**Sudan University of Sciences and Technology**

**College of Graduate Studies and Scientific Research**

# Impact of HACCP System Application on Safety and Quality of Stirred Yoghurt Produced in DAL Diary Plant (CAPO).

# أثر تطبيق نظام تحليل المخاطر والتحكم في النقاط الحرجة علي سلامة وجودة الزبادي المزج المنتج بمصنع دال للألبان (كابو).

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# DEDICATION

To my father for his love and confidence, which help me to continue my educational career

To My mother, (the magic that draws my energy and my strength)

To my brothers (Hafiz, Fatah Elrrahman)

To my sisters (May&Malak)

To my best friend Malaz

With my love

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My best wishes for you `

# Table of Contents

# LIST OF TABLES

# ABSTRACT

This experiment was carried out in January – February 2017 at Dal Dairy Factory (CAPO) - Bahri - Sudan. The main objective of present study is evaluation impact of Hazard Analysis and Critical Control Point (HACCP) application on safety and quality of plain stirred yoghurt. The samples were collected and analyzed within the factory during the yoghurt manufacturing period, starting with raw milk and passing through the manufacturing process until the finished product. Five samples of raw milk and 15 samples of yogurt were collected in duplicate at four different stages during manufacturing for the physiochemical and microbiological analysis. These stages include: raw milk, pasteurization, during fermentation process and final product stage. The results of the sensory evaluation (color, flavor, texture) of raw milk showed its quality and its capability for consumption and processing and all the results were within normal limits. The results of the physiochemical and microbiological analysis of raw milk showed that the product is safe and good for manufacturing by comparing all the results with their limits and requirements required in the Sudanese standard metrology for raw milk and yoghurt. In the final product, the results of the physiochemical and microbiological analysis were as follows: (viscosity 21.9, pH 4.3, temperature 24$^{o}$C, fungi and yeasts 6) as averages for every 10 samples. The results showed that the yogurt was free from the coliforms (*E. coli spp, Kelbsella spp, Citrobacter spp, Enterobacter spp).*The results of the physiochemical and microbiological analysis of the final product confirmed the product's safety from any contaminants and physical, chemical and microbial hazards. Application of HACCP system and GMP, GHP at Dal dairy plant (CAPO) has a good effect on the safety and quality of yoghurt (100%).

# المستخلص

تم إجراء هذه التجربة في شهر يناير – فبراير 2017م بمصنع دال للألبان (كابو) – بحري – السودان. أجريت الدراسة بغرض معرفة وتقييم أثر تطبيق نظام تحليل المخاطر والتحكم في النقاط الحرجة (الهاسب) علي سلامة وجودة الزبادي المزج. تم جمع العينات وتحليلها داخل المصنع أثناء فترة تصنيع الزبادي إبتدءآ باللبن الخام ومروراً بمرحلة التصنيع حتي المنتج النهائي. تم جمع 5 عينة مكررة من اللبن الخام و15 عينة من الزبادي مكررة أيضآ، علي أربع مراحل مختلفة خلال التصنيع بغرض التحليل الفيزيائي والكيميائي والميكروبيولوجي، وتتضمن هذه المراحل ما يلي: (اللبن الخام، البسترة، أثناء عملية التخمير ومرحلة المنتج النهائي). أبانت نتائج التقييم الحسي (اللون، الطعم والقوام) للبن الخام جودته و قابليته للإستهلاك والتصنيع وكانت كل النتائج في الحدود الطبيعية. أوضحت نتائج التحليل الفيزيوكيميائي والميكروبيولوجي للبن الخام أن المنتج آمن للإستهلاك والتصنيع وذلك من خلال مقارنة كل النتائج بالحدود والإشتراطات المطلوبة في المواصفة القياسية السودانية للبن الخام والزبادي. أما بالنسبة للمنتج النهائي كانت نتائج التحليل الفيزيوكيميائية والميكروبيولوجية كما يلي: ( االزوجة 21,6 ، الرقم الهيدروجيني 4,3 درجة الحرارة 24م$^{o}$، الفطريات والخمائر 6 كمتوسطات لكل 10عينة، كما أوضحت النتائج خلو الزبادي من البكتريا الممرضة من نوع Coliforms (*E – Coli spp*، *Kelbsellaspp*، *Citrobacterspp*، *Enterobacterspp*). أكدت نتائج التحليل الفيزيوكيميائي والميكروبيولوجي للمنتج النهائي سلامة المنتج من الملوثات والمخاطر الفيزيائية والكيميائية والميكروبية. من خلال تتبع نظام الهاسب بمصنع كابو للألبان وفحص المنتج وجد أن له أثر فعال في سلامة وجودة الزبادي بنسبة 100%.

# CHAPTER ONE
# INTRODUCTION

Yoghurt is a fermented milk product of creamy texture that can be prepared from milk of many species, but most often made from cow milk. It is rich in protein, calcium and vitamins, and tremendously popular all over Nigeria and the world at large. Yoghurt is made by the controlled thermoduric fermentation of pasteurized non-fat or low-fat milk, carried out around 45$^{o}$C (Akanbi and Oyediji, 2015).

Ahmed; *et al.* (2013), stated that yoghurt is one of oldest fermented milk product, tremendously popular all over the world. It is a very rich source of protein, calcium and vitamins. It fermented by lactic acid producing bacteria (*S -thermophilus and L - bulgaricus*) or some additional bacteria having mutual complementing metabolism. The natural yoghurt is characterized by smooth and viscous gel like texture and has delicate walnuts flavor (Fuquay *et al*, 2011).

In fact, the fermentation of lactose by lactic acid bacteria results in the production of lactic acid, carbon dioxide, acetic acid, diacetyle acetaldehyde and several other component giving a characteristic flavor to yoghurt, however very careful processing is required for production of safe and good quality yoghurt. In fact even a little contamination may have very negative effects on consumer health (Tamime and Robinson, 2007). The quality of yoghurt is governed by a number of factors. In fact, inferior milk quality, unhygienic condition and the use of (wild type) of starter culture give rise to poor grade yoghurt having lower shelf life. In addition, microbiological aspect is one of the most important factors. The microbial quality of yoghurt reflects towards the quality and acceptability of the yoghurt. Due to unhygienic conditions there is possibility microbial contamination (pathogens), which may have serious impact on the health of

consumers. Further, unhygienic vending condition (open packs, higher contamination) also deteriorates the keeping quality of yoghurt (Aziz, 1985).

## Justifications:

Application of HACCP system may lead or assist in product safety. It also may help in the marketing and consumption of the product by increase consumer confidence. The importance of applying the HACCP system in the dairy field, especially milk and its derivatives which are perishable products if they are not maintained properly.

## Objectives:

## General objective:

Evaluation impact of HACCP application on safety and quality of yoghurt produced in DAL Dairy Factory.

## Specific objective:

The main objectives of this study are: -

1. To study the effect of application HACCP system on the safety and quality of yoghurt.
2. To evaluate the current methods of analysis on hazards that appears during the processing and control procedures used in the plant.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1. Yoghurt:

The Food and Drug Administration (FDA) in 2011, described yoghurt as food produced by culturing one or more of the basic ingredients (cream, milk, partially skimmed milk, skim milk or the reconstituted versions of these ingredients may be used alone or in combination) and any of the optional dairy ingredient with a characterizing bacteria (live and active) culture that contains the lactic acid – producing bacteria (*Streptococcus thermophilus* and *Lactobacilus bulgaricus*). The word "live and active cultures" refer to those living organisms which convert pasteurized milk to yoghurt during fermentation (*Streptococcus Thermophiles*and *lactobacillus Bulgaricus*). (Weerathilake; *et al*,2014).

Yoghurt is made by inoculating certain bacteria (starting culture), usually *Streptococcus thermophilus* and *lactobacillus bulgaricus*, into milk. After inoculation, the milk incubated at approximately ($110^{o}\pm5^{o}$F) until fermentation; this coagulated by bacteria – produced lactic acid. Yoghurt may have additional cultures, sweeteners, flavorings, color, additives, stabilizers and emulsifiers and preservation add to it. Yoghurt is one of the oldest produced foods in human history. It is a unique food, which is consumed worldwide without the restriction of any taboo tradition or religion. Cow milk is most commonly used worldwide to prepare yoghurt. However, the milk from the goat, camel and water buffaloes are also employed to make yoghurt. The name of yoghurt is derived from the Turkish word "Jugurt" reserved for any fermented food with acidic taste (Younus, *et al*; 2002).

In 1907, Dr. Metchnikoff co – workers isolated and named one of the yoghurt bacteria, *Lactobacillus bulgaricus* (Jay, 2000).

The popularity of yoghurt soared in 1950 and 1960 with the boom of health food culture. Presently, fermented foods constitute about 25% of the food consumed worldwide. Usually, these foods are considered safe against food borne infections (Adebayo; *et al*, 2014).

## 2.1.1The manufacturing of yoghurt process:

According to the physical nature of the product, commercial yoghurts are classified into three main categories; set, stirred, and drinking, the latter is often referred as stirred yoghurt of low viscosity. The main difference between the products is the type of incubation. Set-yoghurt is incubated in the packaging container while stirred-type yogurt is incubated in the large manufacturing vat prior to packaging (Ozer, 2010). Depending on the manufacturers" preferences, milk used for yogurt manufacture could be either from fresh milk, powder, or combination of both (Robinson *et al.*, 2006 and Tamime and Robinson, 2007).

Although there are no standardized procedures for making yoghurt product, most process agree on a general process. This includes; pre – treatment of milk, heat treatment, homogenization, cooling and starter culture addition, incubation and packaging (Tamime and Robinson, 1999).

### 2.1.1.1 Heat – treatment:

The main purpose of heat-treatment on milk intended for yogurt manufacture is to eliminate pathogens and other competitive microorganisms to create a favorable environment for growth of yogurt cultures. It also reduces the oxygen content as well as provides more readily available amino acids to the cultures (Ozer, 2010).

#### 2.1.1.2 Standardization

As milk composition varies according to the season of the year, standardization of yoghurt base is of critical importance for yoghurt manufacture to maintain consistency of product. As mentioned earlier milk solids non-fat (MSNF) and lipids are important attributes of yogurt profile.

Fat gives the luxury mouth-feel taste while MSNF is important for texture. Typically, fat content and MSNF in yoghurt range between 1-4.5 g/100 ml and 12-18 g/100 ml respectively, but they may be adjusted in order to meet existing or proposed standards or target consumers (Robinson et al., 2006).

According to Ozer (2010), total milk solids of high quality yogurt ranges between 18 and 22% to support the growth of *S. thermophilus* and *L. bulgaricus*, which is optimum at SNF level of 14% and 12% respectively. An increase of milk solids in yogurt is also believed to improve viscosity, mouth-feel, texture and taste (Ozer, 2010).

Addition of milk powder to the product is a common practice applied to increase the milk solids content (e.g. to enrich protein level). Whole milk or skim milk powder can be added but skim milk powder (SMP) is preferred over whole milk powder (WMP) due to potentially lower fat oxidation issues. Typically, the fortification of MSNF with SMP is applied at concentration levels of 3-4% as excessive addition of SMP may result in lumpiness and powdery taste. Although it is not a common practice at industrial scale, addition or reconstitution of skim milk powder with buttermilk powder is possible (Ozer, 2010). Standardization of fat in yogurt can be done easily using the Pearson's square to determine the desired fat content (Tamime and Robinson, 2007).

#### 2.1.1.3 Addition of stabilizer (optional)

The main purpose of adding stabilizers in yogurt is to improve rheological and textural properties. The mode of action of stabilizers in yoghurt is water binding to retard the movement of water within the protein gel network leaving less free water for syneresis. As a result, protein network is stabilized and viscosity is improved. In some cases, hydrocolloids may lead to gel formation. Stabilizers may improve mouth - feel; act as fat substitutes, and thus maintaining low levels of calories of the product (Ozer, 2010).

#### 2.1.1.4 Addition of Sweetener (Optional)

In terms of nutrition, sucrose is one of the three major sugars (lactose and starch) that can be hydrolyzed to glucose and fructose by the enzyme sucrose in the human intestinal tract as source of energy (Fennema, 1996).

#### 2.1.1.5 Addition of Colorant, Flavoring, and Preservatives (Optional)

The addition of colorant, flavoring, and preservatives is discretionary in NZ (FSANZ, 2011). Yogurt is categorized as low risk products in terms of microbial contamination due to its acidic environment that suppresses other microbial growth. However, yeast and moulds can grow in such conditions and are the spoilage microorganisms in yogurt-making. ascorbic acid, sulphur dioxide, and benzoic acid are common preservatives used in yogurt to suppress growth of yeast and moulds. The levels and type of preservatives used differ according to preference of the manufacturer, but the maximum limit of such preservatives should not exceed 50 mg/kg (singly or combination) according to the legal limit set by FAO/WHO (Tamime and Robinson, 2007).

#### 2.1.1.6 Homogenization

The purpose of milk homogenization is to decrease the size of fat globules. In yoghurt making, homogenization using pressures of 15-20 MPA at 65-70°C is critical to stabilize the oil-in-water emulsion. Before homogenization, milk fat globules in their native state (raw milk) are encapsulated within the protein and phospholipids membrane. Homogenization breaks the lipid membrane thus reducing its size. The newly formed small fat globules interact with casein micelles and other milk components to form a new membrane which differs in composition from its native state. This interaction increases water-holding capacity, yoghurt viscosity and enhances light reflection, which makes milk appear whiter (Tamime and Robinson, 2007).

#### 2.1.1.7 Heating

It is still arguable to heat yoghurt mix before or after homogenization due to contamination issue, however homogenization prior to heating is widely practiced to avoid contamination (Tamime and Robinson, 2007)

#### 2.1.1.8 Fermentation

Following homogenization and heating, yoghurt base is cooled to 40-45°C, which is the optimum temperature for growth of the cultures. Although the growth of bacteria may vary between products, inoculation of starter cultures (bulk or freeze-dried) usually consists of a well-balanced ratio (1:1) of *S. thermophilus* and *L. bulgaricus* (Tamime and Robinson, 2007). Fermentation usually takes place at 42-43°C and is stopped when the pH reaches 4.5-4.6. In the case where *Probiotics* are added, incubation temperature may be reduced to 37°C depending on the optimum temperature of the probiotic cultures to facilitate the growth of the probiotic bacteria (Ozer, 2010).

#### 2.1.1.9 Cooling

Chilling is aimed to slow down the growth and metabolic activity of cultures so that excess acid production can be prevented. Another important role of cooling in set-yogurt is to improve the texture. Tamime and Robinson (2007) recommended large installations to cool the yoghurt in two stages to avoid temperature shock, which may increase syneresis during storage. The first cooling commences from incubation temperature to 24°C followed by packaging, then further cooling to 10°C in the first 6 hours and continues to 1-2°C for the remaining cooling period.

#### 2.1.1.10 Packaging and transportation

Packaging is another important step in yoghurt manufacture, not only because it gives protection from contamination but also minimizes the gaseous exchange between inside and outside air. This is crucial in yoghurt containing probiotics, as oxygen exposure may greatly influence the

survival of microorganisms. More importantly, the packaging has to be acid-resistant and prevent loss of volatile flavors. Suitable primary (inner) packaging materials for yogurt include polyethylene (PE), polypropylene (PP), and polystyrene (PS), polyvinyl chloride (PVC) and polyvinyl iodine chloride (PVDC) with or without combination of other materials such as aluminum foil (Tamime and Robinson, 2007).

The other important factors that can be considered in choosing the packaging materials include strength, flexibility, sealing-ability, and resistance to heat and freezing. Aseptic packing may also be applied on line to reduce contamination (Walstra et al., 1999).

Secondary (outer) packaging is also required to ease handling and transportation. The most widely used secondary packaging is semi-rigid plastic crates and cardboard trays, which are stacked in wooden pallets to be transported using fork-lifts. Refrigeration storage and transport are compulsory for transporting the product before it reaches consumers. This ensures minimum biological and chemical reactions which can cause quality defects (Tamime and Robinson, 2007).

### 2.1.2. Health benefits of yoghurt:

Yoghurt is a nutrient – dense food that meets a wide variety of nutritional needs for everyone. It is a good source of protein – an average 8 ounce serving contains between 8 to 10 grams protein, or 16 to 20 percent of the Daily Recommended Value (DRV), because yoghurt is cultured the amount of protein often succeeds liquid milk. Yoghurt is also excellent source of calcium, it may contain up to 35 percent of the Daily Recommended Intake (DRI) for calcium. Yoghurt is low in fat and high in minerals and essential vitamins including riboflavin B2, vitamin B12, phosphorus and potassium. Researchers are currently exploring how live and active yoghurt cultures may have a beneficial effect on the immune system, the potential to lower cholesterol, and how it may combat certain types of cancer – causing

compounds, particularly in the digestive tract. The main health benefits of yoghurt are; may help reduce osteoporosis, can be eaten by people who are suffer from lactose intolerant, diets rich in calcium may help reduce hypertension, may enhance the immunity system of certain individuals, versatile and convenient – use as a substitute for mayonnaise, sour cream and cream cheese to lower calories, It may reduce the risk of colon cancer, is considered meat alternative because it has high protein content and large variety of flavors and styles those can be used to reduce calories(www.westrennationalroundup.org., 2015).

### 2.1.3Quality of yoghurt:

Yoghurt is a versatile food as it an important source of calcium, phosphorus, magnesium, potassium, riboflavin, vitamin A and protein. As a fermented milk product, it is a natural source of Probiotics, which help to maintain a healthy gut and immune system. The popularity of yoghurt has increase due to perceived health benefits resulting in significant increase in consumption. Many types of stirred yoghurt are available in market varying in fat, sugar, texture, flavor and type of fruits. It has keeping quality of 1 – 2 days at ambient temperature and 1 week under refrigerated condition. Microbes in yoghurt may be derived from a variety of sources. The presence of microbes in dairy products including yoghurt are undesirable, at these render the milk products of inferior quality.

Yeast and moulds are mainly responsible for the spoilage of yoghurt, as they are not affected by law pH. Microbiological specifications should be applied to some additives employed in the manufacture of yoghurt. Recently, the Biolimix test method has been developed for rapid detection of coliforms, yeasts and moulds. It is emphasized that the activity of starter culture used for the production yoghurt should be critically monitored periodically to get a product of good quality. Education of food handlers about the importance of

high standards of personal hygiene is very essential for hygienic production of milk products in dairy industries. In addition, the application of Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP) and HACCP programs during the production of yoghurt is a highly imperative from food safety point of view. The changes in the physical, chemical and microbiological structures of yoghurt determine the storage and shelf life of the product (Sofu and Ekinci, 2007).

Fungi, especially the yeasts are a major cause of spoilage of yoghurt as low pH provides a selective environment for their growth. The use of poor quality of milk, unsuitable starter culture, improperly cleaned utensil and unfavorable temperature of incubation, are responsible to lower the quality of yoghurt (De, 1980).

Yoghurt should not be freezes, as it affects the texture and quality. Further; it should be protected from other foods with strong odor by sealing it tightly. Yoghurt should be kept in refrigerator after it is purchased. Clean spoon should be used to take yoghurt into the bowel. In order to avoid contamination, it is imperative not to return unused portion of yoghurt to the original container. Yoghurt produced under good manufacturing practices (GMP) should contain less than 10 yeast cells, and should have a shelf life of 3 – 4 week at $5^{o}$. Yoghurt having initial yeast counts of >100 CFU/g tend to spoil quickly. Hygienic practices in the production can improve the microbial standards of yoghurt. Strict supervision and strength quality control standards are imperative to improve the microbial safety of the product and ultimately reduce the microbial hazards. This communication describes the hygienic and microbiologic quality of yoghurt (El- Bakri and El - Zubeir, 2009).

### 2.1.4 Sudanese standards for yoghurt:

The Sudanese Standards and Metrology Organization approved 28 national standards and 21 adopted standards concerning the yoghurt and fermented milk products through the activities of the milk technical committee (appendix1) (EL Tahir, 2007).

## 2.2 HACCP system:

Hazard Analysis and Critical Control Points (HACCP) is a systematic method that serves as the foundation for assuring food safety in the modern world. The HACCP system is designed to be used to prevent the occurrence of food borne hazards from production through manufacturing, storage and distribution of a food product (Surak, 2006).

### 2.2.1 HACCP History

HACCP has its roots in the late 1950s when National Aeronautics and Space Administration (NASA) contracted with the Pillsbury Company to manufacture safe food for manned space flights. The government placed strict safety requirements for the food that would be consumed by the astronauts. As a result, Pillsbury developed a process that would prevent the occurrence of food safety hazards. This concept was named Hazard Analysis Critical Control points or HACCP (Surak, 2007).

The Safe Food Alliance(SFA) in 2012, explained the history of the HACCP in detail and pointed out that the actual genesis of HACCP began in 1960s when the ANSA, the Pillsbury Company and the U.S Army Laboratories collaborated together to provide safe food for upcoming Space expeditions. It was decided that NASA's engineering management requirements, critical control points, would be used as guideline for this food safety initiative. After the success of NASA providing safe food for their space expeditions, Pillsbury had a recall on product called Farina,

which is a cereal used in infant food. They were finding glass pieces and remnants in the food, which caused contamination. A microbiologist at Pillsbury, Howard Baumann, who also helped in the NASA initiative, advocated for company to adopt a HACP plan. Because of this outbreak and Baumann's success with HACCP, a panel discussion was held in 1971s at the National Conference on Food Protection (NCFP) that examined critical control points and Good Manufacturing Practices (GMP) in producing safe food. The outcome of this meeting leads the Food and Drug Administration (FDA) asking the Pillsbury to establish and manage a training program for the inspection of canned foods for FDA inspectors, the program was first held in 1972 for 21 days with 11 days of classroom lecture and 10 days of canning plant evaluations. The name of this class was titled, "Food Safety through the Hazard Analysis and Critical Control Point System", and this was the first time HACCP was use to educate other food facilities in the industry (Guha and Santhosh, 2013).

In 1985, the National Academies of Sciences (NAC) recommended that all food processing companies in the United States adopted HACCP as the method to prevent foodborne hazards from entering the food supply published, (Bauman, 1992).

Slowly HACCP has been incorporated into the U.S food processing regulations. The USDA Food Safety Inspection Services made HACCP mandatory for the meat, dairy products and poultry industry with issuing of the HACCP / Pathogen Reduction Rules (Cullor, 1997).

The (FDA) has made HACCP mandatory for the safe food and the juice industry, (FDA 2001). In addition, Food processing companies and food distribution companies have made HACCP mandatory for their entire supplier thus extending HACCP beyond the mandatory regulatory requirement (Dunkelberger, 1995).

The Codex alementarius Commission (CAC) incorporated HACCP into Recommended International Code (RIC) of practice – general principles of Food Hygiene. Codex standards play an important role in international trade; however, they adoption by the member nation of the Codex are voluntary(Texts, 2001).

HACCP has become accepted internationally as the best means of ensuring food safety. In 2004 the European Union (EU) adopted several new regulations on the hygiene of foods, including one (852/2004/EC) mandating that effective January, 1n 2006, all food business operators implement procedures based on the HACCP principles. Other government authorities across the globe including Canada, Australia and Japan, have adopted or are adopting the HACCP – based food safety control system (Taylor, 2008).

Today, training for developing and implementing HACCP Food Safety management system are offered by several food safety companies. FDA of California is an accredited HACCP trainer through the International HACCP Alliance and is qualified to perform nationally recognized HACCP training according to Codex Alimentary (CA). Since the signing of the FSMA (Food Safety Modernization Act) in 2011, companies in the food industry has been making drastic changes in order to comply with regulation. The proposed produce safety and preventive controls roles are expected to be finalized in 2014 with staggered dates for compliance. One of the requirements of the rules is "preventive controls (HACCP)." HACCP is important because it prioritizes and controls potential hazards in food production by controlling major food risk such as; microbiological, chemical, and physical contaminations, the industry can better assure consumers that its products are as safe as good science and technology allows. By reducing food borne hazards, public health protection is strengthened "(International HACCP Alliance). HACCP is a

program that government agencies and food facilities have relied on for years and will be a program that continuous to have an impact on food safety and industry for years to come (Arioui; *et al*,2018).

### 2.2.2. Why HACCP?

The food borne diseases continue to be one of the biggest problems for public health throughout the world. The data of the center for control of diseases in USA show that every year 76 million of people suffer food borne infections, of whom 15% undergo hospitalization (Bijo and Malaj, 2008). Food borne diseases can be classified as either infectious or intoxications (Taylor, 2008).

Moreover, some food borne illnesses are caused by as yet unidentified pathogens. Many of most inters today were not recognized as food borne diseases agents prior 1980.e.g, *E.colli* 0157: H 7, L. *Monocytogenes* and *Campylobacter*. Some states may have better surveillance of foodborne illness compared to other because of greater enters, expertise and resources. This can result in a further under estimation of the size of the foodborne disease problem from those states that do not have a good surveillance program. The risk related to the production of food products can be reduced to an acceptable level or eliminated through the application of HACCP system (Scott and Stevenson, 2006).

### 2.2.3. HACCP principles According to NACMCF

According to the national advisory committee on microbiological criteria for food (NACMCF) in 1998, the HACCP system applied based in the following prerequisite programs, five preliminary steps and seven principles (Pierson, 2012).

### 2.2.3.1. Prerequisite programs

The prerequisite programs of HACCP are; training, personnel practices (personnel), premises, equipment and facilities, good manufacturing practices, good hygiene practices, cleaning, sanitation, pest control, receiving, transportation, storage, traceability, recall, supplier control and hazardous material handling.

### 2.2.3.2. HACCP preliminary steps:

**Step 1**

Assemble the HACCP team.

**Step 2**

Describe the products and its distribution.

**Step 3**

Describe the intended use and the users of the product.

**Step 4**

Develop the process flow diagram.

**Step 5**

Verify the process flow diagram.

### 2.2.3.3. HACCP principles.

**Principle 1**

Conduct a hazard analysis.

**Principle 2**

Identify the Critical Control Points (CCPs).

**Principle 3**

Establish Critical Limits for preventive measures associated with each identified CCP.

**Principle 4**

Establish CCP monitoring requirements.

**Principle 5**

Establish the corrective actions which should be taken when monitoring indicates that a deviation from an established critical limits.

**Principle 6**

Establish verification procedures.

**Principle 7**

Establish record – keeping and documentation procedures.

### 2.2.4 HACCP principles according to codex alimentary commission (CAC).

Manikas, and Manos, (2009) clarify; HACCP applies science-based controls from raw materials to finished product. It uses seven principles standardized by the Codex Alimentary Commission (CAC).

**Principle 1**

Identify and analyze hazards associated with the food. Hazards could be biological (ex: foodborne bacterial pathogens); chemical (ex: toxins, allergens); or physical (ex: metal fragments, broken glass).

**Principle 2**

Determine the critical control points (CCPs) these are points of the process at which the hazard can be controlled or eliminated (ex: cooking).

**Principle 3**

Establish critical limits for each CCP. A critical limit is the criterion that should be met to ensure food safety in a product (ex: minimum cooking temperature and time to ensure elimination of harmful bacteria).

**Principle 4**

Establish a monitoring procedure to ensure each CCP stays within its critical limits. Monitoring can be carried out by observations (visual) or by measurement (ex: determine who and how temperature and time will be monitored during cooking). The most common measurements taken are time, temperature and moisture content.

**Principle 5**

Establish corrective actions if the CCP is not within the established limits. By applying corrective actions, the control of hazards is regained (ex: reprocessing or disposing of food if the minimum cooking time and temperature are not met). Corrective action must be taken immediately.

**Principle 6**

Establish verification procedures to confirm that the HACCP plan is operating effectively and according to written procedures. This verification may include reviewing HACCP plans, CCP records, microbial sampling (ex: verify that time and temperature recording devices are calibrated and working properly).

**Principle 7**

Establish record-keeping and documentation procedures that demonstrate that correct procedures have been followed. This includes monitoring documentation, actions taken to correct a potential problem, validation documents (ex: scientific information that supports the use of specific time and temperature for cooking).

## 2.2. 5 HACCP team:

Savage in1995 reported that; The start of any HACCP study needs a multi – disciplinary team who has working knowledge of the process and has been trained in HACCP; the team consist of supervisor or manager, an engineer, microbiologist and any other experts (Savage, 1995).

## 2.2. 6 HACCP plan:

The HACCP team plan include the description of the critical control points at receiving, cold storage, set – up and correct function of cooking, hot holding and chilling equipment. Also the plan must specify the monitoring frequency, method, records, training, responsibilities and accountabilities were. The plan also described detailed steps to ensure corrective actions were executed in the appropriate time frame and methods to verify the efficiency of the system (Savage, 1995).

## 2.3 Hazard and hazards types:

A hazard is defined by NACMCF in 1998, as a biological, chemical or physical agent that is reasonably likely to occur, and will cause illness or injury in the absence of its control. Establishments must consider all three types of hazards – biological, chemical, and physical – at each step of the production process. A "step" is a point or activity in an operation within the production process that is essential to the proper production of the finished product. A food safety hazard that is reasonably likely to occur is one for which a prudent establishment would establish controls because the hazard has historically occurred in the product/process or because there is a reasonable probability that the hazard would occur in the absence of these controls (Panisello and Quantick, 2001).

# CHAPTER THREE

# Materials and Methods

This study was conducted at Sudan University of Science and Technology – Khartoum – Sudan, the experimental and laboratory work was done in the CAPO Dairy plant.

## 3.1 Materials:

The materials used during the yoghurt manufacturing and analysis of samples include; raw milk and yoghurt samples for analysis, stabilizer, starter culture, diluents, buffer solution, detergents for cleaning, alcohols for sterilization and distilled water.

### 3.1.1 Collection of samples:

A total of 40 samples were randomly collected at different stage during yoghurt manufacturing in Khartoum state - Sudan - Dal Dairy Factory (CAPO) under sterilized conditions. Samples were collected during the period from January – February 2017.The different processing stages at which the milk and yoghurt samples were taken included; raw milk, pasteurized milk, during fermentation and cooling process, and in the end after filling the product. The samples were analyzed physiochemical and microbiologically during manufacturing of yoghurt.

## 3.2 Methods:

The work includes three steps: first, a meeting with the head of the company to take his permission and agreement so to meet his staff especially the quality management manager in charge of quality, then an audit was planned and conducted in the organization on the professional practice using a check list based on HACCP system(appendix 2) and it also included follow – up of the yoghurt manufacturing scheme step by step (appendix 3), finally; diagnostic chemical, physical and microbiological

characteristics were conducted during the manufacturing of the yoghurt . All the physiochemical, microbiological and sensory evaluations were carried out during yoghurt production process, starting with raw material through the processing to end of the production. The physicochemical analyses were carried out according to the association of official analytical chemists' methods (AOAC, 2005).

### 3.2.1. The following of product for auditing:

A survey form was developed related to prerequisite programs, five preliminary steps and seven principles of the HACCP system. A literature survey supported by direct observations and face to face interview with all stakeholder of the factory. All of this was recorded taking into account the degree of integration of quality process in the dairy plant. An evaluation rubric was used as the support information collection, it was developed in accordance with the principles of the HACCP system and its prerequisites programs and criteria in a form of open and close questions with balance coupled with evaluation and observations (appendix 2) and (appendix 3).

### 3.2.2 Product sampling:

Samples from raw milk (5) in duplicates were taken and (15) samples induplicate at different stages of yoghurt making were taken also for physiochemical and microbiological analysis (Table:1),Sampling was made during yoghurt manufacturing according to the principles of food safety system (HACCP) and specific standards of factory developed by HACCP team.

**Table (1): Sampling procedures during yoghurt manufacturing for physiochemical and microbiological analysis.**

| Samples | Manufacturing stage | Microbiological test | Physiochemical tests |
|---|---|---|---|
| Raw milk | Individual/ compartment of milk tankers. | Total viable count<br>Lap preliminary count.<br>Coliforms count. | PH, S.G%, T.A%, S.N.F%, T.S%, protein%, antibiotic test, freezing point, appearance, texture, color, flavor, temperature and foreign matter |
| Pasteurized milk | Every silo of pasteurized milk | Coliforms count.<br>Yeast and mould count. | S.G%, FAT%, S.N.F%, T.S%, protein % |
| Fermented yoghurt | The fermentation tank | Coliforms count.<br>Yeast and mould count**.** | Temp, pH (during fermentation).<br>PH, product Temp, tank Temp, viscosity (in cooling process).<br>PH, Temp, viscosity (at the inspection for release). |
| Final product | Filling stirred yoghurt | Coliforms count.<br>Yeast and mould count**.** | PH, temperature,viscosity |

### 3.2.3 Sensory evaluation:

The raw milk samples were evaluated by trained panelists for appearance, texture, color, taste, and flavor; these properties were carried out according to Sudanese standards and metrology organization (SSMO). The term sensory evaluation denotes this type of examination which depends upon man's senses of taste, odor sight and touch (appendix 4) (Abu Elhassan, 2007).

### 3.2.4 Physiochemical Analysis:

The physicochemical analyses were carried out according to AOAC (2005) methods. The physical parameters were taken for the raw and pasteurized milk, fermented yoghurt and end product samples include; impurities, temperature, freezing point (for raw milk only), viscosity (it was taken just at fermentation and end product stages), breaking capability of cup, cup thickness, label position and lid position which were taken in the filling of the end product**,** pH reading and acidity estimation, antibiotic test which measured by antibiotic tester**.**

#### 3.2.4.1 Impurities:

The impurities of raw milk were measured by strainer conductively with the tanker existed in reception unit of the raw milk, and then the milk undergoes cross automatic clarifier to be more clarified.

#### 3.2.4.2 Temperature:

The temperature measured by certified digital thermometer (Lab) model (THER MA 1 and manufactured in UK).

#### 3.2.4.3 Freezing point:

Freezing point measured by Milkoscan device model (Foss FT1 manufactured in Denmark).

#### 3.2.4.4 Viscosity:

The viscosity was measured by using a falling ball Viscometer model (Thermo Haake 6R, manufacturing in Spain) using a glass tube and a

normalized ball equipped with a chronometer at 25$^{o}$C. The viscosity was expressed in Pascal sec (Pas). The manager of methods that are available to measure the viscosity of yoghurt has been discussed by (Sherman, 1970).

**3.2.4.5 Breaking and cup thickness:**

Breaking test to measure the breakability of cups, it measured manually. And cup thickness was done by Nanometer instrument.

Some physiochemical test measured by Milcoscan device which include; pH, specific gravity (S.G %), titratable acidity (TA %), fat percentage (fat %), solid not fat (S.N.F %), total solid (T.S %) protein percentage (protein %).

**3.2.4.6 pH reading and acidity estimation:**

The pH measurement was carried out by a pH meter model (JENWAY 3510, made in UK) calibrated with two solutions: one is basic and another is acidic and at a temperature of 25$^{o}$C. The Doric acidity was determined by titration of 10 ml of yoghurt with 0.1 NaOH using a phenolphthalein as an indicator color. Results were expressed as degree Doric (AFNOR, 1980).

**3.2.4.7 Antibiotic test:**

The antibiotic test was measured by antibiotic tester (Tri – sensor) **(Emko ESM – 3711 – H)** instrument which is a rapid test that allows to simultaneously detecting the presence of β- Lactames, Sulfamides and tetracycline molecules in milk sample. The protocol of tri sensor as follows; Add 200 ml of milk into one reagent micro well and mix to homogeneity, incubated for three minutes at 40$^{o}$c then dip one dipstick into each microwell and read the color intensities.

**Reaction and mechanism of tri – sensor:**

Tri – sensor is a competitive test involving two receptors and generic monoclonal antibodies in one single operation. The test requires the use of two components; the first component is a microwell containing predetermined amounts of both receptors and antibodies linked to gold

particles and the second is a dipstick made up of a set of membranes with specific capture lines. For a valid test, the upper red control line has to be visible after the second incubation. The other three are the specific "test" lines placed below the control line. The line of B – lactam antibiotics (penicillins and cephalosporins) is located below the sulfamide line while the line relating to tetracycline is located above it. When the reagent from the microwell is re – suspended with a milk sample, both receptors and monoclonal antibodies will bind the corresponding analyses if present during first 3 – minute incubation at 40$^{o}$C. Afterwards, when the dipstick is dipped into the milk, the liquid start running vertically on the dipstick and passes through capture zones. When the samples are free of antibiotics, a color development occurs at the specific capture lines, indicating the absence of the targeted analyses in the milk sample. On the contrary, the presence of antibiotics in the sample will not cause the coloured signal to appear at the specific capture lines.

### 3.2.5 Microbiological Analysis:

The microbiological analyses include; Coliforms test (*CitrobacterSpp, Enterobacter SPP, E – coli* and *Klibseilla),* T.V.C (TotalViable Bacterial Count), L.P.C (Laboratory preliminary count) – *mesophilic* and L.P.C – *thermophilic* (in raw milk stage). In the pasteurization and fermentation milk stages the microbiological analysis includes; Coliforms and Yeast and mold test. And in the end product, only yeast and mold was analyzed. Microbiological count data are expressed as colony – forming unit (CFU) per ml of yoghurt.

Total viable count, coliforms count and fungal count was determined by standard plate count method as described by (Coppuccino and Sherman 1996). Each sample was serially diluted using sterile distilled water as diluents as described by (Cai; *et al* 2005). The organisms isolated were characterized and identified using the method described by (Barrow and

Feltham, 1993). Identification of fungi isolates was done by using the method described by (Alakanabi and Oydiji, 2015). Slide culture was carried out to preserve and observe the natural state of the actual structure showing conidiogenous cells and conidia of the fungus as described by Larone (1987).

**3.2.5.1 Preparation of media and glassware:**

Culture media were prepared according to the manufacturer's instructions. The media were sterilized by using Austell autoclave (Austell, model AAJO 40) at 15 IBs pressure (115 – 121$^{o}$C) for 15 – 20 minutes. Plastic containers were washed in running tap water rinsed with distilled water and sterilized in autoclave at 115$^{o}$C for 15 minutes. The glassware was soaked in soap water overnight, washed with running tap water many times, finally rinsed with distilled water and allowed to dry. Graduate pipettes were plugged with cotton wool and Petri – dishes were put into canister and sterilized. All the glassware was sterilized in an oven at 160$^{o}$C for one hour, (Marshall, 1992).

**3.2.5.2. Preparation of media:**

Quarter Strength Ringer solution – 9 ml serial diluents, Violet Red Bile Agar, Plate Count Agar (PCA)/ Pate Count Skim Milk Agar, Malt Extract Agar (MEA)/ YGC Agar (for yeast and mold detection on yoghurt) were prepared.

**3.2.5.3 Apparatus:**

Glass test tubes and suitable cap – o – test caps, autoclave capable of reaching 121$^{o}$C for 15 minutes, incubator at 32$^{o}$C ± 1$^{o}$C, sterile laboratory bottles, sterile sample containers, sterile 1L beaker, balance weighing to 0.1g, sterile 1ml and 10 ml pipettes, 90 mm Petri – dishes, tempering water path 47 – 48$^{o}$C, Bunsen burner, illuminated colony counter and test tube rack.

### 3.2.5.4 Sampling technique:

The milk/yoghurt represented samples were taken aseptically and this step was achieved by either; sampling from an "open" area by using sterile dipper and sample container, or sampling from "closed" area i.e. a pipeline or tank by flaming the sampling – cock, running some product through and then sampling directly was put into a sample container (N.B in case of yoghurt the samples were taken from the enclosed area only).The samples could not have been damaged or changed during transportation or storage and also could not being frozen. In case of final product; the samples were taken from the final packed retail product.

### 3.2.5.5 Preparations:

All possible sources of contamination were cleaned and removed from the bench area, all the samples and apparatus required for analysis were collected together, the bench area was sterilized before starting with microbial analysis, petri – dishes, test tubes and bottles were clearly labeled with the following; sample type, batch code, test media type, plating date and time and any other desired information. The following steps had been taken in the microbiological analysis of milk; Firstly; the sample was agitated thoroughly by rapidly inverting the samples container many times, so that the microorganisms were distributed as evenly as possible, secondly; any foaming was avoided or allowed it to disperse, finally; a ten gram of test sample was weighed into suitable glass vessel (e.g. beaker) and then the powder was added to the dilution bottle containing of 90 ml quarter strength ringer's solution. The test sample was swirl slowly to wet the powder and then the bottle was shaken and stands for 5 min and shaking occasionally.

### 3.2.5.6 The Method:

One ml of milk/yoghurt was pipette aseptically into a test tube containing 9 ml of ringer solution to make the first serial dilution

(subsequent dilutions have been made at this point and the dilution required depended on expected count), 1ml of the required dilution was pipette aseptically into Petri – dish. Due to the rapid sedimentation of spores in the pipette, the pipette was maintained in a horizontal position when filled with appropriate volume of initial sustention and dilutions.

Approximately 12 – 15 ml of the required agar is poured at 44$^{o}$C – 47$^{o}$C, (i.e. VRBA for coliforms analysis, PCA/plate count skim milk agar for TVC and Malt Extract Agar for yeast and molds) into Petri – dishes containing samples and the agar were gently swirled to disperse the sample evenly in the agar, In case of any large bubbles formed in the agar at this stage they were busted by using a sterilized straight wire before the agar sets, or they might be prevented a complete overlay from being poured. A control plate poured for each media and for each batch of agar made. The plates had being allowed to solidify (no long than 10 min) on a level surface. A bout 4ml BRBA medium was poured after completed solidification at 44 – 47$^{o}$C onto the surface of the inculcated medium, and then was put for 10 min to solidify as described above. The plates were invert and shake no more than 3 high, the plates were incubate at 32$^{o}$C±1$^{o}$C for 24 to 48 hours, in case of the milk microbiological analysis where it is suspected the product under examination contains the microorganisms would grow at the surface of the medium; about 4ml of overlay medium was poured at 44 to 47$^{o}$C onto the surface of inculcated medium, and maintained for 10 min to solidify. In case of yoghurt, for yeast and mold detection the samples were incubated at 25 – 28$^{o}$C for 3 – 5 days.

**3.2.5.7 Examination of the plates:**

A white background behind the plates was used when counting VRBA plates, the black background for TVC and the black background also for yeast and mold in case of yoghurt analysis. After the specified period of yoghurt incubation, the dishes containing more than 150 colonies/

prop gules/germs were selected and counted. If fast growing molds are problem, colonies/ prop gules/germs were conducted after 2 days and again after 3 to 5 days of incubation, VRBA – counted colonies, which are dark red and 0.5 mm to 2 mm in diameter, usually surrounded by a reddish zone. **N.B** it was possible for molds to grow giving a cloudy bitty appearance. In yoghurt analysis MAE/YGC – was counted the colonies which take either the form of small round slightly raised colonies(yeast) or classic filamentous growth (molds). In case of milk analysis, PCA/plate count agar – was count the colonies which can take the form of any size between pinpoint and spreader colonies and any mistaking particles of un dissolved or precipitated in dishes for pinpoint colonies were avoided. Doubtful objects was examined carefully, by using a higher magnifier was required, in order to distinguish colonies from foreign materials. The number and type were noted (**N.B** spreader type colonies may interfering plates showing spreader had been interpreted with caution. The actual colony count on the plate(s) was recorded. The microbiological results were expressed in Colony Forming Unit (CFU)/gm of product analyzed. Results interpretation was done according to criteria of Codex Alementarius Commission (IDF, 2003).

### 3.2.6 Statistical analysis:

Statistical analysis was done using Statistical Package for Social Science (SPSS, 2007). The obtained data was analyzed using Descriptive Statistics – Descriptive; to show safety of stirred yoghurt by comparing the means and standards deviations of the sensory evaluation, physical analysis, chemical analysis, microbiological results with the Sudanese Standards and Metrology Organization (SSMO) of yoghurt.

# CHAPTER FOUR

# Results and Discussion

## 4.1 Effect of HACCP application on the quality of stirred yoghurt

The use of food safety and quality assurance in farms and dairy plants is very important to reduce physiochemical and microbiological hazards in milk and dairy products. A regulatory law implementation in milk and dairy industries and long term planning is required to achieve milk safety. The application of HACCP at dairy industry was performed to improve the safety and quality of its products.

In this study the CAPO factory was applied the HACCP system in processing the stirred yoghurt perfectly and was confirmed by the checklist which is done in this study.

The performance of tests of this type on successive occasions (same operator and same conditions) is somewhat variable, hence the need for agreed tolerance, but it is trends away from the norm for any specific piece of equipment that are important. Some suggested standards for plant in content with product prior to pasteurization/heat treatment have been cited by (Harrigan and Mccance, 1976).

### 4.1.1Sensory evaluation and physical test:

The data in table (2) explained that the milk samples under study was met the required standards of the sensory characteristics which is conformed to the results of other researchers (Brussels; *et al.*2005, Allen., 1995., Anon, 1994a and IDFd, 1991).

**Table (2): The sensory properties of raw milk (composite sample).**

| Reads tanker composite sample | Result | Standard |
|---|---|---|
| Appearance/Texture. | Liquid/normal | Liquid/normal |
| Color | white | white |
| Flavor/Odder. | normal | Normal free from off - flavors |
| Temperature control room reading. | $5^{o}C$ | Maximum $10^{o}C$ |
| Foreign matter. | absent | absent |

### 4.1.2 Raw milk analysis:

Data in table (3) showed the physicochemical and microbiological analysis of raw milk samples. The results were in conformity with legal standards of SSMO. These results suggested a positive impact on the shelf – life of the product as well as the overall quality of the product as the chances of customers identifying significant off – flavor's in the product, was also be greatly reduced. The results in table (3) showed that a good hygiene practices (GMP) had being applied (good quality milk) have low acidity 0.015 as average, free of antibiotics and was milked from healthy cows.

The routine measurement of protein is essential in large dairies because, over a typical year, the protein content of cow's milk may vary from 3.2 to 3.6 g 100 $g^{-1}$ and these differences are enough to alter the quality of yoghurt.

Microbiologically, there was a positive impact on the raw milk quality, the maximum of total viable count (TVC) was 20180 CFU/ml as average while in the (SSMO) is 100000cfu/ml ( as a maximum limit), lap preliminary count *(LPC) mesophilic* was 31.70cfu/ml and *LPC thermophilic* was 15.70 (200CFU/ml as a maximum limit), and the Coliforms was 2400 CFU/ml (maximum 10000 CFU/ml as standard).Although the mean value for the freezing point was statistically not significant, it was also within the specification limit with improved regimes, a total colony count of 200 CFU 100 $cm^{-2}$ would be expected nowadays, and below 50 CFU 100 $cm^{-2}$ for any plant containing pasteurized product (Luck and Gavron, 1990).

**Table (3): Physicochemical and microbiological analysis of raw milk samples.**

| Characters | Means ± Std |
|---|---|
| PH | 6.70 ± 0.03 |
| S.G | 1.03 ± 0.01% |
| T.A | 0.15 ± 0.0021% |
| FAT | 3.71 ± 0.19% |
| S.N.F | 9.09 ± 0.02% |
| T.S | 12.94 ± 0.16 % |
| Protein | 3.38± 0.53 % |
| temperature | 4.40±1.20$^{o}$C |
| Freezing point | (-) 0.53 ± 0.000 |
| Coliforms | 2400 ± 0.94(CFU/ML) |
| T.V.C. count | 20180 ± 0.19(CFU/ML) |
| L.P.C. mesophilic count | 31.70 ± 4.70(CFU/ML |
| L.P.C.thermophilic count | 15.70 ± 9.94 (CFU/ML) |

S.G = Specific Gravity, T.A = Titratable Acidity, S.N.F = Solid not Fat,

T.S = Total Solid, T.V.C = Total Viable Count, L.P.C = Lap Preliminary Count.

These results in table (3) were in the line of Hoolasi (2005) who reported that there was statistically significant between the before and after HACCP application on yoghurt; values of PH (6.6 – 6.75) and temperature less than $6^{o}$C, also he was complaint us in the freezing point. According to the food drug administration (FDA, 2001), and a grade milk should contain not less than 8.25% milk solid not fat (M.S.N.F) and not less than 3.25% milk fat exclusive of colostrum, and these results were compatible with that. The term of MSNF refers to all milk constituents excluding milk fat and water, while total solid (T.S) are defined as MSNF plus milk fat (Chandan and O'Rell, 2006).

Depending of the season of the year milk comprises of 3 – 3.5 of fat, 8.5 – 9% M.S.N.F with water making up the remaining constituents. Among the MSNF, around 4.5% is lactose, 3.3% protein (2.6% casein and 0.7% whey proteins) and the remaining being minerals, calcium, magnesium, zinc, etc.) (Robinson *et al*. 2006).

Table (3) show that the temperature on milk arrival, T.A%, Fat %, S.N.F%, protein%, Freezing point, and TVC & Coliforms with the results of raw milk tests were in the acceptable level of principle 3 of HACCP (establish critical limit) because a periodically (physiochemical and microbiological) analysis done from the start to the end of the production in the factory and audited every step by a supervisor quality control (QC) and then reviewed by a head quality manager. The results were in accordance y with (Allen,1995; Hygiene, 1995; Anon, 1994a; IDF1991d) who reported that all the chemical, physical and microbiological of milk analysis could be (Temp.$< 10^{o}$C, T.V.C $\leq$ 100 000cfu $ml^{-1}$ (target) and < 250 000cfu $ml^{-1}$ (may well be accepted in practice), chemical components $\geq$ 3.0 g fat $100g^{-1}$& protein $\geq 3.0g^{-1}$ , somatic cell count $\leq 4.0\times10^{5}$ ml and freezing point depression $\leq .52^{o}$C.).

All of these results indicated that raw milk is safe for processing.

### 4.1.3 Pasteurized milk analysis:

Data in table (4) showed the Physicochemical and mcdicrobiological analysis of pasteurized milk samples. In this table the results indicate that the principle 2 of HACCP had being applied (determination of the pasteurization as (CCP) and monitored automatically (principle 4); if the milk not reach to the required temperature for pasteurization, the milk should be reprocessed immediately, also the absence of coliforms and yeast and moulds in microbiological tests indicates the efficiency of pasteurization.

Many countries have legal standards or at least or professional regulations for tests of milk prepared for yoghurt manufacture. The requirement for a value of S.N.F is reality more decorative than essential because the texture or viscosity of natural yoghurt with an S.N.F below the stipulated minimum would be barely acceptable. An overall measurement of total solid (T.S), could be valuable as a check that the concentration or fortification has been carried out correctly. The modification of the standard gravimetric method for milk has been proposed (Kirk and Sawyer, 1991) as suitable yoghurt.

**Table (4): Physicochemical and microbiological analysis of pasteurized milk samples**

| Parameters | Means ± std |
|---|---|
| S.G | 1.04 ± 0.001 |
| FAT | 3.06 ± 0.03 % |
| S.N.F | 11.66 ± 0.01 % |
| T.S | 14.74± 0.11 % |
| Protein | 4.27 ± 0.05 % |
| Coliforms count | ND |
| Yeast and mould count | ND |

S.G = Specific Gravity

S.N.F = Solid Not Fat

T.S = Total Solid

## 4.1.4 Analysis during fermentation of milk:

Data in table (5) showed the physicochemical, microbiological characteristics during processing steps.

In the processing stage of yoghurt, the different tests were taken at three phases (fermentation, cooling and finishing good inspection for release phase). The main points (CCPs) in this stage are: temperature and pH, because the lactic acid bacteria affected by certain degree of temperature and done well at (40 – 43$^{o}$C).

### 4.1.4.1 pH in fermentation period:

The mean pH values of pasteurized yoghurt in the fermentation tank and yoghurt for release were in their critical limits. The mean pH of the yoghurt in the intermediate fermentation tank was 4.6 (according to the recipe of factory and regulatory standard).

The production of lactic acid during coagulation is monitored principally in relation to consumer preference and hence the selected and end point could vary not only from country to country but also with the type of yoghurt. Also the relationship between titratable acidity and pH is straight forward in highly buffered system like yoghurt (Robinson and Itsaranuwat, 2006).

During the period of fermentation, a highly significant decrease of pH in milk added with starter culture; that might be due to the bacterial activity. This justification was agreed with (Fatiha *et al.* (2016).

According to Robinson (1990), lactic strains have the ability to ferment lactose into lactic acid, with an increase of acidity and decrease in pH of yoghurt.

Cais; *et al*. (2004), indicated that. the pH values of milk under processing from the time it was inoculated with bacterial cultures to the time of the yoghurt manufacturing, decreased from 6.70 to 4.34 (this decreasing is quite confirm with the results in this study see table (3) and table(5). The pH in inspection for release in the final product (table 5) was less than that in the cooling process due to bacterial activity in fermentation process.

### 4.1.4.2 The viscosity:

The viscosity of stirred yoghurt was characterized by a clear increase during fermentation and post acidification (table 5). Similar results were obtained by (Guzel – seydim; *et al*, 2005).

All the results in Table 4 are in the range of critical limits standards of yoghurt characteristics.

**Table (5): Physicochemical, microbiological characteristics during processing steps.**

| Processing steps | Treatment |
|---|---|
| | Means and sd |
| (A)Fermentation process | |
| Temperature | 42.86 ± 0.46$^{o}$C |
| pH | 4.59 ± 0.003 |
| (B)Cooling process | |
| PH | 4.41 ± 0.05 |
| Product temperature | 24.12 ± 0.31$^{o}$C |
| Tank temperature | 24.01 ± 0.56$^{o}$C |
| Viscosity | 28.39 ± 2.10 centipose |
| (C)Inspection for Release | |
| PH | 4.34 ± 0.03 |
| Viscosity | 56.88 ± 1.54 centipose |
| Temperature | 10.34 ± 0. 22$^{o}$C |
| Coliforms count | ND |
| Yeast and mold count | ND |

ND = Not Detected

## 4.1.5 The final product tests:

The data in table (6) explained the physicochemical and microbiological analysis of the yoghurt samples at the end product testing (filling stage).

### 4.1.5.1 The viscosity:

The viscosity in the final product (table 6) is less than in the fermentation (table 4) because the yoghurt after fermentation process has being homogenized via certain pressure machine to be more smoothness. This result may disagree with (Shahid, *et al.*, 2002), who reported that the viscosity of yoghurt is 59, this result is near to that in table (**5**) which probably to non-adherence to (GMP) or due to the final product was not exposed to pressure machine to be smoothness like in this factory.

### 4.1.5.2 pH:

According to the food drug administration (FDA), the maximum limit of the PH on the final product is 4.55 – 4.6. The results showed that the pH was above 4 in all selected samples, this result within the line of (Shahid, *et al*., 2002). The pH is important CCP of yoghurt as a final product because the prolong and uncontrolled fermentation result in lower pH and more acidity, so a proper system of culturing process is required to meet the desirable degree of pH on yoghurt. This results grossed good fermentation because the HACCP team was monitored all the CCPs in the line of the yoghurt production in the factory, especially the adding of starter culture (1:1 from *Streptococcus thermophilus* and *Lactobacillus bulgaricus)* phase according to the principle (2) in HACCP system and the supervisor monitored and verify from it.

### 4.1.5.3 Temperature:

The standard temperature is (22±2) and then the yoghurt refrigerated at ≤ $4^{o}$C for storage and sale.

### 4.1.5.4 Yeast and Mould:

Coliforms were not detected; this indicates the high efficiency of good manufacturing practices (GMP) in the factory.

The maximum limit of yeast and moulds in the final product (yoghurt)is 10 CFU/ml according to SSMO,CAC and FDA, the presence of yeast and moulds in the final product might be refer to probability of spores founding because that the 95$^{o}$C (pasteurization degree) was not enough to kill it; or due to unhygienic personal during starter addition, this result is near or close to finding of (Abdalla, 2018) who reported that the yeast and moulds ranged from 0 to 5.42 cfu/gm and (Salwa *et al*., 2004) who reported that the yeast and mould count increased with the progress of storage in plain yoghurt.

### 4.1.5.5 Coliforms:

In most of yoghurt samples, coliforms bacteria were absent due to pasteurization of milk pre – mix prior to its incubation, this indicates the high efficiency of (GMP). This result was disagree with (Alush *et al.*, 2012) who was found the coliforms was less than 300/0.01g, but it is the range of allowed limits and (Ahmed *et al.*, 2013) who reported that the presence of coliforms counts was varied between (8 – 45cfu/ml) in branded yoghurt sample produced in large industry. The results of viscosity, temperature, coliforms and yeast and moulds in table (6) were within the specification of yoghurt manufacturing.

**Table (6) The Physicochemical and microbiological analysis of the yoghurt samples at the end product testing (filling stage).**

| Parameters | Means ± Std |
|---|---|
| Viscosity | 21.69 ± 0.53 |
| PH | 4.36 ± 0.25 |
| Temperature | 24.13 ± 1.25 |
| Coliforms | ND |
| Yeast and moulds | 6.33 ± 4.90 |

ND = Not Detected

Finally; The hygienic quality of yoghurt is dependent on the effective heat treatment of the milk base, the microbiological quality affected by adding ingredients and packaging materials, the cleanliness of surfaces coming into contact with the yoghurt and the efficiency of the plant sterilization and the absence of coliforms and yeast and moulds is an indication of efficient plant hygiene and sanitation. This contributed to decrease in the number of the customer complaints by implementation of HACCP especially regarding the presence of foreign objects, souring of yoghurt and viscosity of the product. The microbiological quality assessment of yoghurt is mainly concerned with two aspects; protection of consumer against exposure to any health hazard and ensuring that the material is not suffering any microbiological deterioration during its anticipated shelf (Caballero, 2003). In fact, it is helpful in assessing that to what level the hygienic precautions have been adopted during production, which allows the predication of product shelf – life and identification of potential health hazards (pathogens).

# CHAPTER FIVE

# Conclusion and Recommendation

## 5.1 Conclusion:

The present study has been carried to evaluate the impact HACCP system implementation in dairy products in particularly on safety and quality of yoghurt. The investigation has come up with a hold number of information on qualitative management. It appears that the company is 100% for implementation of the 12 steps HACCP system.

Yoghurt is an excellent milk product due to the nutritional and health benefits. Microbial spoilage of milk products results into a great financial loss of the dairy industry. Strict hygienic practices must be followed during production handling and distribution of yoghurt. The authority should issue the license to small dairy producers after the assurance of minimum level of good manifesting practice. The active role of government legislative bodies in monitoring the quality of dairy product including yoghurt is greatly emphasized. It is suggested that simple, easy and less expressive technique should developed for rapid detection of slow growing psychrotrops and fungi in milk product. This mill help in finding the niche environment in processing unit, which lead post process contamination of dairy product including yoghurt.

Finally; evaluation the implementation of the HACCP plan in the factory was considered as one of the most important actions taken by the work team because of the very encouraging results yielded especially in terms of assessment of microbiological situation of the final product which was zero.

## 5.2. Recommendation:

1- Establishment of an efficient and good quality control system during processing in the dairy plants and all food factories processing.
2- Authorities should frequently inspect the dairy plants to confirm that their products comply with required standards.
3- Awareness and understanding the HACCP system as worldwide food safety program which lead to increase consumers' confidence and broad trade competitiveness.

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# Appendixes

# APPENDIX (1)

قائمة بأسماء المواصفات السودانية المجازة من قبل اللجنة الفنية للألبان ومنتجاتها.

| | المواصفة | الإصدار – الرقم |
|---|---|---|
| 1 | اللبن المجفف | 108 – 2002 |
| 2 | الجبن الابيض | 1428 – 2002 |
| 3 | اللبن الخام | 107 – 2002 |
| 4 | طرق إختيار اللبن الفيزيائية والكيمائية | 111 – 2002 |
| 5 | القشدة المعدة للإستهلاك المباشر | 290 – 2002 |
| 6 | اللبن المعقم | 1409 – 2002 |
| 7 | الزبد | 1406 – 2002 |
| 8 | الزبادي | 291 – 2002 |
| 9 | الجبن المضفرة | 1429 – 2002 |
| 10 | السمن الحيواني | 193 – 2002 |
| 11 | الزبادي المنكه والزبادي المعامل حرارياً بعد التخمير | 749 – 2002 |
| 12 | البن المبستر | 189 – 2002 |
| 13 | الطرق الكيميائية لإختبار الجبن | 2414 – 2003 |
| 14 | مواصفة طرق أخذ عينات اللبن المجفف | 112 – 2003 |
| 15 | مواصفة طرق أخذ عينات اللبن المجفف واللبن المبخر | 2321 – 2003 |
| 16 | مواصفة طرق أخذ عينات اللبن السائل | 1288 – 2003 |
| 17 | مواصفة طرق أخذ عينات الزبادي والألبان المخمرة | 1289 – 2003 |
| 18 | مواصفة طرق الفحص الميكروبي للبن المجفف | 1287 – 2003 |
| 19 | مواصفة طرق أخذ عينات الجبن | 2320 – 2003 |
| 20 | مواصفة طرق أخذ عينات بديل لبن الأم المجفف | 2512 – 2003 |
| 21 | مواصفة طرق أخذ عينات المثلجات اللبنية | 1291 – 2003 |
| 22 | اللبن المنكه | 3227 – 2004 |
| 23 | الإختبار الكيميائي للبن المجفف | 1292 – 2004 |
| 24 | اللبنة | 3243 – 2005 |
| 25 | اللبن المعقم (تعديل 2006) | 3615 – 2006 |
| 26 | مصانع الألبان ومنتجاتها | 2914 – 2006 |
| 27 | الجبن القودا | 2910 – 2006 |
| 28 | The packaging material for liquid milk | 2723 – 2007 |

# APPENDIX (2)

Check list of evaluative survey to verify that the HACCP system program doing well in (CAPO) plant.

## GMP & SANITATION AUDIT

**OVERALL CONFORMANCE:**

| S No. | CHECING POINTS | Evaluation | | REMARKS |
|---|---|---|---|---|
| | | conform | Not conform | |
| | | 1 | 0 | |
| **PERSONEL** | | | | |
| 1 | Cleanness and hygiene | | | |
| 2 | Overall and Aprons | | | |
| 3 | white foot protection | | | |
| 4 | Is protective clothing available with chemicals | | | |
| 5 | Do chemicals areas have protective clothing signs | | | |
| 6 | Education and training | | | |
| **EQUIPMENT** | | | | |
| 7 | Designed for easy cleaning | | | |
| 8 | Are all machines adequately guards | | | |
| 9 | Are any machine guards broken / missing | | | |
| 10 | Are all tools stored correctly | | | |
| 11 | Machine clean & in a popper working order | | | |
| STRUCTURAL FACILITY | | | | |
| | **1. Roof ceiling:** | | | |
| 12 | Free of cracks, breaks, opining | | | |
| 13 | Clean, no webbing, dust/ fume accumulation | | | |
| | **2. ceiling** | | | |
| | **Wall junction:** | | | |
| 14 | No webbing, chipped, paints, cracks, extrusion , dirt accumulation | | | |
| | **3. Piping's** | | | |
| 15 | No leaks, in good repair, insulation intact | | | |

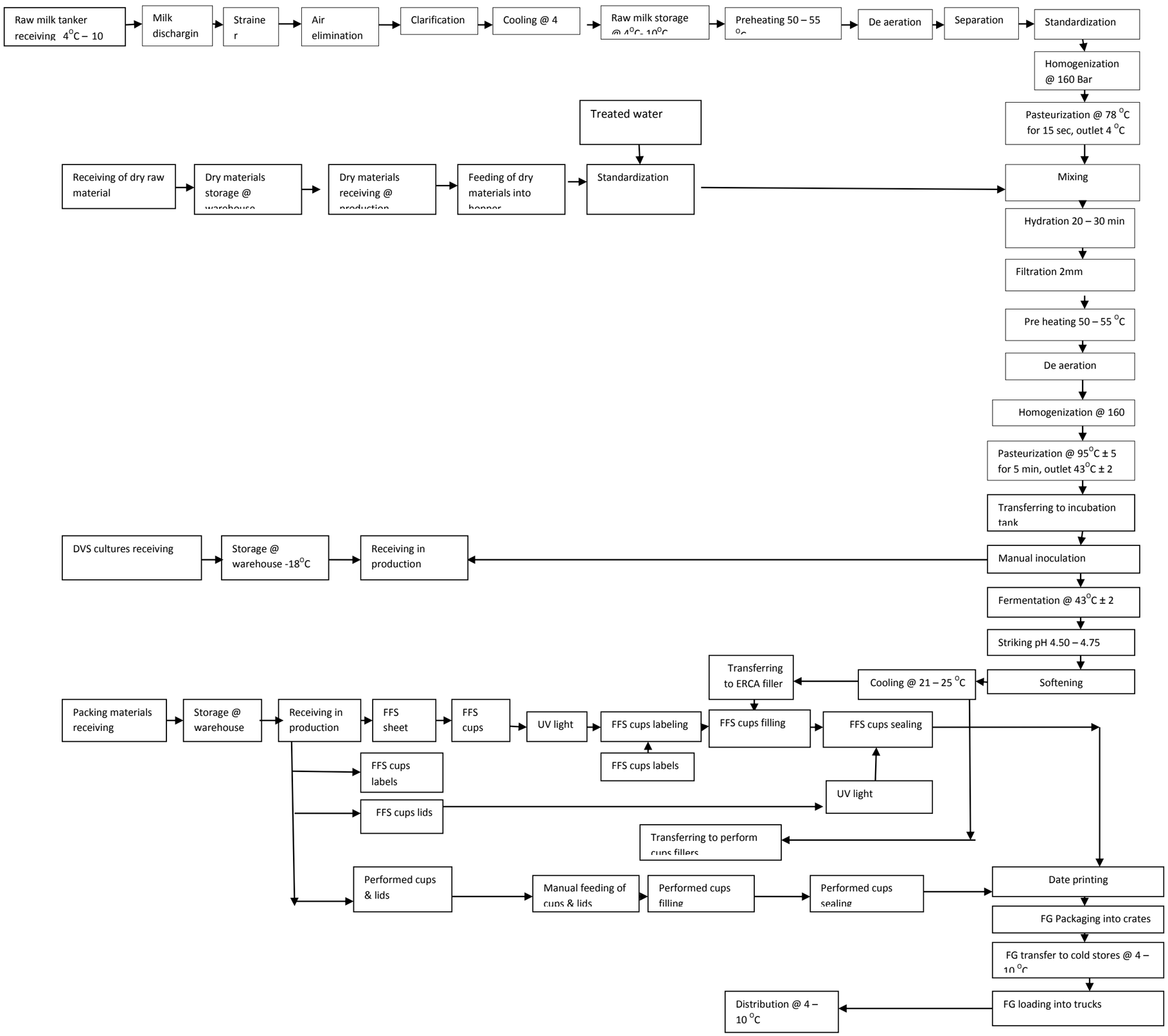
| | | | | |
|---|---|---|---|---|
| 16 | No webbing, dust accumulation, chipped paints, smudge | | | |
| 17 | Installation tightfitting through walls/ ceilings/ roof | | | |
| | **4. Wirings:** | | | |
| 18 | In g good repair, insulation intact | | | |
| | **5. ACUs:** | | | |
| 19 | No drips, leaks or water condensate falling underneath | | | |
| | **6. Wall/ partitions:** | | | |
| 20 | No webbing, chipped paints, broken tiles, | | | |
| 21 | Loose concrete, cracks, cavities, food material smudges | | | |
| 22 | Smooth finish , clean, made of impervious material | | | |
| | **7. Wall – floor Junctions :** | | | |
| 23 | No webbing, chipped paints, cracks, extrusion, dirt accumulation | | | |
| | **Floor** | | | |
| 24 | No pooling of water/ liquid material ( low lying area) | | | |
| 25 | Drains and internal drainage clean, adequate, functional and well covered | | | |
| 26 | Smooth finish , clean, made of impervious material and clean | | | |
| | **8. Door:** | | | |
| 27 | In good repair, smooth non absorbent | | | |
| 28 | Surface no cracks, chipped paints, rusting | | | |
| 29 | Materials is close fitting or adequately proofed to eliminate pest access from outside | | | |
| 30 | Foot bath/ cleaner with sanitizer in use | | | |
| 31 | Equipped with plastic curtain as necessary | | | |
| 32 | Plastic curtain clean and tidy | | | |
| | **9. Window and skylights :** | | | |
| 33 | Non opening; otherwise screened | | | |
| 34 | Clean screen and frame; in good repair | | | |

| | | | | |
|---|---|---|---|---|
| | **10. Ventilation :** | | | |
| 35 | Ambient temp. is controlled | | | |
| 36 | Adequate air circulation to minimize odors, fumes, vapours | | | |
| 37 | Fans, blowers, filters, cabinets clean and dust free | | | |
| | **11. Lights :** | | | |
| 38 | No busted, sufficient and well distributed to satisfy sanitation requirement | | | |
| 39 | Adequately shielded with no cracks or breaks, no dust, fume, web accumulation on surface | | | |
| | **12. Surface of machine equipment, fixtures, fittings :** | | | |
| 40 | No sign of leaks, condensation/ oil dripping | | | |
| 41 | Excessive rusting, flaking points, smudge/ spillage; dirt | | | |
| 42 | No portion potential for accumulating food particles and harboring pest/ microorganism | | | |
| 43 | Made of non-corrosive material, clean, in good condition and kept dry as necessary | | | |
| | **EMERGENCY EQUIPMENT** | | | |
| 44 | Are all symbolic safety signs in good condition | | | |
| 45 | Are all fire appliances identified | | | |
| 46 | Are all fire appliances accessible | | | |
| 47 | Are their seals intact | | | |
| 48 | Is there stacking in front of equipment | | | |
| | **AISLE & STORAGE & REFUSE** | | | |
| 49 | Any stacking in aisle | | | |
| 50 | Is stacking storage safe , tidy and clean area | | | |
| 51 | Bins color coded / marked/ demarcated areas | | | |
| 52 | Are there enough bins | | | |
| 53 | Is any superfluous/ scrap material present | | | |
| | **GENERAL** | | | |
| 54 | Are all doors closed and properly sealed | | | |
| 55 | Rest rooms & toilet in good clean condition | | | |

| | | | | |
|---|---|---|---|---|
| 56 | Pest control inspection up to date | | | |
| 57 | No evidence of pest activity | | | |
| 58 | Control are in a place for monitoring temp of storage areas | | | |
| 59 | Receipt of goods | | | |

# APPENDIX (3)

## Fallow – up of the yoghourt manufacturing scheme step by step



# APPENDIX (4)

Sensory evaluation of raw milk:

1. **Grading chart for raw milk :**

**Directions:** thanks for your cooperation in the evaluation of these milk samples for color, flavor, taste and texture. Use the appropriate scale to show your attitude by checking at the point that best describes your feeling about the sample.

If you there any question please ask.

**Name** ……

| Sample No. | Day | Color | Flavor | Taste | Consistency | Comment |
|---|---|---|---|---|---|---|
| 1 | 3 | | | | | |
| 2 | | | | | | |
| 3 | | | | | | |
| 4 | | | | | | |
| 5 | | | | | | |
| 6 | 6 | | | | | |
| 7 | | | | | | |
| 8 | | | | | | |
| 9 | | | | | | |
| 10 | | | | | | |
| 11 | 9 | | | | | |
| 12 | | | | | | |
| 13 | | | | | | |
| 14 | | | | | | |
| 15 | | | | | | |

**Key of grading chart for raw milk:**

| | Color | Flavor | Taste | Consistency |
|---|---|---|---|---|
| **4** | yellowish | acceptable | acceptable | watery |
| **3** | creamy | Less acceptable | Less acceptable | thick |
| **2** | white | Not acceptable | Not acceptable | gelatinous |
| **1** | other | bad | bad | other |