
133. **Yousef, A.E. and Caurtneg, P.D. (2003)**. Basics of stress adaptation and implications in new. Generation foods , p.1-30 in A.F. Yousef and V.J. Juneja (ed) microbial stress adaptation and food safety. CRC press . Boca Rutsin, FL.
134. **Zhao, I. and Doyle, M.P.(2001).** Evaluation of universal preenrichment broth for growth of heat – injured pathogens. J. Food prof . (64): 1751-1755.

http:// www.medicinenet .com / staph- infection /article.htm

http:// www.medicinenet .com /salmonella//article.htm