Sudan University of Science and Technology

Collage of Graduate Studies

Escherichia coli and *Salmonella spp*. Contamination in yoghurt Manufacturing From Whole Milk - Khartoum - SUDAN

دراسة في التلوث بالبكتريا القولونية و بكتيريا السالمونيلا في تصنيع الزبادي من الحليب الخام بولاية الخرطوم - السودان

A thesis Submitted to the College of Graduate Studies in the fulfillment of the Requirement of Attaining Master of science in Veterinary Preventive Medicine and Public Health

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2019

Dedication

To the soul of my father and sister [may be merciful to them]

To my mother

To my wife

To my beloved son Mohamed,

I dedicate this work.

Acknowledgment

I pray to Allah for giving me the health and the strength to conduct this research
I wish to express my sincere gratitude an thanks to the following persons who have made it possible for me to complete this study.
To my supervisor prof. **Mohamed Abdelsalam Abdallah**. For his professional and personal advise , assistance, guidance, support and patience.
To Dr. **Maha Mubarak**. Co. supervisor, for here kind help support and advice during the whole study.
To the ***Sudan University of Science and Technology*** ,for valuable support.
To all members of Microbiology Section, with special appreciation to ***Nagwa***, for here excellent assistance in the Lab. And special thanks to ***Azza***, at Milk Tech. Lab. For here great support.
TO Department of Veterinary Preventive Medicine and Public Health, Collage of Veterinary Medicine, Sudan University of Science and Technology, for supporting this research.

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ABSTRACT

The aim of this study was to investigate the total viable bacterial load in the different stages of yoghurt processing, and to isolate and identify *Salmonella spp. and Escherichia coli* during the period from November 2015 to October 2017 in yoghurt production unit in Khartoum- Sudan. Sixty samples were collected at different six stages of the processing (Whole milk, pasteurized milk, cold pasteurized milk, milk +starter, after incubation, Cold Yoghurt)where the isolation and identification of the two types of bacteria (*Salmonella spp. and Escherichia Coli*) were investigated. The results revealed that the Total Viable Count (T. V. C.) indicated the highest level of contamination in Whole Milk stage (Mean (Log.CFU/ml) = 6.93±0.20 in which the positive samples for *Salmonella spp*. 2(3.84)% and *Escherichia coli* . 10(19.16)% while the lower Contamination Level was at Hot Pasteurized milk stage (Mean (Log-CFU/ml)=6.34±0.07 in which the positive samples for *Salmonella spp.* 2(3.6) %, and *Escherichia coli* . 3(5.4)%.The statistical analysis of the result revealed there was a significant difference at ($P \leq 0.05$) of total viable bacterial count in different processing stages of yoghurt production

The study concluded that, contamination was reported in all stages of yoghurt production processes with *Escherichia Coli* and *Salmonella spp*.

ملخص الاطروحة

هدفت هذه الدراسة تقصي العدد الكلى للبكتريا في مراحل تصنيع الزبادي المختلفة وعزل ومعرفة بكتيريا السالمونيلا والاشريكية القولونية في الفترة من نوفمبر 2015 وحتي اكتوبر 2017 فى وحدة تصنيع الزبادي بولاية الخرطوم- السودان. جمعت 60 عينة من ست مراحل مختلفة للتصنيع (الحليب الخام, الحليب المبستر الدافئ ،الحليب المبستر البارد، الحليب مع البادئ الحليب بعد التخمير والمنتج الزبادي البارد)وتم عزل ومعرفة نوعين من البكتيريا وهما بكتيريا السالمونيلا وبكتيريا الإشريكية القولونية في ست مراحل خلال عملية تصنيع الزبادي بوحدة تصنيع في الخرطوم- السودان ..أظهرت النتائج ارتفاع عالى لمستوي العد البكتيري بمتوسط وانحراف معيارى(6.93±0.05) فى الحليب الخام وكانت نسبةالعينات الايجابية للسالمونيلا2 (3,84)% وللاشريكية القولونية10(19,16)% و المتوسط والانحراف المعياري هو(0,05 ±6.94) وأقل انخفاض للتلوث كان في مرحلة البسترة الدافئ بمتوسط وانحراف معيارى(6.34±0.07) حيث ان نسبةالعينات الايجابية للسالمونيلا 2 (3,6) % و للاشريكية القولونية 3(5,4)% . أظهر التحليل الإحصائي للنتائج أن هنالك فروق معنوية (P≤0.05)يين مراحل تصنيع الزبادي المختلفة . توصلت الدراسة الى ان التلوث قد تم تسجيله فى كل المراحل التصنيعية لانتاج الزبادى.

Introduction

There is evidence of fermented milk products being produced as food for at least 8000 years. The earliest yogurt were probably spontaneously tormented by wild bacteria living on the goat skin carried by nomadic people. (Yildiz, 2010).

Sudan is large country with different climatic conditions and considered as one of the largest country in animal resources. According to the international classification it is the 1st in the number of camel 3.54 million heads .the forth in the number of goats 43.14 million heads. the sixth in the number of cattle 40 million heads and the seventh in the number of sheep 49.05 million heads. SSMO (2007).Dirar(1993) divided the fermented dairy products of Sudan into two major groups, the truly indigenous which include roub, gariss and mish , and the quasi-indigenous which include Zabadi and Jibnabeida.

HACCP

HACCP system which is science based and systematic identified specific hazards and measures for their control to ensure the safety of food. Roberts, (2001).

As the system of choice for ensuring food safety and is becoming enshrined in national legislation proactive application in food industry will facilitate compliance with developing legislation and demonstrates a diligent approach to food safety . (Jervis 2002).

The HACCP procedure is generally targeted at food safety management (pathogenic micro organisms and their toxins) but as an approaching the context of border quality management.

It can be effectively applied to microbiological spoilage, foreign body contaminations or peptide contamination, it is preferable to conduct

HACCP program with a barrow scope (a single pathogen or possibly pathogens) rather than attempt to cover an extended list of hazard areas.Jervis,2002)

Objectives

1-To identify microbial contamination point for establishing the critical control points (CCP) in yoghurt production process.

2-To evaluate the bacteriological level contamination of yoghurt with ((*Salmonella spp*. and *Escherichia coli* during production process).

Chapter One

Literature review

1.1 Yoghurt as food product

1.1.1Definition:

Yoghurt is a milk product obtained by the fermentation of milk by the action of symbiotic culture of *strep. Thermophiles* and *lactobacillus delbrucckii subsp. Bulgaricus* and resulting in reduction of ph with coagulationFAO/WHO, (2002).*Strep,. thermophiles* and *l.bulgaricus* are cultured with other lactic acid bacteria for taste or health effects , these included lacto bacillus acidophilus (LA) , *lacto bacillus casei* , and*bifido bacteria* species.(Yildiz ,2010).

1.1.2 History of yoghurt processing:

The culinary art of yoghurt making originated thousands of years ago. it is likely , however , that the origin of yoghurt was the middle east , and the evolution of this fermented product through the ages can be attributed to the culinary skills of nomadic people living in that part of the world. (Yildiz. 2010).

During the past two decades there has been renewed interest in the study and understanding of nutritional and therapeutic aspects of dairy products.

Yoghurt and related yoghurt like dairy beverages are probably the first functional foods to be researched by the scientific community.Yoghurt is a very nutritious food and its continued consumption in the western world owes much to the development of its health food image(Early , 1990)..

Consumption of yoghurt is highest in countries around the Mediterranean, Asia, and central Europe(.Bylund ,1995*)*.

Today many different counties claim yogurt as their own invention, yet there is no clear evidence as to where it was first discovered, and it may have been .Independently discovered several times.

The art of making ZABADI (yoghurt) came to Sudan from Egypt, must likely during the time of the Anglo-Egyptian rule (1898-1956).it was prepared by households, in its preparation cow's milk is boiled, cooled and inoculated by back-shopping from previous lot, it is then incubated in worm carrier where it sours and refrigerated and consumed with sugar as a desert or to eat wheat bread with .sometimes it is fed to babies and often turned into sauce for*Aceda*.(Mortada,, 2012).

The first account European encounter with yogurt occurs with Francis 1 who be sufferedfrom a severe diarrhea that no French doctor could cure his ally Suleiman the magnificent , an ottman sultan , sent a doctor ,who alleged by cured the patient with yogurt(Yildiz,2010).

1.-2 Yoghurt processing:

The most important raw material used in yoghurt manufacture is milk. Milk in addition to being nutritious medium presents a favorable physical environment for multiplication of micro organisms and being an animal product is subjected to widely differing production, handling and processing methods results in its contamination by abroad spectrum of microbial types, chemical residues and cellular materials.

(Gilmour , 1990).

Fresh liquid milk is not only dairy product in the country, because some of the milk produced in rural areas is always converted into other production which with stand longer storage conditions such as fermented milk, ghee, cheese, and sour milk .(Payne ,1990).

The quality of yoghurt or any food product can be defined against a wide range of criteria. Including for example the chemical, physical, microbiological and nutritional characteristics.(Hoolasi ,2005).

- Because of that from raw material production to the point of consumption, all dairy products should be subjected to many control measures, and good hygienic practices should be applied throughout the production and processing steps. Most countries have been worried about food borne diseases nearly in developing countries due to food problems reported cases economic and social costs effect around the world(Zhao.etal.,2001**).**
- **The essential procedures in processing of yoghurt:**
- Yoghurt is acidified coagulated product obtained from milk by fermentation with lactic acid producing bacteria of all cultured milk products; yoghurt is the most well known and most popular worldwide.(Early,1998)

1.2.1*:*Manufacturing plants:-

The acidity of yoghurt means that spoilage is often associated with yeasts and moulds and the later in particularly after have their origin in the microbial population of the air so it is important, however, that plants designed to induce air flow through a filling room or production area can also act as a source of contamination. (Tamime and Rohinson, 1985*).*

Different methods and /or techniques have been devised to monitor the hygiene of dairy equipment, surfaces, thus contributing to maintaining production of high quality product, and at the same time ensuring compliance with legal requirements. Whatever tests are employed it is essential that they are applied routinely, for individual readings are in themselves meaningless, only when values for a typical high standard of hygiene have been established for given plant, along with any micro biological hygiene test become valuable. (*Mostert&Hooste,2002).*

Enumeration of total counts of bacteria, coli forms, yeasts and moulds are the common microbiological examinations carried out to assess the bacteriological contamination of surfaces, the types of micro – organisms presents, reflect to same extent, the standard of plant hygiene.

(Tamime and Robinson ,1999*).*

1.2.2: yoghurt production process include:

a- *Whole milk process ,Heat treatment (pasteurization) ,Cooling of milk, Adding starter ,Incubation ,Cooling product (yoghurt).*

***Whole Milk:-**

Preparation of ingredients of whole milk by filtration so as to purifying it from any depress and gives a clear pure product.

Milk standardization:

Robinson, 1999)Codex regulations for yoghurt indicated that the minimum fat content is 15% (Codex. ,2008).The use of stabilizers may help in providing a more uniform consistency and less batch to batch variations.(Vedamuth. ,1991).

Solids – non – fat and total solid levels in milk will vary dependent upon the type of yoghurt to be manufactured as well as the inclusion of other ingredients such as sugar, skimmed milk powder, water and cream. (Hoolasi , 2005).

Homogenization;

Milk is typically homogenized using pressures of 10-20 and 5 mpa. First and second stage pressures, respectively and at a temperature range between 55 and 65 c^o.(Vedamuth ,1991)

***Heat treatment :-**

Types of heat

-*pasteurization temperatures*: 80 – 95 c^o

-*sterilization temperature* : 115 – 170 c^o

-*UHT* : 135 – 140 c^o

Optimum hydrophilic properties of proteins and hence coagulation of the yoghurt milk are obtained when the milk is heated to 85c^o for 30 minutes (Early ,1998).

The advantage of heat treatment:-

Eliminate or reduce food spoilage micro-organisms to acceptable levels.

Reduce the total microbiological population to a level which will not compromise the growth of the starter micro organisms.

Eliminate vegetative food poisoning micro-organisms.

Denature the whey protein in order to improve the texture of the final product and to assist in the prevention of whey separation at any subsequent time during shelf life.

Hydrate certain stabilizers. *(*Hoolasi, 2005).

***Cooling of milk after heat**:-

Heated milk must be cooled to suitable temperature before inoculation.

- Yoghurt manufactured in batch tank or churn, can simply be allowed to cool via cold water jackets or tank (effective in a water bath).(Early, 1998*)*.
- The inoculation temperature will be approximate to 42c^{o}.

According to .(Hoolasi ,2005 *)* if an extended inoculation period is required temperature can be lowered to (30 – 32c^{o}) for short set incubation it is critical to achieve an accurate inoculation temperature since too high temperature can inhibit and ultimately kill starter culture micro-organisms and too low temperature will result in unnecessary extension of fermentation time ,(Early ,1998).

Starter Culture addition and incubation;

The short set method of incubation of yoghurt milk, using traditional starter organisms such as *Streptococcus Salivarinus sub sp.Thermophilus* and *Lactobacillusdelbrucckii sub sp.Bulgareicus*

-Yoghurt curd or (coagulum) begins to form as more lactic acid is produced as the iso-electric point of casein PH 4.6 – 4.7 is approached. A solidity of gel well begins to be seen at approximately PH 5.6 (Hoolasi.,2005).

-Fermentation will be arrested at approximately PH 4.2 – 4.4, sometimes even lower PH 3.8 – 4.0. (Early , 1998).

Cooling product (yoghurt):

Singh et al (1980) reported that the initial taste of stirred yoghurt was maintained during storage for 16 days at 5 c° and the higher the storage temperature, the faster rate of change oftitrable acidity, PH and viable cells of lactic acid bacteria. The shelf-life of any food commodity should combine the two consideration of safety and organoleptic property of the product; it is the latter requires emphasis by the dairy industry before attempts are made to prolong the shelf-life of plain liquid yoghurt beyond five days.

Cooling of the coagulum commences directly after the fermented yoghurt reaches the desired acidity.

The desire acidity will be depended upon type of yoghurt being produced, method of cooling, time taken to empty fermentation vessel and desired final acidity. (Hoolasi,2005) .

On cooling the curd becomes much firmer and if the yoghurt is formulated correctly, surface whey will be re-absorbed after 24 hours chilled storage. (Early,1998).

1.3: Yoghurt:-

Yoghurt is made by introducing specific bacteria strains into milk which subsequently fermented under controlled temperatures ($42c^0 – 43c^0$), and environmental conditions (in fermentation tank).

The bacteria ingest natural milk sugars and release lactic acid as waste product. The increased acidity causes milk proteins to coagulate into solid

mass (curd) in a process called denaturation. The bacterial species *streptococcussalivarius subsp. Thermophiles* (ST) and *lactobacillusdelbrueckiisubsp-bulgaricus*, made the product yoghurt. In United States and European Union countries the product called yoghurt only if live bacteria are present in the final product, in United States non-pasteurized yoghurt can be marketed as live or containing live active culture.

Small amount of live yoghurt can be used to inoculate a new bath of yoghurt pasteurized product, which has no living bacteria may be called fermented milk product.

Pasteurization of yoghurt kill large amounts of essential bacteria beside killing harmful bacteria, *acidopillus,bifidus* and *lactobacillus rhamnosus* were killed.(Yildiz,2010)

1.3.1: Types of yoghurt :-

Recently different types of yoghurt produced worldwide and according to*(Tamime and Robinson, 1999),* grouping of yoghurt based on followings:-

- Legal standards: (ex-according to fat compositions full, semi, skimmed).
- Physical natures (set, stirred, fluid).
- Flavors
- Post fermentation processing (vit-additic or heat treatment).

1- Set yoghurt

A solid set where the yoghurt forms in a consumer container and is not disturbed.

2- Stirred yoghurt

This is the most popular form of commercial yoghurt. In this type consistency of the (set) is broken and the texture is less firm than set yoghurt.

In this type yoghurt is firstly made in a large container and then dispensed into secondary serving containers.

3- Drinking sweet yoghurt :-

Additional milk and flavors are added to stirred yoghurt (milk, fruit syrups, fruits).

Shelf life of this product is (4-10) days because of rising of (PH) by adding of fresh milk.

4- Fruit yoghurt

Adding of fruits and fruits syrups on top or bottom or stirred into the yoghurt.

5- Yoghurt cheese :-

It is fresh cheese made by draining overnight by separating the whey. Its shelf life 7-14 days in refrigerator at $4c^0$.

6- Frozen yoghurt :-

After production, yoghurt frozen by bath or continuous freezers.

7- Dried yoghurt :-

Yoghurt is dried by the sun. This process aiming for longer preservation.

8- There is many other names for fermented milk at many different countries such as :-

- MishtiDahi :
  East Indian Desert
- Dadiah :
  West Sumatean (water buffalo milk)
- Labneh :
  Arab Countries
- Tarator and Cacik :
  Soups from yoghurt in Bulgaria, Mazedonia, Turkey.
- Rahmjoghurt :
  Minimum 10% fat content in Germany.
- Jameed :
  Salted yoghurt and dried in Jordan.
- Raita :
  In South Asian, India, in form of (sauce or dip).
- Zabady :
  In Egypt.
- Bihidasu :
  In Japan (with package of powdered sugar).
- Sour Cream:
  Fat content 12% - 30%.
- Low fat probiotic yoghurt :

commrercial name activiacontain the probiotic bacterium *bifidobacterium animalis*,*Bofidusregularis or bofidusactiregularis* .

(Yildiz ,2010).

Manufacturers of (Activia) claim that the probiotic bacterium helps digestive discomfort and irregularity.

1.3.2: Contents and ingredients of some commercial types of yoghurt and fermented milk products. :-(Almaraey Dairy Company,(K.S.A. 2018).

Fresh yoghurt: Ingredients:-Table -1

Fresh cow`s milk, milk solids, pectin, culture, vit.D3. min 3% fat, min 8-5% milk solids nonfat, fresh yoghurt from 100% pure cow`s milk

Energy (K cal)	68
Protein (g)	4
Carbohydrates (g)	6-2
Of which sugars (g)	6-2
Total fat (g)	3-1
Of which saturated fat (g)	2-1
Sodium (mg)	55
Vit. D3 (I.U.)	40
Vit .A (I.U.)	1-36
Calcium (mg)	130

Fresh LabanIngredients :-Table-2

Natural fresh full fat cow`s milk, laban cultures, vit. D, 3% minimum fat, 8.2% min. non fat solids.

Energy (K cal)	60/25/
Protein (g)	3.0
Carbohydrates (g)	4.6
Total fat (g)	3.3
Of which saturated (g)	2.1
Sodium (g)	0.05
Vit. A (I.U.)	160
Vit. B1 (mg)	0.03
Vit. B2 (mg)	0.16
Vit. B12 (pg)	0.40
Vit. D (I.U.)	40
Calcium (mg)	103
Phosphorus (mg)	85

Turkish Labneh: Table-3

Heat treated labneh "full fat" .

Ingredients:-

Fresh pasteurized full fat cow`s milk, fresh cream, cultures, salt 0.9%, stabilizer (E446) fat 17% min total solids 26% min. **Table -3**

Energy (k cal)	200/853
Protein (g)	5
Carbohydrates (g)	5.5
Fat (g)	17
Of which saturated fat (g)	11
Sodium (g)	0.8
Calcium (mg)	150
Vit. D (I.U)	17
Tran fat	0.1

1.3.3: Ingredients of yoghurt

1- Milk:-

Milk comes from many types of animals has been used for production of yoghurt but cow`s milk is the common one used for industry production of yoghurt in form of :

- Row milk
- Partial skimmed milk
- Skimmed milk
- Cream

Milk used for yoghurt culture must be :

- Not contaminated by bacteriophages.
- Low bacterial count.
- Free from antibiotics, chemicals used for sanitization
- Mastitis
- Colostrums

Milk ingredients

1- lactalbumin

2- B lactoglobulin

3- Borine serum albumin

4- lmmunoglobulins and immune system Components :-

- Cytokines
- Neutrophils
- T. lymphocytes
- Nucleotides

5- lacto ferrin

6- lacto ferricin

7- lacto peroxidase

8- Lysozyme

9- Biotin – binding protein

10- Epidermal growth factor

11- Fibro blast growth factor

12- Riboflarin – binding protein

13- Vitamin biz – binding protein

14- Whey protein peptides includes :

- Casein macro peptide
- & - lactorphin
- B- lactorphin

15- Lipids include :

- Conjugated linoleic acid
- Sphyingomyelin
- Milk fat globule membrane
- Butyric acid,Arachidonic acid

16- Hormones :

- Pituitary hormones
- Steroid hormones
- Leptin hormones
- Thyroid hormones

17- Carbohydrates :

- Oligosaccharides
- Mucins
- Lactose

(Yildiz ,2010)

Starter culture

The starter culture for most yoghurt production is a symbiotic blend of *Streptosalivariussubsp.Thermophiles* and *L. delbrueckiisubsp* –

bulgaricus. Although they can grow independently the rate of acid production is much higher when used together than either of the two organisms grown individually.

(S.T) grows faster and produces both acid and carbondioxide. The formate and carbondioxide produced stimulates (L.B) growth, on the other hand the proteolytic activity of (L.B) produces stimulatory petides and amino acids for use by (ST.1) coagulate of yoghurt mix is due to drop of (PH). ST. initiates the drop of (PH) to (5) and (LB.) responsible for more decrease of (PH) to (4).(Yildiz,2010*)*.

The selected starter ,*L.bulgaricus* isolated from raw milk and *S.thermophilus*in ratio of approximately 1:1 was much higher than commercial yoghurt starter in the acid production and growth of starter, and the yoghurt manufactured with selected starter was better than commercial yoghurt in sensory evaluation such as taste, flavor and all acceptability,(Lee et al.1988).

Yoghurt can be made from any source of milk of any fat content, but mostly fat-free milk yoghurt, skin milk yoghurt, and full fat yoghurt is made with cow`s milk.

1.4: Fermented dairy products benefits:-

- Source of C_a, B_{12}, riboflavin
- Live and active cultures facilitate the digestion of milk.
- Milk proteins and minerals help children and babies to grow.
- Bone density and strength increase strong bone.
- Good bacteria keep bad bacteria away from human digestive system.
- High proteins maintain muscles.

- Flavor, texture and taste are satisfying and give fullness.

1.5: Probiotic bacteria

Yildiz(2010),reported that ,Probiotic bacteria are frequently, but not always chosen from bacteria that normally inhabit the G.I. system of humans, sometimes the term (probiotic) is used as a synonym to (commensally beneficial bacteria) but this is an incorrect usage, commensally flora may be beneficial but until they are isolated. Characterized and shown in human studies to impart a health benefit. They cannot be accurately called (probiotic). Also probiotics must be safe.

Once destined for commercial use these bacteria are purified, grown to large numbers, concentrated to high doses, and preserved. They are provided in products in one of three basic ways:-

*-As a culture concentrate added to a food at medium levels,Inoculated into a milk-based food and allowed to grow to achieve high levels in fermented food,Concentrated and dried cells packaged as dietary supplements such aspowders, capsules, or tablets.

Classification and function of probiotic bacteria

The two most common probiotic genera found in the digestive system are: Lactobacillus and*bifido* bacterium.

- Lactobacillus genus is a group of lactic – acid producing bacteria that play an important role in the digestive tract most specifically in the small intestine.
- *Bifido*bacteria are the major inhabitants of the large intestine. When sufficient numbers of *bifidobacteria*are present they make it

extremely difficult for pathogenic invaders such as the yeast *candida albicans*to exit in the gut.

Bifido bacteria produce acetic and lactic acids.

They also assist in the absorption of B-Comples vitamins.

Species :

Some species belong to *lacto boccillus*genus includes : L . Casei ,L . rhamnosus ,L . plantarum

Bifido bacteria genus include :B . infants, B . longurs,B . breve ,

B . lactic, animals

List of diseases that investigated risk of them is reduced by intake of milk and dairy products.

1- G.I. system disease
2- Cardio vascular diseases
3- Musculo skeletal system
4- Uro-genital system
5- Dermatology
6- Immune system
7- Allergy
8- Nervous system
9- Cognitive system
10- Weight control
11- Aging
12- Nutrigenomics of fermented dairy foods.
13- Dental health

(Yildiz, 2010)

1.6: Fermented milk products and human health

The health benefits of cultured milk products with viable and none viable bacteria are now well recognized but still there is much that needs to discovered.

Epidemiological studies suggest that , overtime adults (Pereira,2002;Yichin,2009*)*. and children (Yildiz,1981; Lovejoy , 2001*)*. who consume a low calcium or low milk product diet fain more weight and more body fat than those with higher intakes .

Dietary calcium markedly accelerates the burning of fat for energy (lipolysis) and helps prevent the conversion of excess carbo hydrates to fat (lipogenesis) resulting in less fat storage.(Sun andZemel,2007*)*.

Individuals on the high – milk product diet three servings of milk products, 1400 mg calcium / day) (*Zemel,.2005)* experienced an almost two – fold greater decrease in fat loss.

The high – milk product diet was associated with the greatest weight loss and the greatest increase in percentage of lean tissue(Novotry ,2004*)*.

The Bulgarian bacillus is in fact *Thermobacteriumbulgaricum*. Later designed as *lactobacillus bulgaricus***.** Currentlyknown as *lactobacillusdelbrueckii sub spbulgarieus* .(Robinson,1999).

Yildiz,(2010) report that the consumption of probiotic cultures may decrease cancer risk,effect of consumption of fermented milks, probiotic bacteria, components of bacteria, or extracts of bacteria have found:-

- A reduction of the activity of fecal enzymes

b- glucoronidase , azoreductase , nitro reductase and 7-&-dehydrogenase) postulated to play a role in colon cancer in human and animal subjects .

- A weakening of mutagenic activity of substances tested in the laboratory .
- A reduction in the incidence of chemic ally induced tumors in rats .
- Degradation of nitrosamines
- Perception of damage to DNA in certain colonic cells .
- Enhancement of immune system functioning
- In vitrobinding of mutagens by cell wall components of probiotic bacteria .

The probiotic cultures may positively influence the G.I. environment to decrease the risk of cancer.

The impact of consumption of milk fermented by *L.casei* strain *shirota* on recurrence of superficial bladder cancer was tested(Kikuchi, 2000*)*.

Milk drinkers have a significantly lower risk of developing breast cancer (Kenkt,1996).Those who drank milk during both childhood and adult hood had a substantially reduced risk of breast cancer(Cho etal.,2004*)*.

Dairy foods (and/ or calcium) appear to be protective against colonic polyps and risk of colorectal cancer , fat identified as potent cancer intlibitor , reduces breast cancer risk in adult women(Parody,1999; Aroetal,2000*)*.

1.7: Other fermented milk beverages:-

Yildiz (2010)reported that, there is another fermented milk beverages include:

-Butter milk

Fermented dairy product produced from cow's milk with a characteristically sour test , the product is made in one of two ways , originally butter milk was the liquid left over from chumming butter from cream . In India butter milk (chaas) is known to be the liquid left over after extracting butter from churned curd (dei) . Today this is called traditional butter milk.

On the other hand artificially mode butter milk also known as cultured butter milk , is a product where lactic acid Bactria called *Str. lactis* have been added to milk .

Whether traditional or cultured the tartness of butter milk is due to the presence of acid in the milk .

-Koumiss (kumis) (Turkish = kimizi) :-

Fermented dairy product traditionally made from mare's milk, the drink remains important to the people of central Asia. Stepps , including the Turks, Bashkirs , kazakha , Kyrgyz , Monogls , yakuts and Uzbks .

Koumiss is a dairy product similar to kefir but is produced from a liquid starter culture in contrast to solid kefir (grains). Because mares milk contains more sugars than the cow's or goat's milk fermented, two kefir koumiss has a higher, though still mild, alcohol content .

Even in the areas of the world where koumiss is popular today , mares milk remains Avery limited commodity , industrial – scale production koumiss there for generally uses cow's milk which is richer in fat and protein but lower in lactose than the milk from mares.

Cow's milk is fortified before fermentation in one of several ways; sucrose may be added to allow a comparable fermentation.

Many of these products have developed in regional areas and depending on the starter organisms used , have various flavor , textures and components from fermentation process such as gas or ethanol .

1.8: Yoghurt Microbiology

1.8.1: *Salmonella spp.* and *Escherichia coli.* In yoghurt:

1.8.1.1: Escherichia Coli.

Since detection and enumeration of coli forms is one of the standard tests required by the International Dairy Federation(Mossel*et al*,1995).

The ability of *Escherichia Coli*. to survive in high –acid food as in case of yoghurt is of public health significance *Escherichia Coli* a natural inhabitant of the tracts of humans & worm-blooded animals, is used as an indicator bacterium because it acquires antimicrobial resistance faster than other conventional bacteria .(Miranda *et al*,2007*).*

1.8.1.1.1: Classification of *Escherichia Coli*:-

Kingdom: *bacteria*

Phylum : *proteo bacteria*

Class : Gamma proteo bacteria

Family : *Enterobacteriaceae*

Species :*Coli. N0minal name Escherichia coli*

Genus : *Escherichia*

(M0rris, 2013)

1.8.1.2: Salmonella spp. ;-

Food-borne diseases caused by *non-typhoid salmonella* an important public health problem worldwide, nearly 1-4 million cases of *salmonellosis* in human occur each year in the United States.(David et al.,2001).

Salmonella are often pathogenic for humans and animals when acquired by oral route (Jawetz*et al* ,2002)

1.8.1.2.1: Classification of *Salmonella*:-

Domain : Bacteria

Kingdom*: Eubacteria*

Phylum : *Proteo bacteria*

Class : *Gamma proteo bacteria*

Order : *Entero bacteria*

Family : *Enterobacteriaceae*

Genus : *Salmonella*

Spsecies*S. benogori*

S. enterica

Non spore forming predominantly motile entero bacteria

(Morris ,2013).

Salmonella gram negative anaerobic mobile short rods or non spore forming rods that ferment glucose usually with gas motile in micro scope

Salmonella spp. Isolated in MacConkey,s Media Culture gives yellow non lactose fermented colonies.

While in C.L.E.D media gives blue non lactose fermented colonies.

In D.C.A media salmonella spp. Gives large pale yellow non lactose or sucrose fermented with black centre colonies.

Lactic Acid Bacteria commonly found in dairy products include strains of *Streptococcus,lactococcus, lactobacilli,Bifidobacteria, Enterococcus, and Pediococci*, within these species there are numerous strain types which can be used in fermentation processes to give specific acidification and flavor profiles to the final product,bacteria associated with dairy fermentations can grow over a wide temperature range from 4-50 c. Mesophilic bacteria have optimum growth range of 25-35c^{o}, while thermophilic species have an optimum range 37-45c^{o}.(Jonson and Steele, 2013).

The growth of bacterial cells within dairy foods is heavily influenced by parameters such as PH, water activity and salt-in-moisture levels as well as temperature.(Cian, 2015).

1.9: HACCP system:

1.9.1: History and identify: The HACCP system which is science based and systemic identified specific hazards and measures for their control to ensure the safety food.(Zaho, 2001***).***

1.9.2: Develop of HACCP Concepts:-

HACCP concepts was pioneered in 1960 by (Pillsbury Company), the United State army and United States National Aeronautics and Space administration (NASA) as (collaborative) development for the production of safe foods. (Abdalla, 2010).

The use of HACCP principles in the promulgation of regulations of low acid carried foods was completed in 1974 by the United States food and Drug Administration. (F.D.A). (Abdalla, 2010*)*.

The consumers expect to a great extent,unconditionally, that the manufactures has ensured that the product is safe for human consumption with respect to both chemical and microbial contamination,conform to any regulations enshrined in law or statutory requirements lay down by health or local authorities,itIs capable of achieving as benefited shelf –life without spoilage,has the highest possible organolyptic standard that can be achieved within the existing constraints of manufacture or marketing.(Tamime and Robinson,1985).

Good hygienic practices should be applied throughout the production and processing chain so that the milk proudest is safe and suitable for their intended use, wherever appropriate hygienic practices for milk and milk products should be implemented following the annex to the codex recommended international code of practice general principles of food hygiene **.**(IDF/FAO 2004)**.**

The HACCP system offers as structured approach to the control of hazards in food processing and property applied , identifies areas of concern and appropriate control measures before product failure experienced . It is represented a shift from retrospective quality control

through end – product testing to a preventative quality assurance approach .The end product testing against microbiological criteria is shifted to role of verification in HACCP program. (Jervis ,2002) .

Although modem large scale production is designed to handle thousands of liters per day using highly sophisticated technology with mechanization and auto mastication , the basic principles under lying the manufacturing process have altered with time .(Tamime and Robinsan, 1985).

The HACCP procedure is generally targeted at food safety management (pathogenic micro organisms and their toxins) but as an approaching the context of border quality management .

It can be effectively applied to microbiological spoilage , foreign body contaminations or peptide contamination , it is preferable to conduct HACCP program with a barrow scope (a single pathogen or possibly pathogens) rather than attempt to cover an extended list of hazard areas when do cementation will become complex . However , an experienced team might choose to cover the whole spectrum of hazard areas depending on :(a) the resources available to produce and maintain a composite HACCP plan (b) the way in which it is to be in corporate system – (Jervis, 2002) .

From raw material production to the point of consumption all dairy products should be subject a combination of control measures. These measures should meet the appropriate level of public health protection for the application of the HACCP system some guidelines must take place :-

* prior to application of HACCP to any sector of the food chain , that sector should be operating according to the codex general principles of

food hygiene , the appropriate codex codes of practice and appropriate safety legislation .

* during hazard identification , evaluation and subsequent operations in designing and applying HACCP system , consideration must given to the impact of raw materials , ingredients , food manufacturing practices , role of manufacturing processes to control hazards , likely and use of the product , categories of consumers of concern, and epidemiological evidence real-time to food safety .(Hoolasi,2005).

1.9.3:Advantages of HACCP :-

- The HACCP system as it applies to food safety management uses the approach of controlling critical points in food handling to prevent food safety problems .
- Identifies specific hazards and measures for their control to ensure the safety of food.
- Prevent and reduces the reliance on end product inspection and tests.
- The system can be applied throughout the food chain from primary production to the consumer.
- HACCP enhances the responsibility and degree of control at the level of the food industry.
- The application of HACCP system can aid inspection by food control regulatory authorities and promote international trade by increasing buyer`s confidence **.**(Abdalla, 2010).

HACCP is a systemic approach relevant to all stages of food processing covering agriculture and horticultural practices,harvesting,processing, product distribution and customer practices. It is move to a preventive

quality assurance approach enabling the manufacturer to get it right the first time and reduce reject wastes.Is the preferred risk management tool in the total quality management ,recognized and promoted by international bodies (such as the codex Alimentarius Commission).

As the system of choice for ensuring food safety and is becoming enshrined in national legislation proactive application in food industry will facilitate compliance with developing legislation and demonstrates a diligent approach to food safety . .(Jervis , 2002).

The HACCP system offers a structured approach to the control of hazards in food processing and properly applied, identifies areas of concern and appropriate control measures before product failure is experienced. (Jervis ,2002).

Chapter Two

Materials and Methods

2.1;Microbiological, chemical and physical analysis:-

2.1.1: Sampling:-

Samples for microbiological analysis were taken aseptically and kept between 1-5c^{o}. Samples for chemical analysis analyzed immediately. (Hoolasi, 2005).

Sixty samples taken at different stages of yoghurt manufacturing process, inyoghurt production unit in Khartoum –Sudan and analyzed.

2.1.2: Total of 60 samples were collected in test tubes from (6) stages of yoghurt production process, in yoghurt production unit,Khartoum.Sudan.They weredividedinto 2 groups, first group 30 samples, and then 30 samples repeated for the same stages.

- Samples were collected from whole milk stage, pasteurized milk stage, cold pasteurized milk stage, milk+starter stage, after incubation stage , andcold yoghurt stage)
- These samples were transferred to laboratory forbiological and chemical tests to determine the general health condition of yoghurt and to detect the critical points in the process.

2.2: Microbiological Analysis

2.2.1: Preparation of dilutions:

1ml. of each milk samples and 1gm. of yoghurt samples was diluted in 9ml. sterile quarter – strength ringers buffer solution. Appropriate serial

decimal dilutions were made using sterile ringer's solution (Hoolasi,2005).

Total plate counts:(T.B.C.) standard plate count (Agar Merck 1-05463) was used to determine the total bacterial count.

2.2.2: Procedure Process

A) Adding samples to nutrient broth in test tubes to make a solution.

b) 5ml of this solution incubated over night for (18 – 24 hours) at 37c^{o} to obtain bacterial growth.

c) 9ml normal saline in sterile tubes for serial folds dilutions prepared.

d) By micro pipette, 1ml from inoculated nutrient broth add to first tube of 9ml normal saline to make dilution 1/10 dilute (1).

e) From 1/10 dilution 1ml by micro pipette added to second tube of 9ml normal saline this make dilution of 1/100 dil. (2). This process continues till dilute (5) which is 1/100000.(Mile and Misera) method.

f) 1ml from dilution (4) (1/10000) added to surface of Petri-dish contain Nutrient Agar Media, incubated 37c^{o} for 18-24 hours (overnight). This test is done again using dil. (5) (1/100000).

- The media was prepared according to the manufactures instructions and the pour plates. Incubated for 72 hours at 30c^{o}.

2.3 Media:-

2.3.1:*Mac*ConKeysAgar media:-

The media was prepared according to manufacture instructions by dissolving 51.1 gm in distal water mixed and sterilized by auto clave at

121c^{o} for 15 minutes. After that distributed in sterile Petri-dishes in 25ml volume, left to solidity and stored at 4c^{o} before used.

2.3.2: Nutrient Agar media:

It is a basic culture medium used to sub culture organisms for maintenance purpose or check the purity of sub cultures from isolation plate's prior biochemical or serological test.

2.3.3*:* Selective Media:

DeoxycholatecholateSitrate lactose Sucrose Agar,*(DCLS) Agar*Very versatile solid medium, originally developed by Levine for differentiation of *Escherichia coli* and *Aero bacter***,** it turnedout to be effective for the rapid identification of *Candida Albicans* and was found use full for identification of Coagulase – positive *StaphyloCocci.*

2.4: **Bacterial Count**:-

2.4.1:*:* Total Viable plate count*: (T.V.C)*

One of the most common methods of determines cell number is viable plate count. A sample to be counted is diluted in a solution that will not harm the microbe, yet doesn`t support its growth.

A standard plate Count method used to determine the numbers of viable bacterial cells per unit volume of the sample.

The number of viable bacterial cells per milliliter of liquid samples was determined by taken affixed volume of sample transferred to a plate, the solution spread across the plate and the colonies counted after incubation, the colonies were referred to as colony forming unites (CFUs)on the plate was determined, it was divided by the volume plated determined the

concentration of the cells in the sample , the sample contained over one thousand cells per unit volume then it produced to many CFUs to a count accurate on the plate. These samples diluted in sterile media before was transferred to plate media so that accountable number of colonies appeared , since the actual concentration of the sample was unknown it was common practice to dilute the sample serially(for example).sample transferred to a plate. Spread across the plate and colonies counted after incubation over night at 37c^{o} for 18-24 hours. using standard Petri – dishes , Colonies were referred to CFUs (Colony Forming Unites), accountable plate was one with between 30 and 300CFUs,dilutions with fewer than 30 colonies are easily counted .The dilution determined by dividing the amount plated once the concentration of the cells at specific dilution was determined ,the concentration in the origin sample was calculated after dividing by the total dilution (using aseptic technique)

Microbial analysis procedures;

Samples were put in nutrient broth in test tubes ,5ml of this solution incubated for 18-24 hours at 37 c°(overnight) for bacterial growth, Serial dilutions from normal saline included bacteria to be diluted, serial folds dilution in sterile test tubes ,every one contain 9ml normal saline were prepared ,1ml from nutrient broth included bacteria added in the first 9ml tube, dilution 1= 1/10, the procedure continue till dilution 5=(1/100000),from dill- 4 and dill-5 ,1ml taken and added to surface of Petri-dish contain Nutrient Agar Media ,incubated (overnight) at 37 c° for 18-24 hours shows the colonies. This method called (Mile and Misera). After that colonies counted.

3.5: Isolation of *Salmonella spp. and* *Escherichia* ***coli***

Escherichia coli isolated in ManCconkey Agar Media, E-coli fermenting lactose (pink color) , that not fermenting lactose gives(color less).

In D.C.A. culture *Escherichia coli* gives pink and opaque colonies.

Salmonella Isolated in MacConkey Media gives pale yellow non lactose fermented colonies.

Salmonella in D.C.A gives large pale yellow non lactose or sucrose ferment with black center colonies.

In C.L.E.D media gives blue non lactose ferment colonies.

2.6: Biochemical Tests :-

2.6.1: Primary tests:

2.6.1.1: oxidase test

To detect the presence of cytochrome oxidase enzyme called Indophenole Oxidase, reduced color less reagent becomes an oxidized colored product.

2.6.1.2: Oxidation fermentation test:

Determine if gram-negative bacteria metabolize carbohydrates oxdatively, by fermentation and there for have no ability to use the carbohydrate in the media.

2.6.1.3: Motility test:

Immersion oil in high pour lens, motility was characterized by fast unidirectional movement as compared to the Brownian motion whereby the cells move round in one particular point.

2.6.1.4: Catalase test:-

In this test the bacteria which are catalase positive is detected by HydrosePeroxide**,** due to presence of catalase enzyme.

2.6.2: Secondary Test

2.6.2.1: Citrate test

The citrate test is often part of a battery of tests used to identify gram-negative pathogens and environmental isolates, positive diagnostic test depend on generation of alkaline by product of citrate metabolism.

Increase in PH of medium was demonstrating by the color change of PH indicator.

2.6.2.2 :Indol test*:*

Indol : Methyl Red, vogues Proskauer, and Citrate were used to differentiate the gram-negative bacilli in family *Enterobacteriaceae.*

Positive isolates were cherry red coloration in oil layer on top of broth while negative isolates had no red color in oil layer on top of broth.

2.6.2.3: Urease test

To check for purity of organism

Selective agar medium stab with nichrome wire incubated at 35-37c^{o}in water both, examined after 4 hours and overnight:

Positive isolates were pink color whilenegativeisolates had No change

2.7: Chemical Tests:

2.7.1:Primary chemicaltests:**Table-4**

	Catalase test	**Oxidase test**	**O.F. test**	**Sugar test**	**Motile test**
Salmonella	-	+	F	+	Motile
Escherichia coli	-	+	F	+	Motile

2.7.2*:* Secondary chemical tests*:***Table-5**

	Indol (Kovac-s)	**Citrate test**	**Urease test**	
Salmonella	-	+	-	
Escherichia coli	+	-	-	

Kia Kilgarn Iron Agar test;**Table -6**

Slope	**H2S**	**Batt**	**Gas**	
Red	Yellow	+	+	
Yellow	Yellow	-	+	

2.8: Statistical Analysis:

The data were analyzed with statistical package for Social Science (SPSS), version software (SSPS Inc, and Chicago IL, USA). All bacterial counts were converted tolog 10CFU/ml (g) for analysis and ANOVA was performed. Statistical significance was set at a p-value of $p<0.05$.

Chapter Three

Results

The study on yoghurt at different stages of productions from the first stage (whole milk) up to last stage of production (yoghurt), showed isolation and identification of two types of bacteria spp.*Escherichiacoli* and *salmonella*. spp.

Total Viable Bacterial Count of **60** samples from all stages of yoghurt production process; Table -7

NO	STAGE	TVBC	NO	STAGE	TVBC
1	Whole milk	2150000	31	Adding starter	7150000
2	Whole milk	9750000	32	Adding starter	6800000
3	Whole milk	7650000	33	Adding starter	7300000
4	Whole milk	8900000	34	Adding starter	7200000
5	Whole milk	6250000	35	Adding starter	6750000
6	Whole milk	6650000	36	Adding starter	8550000
7	Whole milk	9300000	37	Adding starter	5750000
8	Whole milk	9650000	38	Adding starter	6200000
9	Whole milk	7400000	39	Adding starter	8400000
10	Whole milk	10550000	40	Adding starter	6200000
11	Pasteurized	2350000	41	After	7350000

	milk			incubation	
12	Pasteurized milk	2280000	42	After incubation	8450000
13	Pasteurized milk	2200000	43	After incubation	7350000
14	Pasteurized milk	1800000	44	After incubation	7650000
15	Pasteurized milk	2350000	45	After incubation	7400000
16	Pasteurized milk	2300000	46	After incubation	9500000
17		2300000	47	After incubation	6950000
18		2300000	48	After incubation	7600000
19		1800000	49	After incubation	6400000
20	Pasteurized milk	1700000	50	After incubation	8550000
21	Cold pasteurized milk	9500000	51	Cold yoghurt	7250000
22	Cold pasteurized milk	8500000	52	Cold yoghurt	6850000

23	Cold pasteurized milk	7500000	53	Cold yoghurt	7350000
24	Cold pasteurized milk	6750000	54	Cold yoghurt	9450000
25	Cold pasteurized milk	6700000	55	Cold yoghurt	8000000
26	Cold pasteurized milk	7350000	56	Cold yoghurt	6450000
27	Cold pasteurized milk	6450000	57	Cold yoghurt	7950000
28	Cold pasteurized milk	7300000	58	Cold yoghurt	6800000
29	Cold pasteurized milk	6700000	59	Cold yoghurt	7950000
30	Cold pasteurized milk	7300000	60	Cold yoghurt	6250000

In this study isolation of *Salmonella spp*. and *Escherichia coli* was obtained in all stages of yoghurt manufacturing process (Table 7).

Biochemical Tests:

Table1: P**rimary chemical tests**

	Catalase test	**Oxidase test**	**O.F. test**	**Sugar test**	**Motile test**
Salmonella	-	+	F	+	Motile
E-coli	-	+	F	+	Motile

Secondary chemical tests*:2*

	Indol (Kovac-s)	**Citrate test**	**Urease test**
Salmonella	-	+	-
E-cali	+	-	-

Kia Kilgarn Iron Agar test:

Slope	**Hzs**	**Batt**	**Gas**
Red	Yellow	+	+
Yellow	Yellow	-	+

The results in table 7(Fig1) showed the number of isolated bacteria(*Salmonella spp. and Escherichia coli*) in different points in yoghurt processing as follows:

Whole milk: The number of isolated bacteria 2(3.84)% samples were positive for *Salmonella* and 10(19.16) for *Escherichia coli*

Pasteurized milk: The number of isolated bacteria 2(3.6)% samples were positive for *Salmonella* and 3(5.4)% for *Escherichia coli*

Cold pasteurized milk : The number of isolated bacteria 4(7.6)% samples were positive for *Salmonella* and 6(11.4)% for *Escherichia coli*

Milk+starter: The number of isolated bacteria 3(5.63)% samples were positive for *Salmonella* and 5(9.37)% for *Escherichia coli*

After incubation: The number of isolated bacteria 3(5.63)% samples were positive for *Salmonella* and 3(5.4)% for *Escherichia coli*

Cold yoghurt: The number of isolated bacteria 3(6)% samples were positive for *Salmonella* and 6(12)% for *Escherichia coli*

Table 8 : Number and Percentage of *Salmonella spp. and E-coli* in different stages of yoghurt processing

No	**CCP**	***Salmonella Spp.%***	***Escherichia coli%***	**Total %**
1	Whole milk	2(3.84)%	10(19.16)	12(23)%
2	Pasteurized milk	2(3.6)%	3(5.4)%	5(9)%
3	Cold pasteurized milk	4(7.6)%	6(11.4)%	10(19)%
4	Milk+starter	3(5.63)%	5(9.37)%	8(15)%
5	After incubation	3(5.63)%	5(9.37)%	8(15)%
6	Cold yoghurt	3(6)%	6(12)%	9(18)%
	Total	17(32.3)%	35(66.7)%	52(99)

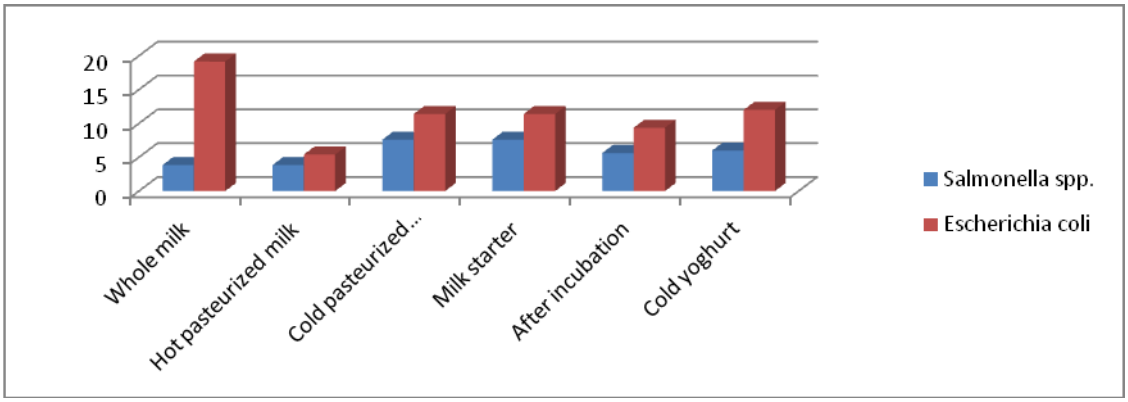

Figure 1: **Numbers and percentages of (*Salmonella spp*. and *Escherichia Coli*.) in different stages of yoghurt processing.**

Isolation and identification of bacteria at different operation process under investigation showed that Whole milk stage as high level of contamination and the T.V.C. with *Salmonella spp.* was 23.84% and *E-coli* 19.16% ($p \leq 0.05$).While the lower level of contamination in hot pasteurized milk and the T.V.C. with *Salmonella s*

pp. was 3.6% and *Escherichia coli* 5.4%**.** Table 8 (Fig 2)

Table 9: Isolation and Identification of *Escherichia coli* and *Salmonella Spp*.:

Stage	*Salmonella spp.* %	*Escherichia coli%*	Total %
Pasteurized milk hot	2(3.6) %	3(5.4) %	5(9) %
Whole milk	2(3.84) %	10(19.16) %	12(23) %

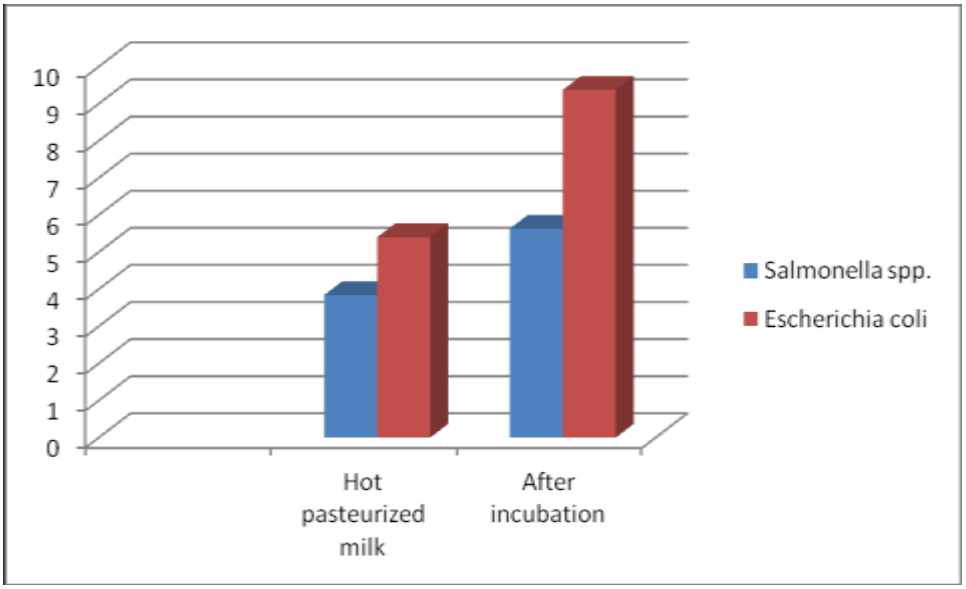

Figure 2: Percentage of *Salmonella spp. and E-coli.* In high and low contamination in yoghurt process

Whole milk Point showed contamination (mean (log_{10} cfu/ml) 6.93 ± 0.20a.

Hot pasteurized milk Point showed contamination (mean (log_{10} cfu/ml) 6.34 ± 0.07^{b}

Cold pasteurized milk **Point showed contamination (mean (log_{10} cfu/ml)** 6.87 ± 0.05^{a}

Milk + starter Point showed contamination (mean (log_{10} cfu/ml) 6.85 ± 0.06^{a}

After incubation 6.89 ± 0.05^{a} **Point showed contamination (mean (log_{10} cfu/ml)**

Cold yoghurt Point showed contamination (mean (log_{10} cfu/ml) 6.87 ± 0.05^{a}

Table 10 : Means (Total viable count of Bacteria (log-CFU/ml) at different points of processing yoghurt product:

Point of Stage	TBC (log CFU/ml.)
Whole milk	6.93 ± 0.20a
Hot pasteurized milk	6.34 ± 0.07[b]
Cold pasteurized milk	6.87±0.05[a]
Milk + starter	6.85±0.06[a]
After incubation	6.89±0.05[a]
Cold yoghurt	6.87±0.05[a]
Significant level	**

** a P-value ≤0.05

Statistical analysis:

The data were analyzed with SPSS software (Statistical Package for Social science version 20, all TVCs bacteria were converted to log10 CFU/cm2 for analysis .NOVA was performed. Statistical significance was set at P-value of ≤0.05

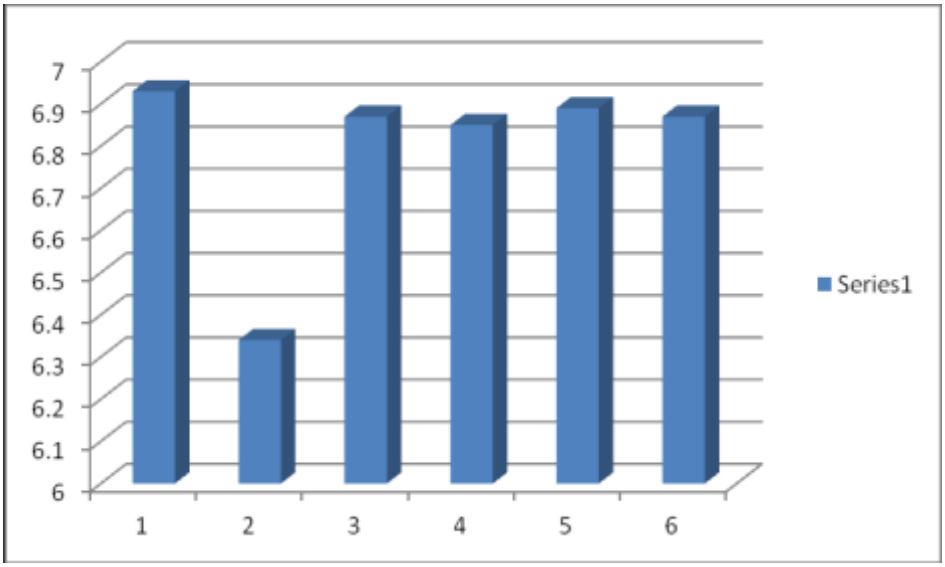

Figure 3: **Mean** (Total viable count of Bacteria (log-CFU/ml) **at different point of yoghurt production process .**

Chapter Five

Discussion

In this study bacterial contamination was obtained in all stages of yoghurt process and this is in agreement with Montville, (2005) who stated that fermented dairy products are often not manufactured under sterile conditions or with sterile milk (unpasteurized) and this can allow non-starter Lactic Acid Bacteria (LAB) as well as spoilage or pathogenic bacteria to gain access to the fermenting food system. In general, within the *Escherichia* genus, pathogenic *Escherichia coli* organisms are significantly coli forms is one of the standard tests required by the International Dairy Federation (Mossel et al. 1995), the ability of *Escherichia coliO157 :H7* to survive in high-acid food is of public health significance.

Also the present results agree with Johonson(2013) who stated that bacteria associated with dairy fermentations can grow over a wide temperature range from 4 to 5 C°.Mesophilic bacteria have an optimum growth range of 25 -35 C°., while Thermophilic species have an optimum range of 37 -45 C°.

Also the present research agrees with Yang et al. (2012) who stated that bacteria are naturally present and are used extensively across all areas of dairy and food fermentation either as natural micro- flora or as starter culture added under controlled conditions.

Morgan *et al* (1993) reported that number of potential problems could be identified at dairies : the milk might be inadequately pasteurized, or contaminated after pasteurization either because of inadequate cleaning of systems or by farm yard matter. In present results the Total Viable

Count (T.V.C.) showed the lower contamination stage is after pasteurization hot milk, and this agrees with FAO(2006) which reported that the heat treatment using sterilization methods completely inactivates enzymes and destroyed the most heat resistant micro organisms

D'aost (2001) who stated that standard methods of pasteurization both via pasteurization and high-temperature, short time pasteurization, are very effective in destroying Salmonella spp. and this agrees with the present findings.

Crow *et al*. (2001) agrees with this study and stated that pasteurization inactivates pathogenic bacteria ,but also the results in significant reduction or in activation of naturally occurring micro-flora population.

On conclusions reported that contamination was detected in all yoghurt production process. *Escherichia Coli* and *Salmonella spp.* were detected and isolated from 6 stages of yoghurt production process, the lower contamination stage was after pasteurization hot milk, the highest contamination stage was in whole milk

Conclusions

This study conducted that, contamination is reported in all yoghurt production process. *Escherichia Coli* and *Salmonella spp.* were detected and isolated from 6 stages of yoghurt production process, the lower contamination stage was after pasteurization hot milk, the highest contamination stage was in whole milk

Recommendations

1- Care should be taken during milk process to reduce the possibility of fecal contamination of row milk.

2- Hygiene managements of worker equipment should always been taken.

3- Adequate pasteurization of raw milk.

4- HACCP system provides a functional tool to minimize contamination of yoghurt production and gives a product of standard value.

5-Milk used for yoghurt production must be obtained from granted sources.

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