بسم الله الرحمن الرحيم

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Evaluation and Implementation of Hazard Analysis and Critical Control Points System (HACCP) in Stirred Yoghurt Plant

تقويم وتطبيق نظام تحليل المخاطر ومراقبة النقاط الحرجة في مصنع الزبادي الممزوج

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الآيـة

قال تعالى:

(وَإِنَّ لَكُمْ فِي الْأَنْعَامِ لَعِبْرَةً نُسْقِيكُمْ مِمَّا فِي بُطُونِهِ مِنْ بَيْنِ فَرْثٍ وَدَمٍ لَبَنًا خَالِصًا سَائِغًا لِلشَّارِبِينَ).

صدق الله العظيم

سورة النحل الآية (66)

Dedication

To My

Family ***for their kind support and encouragements,***

Teachers

And all my Friends.

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I would like to express my thanks to my supervisor ***Dr. Barka Mohammed Kabier Barka*** *For helpful assistance, guidance, patience and keen supervision during this work.*

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Abstract

This study was carried out to evaluate the Hazard Analysis and Critical Control Point (HACCP) system implemented in stirred yoghurt processing line in CAPO dairy products. HACCP plan was set, the critical control point (CCP_S) were identified based on the flow diagram of stirred yoghurt production line. The CCP_S including raw milk, after pasteurization process, during mixing, fermentation process, packaging and distribution were defined. The study period of HACCP implantation was in April, May, June and July (2014). Raw milk samples were collected from milk collection centers (MCCs) including Alifoun, Tibna and Sayegh. Control measures including, physio-chemical and microbial were determined to samples, raw milk samples and samples collected from different steps of stirred yoghurt production and final products. Critical limits, monitoring procedures were set and the results obtained were statistically analysed. The results obtained of store milk temperature from three MCC_S during the study period revealed significant ($p<0.05$) decrease in temperature. The pH was not significant ($p<0.05$) difference during the study period (April- July) as well as titarable acidity and specific gravity. While the fat and solid non fat content significantly ($p<0.05$) increased in Alifoun MCC as well as samples were nilled of antibiotic during the study period. The microbiological results indicated that Coliform bacterial count (log_{10} cfu/ml) were significantly ($p<0.05$) decreased in Alifoun MCC compared with Tibna and Sayegh MCC. The total bacterial count was significantly ($p<0.05$) difference among the three MCC_S. Lab Pasteurization Count (LPC) for mesophilic and thermophilic (log_{10} cfu/ml) was significantly ($p<0.05$) low in Alifoun compared with that of Tibna and Sayegh MCC.

During the mixing process the fat, solid non fat content, total solid content, titarable acidity and specific gravity were not significantly ($p<0.05$) different

during the study period. After the pasteurization process the specific gravity, solid non fat and total solid content were not significant ($p<0.05$) difference during the study period. During fermentation process the incubation temperature were not significantly ($p<0.05$) difference as well as the pH value, viscosity and cooling temperature. While the total solid content of fermented milk yoghurt was significantly ($p<0.05$) deference. The pH of final stirred yoghurt was not significantly ($p<0.05$) difference. Whereas the viscosity of final product was significantly ($p<0.05$) difference as well as the final product was null of Yeast and Moulds during the study period. Overall quality parameters defined during the study were within the standard range of stirred yoghurt plant. Therefor, application of the HACCP could contribute to safe and high quality stirred yoghurt.

ملخص البحث

أجريت هذه الدراسة بغرض تقويم نظام تحليل المخاطر والتحكم في النقاط الحرجة (الهسب) المتبع في خط إنتاج الزبادي المزج بمصنع كابو لمنتجات الألبان. تضمنت النقاط الحرجة الحليب الخام، البسترة، عمليه الخلط والتكوين، التخمير، التعبئة والتوزيع كذلك تم تحديد نقاط التحكم، الحدود الحرجة، إجراءات الرقابة والأفعال التصحيحية لكل النقاط الحرجة في مخطط التصنيع.تم إجراء الدراسة خلال فترة زمنية تضمنت أربعة أشهر وهي أبريل، مايو، يوليو ويونيو 2014م أجريت خلالها اختبارات الخواص الفيزيوكيميائية والمايكرو بيولوجية لعينات اللبن المتحصل عليها من مراكز تجميع الألبان وهي العيلفون، التِبنة والصايغ والتي يعتمد عليها المصنع في إنتاج وتصنيع منتجاته خاصة الزبادي وتم تحليل النتائج المتحصل عليها إحصائيا.

أظهرت النتائج وجود زيادة معنوي $P<0.05$ في درجة حرارة الحليب بيمنا لم يكن هنالك تأثير معنوي $P<0.05$في رقم الـ pH، الحموضة والكثافة النوعية مع وجود زيادة معنوية $P<0.05$ في محتوي الدهن، الماد الصلبة غير الدهنية والمواد الصلبة الكلية في عينات الحليب الخام المتحصل عليها من مركز العيلفون. كما أظهرت نتائج التحليل خلو عينات الحليب من المضادات الحيوية.

أظهرت التحاليل المايكروبيولوجية للحليب الخام وجود انخفاض معنوي$P<0.05$ في بكتيريا القولون لعينات اللبن المتحصل عليها من مركز العيلفون بينما كانت هنالك اختلافات معنوية $P<0.05$ في العد الكلي للبكتيريا بين عينات اللبن المتحصل عليها من مراكز تجميع الألبان كما أظهرت الإختبارات المعملية وجود إنخفاض معنوي في بكتيريا الـMesophilic والـThermophilic لعينات اللبن المتحصل عليها من مركز العيلفون لتجميع الألبان.

أظهرت نتائج التحليل خلال عملية الخلط والتكوين عدم وجود اختلافات معنوية $P<0.05$ في كل من محتوي الدهن، المواد الصلبة غير الدهنية، المواد الصلبة الكلية، الحموضة والكثافة النوعية لخليط اللبن المتحصل عليه من مراكز تجميع الألبان الثلاثة.بعد بسترة الحليب أظهرت نتائج التحليل عدم وجود إختلافات معنوية $P<0.05$ في الكثافة النوعية، المواد الصلبة غير الدهنية والمواد الصلبة الكلية خلال فترة الدراسة.

خلال فترة التخمير لم تكن هنالك إختلافات معنوية$P<0.05$ في درجة حرارة التحضين، الـpH واللزوجة بينما كانت هنالك إختلافات معنوية$P<0.05$ في المواد الصلبة الكلية. أما المنتج النهائي فقد أظهرت النتائج عدم وجود علاقة معنوية في الـpH بينما كانت هنالك زيادة معنوية $P<0.05$ في اللزوجة مع خلو المنتج من الأعفان والخمائر. صفات الجودة الكلية التي تم تحديدها أثناء الدراسة كانت في حدود مواصفة الزبادي المزج بالمصنع. لذلك تطبيق نظام تحليل المخاطر والتحكم في النقاط الحرجة (الهسب) يمكن أن يساهم في السلامة والجودة العالية للزبادي المزج.

CHAPTER ONE

INTRODUCTION

The growing awareness of the relationship between diet and health has lead to an increased demand for quality food products that support health, above and beyond providing basic and nutritional needs. Milk is a natural and highly nutritive and balanced daily diet. It is one of the best sources of calcium and provides high quality protein, vitamins and other minerals (Smit, 2005). The hygienic quality problems of milk and milk products may arise from raw milk of diseased animals (Murphy and Boor, 2000). Bacterial contamination of raw milk can originate from different sources, including low quality raw milk, improper refrigeration and an inadequate packaging system (Tokar and Teger, 2008).

Yoghurt is one of the most widely distributed dairy products. It is made from milk yoghurt essentially has all the nutritional components of milk in different forms with diverse local names is made throughout the world (Vedamuth, 1991; Tarakci and Erdogan, 2003).

The natural yoghurt is characterized by a smooth and viscous gel like texture and has a delicate walnutty flavor (Fuquay, 2011). The fermentation of lactose sugar by lactic acid bacteria results in the production of lactic acid, carbon dioxide, acetic acid, diacetyl, acetaldehyde and several other components giving a characteristic flavor to yoghurt (Tamime and Robinson, 2004).

Yogurt quality is difficult to standardize because of many forms, varieties, manufacturing methods, ingredients and consumer preferences that exist (Kroger *et al.*, 1989). Therefore, very careful processing is required for the production of safe and good quality yoghurt to satisfy the highest expectations of the consumers.

Good agricultural practice (GAP) and good manufacturing practice (GMP) together with HACCP program should meet the appropriate level of public health protection. Good hygienic practices (GHP) should be applied throughout the production and processing chain so that the milk product is safe and suitable for their intended use. The GHP should be implemented following the Annex to the Codex Recommended International Code of Practice– General Principles of Food Hygiene (IDF/FAO, 2004).

During manufacturing there are certain stages where there is a potential for microbiological and foreign object contamination that results in many problems related to quality and safety of the products.

This could have a negative impact on the quality and image of the product. To address these issues the implementation of a system based on technical and scientific principles, such as HACCP system, is crucial.

Marketing of yoghurt and dairy products made of raw milk are the common futures of dairy products in Sudan. Nowadays, many private dairy factories are invested in yoghurt processing in Sudan such as Premier Milk Company, BEST Milk Company and FAABY Milk Company. Among them CAPO milk company is one of the most famous and the company had ISO certificate. However, implementation of safety programs based on HACCP system at CAPO Milk Company did not exist. Therefor, the present study is carried out with the following objectives:

1. To establish a HACCP plane for implementation in yoghurt processing at CAPO stirred yoghurt plant.
2. To define the microbiological and chemical risks associated with different steps of stirred yoghurt processing at the plant.

3. To define the critical points based on expected chemical and microbiological contamination during processing of stirred yoghurt.
4. To evaluate HACCP implementation on chemical, physical and microbial quality of stirred yoghurt.

CHAPTER TWO

LITERATURE REVIEW

2.1 Milk

Milk has been defined as the normal secretion (excluding colostrums) which can be gained by normal milking method from the lactating mammary gland of the healthy normally fed cows. Milk can be considered as a three basic components water, fat, and solid-non-fat (SNF). The organic matter in the non-fatty portion consists mainly of the protein casein, albumin and globulin, lactose and citrates. However, milk from individual cows may show a day-to-day variation. Such fluctuation may be influenced by the mental and physical conditions of the animal. Excitement, worry or discomfort is liable to have a diverse effect on both the quantity and quality of milk produced (Johnson, 1986). Table 1 display the chemical component of different mammal's milk.

Table (1): Chemical composition of cow's milk and other mammals.

Species	Composition (%)						
	Water	**Fat**	**Protein**	**TS**	**SNF**	**Lactose**	**Ash**
Human	87.43	3.75	1.63	12.57	8.82	6.89	0.21
Cow	87.20	3.70	3.50	12.80	9.10	9.40	0.70
Buffalo	82.76	7.38	3.60	17.24	9.82	5.48	0.78
Camel	87.61	5.38	2.98	12.39	7.01	3.26	0.70
Ewe	80.71	7.90	5.23	19.29	11.39	4.81	0.90
Goat	87.00	4.25	5.23	13.00	8.75	4.81	0.86
Mare	89.04	1.59	2.69	10.96	9.37	6.14	0.51

Source: Johnson (1980).

2.1.1 Nutritive value of milk

Milk is an excellent source of high biological value protein because it contains, in varying amount, all essential amino acid that human body cannot synthesize and in proportion, resembling amino acid requirement. Milk and milk product provide significant amount of micronutrients; including calcium, b-group vitamins, (particularly riboflavin B_{12}, also thiamin, niacin and vitamin B_6, vitamin a, iodine, magnesium, phosphorus, potassium and zinc (Anita, 2001).

Although, the salts of milk are quantitatively minor constituents, they are of major significance to its technological properties especially to the stability and properties of the milk's protein system (Fox and weeny, 1998). Calcium from dairy products has greater bio-availability than calcium from vegetables (Bordy, 1999). The components of milk color are due to milk's natural pigment concentration; from carotenoids, protein and riboflavin (Noziere *et al., 2000b*).

Milk lipids are considered to be one of outstanding milk constituents with respect to presence of lipids classes, variety and number of identified fatty acid (Jensen *et al.,* 1991). Milk lipids include anti carcinogenic compounds such as conjugated linoleic acid, sphingomyelin and butyric acid (Parodi, 1999).

Lactose (β-galactosyl-glucose) is the carbohydrate source in milk. It was probably the first prebiotic compound that nature developed in order to support the healthy growth of infants (Harju, 1991). Lactose cannot be absorbed unless being hydrolyzed to monosacchariedes; glucose and galactose by intestinal lactase, which declines early in life to the point of virtual absence in adulthood, making them intolerant (Vesa, 1999). In populations with an old dairying tradition, lactose malabsorption is more rare (Harju, 1991). The nutritional value of milk as a wholes greater than the value of its individual nutrient because of its unique nutritional balance (Wattiaux, 2000).

2.1.2 Microorganisms in milk

The ability of any microorganisms to grow in food products depend on a number of limiting factors such as temperature, radix potential, pH, water activity, added preservatives and competitive microflora (Gaze, 1992).

2.1.3 Spoilage microorganisms

During cold storage after milk collection, psycrotrophic bacteria population dominate the microflora and their extracellular enzymes, mainly proteases and lipases contribute to the spoilage of dairy products (Hantsis-Zacharov and Halpern, 2007). The numbers of psychrotrophs that develop after milk collection depend on the storage temperature and time. Under sanitary condition <10% of the total microflora is psychrotrophs in contrast to >75% under unsanitary condition (Cousin, 1982).

The most common spoilage microorganisms of milk and dairy products are gram negative rod shaped bacteria, gram positive spore forming bacteria, lactic acid forming bacteria, yeast and moulds (IDF, 1994).

Psudomons spp. are the most important group of psychrotrophs associated with spoilage. They may however, produce extracelluar enzymes, which are particularly destructive, if high numbers are present (IDF, 1994). Psedomonas species are the most common organisms in raw or pasteurized milk at the time of the spoilage (Sorhaug and Stepaniak, 1997), and they constitute the predominant microorganisms limiting the shelf life of processed fluid milk at 4°C (Gilmour and Rowe, 1990). Also, lactic acid producing microorganisms (*Streptococcus* spp., *Lactobacillus* spp. And *Leuconostoc* spp.)spoil milk by fermenting lactose to produce lactic acid (IDF, 1994).

The defects that can occur in milk due to microbial growth are off flavor, lipolysis with development of rancidity, gas production, souring due to fermentation, coagulation of milk protein, viscous or ropy texture and discoloration (Banwart, 1981).

2.1.4 Fermentative microorganisms

Eight main genera of lactic acid bacteria (LAB) each genus and species has different characteristics but they are generally chained cocci or rod shaped gram positive, non sporulating bacteria that produce lactic acid as a major or sole product of fermentative metabolisms (Salminen and Wright, 1993). Lactic acid bacteria ferment carbohydrates into energy and lactic acid (Jay, 2000).

Depending on the microorganisms, metabolic pathway differ, when glucose is the main carbon source: homo-fermentative bacteria such as Lactobacillus and Streptococcus yield two lactose form one glucose molecule, whereas the hetro-fermentative bacteria such as Leuconostoc and Weissella, transform glucose molecule into lactate, ethanol and carbon dioxide (Caplice and Fitzgerald, 1999; Jay, 2000; Kuippers *et al.*, 2000).

Lactic acid bacteria occur naturally in fermented food, they can produce small organic compound that give the aroma and flavor to fermented product (Caplice and Fitzgerald, 1999). Lactic acid bacteria and their metabolites have been shown to play an important role in improving microbiological quality and shelf life of many fermented food products and provide good example of biological preservation (Zottola, 1994).

The presence of natural indigenous lactic acid bacteria derived from fermented milk has a competitive exclusion of contamination and pathogen (Urano, 2003). Pathogen inhibition by lactic bacteria may provide significant protection against

pathogenes, either through a natural competitive barrier against pathogens in gastrointestinal tract or as a method of decontaminate bacteria toxic.

The use of bacteriocin producing microorganisms, such as lactic acid bacteria (LAB) in the food industry is attractive because there is an increasing demand for natural products and increasing concerns about food borne diseases (Cleveland *et al.*, 2001). Lactic acid bacteria have been used as flavoring and texturizing agents as well as preservative in food for centuries, are now added as starters (Caplice and Fitzgerald, 1999).

Lactic acid bacteria such as *Lactobacilli, Lactococus lactis*, and *Streptococcus thermophillus,* inhibit food spoilage and pathogenic bacteria and preserve the nutritive qualities of raw food materials for an extended shelf life (Heller, 2000; O'sullivan *et al.;* 2002).

2.1.5 Pathogenic microorganisms

Pathogens involved in food borne outbreaks that associated with consumption of milk include *Salmonella, Listeria monocytogens*, *campylobacter, staphylococcus aureus*, *Bacillus cereus* and *colostridum botulinum* (Ryser, 1998).

The most common contagious mastitis pathogens are *staphylococcus aureus*, *streptococcus agalacitiae* and *Mycoplasma bovis*, but some strain of *streptococcus ubreis* may be transmitted by milk (Zadoks, 2003).

Other milk borne pathogens that have resulted in food poisoning among the human population are *yersinia enteropathogenic E. coli* and *salmonella* (Shewmake and Dillon, 1998).

2.1.6 Fermentation of milk

Acidification of milk by fermentation is one of the oldest methods of preserving milk and imparting to it special favorable organoleptic qualities (Tamime and Death, 1980).

Fermentation is the process leading to the anaerobic breakdown of carbohydrates. Fermentation is an energy-yielding, oxidation-reduction process (Kosikowski, 1982).

The major sugar and citric acid fermentation in milk summarized by (Kosikowski, 1982) include:

- Lactic acid fermentation: The lactic acid fermentation is the most important one in milk, for it is required in all instances.
- Propionic acid fermentation: In emmental, swiss and certain sweet curd and ripened cheeses, the propionic acid fermentation leads to the typical cheese, flavour.
- Citric acid fermentation: It is responsible for the delicate aromatic flavour of buttermilk, sour cream, cream cheese and some cottage cheeses.
- Alcoholic fermentation: As in kefir and koumiss which contain 1- 3% ethyl alcohol.
- Butyric acid fermentation: It generates large amount of carbon dioxide and hydrogen gas in the product.

However, in Sudan there are four types of traditionally fermented dairy products: rob, gariss, laban- gedim and mish. In addition, there are two quasi-indigenous products, namely, Jibna Beida and zabadi (local name for yoghurt), (Dirar, 1993).

2.2 The therapeutic effect of fermented dairy products

The significant increase in the consumption of cultured dairy products during

recent years notably that of yoghurt, has been ascribed to their image as wholesome high-protein convenient, healthy or low fat products.

Cultured products are digested more easily and therefore, are nutritious because the proteins, carbohydrates and fat are predigested by culture bacteria in their manufacture (Shahani and Chardan, 1979).

Death (1984) studied the most widely accepted therapeutic use of these cultured products in the treatment and prophylaxis of gastrointestinal disorders. The fermented milk aids in the regeneration of the natural flora through the creation of a favorable growth environment, and with some products, their bacteria may also colonize the gut.

Curiously, cultured milk products have been credited with the ability of alleviating both constipation and diarrhea (Death, 1984).

Fermented milk products make excellent foods, particularly for elderly people. Extremists claim a longer life expectancy for the consumers where these foods are staple. They point to the high percentage of centenarians in regions where fermented milk products are consumed. Others see nothing more in fermented milk products than good basic foods (Kosikowski, 1982).

Death (1984) showed that cultured products vary considerably and are dependent on the following: method and extent of fortification, heat treatment, time and temperature of incubation, content of additives such as fruit and stabilizers and the conditions of storage.

Table (2): Different types of fermented milk products

Product	Origin	Starters used
Yoghurt	Nomadic tribes of Eastern Europe	*S. thermaphilus* *L. bulgaricus*
Kefir	Caucasian Mountains	*Saccharomyces kefir* *Torula kefir* *L. caucasicus* *Leuconostoc spp.* *Lactic acid streptococci*
Koumis	Russia	*L. bulgaricus* *Torula yeast*
Acidophilus	U.S.A.	*L. acidophilus*
Bulgarian buttermilk	Bulgaria	*L. bulgaricus*
Cultured buttermilk	U.S.A.	*Leuc. citrovorum* *Leuc. de frohicum* *L. lactis spp. lactis* *L. lactis ssp. Cremoris* *L. lactis gip. diacetylactis*
Cultured cream		*Lactic acid streptococci* *Leuconostoc bacteria*

Source: Kosikowski (1982)

2.3 The History of Zabadi in Sudan

Zabadi is a kind of yoghurt which in the Sudan is made from Cow's milk, until the 1950s, zabadi was only known to the inhabitants of Khartoum and few relatively large towns. There is little doubt that the part of zabadi making come to the Sudan from Egypt through the mediation of such ethnic groups as Egyptians', Syrians, Greeks and Turkish (Dirar, 1993).

2.3.1 Zabadi preparation

The milk is first boiled and after cooling it is inoculated with a previous batch of zabadi. It's then incubated in a warm place in the house or kitchen, at varying room temperature. The milk takes between 8-12 Hours to finally sour. Compare with

western yoghurt, zabadi is much sour and has a variable flavor, the product not being standardized (Dirar, 1993).

From 1980 to the present, the market of zabadi in the capital has been dominated by the product of big companies imported pure starter culture, consisting of strain of *streptococcus thermophilus* and *lactobacillus* bulgaricus. The production of these companies replaced the traditional zabadi in Khartoum and is already spreading out to other nearby towns. This modern yoghurt is also referred to as zabadi and the consumer does not seem to differentiate between the two products. In fact the new zabadi is liked better for its heavier body and lower acidity. Nevertheless, traditional zabadi dominates the markets of small towns in the country (Dirar, 1993).

2.4 Yoghurt

Yogurt is a product made from heat treated milk that may be homogenized prior to the addition of lactic acid bacteria (LAB) cultures containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (C.F.R.S, 2011). Similarly, Tamime *et al.,* (2002) defined yoghurt as a product of the lactic fermentation of milk by addition of a starter culture, which results in a decrease of milk pH to less than or equal to 4.6. Table 3, showed the variety names of yoghurt in different countries.

Yoghurt is more nutritious than many other fermented milk products because it contains a high level of milk solids in addition to nutrients developed during the fermentation process. Its name differs from one country to another. Different forms of yoghurt are now available in the market like stirred, set, frozen and liquid yoghurt (Saint and Souchon, 2008). 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 the nomadic people living in that part of the

world. Although modern large-scale production is designed to handle thousands of liters per day, using highly sophisticated technology with mechanization and auto mastication, the basic principles underlying the manufacturing process, have altered little with time (Tamime and Robinson, 1999).

Table (3) The Varity names of yoghurt in different countries:

Traditional	Country
Jugurt	Turkey
Kissel	Balkan
Kefir	Russia
Busa	Turkistan
Lebel	Some Arab countries
Zabady	Sudan /Egypt
Mast/Dough	Iran
Dahi	India
Mazam	Arminia
Tiaodu	Greece
Cieddu	Italy
Giodu	Sardinia
Tahro	Hungary

Source: Tamime and Death (1980).

2.4.1 Types of yoghurt

The types of yoghurt that are produced worldwide can be divided in to various categories and sub-division is usually erected on the basis of:

2.4.1.1 Legal standards

Legal standard for yoghurt are mainly based on the chemical composition of the product, i.e. percentage of fat content , solids non fat(SNF) or total solid(TS). A minimum specification of SNF or TS is included by some countries, but the main division is on the basis of fat content. According to FAO/WHO, yoghurt may designated as full (above 3.0%), medium (3.0 – 0.5%) or low (0.5% or below) fat (Sara, 2010).

2.4.1.2 Post-incubation processing

Varies types of modified yoghurt can be found in the market. Nonfat and low fat yoghurts were introduced to meet consumer needs. During the past decade, many attempts have been made to produce nonfat and low fat yogurts which are similar in quality to full fat yogurt. Pasteurized yoghurt is processed by convectional method of manufacture, but after fermentation, the yoghurt is heat treated in order to extend the shelf life. Frozen yoghurt is prepared in convectional manner, but is then deep-frozen to at least (20^{o}C). It may in addition require a higher than normal level of sugar and stabilizers in order to maintain the integrity of the coagulum during freezing. Dietetic yoghurt may include low calorie yoghurt, low lactose yoghurt or vitamin/protein fortified yoghurt. Concentrated and dried yoghurt must be mentioned where the former product has total solid of around 24% and the latter type between 90-94% (Tamime and Robinson, 1999).

Industrially, yogurts can be largely divided into two types. Set style yoghurt is made in retail containers giving a continuous undisturbed gel structure in the final product (Tamime and Robinson, 1999). On the other hand, stirred yogurt has a delicate protein gel structure that develops during fermentation (Benezech and Maingonnat, 1994). In stirred yogurt manufacture, the gel is disrupted by stirring before mixing with fruit and then it is packaged. Stirred yogurts should have a smooth and viscous texture (Tamime and Robinson, 1999). In terms of rheology, stirred yoghurt is a viscoelastic and pseudoplastic product (De Lorenzi, 1995).

Yoghurts come in a variety of textures (e.g. liquid, set, and smooth), fat contents (e.g. luxury, low-liquid, virtually fat-free) and flavors (e.g. natural, fruit, cereal), can be consumed as a snack or part of a meal, as a sweet or savory food, and are available all year round. This versatility, together with their acceptance as a

healthy and nutritious food, has led to their widespread popularity across all population subgroups (McKinley, 2005).

2.4.2 Factors effect nutritional value of yoghurt

Early(1998) suggested that the nutritional value of yoghurt is clearly depend upon it's composition, row materials used, ingredients added and the manufacture process will have effects on vitamin, protein, fat and mineral matter.

2.4.2.1 Lactose Intolerance

Lactose, the main carbohydrate found in milk and dairy products, is a disaccharide sugar composed of two monosaccharides, glucose and galactose, joined together by a condensation reaction. It can be hydrolysed in the body by the enzyme lactase (β-galactosidase) and the resultant simple sugars can then be absorbed in the small intestine and used as fuel by the body (BNF, 2002).

Lactose intolerance user can digest a sour milk product much better than plain milk; the lowered lactose content of sour milk is easily digested by activity of the yoghurt bacteria as well as the stimulation of lactose activity of the intestinal mucsa by yoghurt. Alternatively the depletion of the stomach content in the duodenum may be related to consumption of milk, thereby the contact time of lactose hydrolyzing enzyme with the substrate would be extended, resulting in better digestion of lactose (Walstra, 2006).

2.4.2.2 Functional properties of milk fat

Conjugated linoleic acid (CLA), a type of essential fatty acid found almost exclusively in the fat of dairy products, can be obtained only through the diet because it is not produced by the human body. CLA has been shown to be a powerful natural anti-carcinogen that also can reduce the risk for cardiovascular disease, help fight inflammation, reduce body fat especially abdominal fat lower

cholesterol and triglycerides, increase metabolism, lower insulin resistance and enhance the immune system (Walstra, 2006).

2.4.2.3 Bioavailability of Vitamins and Minerals in yoghurt

Vitamins and minerals naturally found in milk are better assimilated by the human body when in the form of yogurt. This is due to the fermentation process involving Lactobacillus bulgaricus, L. acidophilus and Streptococcus thermophilus, among other types of probiotic bacteria. Yogurt is a good source of calcium, phosphorus, iodine and vitamin B_2. It is also a prime source of protein, conjugated linoleic acid (CLA), vitamin B_{12}, tryptophan (an essential amino acid), potassium, vitamin B_5, zinc and molybdenum (a necessary mineral).

2.5 Preparation of Traditional yogurt

Although, yoghurt was accidentally discovered in ancient times through natural processes, yoghurt manufacturing procedures are now highly developed. Before yoghurt bacteria were discovered, no one knew what caused milk to coagulate. Yoghurt was traditionally made from boiled milk inoculated with yoghurt from the previous day. Inoculated milk and keeping overnight at room temperature (Tamime and Robinson, 1985).

2.6 Microbiological spoilage of yoghurt

Yoghurt spoilage associated with yeasts and moulds and the latter in particular often have their origin in the microbial population of the surrounding air. The control of the atmosphere within the factory environment will depend on the level of air cleanliness. It is important, to know that plants designed to induce air flow through a filling room or production area can also act as a source of contamination (Tamime and Robinson, 1985).

Packaging materials stored adjacent to the filling line and the unnecessary movement of personnel can also cause problems in plant. Although yeasts and moulds of atmospheric origin can be important threat, especially at certain times of the year, it is the product contact surfaces of the plant that usually pose the greatest threat to product safety (Tamime and Robinson, 1999).

Different methods and/or techniques have been devised to monitor the hygiene of dairy equipment surfaces, to produce high quality products, and at the sometime ensuring compliance with legal requirements. It is essential that values for a typical, high standard of hygiene to be established for a given plant based on microbiological test (Mostert and Jooste, 2002).

Enumeration of total counts of bacteria, coliforms, yeasts and moulds are the most common microbiological examinations carried out to assess the bacteriological contamination of plant surfaces.

The types of micro-organisms present reflect to some extent, the standard of plant hygiene(Tamime and Robinson, 1999). Selective and differential culture media may also be used to test specifically for given groups of organisms although a given method may not remove all the organisms. However it's consistently used in specific areas to provide valuable information. The most commonly methods for surface assessment are outlined by Mostert and Jooste (2002) and include the swab/ swab-rinse, surface rinse, agar flooding and agar contact plate methods.

2.7 Yoghurt Raw materials

The first stage of yoghurt manufacture is to combine all the ingredients which are included in the fermentation base material. Solids-non-fat and total solid levels 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. Dry ingredients require sufficient time for hydration and de-aeration and these ingredients are milk powder, stabilizer, and sugar (Early, 1998).

Yogurt was traditionally made from milk with no added ingredients. To improve yoghurt texture, milk or skim milk is fortified with other materials such as nonfat dry milk (NDM), whey protein concentrate (WPC) and some other dairy or plant-based ingredients. Different sources of milk are commercially in yoghurt production used including: cow, mare, ass, goat, buffalo, yak, ewe, reindeer and camel (Tamime and Robinson, 1985).

2.8 Homogenization

Homogenization is the breaking down of fat into smaller globules which prevents the formation of a cream. Homogenization improves the consistency and viscosity of yoghurt, thus a greater stability to syneresis can be obtained (Tamime and Robinson, 1985). Furthermore, homogenization of yoghurt mix breaks up powdered ingredients resulting in uniform distribution of the ingredients (Vedamuthu, 1991). According to Schmidt and Bledsoe (1995), homogenization has an adverse impact on yoghurt with a lower fat content, because it increases syneresis or reduces water holding capacity due to empty spaces between casein matrices, and lack of native milk fat globule membrane (FGM). In higher fat yoghurts clusters of fat globules can fill up these spaces, thus syneresis can be minimized.

2.9 Heat treatment of yoghurt

The objectives of heat treatment of yoghurt mix are to kill pathogenic microorganisms, minimize spoilage microorganisms and inactivate lipase and hence prevent lipolysis (Rasic and Kurmann, 1978).

Yoghurt mix is normally heated at a higher temperature and longer time than normal pasteurization, ranging from 90 to 95ºC for 5 to 10 min, to help improve product consistency through whey protein denaturation (Tamime and Robinson, 1985). The degree of denaturation depends on the intensity of heat applied. Low TS yoghurt may require more whey protein denaturation than high TS yoghurt (Rasic and Kurmann, 1978). Whey proteins which participate in casein aggregation in yoghurt are α- lactalbumin **(**α-LA) and β-lactoglobulin **(**β-LG). The former has a denaturation temperature of 62°C and the latter, 78ºC (Wong, 1988). The effect of heat on protein is a two-staged process (Early, 1998). Firstly, the structure is altered, causing denaturation and secondly, aggregation takes place followed by coagulation. This is dependent upon degree and duration of heating. Pasteurization temperatures (80–95ºC), Sterilization temperatures (115–120ºC) or UHT temperatures (135–140ºC) have also been used to heat treat yoghurt milk.

2.10 Cooling of milk

After heat treatment the milk is required to be cooled to a suitable temperature prior to inoculation. In most cases this will be carried out in the regenerative section of the plate heat exchanger. Yoghurt, manufactured in a batch tank or churn, can simply be allowed to cool via cold water 30 jackets or tank (effectively in a water bath) (Early, 1998). The inoculation temperature for short set method will approximate to 42ºC. This temperature can be lowered if an extended incubation period is required (approximately30– 32ºC). Allowances need to be made for incubation tank wall temperature, cold starter addition and latent heat effects and, therefore, the actual cooling temperature is measured on exiting cooling (regeneration) section, is likely to be 1–2ºC higher than required, dependent upon volume, agitation system, distance travelled, etc. For short set incubation it is critical to achieve an accurate inoculation temperature since too high a temperature can inhibit and ultimately kill starter culture micro-organisms

and too low temperature will result in unnecessary extension of fermentation time (Early, 1998).

2.11 Fermentation

In modern automated plants, stirred type and set type yoghurts are often produced concurrently. As has already been indicated, the short set method of incubation of yoghurt milk, using traditional starter organisms such as *Streptococcus salivarius sub sp. thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*, will require the incubation temperature environment for the microorganisms to metabolize synergistically. There should be no agitation during incubation. The 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 the gel will begin to be seen at approximately pH 5.6. Since the protein is most insoluble at its iso-electric point and has lowest water binding properties, the yoghurt gel is very sensitive at this pH. In the case of stirred manufacture, the point at which incubation is stopped is dependent upon a number of factors, such as volume of fermentation vessel and therefore time taken to empty the tank, final pH required and time taken to completely arrest further acidity development. This last point is particularly important in batch (churn) manufacture and with reference to the partial cooling technique employed in continuous manufacture where coagulum may be filtered through a fine mesh screen prior to fruiting, filling and secondary cooling in retail container. Fermentation will be arrested at approximately pH 4.2–4.4, sometimes even lower pH 3.8–4.0 (Early, 1998).

2.12 Striking

This step applies only to stirred and liquid/drinking type yoghurt and is essentially the operated to break the warm gel/curd and re-incorporat the whey. Slow speed paddle agitation (e.g.2 - 4 rpm) for approximately 5 - 10 minutes is usually

sufficient to obtain a homogeneous mix. Agitation also tends to inhibit the culture activity and slows the rate of acidity development (Early, 1998).

2.13 Cooling of the coagulum

Cooling the coagulum commences directly after the fermented yoghurt reaches the desired acidity. The desired acidity will be dependent upon type of yoghurt being produced, method of cooling, time taken to empty fermentation vessel and desired final acidity. This will take place at approximately pH 4.5- 4.6. Cooling is achieved in stirred/liquid yoghurt by pumping the yoghurt via a gentle action positive displacement pump, through a plate or tubular cooler in order to achieve a temperature which is low enough to retard starter culture activity. The capacities of pump and cooler are dimensioned in order that a large fermentation tank will take approximately 20 minutes to be emptied in order to maintain uniform product quality (Early, 1998).

Localized protein precipitation often referred to as ‘nodulation’ can be a major characteristic fault in stirred or liquid yoghurt. There has been significant research into the cause of nodulation in an attempt to prevent its development or reduce the degree of nodulation. Increased ratio of lactobacilli to streptococci in the culture addition, too high a temperature of incubation, excessive pH development and high inoculation levels have all been cited as examples reason for modulation.

One physical method of eliminating this problem is to pass fermented yoghurt through a fine screen in order to break up the ‘nodules’ and produce a smooth consistent product. This operation can be done when the yoghurt base is cooled. However, this can result in whey release and loss of viscosity in the final product. This is why some manufacturers conduct two-stage cooling. By passing partially cooled-yoghurt at approximately 20-25°C through the screen, any physical damage, including wheying off and reduction in viscosity can be healed to a large

extent by reforming of the coagulum on final cooling in the retail container. Care needs to be taken, however, in allowing for some acidity increase if the yoghurt is only partially cooled for as long as it stays at this temperature before it is finally chilled (Early, 1998).

Yoghurt coagulum is broken by the treatment it receives in the pump and cooler. The mechanical treatment will decrease the viscosity but in a well-designed plant, the yoghurt viscosity will increase again after some hours in the chill store. It is vitally important that tanks, pipes and heat exchangers are designed with consideration to the total permitted mechanical treatment of yoghurt. Cooling temperatures vary and are dependent upon the:

- Composition of yoghurt and its inherent ability to withstand cold mechanical handling.
- Filling capability of the process.
- Duration of intermediate storage.
- Efficiency of refrigeration plant.
- Cooling capability after post-filling, i.e. chill room temperature, air circulation, etc.

Traditional continuous manufacture involves single stage cooling to 8 - 10°C, holding in intermediate tanks and then blending with fruit preparation. The stabilization requirements for these manufacture extremes are very different and attention must be paid to stabilizer activity in order for the stabilizer to be effective and avoid any quality problems. In the case of set yoghurt, cooling takes place inside the retail container and is generally started before the final pH is reached. Care must be taken when transferring the retail containers from the incubation room to the chill store because of the fragility of the coagulum at this point. The main problem to be avoided is ‘wheying off’ or syneresis and any unnecessary

physical movement which will encourage this problem. 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).

2.14 Intermediate storage of stirred yoghurt

Intermediate storage is often necessary due to yoghurt production rate and filling rate incompatibility. It is also a necessary requirement to have cooled yoghurt available to fruit, so production should have the capacity to hold cooled yoghurt as a stock. However, it is important that these stocks are not too great and that storage times are shorter than 24 hours and, ideally much less than this, nominally, a few hours. Ideally a temperature of 8- 10°C is optimal, depending upon storage time. Intermediate storage should be as short as possible since physical changes take place that can affect final yoghurt quality. The product may release whey that is difficult to re-incorporate, resulting in loss of yield. Viscosity and body will develop that will largely be lost when the yoghurt is disturbed again. The ability of the yoghurt to bind whey will be reduced by cold disturbance (Early, 1998).

2.15 Packaging

Primary packaging will include glass polyethylene, polypropylene, polystyrene, polyvinyl chloride, polyvinylidene chloride, plastic sachets and paper cartons.

Aluminum foil is widely used to seal yoghurt containers. Due to the acidity of yoghurt and the requirement for the foil to be heat sealed to the plastic container, the aluminum foil is normally coated with a layer of plastic. Individual packages may be further collated into packs of four, six, twelve etc. and the most popular secondary packaging is either cardboard in the form of an outer sleeve or tray or semi rigid plastic crates. Cardboard trays can be over wrapped with heat seal material. Current EEC labeling regulations (1984 Food Labeling Regulations)

require that all pre-packed foods exhibit nature of food, ingredients list, name of manufacturer, packer or seller and expiry (Early, 1998).

2.16Contamination of yoghurt during packaging

Despite all the possible sites in the processing chain at which bacteria can be introduced, the step that has the greatest influence on the keeping quality of heat-treated dairy products is the filling operation.

The new generation of packaging machines incorporates features such as exclusion of air from the filler (by using bellows instead of pistons) and carton sterilization by UV light and hydrogen peroxide. Real contamination may also occur at the filling stage from condensation formed on the machines as well as from smearing of products by moving parts of filling valves (Nriagu and Simmons, 1990). Packaging material, such as properly prepared plastic and laminated plastic materials, are not considered an important source of bacteria (Lück, 1981).

2.17 Chill storage of yoghurt

Yoghurt which has not been subjected to any form of heat treatment via pasteurization, sterilization or UHT processes; in its final product form needs to be kept cold until it reaches the customer. This includes the majority of yogurts which will have a shelf life of approximately15-21 days. Temperature variation will affect texture, viscosity, syneresis as well as improving the environment for potential food spoilage and food poisoning micro-organisms. Exposure to higher temperatures than recommended below can increase biochemical reactions such as fat oxidation, hydration of protein constituents in yoghurt, slight dehydration of exposed yoghurt surface and changes in color of fruit (Early, 1998). Chill storage should be between 2 and 5°C, with no rise above 10°C at intermediary stages in distribution, i.e. palletized transport, non-refrigerated stockholding, and retail cabinet exposure. Although classified as a ‘low-risk food’, attention requires to be

paid to good manufacturing practice and temperature control legislation has to be adhered to (Early, 1998).

2.18 Distribution of yoghurt

Quality assurance principles should extend to monitoring food products throughout the distribution chain. Although the final yoghurt is likely to be stored for only a short period of time prior to distribution to customers' premises, any identified hazards such as rodent/insect infestation, exposure to temperature increase, potential for physical damage etc., need to be monitored and preventative action taken where appropriate. During the first 24 to 48 hours of cold storage, improvements in the physical characteristics take place, mainly as a result of hydration and/or stabilization of the casein micelles. If practically possible, it would therefore be an advantage to retain yoghurt in chill storage for at least 24 hours before commencing distribution (Early, 1998).

In recent years it became increasingly important, for various reasons, to manage and control all the elements of food manufacturing processes. One approach in this regard, that is well established and implemented world-wide is the HACCP system (Tamime *et al*., 2002).

2.19 HACCP background

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. The application of HACCP is systematic because structured hazard analysis and implementation are provided. The process is also logical in that each processor understands it own operation and is able to assess controlling the specific process optimally (Jervis, 2002).

The origins of HACCP are traced to the 1960's and the United States of America when the Pillsbury Company, the United States Army Laboratories at Natick, and the National Aeronautics and Space Administration collaborated to develop the system as a means of managing safe food production for manned space flights. The outcome was the HACCP concept, which has been adopted and developed to its current status as the food safety management tool recommended by the Codex Alimentarius Commission to a devise on consumer protection under Sanitary and Phytosanitary Measures (1994) agreed at the Uruguay round of GATT negotiations. As such, HACCP is a reference point in international trade disputes, and it is increasingly enshrined in national legislation.

The HACCP procedure is generally targeted at food safety management (pathogenic microorganisms and their toxins), but, as an approach in the context of broader quality management, it can be effectively applied to microbiological spoilage, foreign-body contaminations or pesticide contamination. It is preferable to conduct a HACCP program with a narrow scope (a ksingle pathogen or possibly pathogens) rather than attempt to cover an extended list of hazard areas when documentation 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 incorporated into the local quality plan and quality system (Jervis, 2002).

2.20 Benefits of HACCP

The key benefits of HACCP in the food and dairy industry are many, and can be summarized as follows:

- HACCP has the potential to identify all hazards in the manufacturing process so that controls can be established to assure food safety/quality.
- HACCP is a systematic approach relevant to all stages of food processing covering agriculture and horticultural practices, harvesting, processing, product distribution, and customer practices.
- HACCP is the preferred risk management tool in total quality management.
- HACCP focuses technical resources on critical parts of the process and provides a cost-effective control of food-borne hazards.
- HACCP facilitates the move from retrospective end-product testing to a preventative quality assurance approach enabling the manufacturer to get it right the first time and reduce reject waste.
- HACCP 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 the food industry will facilitate compliance with developing legislation and demonstrates a diligent approach to food safety (Jervis, 2002).

2.21 Pre-requisite programmes

Pre-requisite programmes such as GAP, GMP and GHP must be working effectively within a commodity system before HACCP is applied. If these pre-requisite programmes are not functioning effectively then the introduction of HACCP will be complicated, resulting in a cumbersome, over-documented system (FAO and IAEA, 2001).

Good agricultural practices primary in food production should be managed to ensure that food is safe and wholesome for the consumer. Farmers should control production so that contamination of the crop, proliferation of pests, and diseases of

animals and plants, do not compromise food safety. Good Agricultural Practices (GAP), including Good Hygienic Practices (GHP) where appropriate, should be adopted to make sure that the harvested commodity will not present a food hazard to the consumer (FAO and IAEA, 2001).

Good manufacturing practices showed establishment design and facilities. The structure and location of a processing plant needs to be considered in relation to the nature of operations and risks associated with them (FAO and IAEA, 2001).

Effective control of operation should be in place to reduce the risk of contamination of the commodity or food supply such that it is safe and fit for purpose:

- Adequate time, temperature or humidity controls
- Food grade packaging
- Potable water supplies
- Maintenance of equipment (FAO and IAEA, 2001).

Maintenance and sanitation procedures and work instructions should exist to demonstrate an adequate level of maintenance of an establishment as well as efficient practices for cleaning, waste management, and pest control (FAO and IAEA, 2001). Personnel hygiene measures need to be in place to ensure that food handlers do not contaminate food. This objective can be attained by maintaining an appropriate level of personal cleanliness and following guidelines for personal hygiene (FAO and IAEA, 2001).

Transportation method should be such that measures are taken to prevent any contamination or deterioration of the commodity (FAO and IAEA, 2001). Food handlers should be trained in personal hygiene, as well as in the specific operation with which they are working, to a level commensurate with their duties (FAO and IAEA, 2001). Product information and consumer awareness should be accompanied by adequate information to ensure that personnel at the next stage in

the food chain will handle, store, process, prepare and display the product safely (FAO and IAEA, 2001).

2.22 Application of HACCP

This detail what needs to be done at each of the HACCP stages, and it refers to generic flow diagrams and HACCP plan records that have been produced in order to illustrate the points made. It is essential that each HACCP study be based on the specific process and product details, and generic plans should never be adopted as a shortcut to save time and resources. The different sequential stages are as follows (Jervis, 2002).

2.22.1 Definition terms of reference

Terms of reference should clearly define the scope of the intended HACCP study and address the following points:

- The product to be considered.
- The process site and, if relevant, the process line within that site. It is not advisable to group together apparently similar products and processes where what might be minor variations in formulation and/or process conditions could significantly change the preservation characteristics of the product.
- What the study will cover- biological, chemical, or physical hazards (or combinations of these)- and whether the study will be limited to food safety considerations or cover broader quality issues (i.e., spoilage). The study will proceed more quickly if the terms of reference are limited to biological food safety issues, or even the consideration of one pathogen relevant to the food.
- The point in the process at which safety or other quality attributes are to meet: at point of manufacture or at point of consumption?

2.22.2 Selecta HACCP team

It is important that senior management in the company be made aware of the resources necessary to carry out an effective HACCP study (personal time, appropriate meeting room, secretarial support, and the need to consult outside resources for information) and are committed to providing these resources. The time required to complete the study will depend on the complexity of the process and the terms of reference agreed as Stage 1. If resources cannot be assured to meet the study defined in Stage 1, then the study should not be progressed. HACCP requires a multidisciplinary approach, and the HACCP team should include the following skills:

- A quality assurance/quality control specialist who understands the hazards and risks for the product and process under study. Depending on the study terms of reference, this might involve a microbiologist or chemist; and, if this resource is not available in-company, consultation with an eternal resource might be necessary to obtain information relating to microbiological risk and hazards.
- A production specialist to contribute details of what actually happens on the production line throughout all shift patterns.
- An engineer to provide information on:

(a)The operating characteristics of the process equipment under study.

(b) The hygienic design of equipment and buildings.

- Others co-opted onto the team as necessary. These might include specialist equipment operators, hygiene manager, ingredient and packaging buyers, and distribution managers. It might also be appropriate to consider co-opting specialist technicians from companies to which various scheduled maintenance and calibration functions are contracted (e.g., temperature

measurement equipment, pasteurizer plate and jacketed silo integrity, clean-in place systems).

An individual experienced in HACCP should be nominated as chairman to be responsible for managing the study. The chairman should have received training in the principles of HACCP and be experienced in HACCP team work. While HACCP team members will be selected for their specialist knowledge, it is important that they will also have a working knowledge of the HACCP procedure so that they can contribute effectively to the study. Team members may need some training before commencement of the study, and this can be provided either internally by the HACCP team or externally.

It is important that a HACCP team member or co-opted person is identified to keep notes as the work progresses and from which both the HACCP plan and the HACCP study notes can be derived. HACCP study notes should record background information and the basis for conclusions reached in sufficient detail to be helpful when the HACCP plan is reviewed. The HACCP study notes might also be used as background information in trouble-shooting in the event of product failure or inadequate outcome from the verification program (Hoolasi, 2005).

2.22.3 Description of the product

The product under study should be fully described. This stage often tends to be inadequately covered, but diligent attention to detail here is crucial to the identification of hazards. The product description should be considered against the following headings and recorded as HACCP study notes:

Composition: All factors that might influence the preservative characteristics of the food should be recorded. Basic compositional data should be noted including that on solids/moisture levels, fat levels, type of preservative, if used, etc.

Compositional data should also be recorded for any additives used, particularly where these are supplied as fresh, hydrated materials.

Processing: All relevant processing parameters should be recorded. They should be validated as giving the required effect with respect to micro-organisms of concern and the appropriate operating conditions recorded at this stage in a HACCP study.

Packaging system: The type of packaging should be noted. This note will include differentiation between shrink wrapping, vacuum packing, and sealed plastic tub packing. Aseptic or ultra clean packaging regimes should also be noted where appropriate. In the context of dairy products, it is useful to record the conditions of storage of intermediate stages of production. The degree of exposure to the process plant environment during filling should also be recorded.

Storage and distribution conditions: The storage temperature regimes (ambient, chilled, and frozen) throughout the product shelf life should be recorded where possible, and this should include anticipated variations (e.g., retail display, customer's shopping bag, and home storage conditions).

Required shelf life: The total shelf-life requirement together with "life after opening" where appropriate, should be recorded.

Instruction of use: Dairy products are usually consumed without further processing (heating), so that this section should record instructions given with regard to refrigerated storage (where appropriate) and 'use within' times, after opening, together with overall "use by or best before" dates(Hoolsi, 2005).

2.22.4 Intended use identification

The consumer target group for the product should be noted, different consumer groups may have varying susceptibilities to the potential hazards (Carol, 2012).

2.22.5 Construction of flow diagram

The purpose of a flow diagram in a HACCP study is to elicit a thorough examination of the process, which is recorded in a way that assists and directs subsequent stages. There is no specified format to be used in HACCP flow diagrams, but they should sequentially set out all steps in the process together with relevant technical data. Consideration should be given to the following:

- The sequence of all process steps within the scope or the study including rework/recycle loops.
- Interaction of services (e.g., cooling water, air, compressed air, clean-in-place systems).
- Temperature/time history for all raw materials, intermediate products, and final products within the scope of the study, together with microbiological and analytical data with appropriate floor plans and equipment.
- Equipment design with particular attention to ease of cleaning and presence of void spaces that might accumulate contamination.
- Personnel and hygiene disciplines.

2.22.6 On-site confirmation of flow diagram

The flow diagram produced should be confirmed, on site, by the HACCP team. Points to be confirmed are that any effect of shift patterns and weekend working are included on the flow diagram, together with circumstances of any reclaim or rework activity that might be introduced from time to time. If the HACCP study is being applied to a proposed new process line/product, flow diagram confirmation will not be possible. In this case the HACCP plan can be completed, but it must be subject to review as the line/product is finalized (Hoolasi, 2005).

2.30 Principles of HACCP

In theory, the only way of ensuring that every package of yoghurt from a given production line is safe, from a chemical or microbiological standpoint, is to test every package. Clearly, such a suggestion is totally impractical, so that instead, a representative group of packages is withdrawn against a sampling plan appropriate for the product and the history of the plant. However, whilst this approach is essential to confirm that preset standards of hygiene are being met and that potential contaminants are at a low level or absent, the procedure can never prevent some spoiled packages from reaching the consumer. Consequently, the emphasis within quality assurance has turned to the avoidance of problems, a concept that forms the basis of HACCP. In particular, the system identifies seven aspects of production that merit constant attention and these aspects are enshrined in seven principles (Tamime and Robinson, 1999).

2.23.1 Listing of all hazards associated with each step and consideration of any control measure to eliminate or minimize hazard (principle 1)

2.23.1.1 Raw milk

The presence of chemical contaminants in milk can be traced to feeding practices (aflatoxins, nitrates), animal husbandry practices (pesticides), veterinary therapy (antibiotics), pollution (lead, radioactive elements) or accidents. These factors must be controlled at the farm level by the farmers, their associations or cooperatives, agricultural services and inspection agencies.

Microbiological hazards include the classical zoonotic agents in certain regions of the world, or the more common contaminants such as *salmonella, campylobacter, listeria, E. coli Staphylococcus aureus, Bacillus cereus* etc. Moulds, viruses and parasites are of less or no concern. Almost all potential microbiological hazards can be eliminated with a heat treatment (pasteurization or sterilization). *B. cereus*is

an exception; however, illness due to this organism is unlikely to be caused by the normal use of dairy products (Christiansson, 1993).

2.23.1.2 Additives and supports

The use of fruits and other ingredients in products such as ice-cream, yoghurt and white cheeses is common nowadays. They should be carefully looked at, because dairy people may be unfamiliar with their microbiology and chemistry, and because they are often added after pasteurization. Fermentation is not very effective in reducing potential microbiological hazards. Proper selection of the suppliers (based on their application of the HACCP system) and careful choice of purchasing specifications is therefore very important.

2.23.1.3 Environment of dairy lines

The environment of the dairy lines is an important potential source of hazards. A line is rarely a closed operation, i.e. with a continuous barrier between product and line environment from pasteurization until packing. Consequently, the potential hazards in the line and in environment need to be listed. Salmonellosis, staphylo-enterotoxicosis and listeriosis epidemiologically linked to dairy products. However, since the production technologies vary widely, the causative microorganisms are potential hazards only in some dairy lines. Listeriais not commonly found in dry, warm environments (Cox**,** ***et al.,*** 1989) Staphylococci can be found, but are rarely a potential hazard (Kleiss, ***et al.***, 1994). Preventive measures are, in all these cases, included in Codes of Good Manufacturing Practices (IDF, 1994). In critical environments, specific measures may be necessary to control potential hazards. It is worth mentioning here that a HACCP system is effective only in lines where good manufacturing practices (GMP) are followed.

2.23.1.4 Packaging material

Other hazards which are less important, but should not be overlooked, are physical hazards and the hazards related to packaging material. The possibility of the presence of physical hazards can, of course, never be excluded. Its occurrence is unlikely because of the hygienic way in which milk is handled. Packaging material should not be a source of hazards, although returnable glass, as environment-friendly material, may pose some problems. For each of the hazards concluded to be significant in the hazard analysis, the HACCP team should identify control measures that will eliminate the hazard or reduce it to an acceptable level. There may be more than one control measure required to control a hazard. In other cases, one control measure at a single point can control more than one hazard (e.g., pasteurization eliminates all vegetative pathogens and spoilage micro-organisms).

2.23.1.5 Cleaning

One control measure can be relevant to several process steps where a hazard is repeated (e.g., application of CIP cleaning or environmental cleaning to control recontamination). Where no control measure can be identified to control a hazard, redesign or modification of the process or product formulation may need to be considered. A final point to note is that in identifying control measures in a HACCP study on an established product and process, the team should not restrict consideration to measures already in place but should be prepared to propose other control measures that might be appropriate.

2.23.2 Establishment of critical control points (principle 2)

The objective of determine CCPs is to systematically assess the hazards and related control measures identified by considering each process step (as recorded in the flow diagram) in turn and reaching a conclusion on its “CCP” status before moving on to the next process step- that is, to identify process steps at which control can be

applied and which are essential to prevent or eliminate a hazard or reduce it to an acceptable level. It is useful to be guided by a CCP decision tree as shown in Figure 1.

2.23.3Establishment of critical limits for each CCP (principle 3)

A critical limit is a criterion that separates acceptability from unacceptability at each CCP. It should be measurable in real time (while the process is running) and might include measurements of temperature/time/pH or acidity, moisture, the phosphates test for pasteurized milk, ATP methodology to assess cleaning efficiency, or other observations. A critical limit might be mandatory (e.g., pasteurization temperature and time) or based on data collected under good manufacturing practice where a specific target level and tolerances are set (Hoolis,2005).

2.23.4 Establishment of a monitoring system for a CCP (principle 4)

Monitoring involves a planned sequence of observations or measurements against critical limits to assess whether a CCP is under control. Ideally, monitoring should identify a trend toward a critical limit maximum or minimum so that corrective action can be taken before the process is out of control and, in any event, should aim to identify violation of critical limits as soon as possible to minimize the amount of embargoed/rejected product. Monitoring can be on-line with automated corrective action (e.g., flow diversion systems on pasteurizers), or they can be off-line when corrective action might involve the rejection of any product implicated. Physical and chemical measurements are preferred to microbiological testing because they can be completed rapidly and often is indicative of conditions that control the microbiology of the product (e.g., phosphatase test on pasteurized milk). All record and documented associated with monitoring CCPs must be

signed by the person (s) doing the monitoring and by responsible reviewing official(s) of the company (WHO, 2008).

2.23.5 Establishment of a corrective action plan(principle 5)

This specifies the action(s) necessary when monitoring shows a potential or actual loss of control at a CCP. The action(s) will aim to bring the process back into control before critical limits are reached (e.g., a temperature drift from a target of 5ºC to near the tolerance value of 7ºC will call for an engineer to adjust the refrigerator plant), or it will specify the disposal of product that has breached a critical limit. Monitoring requirements and corrective action plans should be considered together by the HACCP team, and a clear decision should be reached and recorded on responsibilities for corrective actions(Hoolasi, 2005).

2.23.6 Establishment of verification procedures (principle 6)

Verification applies methods, procedures, product tests, and evaluations other than monitoring, to determine compliance with the HACCP plan. It demonstrates that the HACCP plan and its application are consistently controlling the process so that product meets the food safety or quality requirements. The HACCP team should specify methods and frequency of verification procedures which might include the following:

- Microbiological examination of intermediate and final product samples.
- Review of complaints from consumers or regulatory bodies and outcomes of investigations into these complaints, if they were substantiated, indicating that the HACCP plan did not completely control the process.
- Auditing all monitoring and corrective actions records to establish whether the HACCP plan is fully implemented and demonstrates control.

- Review of validation records and, if appropriate, the application of more searching tests at selected CCPs to confirm the efficacy of the control measure.
- Another important aspect of verification is initial validation of the HACCP plan to determine that the plan is scientifically and technically sound that all hazard have been identified and that if the HACCP plan is properly implemented these hazard will be effectively controlled.

2.23.7 Establishment of documentation (principle7)

The complexity and quality of documentation necessary will depend on the size and type of operation. The key point is that the manufacturer must be able to demonstrate that the seven principles of HACCP have been correctly applied. To be effective, HACCP must be fully integrated into the unit quality systems as an element of total quality management.

The following documentation should be issued as controlled documents:

- The finalized HACCP plan. Process steps assessed as not being CCP's should also have critical limits, monitoring procedures, and corrective actions identified on the HACCP plan, and they can be designated as control points that contribute to good manufacturing practice.
- Guidelines, procedures and work instructions/records sheets.

Guidelines on good hygienic practice (GHP) are an essential element of the documentation required. Any issues specific to the HACCP study that are missing can be covered either by amendment of the guidelines or by inclusion in the HACCP plan.

Procedures cover the following:

- Training for hygiene and operation.
- Personnel hygiene and sickness reporting.
- On-site food services.

CHAPTER THREE

MATERIALS AND METHODS

3.1 The study area

This study was conducted in DAL Dairy Factory is the name for Blue Nile Dairy's with new manufacturing facility and offices. The original plant was in Soba (just outside Khartoum) constructed in 1996. As the business grew, it was expanded to meet the demands of the market for delicious tasting, natural dairy products. Eventually, in 2009, the old plant reached its expanded capacity and plans designed for the future. A much larger manufacturing facility was therefore envisioned and in early 2009 an agreement was signed with Tetra Pak of Sweden to build a new dairy plant.

The new plant (CAPO) was opened in September 2010. It is situated in Bahri Industrial Area (North Khartoum). CAPO is Sudan's favourite dairy brand, producing a wide range of tasty and nutritious products, such as yoghurt, fresh and long life milk, cream and mish. Its own a dairy farm and milk collection centers (MCC_S) for their dairy processing. The raw milk was brought from the farms through a cooled bulk tank, transferred into the holding tank, and then milk was pasteurized at 72°C for 15 seconds using HTST plate heat exchanger pasteurizer.

3.2 HACCP plan

Theoretically, the way of ensuring that every package of yoghurt from a given production line is safe from chemical or microbiological stand point and it is very difficult to test every package. However whilst this approach is essential contaminant are at low level or absent. This can never prevent some spoiled packages from reaching the consumer. The HACCP system aims to identify specific hazard that, if they arose could adversely affect the safety of stirred

yoghurt and to put in place a procedure that will either prevent a hazard arising or will be able to control the situation in a manner that reduce the risk to the consumer. In HACCP plan the system identified different aspect of production that merit constant attention and these aspects are applied practically

First:

Any potential hazard associated with stirred yoghurt production from the growth/ collection of row material through the manufacturing and final product has been identified.

Second:

The precise points in the above sequence that can be controlled in order to eliminate a hazard or minimize the risk to public health were defined during stirred yoghurt production then the step in the process is regarded as critical control point (CCP). If no major risk is involved, the step identified as critical point (CP).

Third:

Targets have been established in order to claim control over stirred yoghurt process e.g. total count on product control surface (CCP) or the viscosity of stirred yoghurt with agreed tolerance (CP).

Fourth:

A monitoring system has been established to record these particular facets of stirred yoghurt production are under control.

Fifth:

In the case of the (CCP) or (CP) during stirred yoghurt production is not under control, the corrective action should be implemented immediately.

Sixth:

All results during stirred yoghurt production have been kept for documentation. In addition to the details of all potential, e.g. times/temperatures and microbiological parameter, but also the responsibilities of the process.

3.3 Materials

Samples were collected at different stage of stirred yoghurt processing as follows:

1- Fresh milk samples: Samples were collected from raw fresh milk supplied to the factory from each (MCC_S)including Alifoun, Tibna and sayegh replicated every 5 days in four Months (April–July) in cleaned and steriled bottles for physiochemical and microbial tests.
2- After pasteurization stage: Samples from the milk tanks were collected immediately after pasteurization replicated every 5 days in four Months (April - July) in cleaned and steriled bottles for chemical tests.
3- During mixing process: Samples from mixed milk were collected for chemical tests.
4- During fermentation process: Samples were collected from fermented milk in cleaned and steriled bottles for chemical tests.
5- Final product: 4samples of stirred yoghurt were collected and stored at refrigeration prior to analysis.

3.4 Chemicals and reagents

- Sulfuric acid (H_2SO_4 conc., density 1.815 and 1.86gml/ml) (BDH).
- Amyl alcohol (MERCK) Catalyst tables from BDH Laboratory reagents-BDH Chemicals Ltd Poole England.
- Sodium hydroxide (NaOH) (Scharlau-SO0420).
- Phenolphthalein (BDH Chemicals Ltd Poole England).

3.5 Product description and intended use

The description of stirred yoghurt in CAPO milk company and the intended use are shown in table (4).

Table (4) product description of stirred yoghurt and the intended use of it:

Process/ Product type name	Description
1- Product name(s)	Plain stirred yoghurt
2- Composition	Whole fresh milk; Whole milk powder; skimmed milk powder; bacterial culture and pectin.
3- Importance product characteristics	pH
4- How it is be use	Direct consumption
5- Packaging	Plastic cup and foil sealed
6- Shelf life	10 days
7- Where it well be sold	Retailers
8- Legal requirement	Fat – Total solid
9- Special storage and distribution control	Refrigeratored at $\leq 10^{\circ}C$

3.6 Flow diagram of stirred yoghurt produced at CAPO dairy Company

The manufacturing steps of yoghurt are shown in figure (1). All stages starting from raw material to end product are presented.

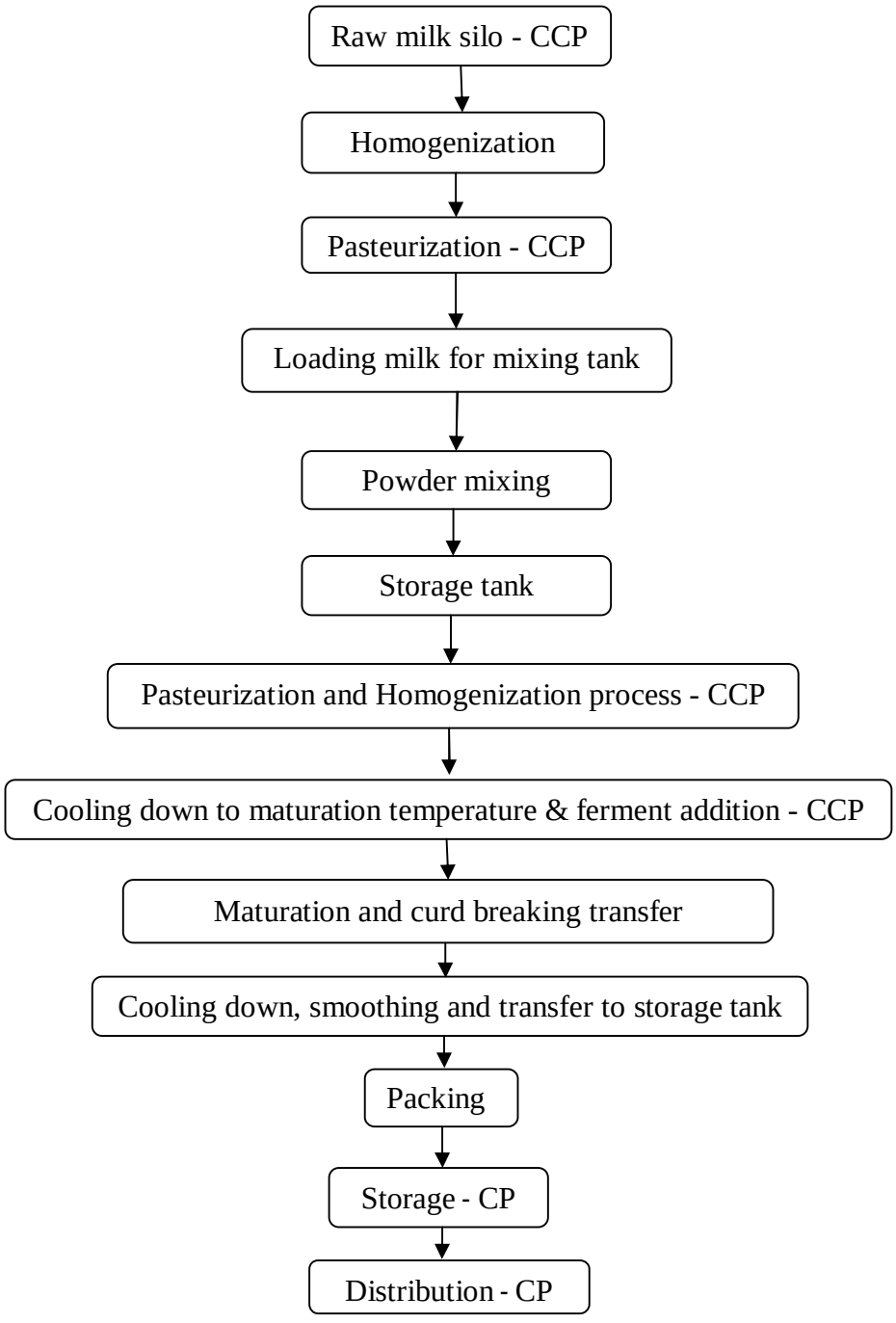

Figure (1): Flow diagram for the manufacture of stirred yoghurt at CAPO dairy Company.

3.7 Determination of the Critical Control Points

The CCP_S throughout stirred yoghurt production were identified according to the HACCP decision tree recommended by codex (2003) shown in figure (2).

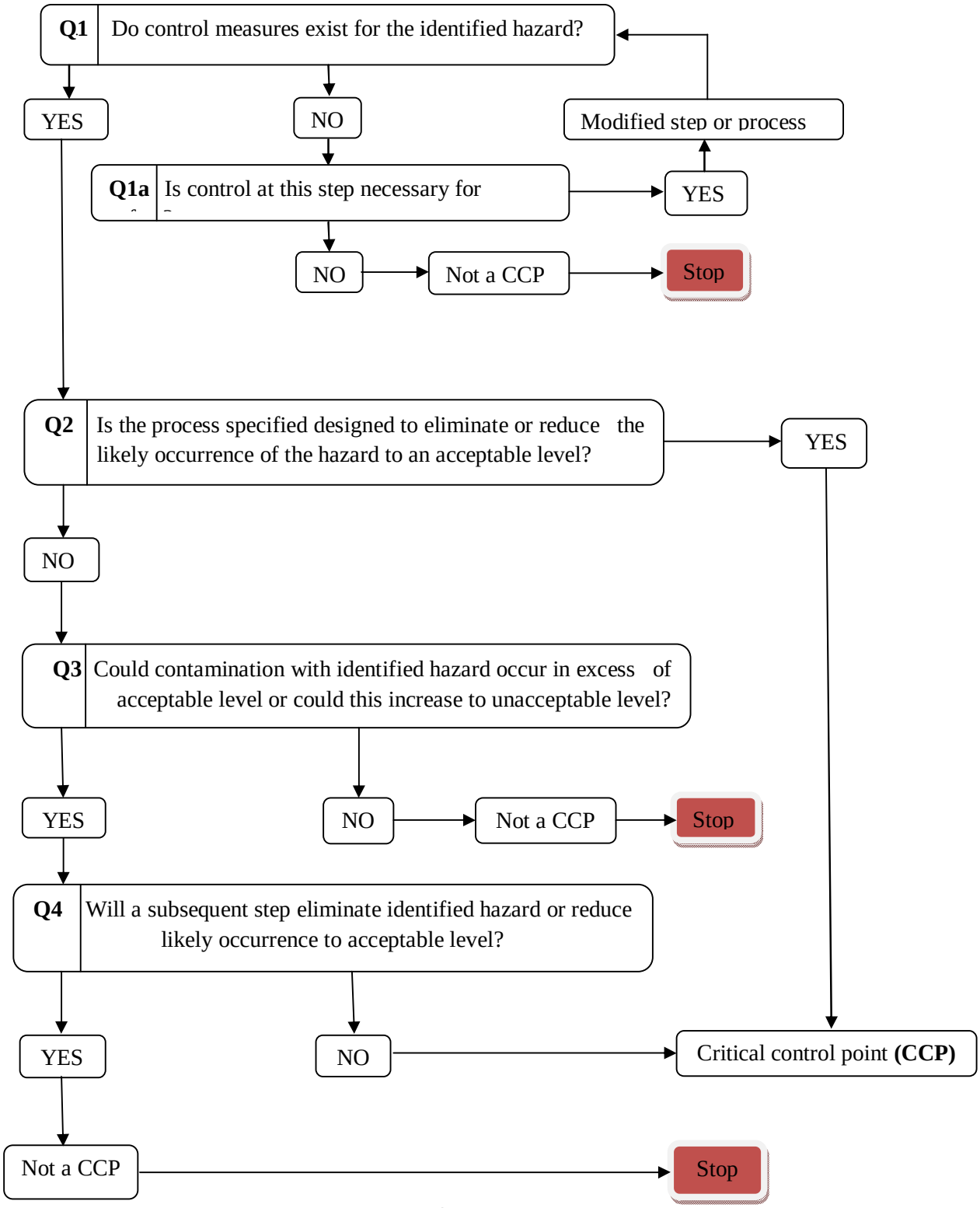

Figure (2) Decision tree to identified CCP_S

Source: Codex (2003).

3.8 Monitoring tests

Total of seven hundred and eighty four analyses were performed physically, chemically and microbiologically. The fresh milk was collected from milk collection centers (MCC_S) Alifoun, Tibna and Sayegh. The authoritative milk supply centers. The milk was received in factory via aseptically container truck daily and physiochemical analyses were immediately carried out under aseptic condition.

Samples were taken at different stages during yoghurt manufacturing process for analyses. For microbiological analysis samples were taken aseptically, kept at 1 – 5˚C and analyzed within one hour.

3.8.1 Media preparation

All media were prepared following the instruction and advance of the manufacture.

3.8.2 Plate account agar

Plate account agar was prepared by dissolved 23.5gm of the media (powder) in 1000ml distilled water. Heated to boiling and sterilized by autoclaving at 121°C for 15 min.

3.8.3 Potato dextrose agar

39gm of powder were dissolved in 1000ml distilled water. Heated to boiling and sterilized by autoclaving at 121°C for 15min.

3.8.4 Violate red bile agar

41.53gm of powder were dissolved in1000ml distilled water, sterilized in an autoclave at 121°C for 15 min, cooled to 45°C.

3.8.5 EMB (Eosin Methylene Blue agar)

36gm of powder were dissolved in 1000ml distilled water, sterilized in an autoclave at 121°C for 15 min and Overheating was avoided.

3.9 Serial dilution of samples

One ml of each milk sample and1gm of yoghurt sample was weighed aseptically and added to test tube containing 9ml of sterile diluents and well mixed to give 10^{-1}; using sterile pipette 1ml of the last dilution was transferred to test tube containing 9ml of sterile diluents and well mixed to give 10^{-2} in the same way continued to the prepare other serial dilution (Harrigan, 1998).

3.9.1Antibiotic test

Trisensor is a new and completely unique rapid test in a dipstick format for multi-antibiotic detection in one single operation. It is a receptor assay allowing the detection of every ß-lactam, Tetracycline and Sulfonamide in milk. This unique test is extremely user-friendly, fully automated, reliable and takes only 6 minutes to get the result at 40°C ± 3°C.

3.9.2 Total bacterial count

One ml of each serial dilution was transferred aseptically in to sterile Petri dishes. 15ml of plate count agar were added. The inoculums was mixed with medium and allowed to solidify. The plates were then incubated at 37˚C for 24 hrs. Plates were examined and the colonies on every plate were counted then the total viable count was determined as colony forming unit per ml (cfu/ml) (Harrigan 1998).

3.9.3Total Coliform count

One ml from each sample serial dilution was transferred aseptically in to sterile Petri dishes. 15ml of violate red bile agar were added to the Petri dishes. The

inoculums was mixed with medium and were allowed to solidify. The plates were then incubated at 37˚C for 48hrs. Colonies with dark red showed appositive test (Harrigan, 1998).

3.9.4 Yeast and Moulds

One ml from each serial dilution was transferred aseptically in to sterile Petri dishes. 15ml of potato dextrose agar were added to Petri dish. The inoculums was mixed with medium and allowed to solidify. The plates were then incubated at 25°C for 72 hrs. The colonies were counted to determine the viable count of yeast and moulds (Harrigan, 1998).

3.9.5 Swab rinse method

This method was used for the assessment of effective cleaning of milk contact surfaces e.g. milk transferring pipes, milk valves, filling machine, and milk silos by transferring the swab in to Petri dish containing plate count agar (Mostert and Jooste, 2002).

3.10 Chemical and physical analyses

3.10.1 Determination of Total solids

Total solids is consists especially from fat, protein, lactose and minerals. There are some methods to determine the total solids but the most popular and the easiest in the use is the equation according to the use of fat content and solids non fat of the milk or yoghurt. The solids non fat were determine by using the lactometer.

- Milk was well homogenized and transferred to the measurable cylinder and immersed the lactometer use in the milk.
- The lactometer number in the surface of the milk was recorded and added for the reading number 0.5(surface tension).

- The temperature of the sample was obtained by the thermometer and standardized the lactometer reading by (+) or (-) 0.2 for any plus or minus of sample temperature which the lactometer was designed to read according to it (15.5 ±5˚c).

The total solid were calculated according to the following formula:

S.N.F = 0.3 × Fat + $\frac{lactometer\ readind}{4}$ (Babkoak equation).

T.S content = S.N.F + Fat

Were:

S.N.F ≡ Solids Non Fat.

T.S ≡ Total Solids.

3.10.2Titratable acidity

Titratable acidity was determined according to AOAC (1990) method. Ten milliters of each samples were placed in a white porcelain dish and four drops of phenolphthalein indicator were added. Titration was carried out using 0.1N NaOH until a faint pink color appeared. The titration figure was divided by ten to get the percentage of lactic acid(1 milliter of 0.1N NaOH sodium hydroxide = 0.009gm of lactic acid).

3.10.3 pH

The pH was directly measured using a jenway 3510 pH meter at room temperature.

3.10.4 Temperature

The temperature was directly measured by a Digital Thermometer WT-1 WT-1B.

3.10.5 Viscosity

Viscosity was directly determined by the use of the digital Visco Lab – Alpha.

3.10.6 Butterfat

The fat content was determined by Gerber method according to AOAC (1990) method as follows:

Ten ml of sulfuric acid (density 1.815gm/ml at 20°C) was poured into a clean Gerber tube, followed by the addition of 10 ml of milk sample. Then 1ml of amyl alcohol and distilled water at 20°C was added. The tubes were thoroughly mixed till no white particles were seen. The tubes were centrifuged at 1100 revolution per minute (rpm) for 5 minutes. The tubes were then transferred to water bath at 65°C for 3 minutes. The fat content was immediately read.

3.11 Statistical analysis

One way ANOVA test was performed to examine significant difference between normally distributed data. Tukey's –test was used to perform multiple comparisons between mean within each specific parameter. Probability level of less than 0.05 was considered significant ($p<0.05$). All data were analyzed using MINITAB statistical software for window (2006).

CHAPTER FOUR

RESULTS AND DISSCUSION

4.1 Identification of possible hazards in stirred yoghurt

The hazards (microbial, chemical and physical) during steps of stirred yoghurt are presented in Table (4.1).

4.2 Control measures of hazards

The control measures during processing of stirred yoghurt are presented in Table (4.1).

4.3 Identification of control point and critical limits

The critical control point was established according to decision tree and divided to CP (critical point) and CCP (critical control point), also critical control point included GMP (Good manufacturing practices) according to stirred yoghurt production step. While critical limits include the parameters limits such as pH > 6.1%, solid non fat >11.2, fat content > 3.0%, antibiotic (negative) and milk cooling temperature < 5°C Table (4.1).

4.4 Monitoring procedure

4.4.1 Physical quality of fresh milk

4.4.1.1 Temperature of fresh milk

The exposure of milk to high temperature during transportation may favor the growth of pathogens and the production of heat resistance toxins. There for, this stage is a CCP because the reception test stands for an acceptance test.

The temperature of fresh milk samples collected from milk collection centers (MCC_S) including: Alifoun, Tibna and Sayegh during the study period (April, May, June and July) are show in Table (4.2).

Statistical analysis showed that there was significant different ($p<0.05$) in temperature of Alifoun MCC in May 5.4°C as compared with Tibna and Sayegh MCC. There was no significant ($p<0.05$) different between different MCC during April, June and July, except in May milk from Alifoun differ (Table 4.2). The differences might be due to variation in the milk handling equipments, milk handling techniques and lack of cooling system. Nevertheless overall results are in agreement with that reported by Tamime and Ropenson (1999) who stated that the temperature of milk in collection center is 0 – 10°C±1°C temperature tolerance.

The pH of fresh milk in MCC_S (Alifoun, Tibna and Sayegh) was found to be in the range of 6.6 to 6.7.as shown in Table (4.3). During the study period (April – July) there was no significant ($p<0.05$) different in pH of all different MCC_S. These results are in agreed with Hoolasi (2005) who stated that the pH of raw milk is 6.73. While FAO (1999) reported that the pH of fresh caw milk has a pH value that ranges from 6.6 to 6.8.

Table 4.1: Shows the possible hazards during the manufacturing stirred yoghurt:

Process step	Hazards	Control measures	Control point	Critical limits	Monitoring procedures	Corrective action
Raw milk (Received milk)	Presence of micro-organisms.	Microbiological analysis.	CP	6 $\log_{10}$ cfu/ml	Coliform 4.10±0.20$\log_{10}$cful/ml	
		pH.	CP	5 $\log_{10}$ cfu/ml	TVBC 4.77±0.45 $\log_{10}$ cfu/ml pH 6.71%	Not required
	Chemical	Fat content.	CP	> 3.0%	3.91 %	Not required
		Total solid content.	CP	> 11.2%	12.74 – 13.82%	
Raw milk (Released: tests)	Microbial hazard (Antibiotics)	Antibiotic release test.	CCP	Negative	Negative	Not required
	Physical hazard (Foreign material)	.In-line filter. Inspect tanker Before offloading. Quality of Gaskets.	CCP	No damaged gaskets	Raw milk procedure	Daily tanker inspections
Raw milk (Cooling)	Microbial	Temperature	CP	< 10°C	Measured temperature 5.4 –8.18 °C	Not required
Raw milk (Silo release)	Microbial	Temperature	CP	Milk to be kept at minimum of 3°C. Within parameters	Measured temperature 5.4 –8.18 °C	Re cooling.
Homogenization	Chemical risk	Cleaning	GMP	Good quality and homogenous	Checked for homogeneity	Not required

Process step	**Hazards**	**Control measures**	**Control point**	**Critical limits**	**Monitoring pr**
Pasteurization	Microbial	Temperature and time	CCP	95 °C, 10 mint	Visual on PC d 10 mint
Pasteurization (Transfer of milk to holding tank)	Microbial	Microbiological analysis.	CCP	2.3 $\log_{10}$ cfu/ml	LPC Mesophil cfu/ml LPC Thermo $\log_{10}$ cfu/ml
Loading milk for mixing	Microbial	Microbiological analysis.	GMP	5 $\log_{10}$ cfu/ml	TVBC 4.46- 5.
Powder mixing	Physical foreign Material chemical	Production and process control. TS SNF	GMP CP CP	Wash not more than 24 h before. 14 – 15%. 12%.	Visual on PC d 15%. 12.11%
Storage tank	Microbial	Cleaning	GMP	Wash not more than 24 h before	Visual on PC d
Pasteurization And homogenization process	Microbial	Time and temperature	CCP	95 °C 10 mint	Visual on PC d 10 mint
Cooling down to maturation temperature and ferment addition	Microbial	Time and temperature	CCP	43 - 45°C 3 – 5 hr.	43 °C.

Process step	Hazards	Control measures	Control point	Critical limits	Monitoring pr
Maturation and breaking of curd	Microbial chemical	Cleaning pH	GMP CP	Wash not more than 24 before. 4.2 – 4.4%	Visual on PC d 4.4%
Cooling down, smoothing with filter and transfer to storage tank	Microbial	Temperature	CP	< 20 ºC	19 ºC.
Packing	Microbial	Cleaning	GMP	Wash 24 h before	Visual on PC d
Storage	physical Microbial	Temperature Viscosity Yeast and mould	 CP	< 10ºC 24 h 46.60–57.83 mPas. Negative	8.85 ºC. 53.17 – 59.68 r Negative
Distribution	Microbial	Temperature	CP	< 10ºC	8.85 ºC.

4.4.1.2 pH of fresh milk

Table 4.2: Temperature of fresh milk samples collected from different Milk Collection Centers

Month	Temperatures C°		
	Alifoun	Tibna	Sayegh
April	$6.90 \pm 0.58^{A}_{B}$	$7.81 \pm 1.40^{A}_{B}$	$7.45 \pm 0.78^{A}_{B}$
May	$5.4 \pm 0.60^{B}_{C}$	$7.61 \pm 0.90^{A}_{E}$	$7.64 \pm 0.69^{A}_{E}$
June	$7.71 \pm 0.56^{A}_{F}$	$8.18 \pm 0.76^{A}_{F}$	$7.41 \pm 0.81^{A}_{F}$
July	$7.05 \pm 1.24^{A}_{G}$	$7.28 \pm 1.49^{A}_{G}$	$7.07 \pm 1.45^{A}_{G}$

* Values are mean ± SD for replicate independent runs.
* Values that bearing different superscript letters in the same column are significantly different at $P < 0.05$.
* Values that bearing different subscript letters in the same rows are significantly different at $P < 0.05$.

Table 4.3: pH of fresh milk samples collected from different Milk Collection Centers

Month	pH		
	Alifoun	Tibna	Sayegh
April	$6.71 \pm 0.01^{A}_{B}$	$6.71 \pm 0.02^{A}_{B}$	$6.69 \pm 0.02^{A}_{B}$
May	$6.71 \pm 0.03^{A}_{C}$	$6.66 \pm 0.02^{A}_{E}$	$6.71 \pm 0.02^{A}_{E}$
June	$6.71 \pm 0.35^{A}_{F}$	$6.69 \pm 0.02^{A}_{F}$	$6.70 \pm 0.05^{A}_{F}$
July	$6.68 \pm 0.04^{A}_{G}$	$6.68 \pm 0.07^{A}_{G}$	$6.68 \pm 0.02^{A}_{G}$

* Values are mean ± SD for replicate independent runs.

* Values that bearing same superscript letters in the same column are no significantly different at $P < 0.05$.

* Values that bearing different subscript letters in the same rows are significantly different at $P < 0.05$.

4.4.1.3 Titratable acidity (TA) of fresh milk

Table (4.4) shows the Titratable acidity of the three MCC_S during the study period. There was no significant ($p>0.05$) difference in TA of milk collected from different (MCC_S) Alifoun, Tibna and Sayegh. The TA ranged between 0.15 – 0.154% in all samples (Table 4.4).

These finding are within the range reported by O" Connor (1994) who stated that the normal fresh milk has an apparent acidity of 0.14 to 0.16% as lactic acid.

4.4.1.4 Specific gravity (SG) of fresh milk

Referring to results presented in Table (4.5), There was no significant ($p<0.05$) difference in SG of milk collected from MCC_S.

Tamime (2009) stated that specific gravity of normal milk ranges from 1.027 – 1.035 g per ml with a mean value of 1.032 g per ml. Therefor the results of SG of milk samples collected from the three MCC_S fall within the ranges of Tamime (2009) finding as show in Table 4.5.

4.4.1.5 Fat content of fresh milk

Table (4.6) shows the fat content of raw milk collected from different MCC_S during the study period. Statistical analysis showed no significant ($p<0.05$) difference in fat of milk samples collected in each MCC_S. However, there was significantly ($p<0.05$) different in fat of Alifoun collection center in May and July as compared to Tibna and Sayegh MCC_S (Table 4.6). This difference might be due to variability among the breeds of cows, within a bread and stage of lactation and feeding.

The average of fat content of milk samples from Alifoun, Tibna and Sayegh during study was 3.97±0.17, 3.86±0.1 and 3.86±0.13% respectively. According to European Union quality standard for unprocessed whole milk fat content should not be less than 3.5% (Tamime, 2009).Consequently the average fat content of milk in this study is within the range of defined standard.

Table 4.4: Titarable Acidity (TA) of fresh milk samples collected from milk Collection Centers

Month	TA%		
	Alifoun	Tibna	Sayegh
April	$0.151\pm0.03^{A}_{B}$	$0.150\pm0.03^{A}_{B}$	$0.152\pm0.03^{A}_{B}$
May	$0.152\pm0.03^{A}_{C}$	$0.154\pm0.02^{A}_{C}$	$0.151\pm0.03^{A}_{C}$
June	$0.152\pm0.04^{A}_{D}$	$0.153\pm0.04^{A}_{D}$	$0.153\pm0.04^{A}_{D}$
July	$0.151\pm0.02^{A}_{E}$	$0.150\pm0.02^{A}_{E}$	$0.152\pm0.03^{A}_{E}$

* Values are mean ± SD for replicate independent runs.

* Values that bearing same superscript letter in the same column are not significantly different at $P<0.05$.

* Values that bearing same subscript letter in the same rows are not insignificantly different at $P< 0.05$.

Table 4.5: Specific Gravity (SG) of fresh milk samples collected from different Milk Collection Centers

Month	SG		
	Alifoun	Tibna	Sayegh
April	$1.030\pm0.08^{A}_{B}$	$1.03\pm0.05^{A}_{B}$	$1.03\pm0.06^{A}_{B}$
May	$1.030\pm0.06^{A}_{C}$	$1.029\pm0.05^{A}_{C}$	$1.029\pm0.05^{A}_{C}$
June	$1.029\pm0.05^{A}_{E}$	$1.03\pm0.06^{A}_{E}$	$1.03\pm0.08^{A}_{E}$
July	$1.028\pm0.03^{A}_{F}$	$1.03\pm0.02^{A}_{F}$	$1.03\pm0.02^{A}_{F}$

* Values are mean ± SD for replicate independent runs.

* Values that bearing same superscript letter in the same column are not significantly different at $P< 0.05$.

* Values that bearing same subscript letter in the same rows are not significantly different at $P< 0.05$.

Table 4.6: Fat content of fresh samples collected from different Milk Collection Centers

Month	Fat%		
	Alifoun	Tibna	Sayegh
April	$3.94\pm0.25^{A}_{B}$	$3.91\pm0.10^{A}_{B}$	$3.92\pm0.05^{A}_{B}$
May	$4.14\pm0.16^{A}_{C}$	$3.86\pm0.04^{A}_{D}$	$3.90\pm0.15^{A}_{D}$
June	$4.02\pm0.18^{A}_{E}$	$3.79\pm0.11^{A}_{F}$	$3.83\pm0.20^{A}_{F}$
July	$4.13\pm0.10^{A}_{H}$	$3.91\pm0.15^{A}_{S}$	$3.96\pm0.15^{A}_{S}$

*Values are mean ± SD for replicate independent runs.

* Values that bearing same superscript letter in the same column are insignificantly different at $P< 0.05$.

* Values that bearing different subscript letter in the same rows are significantly different at $P< 0.05$.

4.4.1.6 Solids non fat (SNF)

In the current study, statistical analysis showed insignificant ($p<0.05$) difference in the SNF milk samples collected from each collection centers during the study period (Table 4.7).

SNF content of milk samples which were collected from Alifoun center in May (9.17±0.10%) and June (9.50±0.18%) were significantly ($p>0.05$) higher than samples which were collected from Tibna and Sayegh. The difference observed in SNF content of milk could be due to difference in the feeding practices, season, milking methods and lactation period exerted (suman *et al,* 1998). While there was no significant ($p<0.05$) difference among milk samples collected within each MCC during April and July (Table 4.7).

The average SNF content of milk samples collected from Alifoun, Tibna and Sayegh was 9.22±0.6, 8.98±0.50 and 8.98±0.65% respectively. According to European Union quality standards for unprocessed whole milk, Solid-not fat content should not be less than 8.5% (Tamime, 2009). Accordingly, the pooled average SNF content 9.06% observed from the three different milk collection centers were within the recommended standard.

4.4.1.7 Total Solids (TS)

The nutritional as well as economic value of milk is directly associated with its solids content. The higher the solid content, the better its nutritional value and greater the milk product yield (Shearer *et al*., 2003).

The data in Table 4.7showed no significant ($p<0.05$) difference in the total solids (TS) content among milk samples collected from milk collection centers, (Tibna and Sayegh) throughout the study period, except in July. However there was significant ($p<0.05$) difference in TS of milk collected from Alifoun, as compared

with that of Tibna and Sayegh collection centers during the study period. On the other hand, TS of milk samples collected from different collection center differ significantly ($p<0.05$) during the study period (Table 4.8).The variation could be due to difference in breed, feeding and management practices which have important effects on milk composition and quality (O" Connor, 1994).

Total solids content of milk samples collected from Alifoun was significantly ($p<0.05$) high than samples collected from Tibna and Sayegh during May, June and July, except in April samples from sayegh center was the highest total solid. The average total solids content of milk samples collected from Alifoun, Tibna and Sayegh milk collection centers was 13.9±0.34, 13.08±0.24, 13.12±0.3 and 13.15±0.25% in April, June and July respectively. European Union established quality standards for total solids content of cow milk is not less than 12.5% (FAO/WHO, 2007). Therefor, the average total solids content of milk samples in the present study was within the recommended standard.

Table 4.7: Solid Not Fat (SNF) of fresh milk collected from different Milk Collection Centers

Month	SNF%		
	Alifoun	Tibna	Sayegh
April	$9.17\pm0.19^{A}_{B}$	$9.03\pm0.08^{A}_{B}$	$9.11\pm0.18^{A}_{B}$
May	$9.17\pm0.10^{A}_{C}$	$9.03\pm0.06^{A}_{D}$	$9.00\pm0.10^{A}_{D}$
June	$9.50\pm0.18^{A}_{E}$	$9.01\pm0.12^{A}_{F}$	$8.90\pm0.23^{A}_{F}$
July	$9.07\pm0.18^{A}_{G}$	$8.85\pm0.24^{A}_{G}$	$8.91\pm0.14^{A}_{G}$

* Values are mean ± SD for replicate independent runs.

* Values that bearing same superscript letter in the same column are insignificantly different at $P< 0.05$.

* Values that bearing different subscript letter in the same rows are significantly different at $P< 0.05$.

Table 4.8: Total Solid content (TS) of fresh milk collected from different Milk Collection Centers

Month	TS%		
	Alifoun	Tibna	Sayegh
April	$13.11\pm0.24^{B}_{C}$	$13.10\pm0.44^{A}_{C}$	$13.36\pm0.36^{A}_{C}$
May	$13.46\pm0.40^{AB}_{D}$	$12.89\pm0.06^{B}_{E}$	$12.90\pm0.13^{B}_{E}$
June	$13.82\pm0.73^{A}_{F}$	$12.81\pm0.12^{B}_{G}$	$12.74\pm0.28^{B}_{G}$
July	$13.54\pm0.32^{AB}_{F}$	$12.929\pm0.17^{B}_{H}$	$13.050\pm0.27^{AB}_{H}$

* Values are mean ± SD for replicate independent runs.

* Values that bearing different superscript letters in the same column are significantly different at $P< 0.05$.

* Values that bearing different subscript letter in the same rows are significantly different at $P< 0.05$.

4.4.2. Microbiological Analysis

All microbial counts were presented as colony forming units per ml (log cfu/ml) of milk samples.

4.4.2.1 Coliform count

Table(4.9) displayed total Coliform bacteria count of raw milk collected from different collection centers MCC_S (Alifoun, Tibna and Sayegh) during the study period in April, May, June and July 2014.

The acceptable limits of Coliform counts in milk should be less than 100 cell/ml (2 log/ml) as stated by Shojaei and Yadollahi (2008). However, Muhammed *et al.* (2009) reported higher Coliform count in hot season. Statistical analysis of our results showed that there was no significant ($p<0.05$) difference in samples collected from each MCC during the study period (Table 4.8). Nevertheless, samples collected from Alifoun milk collection center was significantly ($p<0.05$) different as compared to samples collected from Tibna and Sayegh center during the study period (April – July). The coliform count obtained in this study is in agreement with that reported by Fekadu (1994) who found coliform counts of 3.8 – 4.0 $\log_{10}$ cfu/ml. Zelalem and Bernard (2006) obtained higher coliform count of 6.57 $\log_{10}$cfu/ml for cows' milk collected from different producers. The higher coliform count explained by the initial contamination of the milk samples either from the cows, the milkers, milk containers and the milking environment. Thus, extension services and training of farmers in milk handling practices are required to improve the quality of raw milk. In addition, the high number of coliform count indirectly revealed the farms were not properly mange due to poor personal hygiene practices throughout the milking process. Furthermore, the contaminated utensils especially the milking churns, teats cup or any utensils that intact with raw milk might influence the microbial quality of raw milk along the chain. Coliform count regularly in excess of 100 cfu/ml is considered by some authorities as

evidence of unsatisfactory production hygiene. Sporadic high coliform count may also be a consequence of unrecognized coliform mastitis, mostly caused by *E.Coli*. The coliform micro-organisms are found also on the surface of the under washed or moisture milking equipment (Barmley, 1990).

The existing results showed that the microbial quality of raw milk obtained from Alifoun MCC was high as compared with Tibna and Sayegh MCC during the study period (Table 4.9).

Table 4.9: Coliform bacterial count of different Milk samples collected from milk Collection Centers

Month	Coliform (log_{10} cfu/ml)		
	Alifoun	Tibna	Sayegh
April	$3.26\pm0.45^{A}_{B}$	$4.56\pm0.15^{A}_{C}$	$4.48\pm0.09^{A}_{C}$
May	$3.02\pm0.14^{A}_{D}$	$4.61\pm0.04^{A}_{E}$	$4.51\pm0.15^{A}_{E}$
June	$3.35\pm0.67^{A}_{F}$	$4.63\pm0.05^{A}_{G}$	$4.56\pm0.04^{A}_{G}$
July	$3.29\pm0.65^{A}_{H}$	$4.60\pm0.10^{A}_{K}$	$4.42\pm0.11^{A}_{K}$

* Values are mean ± SD for replicate independent runs.

* Values that bearing same superscript letter in the same column are insignificantly different at $P< 0.05$.

* Values that bearing different subscript letter in the same rows are significantly different at $P< 0.05$.

4.4.2.2 Total viable bacterial count (TVBC)

Table (4.10) illustrated total viable count of raw milk samples collected from different MCC_S (Alifoun, Tibna and Sayegh) during the study period in April, May, June and July 2014. Statistical analyses did not reveal any significant ($p<0.05$) difference in TVBC of milk samples collected from Tibna and Sayegh centers except in June. While the milk samples collected from Alifoun center was significantly ($p<0.05$) different from samples collected from Tibna and Sayegh center, except in April. Total viable count of raw milk samples collected from each center (Alifoun, Tibna and Sayegh) within the study period were significantly ($p<0.05$) different.

TVBC of milk samples collected from Alifoun in April (4.7±0.11 log_{10} cfu/ml) and June (4.75±0.16 log_{10} cfu/ml) were similar and that in July and May significantly ($p<0.05$) different. While milk samples collected from Tibna center in May (5.06±0.12 log_{10} cfu/ml) and July (5.23±0.34 log_{10} cfu/ml) are reached the highest TVBC among MCC_S during the study period. Milk samples collected from Sayegh center showed the high TVBC reading in May 5.04±0.07 log_{10} cfu/ml. The higher TVBC of milk may be due to in efficiency of cooling system and cross microbial contamination. According to the Regulative EU (Regulation 853, 2004) the average total number of micro-organisms should not exceed 100 000 per ml (5 log_{10} cfu/ml) of raw cow's milk from primary production. To avoid the increase of the number of micro organisms the European Regulative 853 (2004) recommends that immediately after milking, milk must be held in a clean designed place equipped well to avoid contamination. The milk must be cooled immediately to not more than 8 °C in the case of daily collection, or not more than 6 °C if collection is not daily. During transport the cold chain must be maintained and on arrival at the establishment of destination, the temperature of the milk must not be more that 10°C (Regulation EC 853, 2004).

Fekadu (1994) reported that the minimum and maximum total bacterial count of raw cows' milk to be 6 to 8.8 $\log_{10}$cfu/ml. The results of raw milk samples collected from the three different sources were less than the ranges stated by Fekadu (1994) finding as shown in (Table 4.10).

Table 4.10: Total Viable bacterial count (TVBC) of different Milk samples collected from milk Collection Centers

Month	TVBC (log_{10}cfu/ml)		
	Alifoun	Tibna	Sayegh
April	$4.72\pm0.11^{AB}{}_{E}$	$4.98\pm0.44^{BC}{}_{E}$	$4.88\pm0.27^{BC}{}_{E}$
May	$4.60\pm0.06^{B}{}_{G}$	$5.06\pm0.12^{C}{}_{F}$	$5.04\pm0.07^{A}{}_{F}$
June	$4.75\pm0.16^{AB}{}_{K}$	$5.23\pm0.34^{C}{}_{R}$	$4.63\pm0.45^{C}{}_{K}$
July	$4.83\pm0.23^{A}{}_{N}$	$4.46\pm0.43^{B}{}_{S}$	$4.42\pm0.11^{B}{}_{S}$

*Values are mean ± SD for replicate independent runs.

* Values that bearing different superscript letters in the same column are insignificantly different at $P< 0.05$.

* Values that bearing different subscript letters in the same rows are significantly different at $P< 0.05$.

4.4.2.3 Mesophilic bacteria lab pasteurization count

Table (4.11) show Lab pasteurization count (Mesophilic bacteria) of milk samples collected from different MCC_S during the study period April – July 2014.

Statistical analysis of results showed no significant ($p<0.05$) difference in Mesophilic bacteria of milk samples collected from each specific MCC whiten the study period. While Mesophilic bacteria from Alifoun center was significantly ($p<0.05$) differences compared to Tibna and Sayegh centers in April, May and June. Mesophilic bacteria of milk sample from Alifoun was lower than Tibna and Sayegh MCC_S (Table 4.11).

According to the national sanitary standards, the acceptable amount of total Mesophilic bacteria count in pasteurized milk is less than 4.699 log cfu/ml (Berry 2004). There for, the Mesophilic bacteria results of raw milk samples collected from three MCC_S (1.08 – 1.59 $\log_{10}$ cfu/ml) was less than that reported by Berry (2004) finding (4.699 $\log_{10}$ cfu/ml).

4.4.2.4 Thermophilic bacteria lab pasteurization count

Table (4.12) presented Lab pasteurization count (Thermophilic bacteria) of milk samples collected from different MCC_S during the study period April – July 2014. There was no significant ($p<0.05$) difference the Thermophilic bacteria of milk samples collected from each specific MCC within the study period (Table 4.11). Despite the fact that there is significant ($p<0.05$) difference in Thermophilic bacteria of milk samples collected from different MCC_S (Alifoun, Tibna and Sayegh) within each month of study. Thermopilic bacteria of milk samples collected from Tibna and Sayegh MCC_S was high than that from Alifoun. There for, Alifoun milk collection present the best center on milk microbial quality. One the other hand swap total plat count for surfaces and manufacture unit was negative throughout the study period (Table 4.13)

Table 4.11: Total lab pasteurization count (LPC) for Mesophilic bacteria of different milk samples collected from milk collection centers (MCC_S)

Month	LPC mesophilic (log_{10} cfu/ml)		
	Alifoun	Tibna	Sayegh
April	$1.33\pm0.20^{A}{}_{C}$	$1.71\pm0.21^{A}{}_{D}$	$1.57\pm0.26^{A}{}_{D}$
May	$1.32\pm0.21^{A}{}_{E}$	$1.59\pm0.12^{A}{}_{F}$	$1.57\pm0.15^{A}{}_{F}$
June	$1.20\pm0.14^{A}{}_{G}$	$1.64\pm0.17^{A}{}_{H}$	$1.58\pm0.10^{A}{}_{H}$
July	$1.08\pm0.29^{A}{}_{K}$	$1.66\pm0.24^{A}{}_{K}$	$1.78\pm0.25^{A}{}_{K}$

* Values are mean ± SD for replicate independent runs.

* Values that bearing same superscript letter in the same column are insignificantly different at $P< 0.05$.

* Values that bearing different subscript letter in the same rows are significantly different at $P< 0.05$.

Table 4.12: Total lab pasteurization count (LPC) for Thermophilic bacteria of different milk samples collected from milk collection centers (MCC_S)

Month	LPC Thermophilic (log_{10} cfu/ml)		
	Alifoun	Tibna	Sayegh
April	$1.07\pm0.24^{A}_{C}$	$1.33\pm0.16^{A}_{D}$	$1.17\pm0.37^{A}_{C}$
May	$1.00\pm0.11^{A}_{F}$	$1.28\pm0.06^{A}_{G}$	$1.23\pm0.09^{A}_{G}$
June	$0.92\pm0.56^{A}_{H}$	$1.34\pm0.17^{A}_{B}$	$1.24\pm0.11^{A}_{H}$
July	$0.53\pm0.45^{A}_{M}$	$1.22\pm0.17^{A}_{K}$	$1.37\pm0.13^{A}_{K}$

* Values are mean ± SD for replicate independent runs.

* Values that bearing same superscript letter in the same column are insignificantly different at $P< 0.05$.

* Values that bearing different subscript letter in the same rows are significantly different at $P< 0.05$.

Table 4.13: Total plate count (TPC) for swap surface and rinse water

Month	TPC (cfu/ml)	
	Rinse water	surfaces
April	-ve	-ve
May	-ve	-ve
June	-ve	-ve
July	-ve	-ve

4.4.3 During mixing process

4.4.3.1 Titarable acidity (TA) of milk yoghurt

Table (4.14) showed Titarable acidity of milk yoghurt during mixing process. The TA during mixing throughout the study period ranged between 0.21 – 0.22%. There were no significant ($p>0.05$) difference in TA of samples collected from each specific month during the study period.

4.4.3.2 Total solid (TS) content of milk yoghurt

Table (4.15) presented the total solid of mixed milk yoghurt during the study period. The TS during mixing throughout the study period ranged between 15.15 – 15.25%. Statistical analysis did not revealed significant ($p<0.05$) different in total solids of samples at mixing stage in each specific month of the study period (Table 4.15). These results were similar to Tamime and Robinson (1999) who stated that milk solids contents of many commercial yoghurt products ranged between 14 – 15%.

4.4.3.3 Solids non fat (SNF) content of milk yoghurt

SNF during mixing throughout the study period ranged between 12.11 – 12.21%. The solids non fat did not differ significantly ($p>0.05$) during mixing stage in each month of the study period (Table 4.16).

4.4.3.4 Fat content of milk yoghurt

Table (4.17) showed fat content of milk yoghurt during mixing process. Statistical analysis did not indicate any significant ($p>0.05$) difference during mixing stage in each month throughout the study period.

4.4.3.5 Specific gravity (SG) of milk yoghurt

Specific gravity of milk yoghurt explain did not show significant ($p<0.05$) different during mixing process in each month throughout the study period (Table 4.18).

Table 4.14: Titarable acidity (TA) of mixed milk during mixing process

Month	TA%
April	0.22 ± 0.03^{A}
May	0.22 ± 0.2^{A}
June	0.22 ± 0.02^{A}
July	0.21 ± 0.02^{A}

*****Values are mean ± SD for replicate independent runs.

* Values that bearing same superscript letter in the same column are not significantly different at $P< 0.05$.

Table 4.15: Total Solid (TS) of mixed milk during mixing process

Month	TS%
April	15.15 ± 0.08^{A}
May	15.15 ± 0.15^{A}
June	15.2 ± 0.14^{A}
July	15.25 ± 0.07^{A}

* Values are mean ± SD for replicate independent runs.

* Values that bearing same superscript letter in the same column are not significantly different at $P< 0.05$.

Table 4.16: Solid Non Fat (SNF) of mixed milk during mixing process

Month	SNF%
April	12.11 ± 0.07^{A}
May	12.11 ± 0.11^{A}
June	12.17 ± 0.14^{A}
July	12.21 ± 0.07^{A}

* Values are mean ± SD for replicate independent runs.

* Values that bearing same superscript letter in the same column are not significantly different at $P < 0.05$.

Table 4.17: Fat content of mixed milk during mixing process

Month	Fat%
April	3.04 ± 0.07^{A}
May	3.04 ± 0.05^{A}
June	3.02 ± 0.05^{A}
July	3.06 ± 0.08^{A}

* Values are mean ± SD for replicate independent runs.

* Values that bearing same superscript letter in the same column are not significantly different at $P< 0.05$.

Table 4.18: Specific gravity (SG) of mixed milk during mixing process

Month	SG
April	1.043 ± 0.02^{A}
May	1.042 ± 0.03^{A}
June	1.040 ± 0.04^{A}
July	1.042 ± 0.01^{A}

* Values are mean ± SD for replicate independent runs.

* Values that bearing same superscript letter in the same column are not significantly different at $P < 0.05$.

4.4.4 After pasteurization process

The pasteurization process is important step as it plays a role in the destruction and /or changes in the physic-chemical properties of the milk constituents and fermentation temperature will impact on the physical characteristics and sensory properties of the end product (Hoolasi, 2005).

Specific gravity, Solid non fat, total solid and overall temperatures after pasteurization process are shown in Table 4.19, 4.20, 4.21 and 4.22 respectively.

Statistical analysis did not revealed any significant ($p<0.05$) difference in specific gravity, solid non fat, total solid content and overall temperature after pasteurization stage in each specific month throughout the study period in April – July 2015.

4.4.5 During fermentation process

Total solid content, pH, viscosity and cooling temperature are shown in Tables 4.23, 4.24, 4.25 and 4.26 respectively.

Statistical analysis showed significant ($p<0.05$) difference in total solids during study period. The average total solids content of stirred yoghurt was 16.50. These results are in line with findings of Muhammad *et al*. (2005) who reported that the highest range of total solids in yoghurt to reach17.1%, due to addition of skimmed milk powder during the mixing of milk yoghurt. According to Soukoulis *et al.*, (2007) the addition of skim milk powder tends to improve the textural quality and decrease the vulnerability of yoghurt to syneresis. Whereas the pH, viscosity and cooling temperature of stirred yoghurt during fermentation process was not significantly ($p>0.05$) difference during the study period (Table 4.23, 4.24 and 4.25). These results were in agreement with Tamime and Robinson (1999), who reported that when yoghurt have reached the desired pH of 4.6,it is partially cooled to 20°C before fruit or flavoring ingredients are added.

Table 4.19: Specific gravity of milk after pasteurization process

Month	SG
April	1.039 ± 0.004^{A}
May	1.042 ± 0.004^{A}
June	1.043 ± 0.002^{A}
July	1.042 ± 0.001^{A}

* Values are mean ± SD for replicate independent runs.

* Values that bearing same superscript letter in the same column are not significantly different at $P < 0.05$.

Table 4.20: Solid Non Fat content (SNF) of milk after pasteurization process

Month	SNF%
April	12.13 ± 0.06^{A}
May	12.15 ± 0.11^{A}
June	12.22 ± 0.12^{A}
July	12.11 ± 0.03^{A}

* Values are mean ± SD for replicate independent runs.

* Values that bearing same superscript letter in the same column are not significantly different at $P< 0.05$.

Table 4.21: Total Solid content (TS) of milk after pasteurization process

Month	TS%
April	15.16 ± 0.03^{A}
May	15.20 ± 0.15^{A}
June	15.26 ± 0.11^{A}
July	15.17 ± 0.08^{A}

* Values are mean ± SD for replicate independent runs.

* Values that bearing same superscript letter in the same column are not significantly different at $P< 0.05$.

Table 4.22: Temperature of milk after pasteurization process

Month	Temperature C°
April	43.0 ± 0.57^{A}
May	42.4 ± 0.53^{A}
June	42.8 ± 0.69^{A}
July	43.0 ± 0.81^{A}

* Values are mean ± SD for replicate independent runs.

* Values that bearing same superscript letter in the same column are not significantly different at $P< 0.05$.

Table 4.23: Total Solid of stirred yoghurt during fermentation process

Month	TS%
April	17.48 ± 2.03^{A}
May	17.08 ± 1.90^{AB}
June	16.31 ± 1.20^{AB}
July	15.16 ± 0.12^{B}

* Values are mean ± SD for replicate independent runs.

* Values that bearing different superscript letters in the same column are significantly different at $P< 0.05$.

Table 4.24:pH of stirred yoghurt during fermentation process

Month	pH
April	4.6 ± 0.032^{A}
May	4.6 ± 0.030^{A}
June	4.9 ± 0.77^{A}
July	4.6 ± 0.033^{A}

* Values are mean ± SD for replicate independent runs.

* Values that bearing same superscript letter in the same column are not significantly different at $P< 0.05$.

Table 4.25: Viscosity of stirred yoghurt during fermentation process

Month	Viscosity mPas
April	30.2 ± 8.12^{A}
May	29.6 ± 4.01^{A}
June	33.0 ± 4.3^{A}
July	32.4 ± 2.95^{A}

* Values are mean ± SD for replicate independent runs.

* Values that bearing same superscript letter in the same column are not significantly different at $P < 0.05$.

Table 4.26: Cooling Temperature of stirred yoghurt during fermentation process

Month	Temperature C°
April	19.85 ± 1.46^{A}
May	19.00 ± 1.00^{A}
June	20.7 ± 1.97^{A}
July	19.57 ± 1.93^{A}

* Values are mean ± SD for replicate independent runs.

* Values that bearing same superscript letter in the same column are significantly different at $P < 0.05$.

4.4.6 Analysis of final product

4.4.6.1 pH of final stirred yoghurt

Table (4.27) showed the pH of final stirred yoghurt product. Statistical analysis did not showed significant ($p>0.05$) difference in pH of final product during the study period in April – July. These results were in agreement with Early (1998), who declared that fermentation will be arrested at approximately pH 4.2 – 4.4, or even at lower pH of 3.8 – 4.0.

4.4.6.2 Viscosity of final stirred yoghurt

Table (4.28) showed the viscosity of final stirred yoghurt during the study period. Statistical analysis showed significant ($p<0.05$) increasely. The highest score of viscosity was in July (59.68±1.77 mPas (millipascal seconds) followed by June, May and then April in descending order.

Viscosity values of stirred yoghurt ranged from 57.83 mPas after 30 sec. to 46.60 mPas after 120 sec. Viscosity decreased by 11.23 mPas during shearing. Denin *et al* (2002) concluded that the hydrophilic properties of acid casein gel is produced from milk heat treated at 90ºC and tends to increase during storage, which has positive influence on the viscosity of stirred yoghurt.

4.4.6.3 Yeast and moulds

Table (4.29) shows that yeast and moulds of samples collected from CAPO stirred yoghurt during each month of the study period. Total yeast and moulds in stirred yoghurt were to be nill. Abdelrazig (2008) stated that the yeast and mould were found to be nill, but Abdall (1997) reported that 100cfu/ml of yeast and mould has been given the good quality.

4.4.6.4 Temperature of final product

Table (4.30) shows the temperature of final stirred yoghurt. Statistical analysis showed no significant ($p>0.05$) difference in temperature of final stirred yoghurt in each month during the study period, which ranged between 8.14 – 8.85°C This result was in agreement with Early, (1998) who stated that a temperature of 8 – 10°C is optimal, depending upon storage time. Intermediate storage should be as short as possible since physical changes take place can affect the final yoghurt quality.

4.5 Corrective action

As shown in Table 4.1 and based on critical limits and monitoring procedure no correction action require to modify the process.

Table 4.27: pH of final stirred yoghurt

Month	pH
April	4.43 ± 0.04^{A}
May	4.42 ± 0.06^{A}
June	4.43 ± 0.02^{A}
July	4.43 ± 0.04^{A}

* Values are mean ± SD for replicate independent runs.

* Values that bearing same superscript letter in the same column are not significantly different at $P< 0.05$.

Table 4.28: Viscosity of final stirred yoghurt

Month	Viscosity(mPas)
April	53.17 ± 4.19^{B}
May	56.92 ± 5.64^{AB}
June	58.65 ± 3.48^{AB}
July	59.68 ± 1.77^{A}

* Values are mean ± SD for replicate independent runs.

* mPas (millipascal seconds). 1mPas = 1CP.

* Values that bearing different superscript letters in the same column are significantly different at $P < 0.05$.

Table 4.29: Yeast and moulds of final product

Month	Yeast and mould cfu/ml
April	-ve
May	-ve
June	-ve
July	-ve

Table 4.30: Temperature of final stirred yoghurt

Month	TemperatureC°
April	8.85 ± 1.34^{A}
May	8.42 ± 2.76^{A}
June	8.14 ± 1.77^{A}
July	8.85 ± 1.57^{A}

* Values are mean ± SD for replicate independent runs.

* Values that bearing same superscript letter in the same column are not significantly different at $P< 0.05$.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIOS

5.1 Conclusions

The findings of the present study evaluate implementation of HACCP in stirred yoghurt plant in Capo Company during the study period. Sample was successfully collected to indicate proper control during application of HACCP in the production line. All results recorded during monitoring step (physical, chemical and microbiological) did not deviate from standard values of each specific quality parameter (Raw milk pH 6.6%, TA 0.151%, fat 3.9%, SG 1.030% and TVBC 4.72 log_{10}cfu/ml. During mixing step TS 15.5%, SNF 12.11%. After pasteurization SG 1.039%, SNF 12.11% and TS 15.16%. During fermentation pH 4.6%, viscosity 30.2 mPas and Temperature 43°C. Final stirred yoghurt pH 4.43%, viscosity ranged between 53.17 – 59.68 mPas, negative yeast and mould and cooling temperature 8.14°C.

According to microbial results the tested samples which collected from Alifoun MCC showed best results during the study period. This may be attributed to the applied proper sanitation and good manufacturing practice (GMP).

5.2 Recommendations

From this study it can be recommended that:

1. The application of HACCP system and identifying critical points is important and should be a comprehensively exercised and approached on a scientific basis in dairy food factories.
2. In the case of stirred yoghurt manufacturer, the quality of raw milk can be improved by the implementation of HACCP at farm level (milk suppliers to the company).
3. Adequate sanitary measures should be taken at stage from production of raw milk to processing of stirred yoghurt especially during collection of raw milk from Tibna and Sayegh MCC_S. These measures include proper handling of the cow, personnel hygiene, use of hygienic milking and processing equipments.
4. Ongoing education, training and motivation of all personnel on HACCP principles are essential, especially when employee turnover is high. The implementation process should be undertaken as any other project in the company such as the installation of a new production line, the launch of a new product, and others.
5. A suggestion for future research in stirred yoghurt production is to be established, in detail for best investment in Sudan.

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