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**Evaluation of Hazard Analysis and Critical Control Points
(HACCP) Implementation in the Pasteurized Milk Plant**

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Dedication

To my beloved

*Mother and father for their support
and encouragements, to my brothers and
sister*

And all my friends.

To the soul

Of my uncle Osman

To all my teachers in all education levels.

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Abstract

This study was designed to evaluate the Hazard Analysis and Critical Control Points (HACCP) system implemented in pasteurized milk processing line.

The critical control points (CCPs) were including raw milk, immediately after pasteurization, after packaging, after two days, four days and six days of storage. Then physicochemical and microbial tests were carried out for the samples taken.

The results obtained revealed that the pH value of raw milk was 6.63, and acidity recorded 0.17 as lactic acid, and density recorded 1.03. Protein, fat, lactose, solids not fat and soluble solids recorded 3.00%, 3.50%, 4.42%, 9.90% and 13.10% respectively.

Total bacterial count was 5.7×10^5 cfu/ml, total coliforms 5×10^2 MPN/ml, *E. coli* 7.0 MPN/ml, *staphylococcus* recorded 5.7×10^2 cfu/ml, *salmonella*, yeasts and moulds were not recorded in raw milk samples.

Immediately after pasteurization of milk pH, acidity, density, fat, protein, lactose, solids not fat and total soluble solids recorded 6.63, 0.17%, 1.03%, 3.30%, 3.00%, 4.30%, 9.50% and 13.10% respectively.

The microbial analysis recorded values of total bacterial count as 3.8×10^3 cfu/ml, while the samples are free from total coliforms, *E. coli*, *staphylococcus*, *salmonella*, yeast and moulds.

After packaging of pasteurized milk pH, acidity, density, fat, protein, lactose, solids not fat and total soluble solids recorded 6.60, 0.17%, 1.03, 3.10%, 3.00%, 4.30%, 9.35% and 13.10%, respectively. On the other hand, total bacterial count was 3.8×10^3 cfu/ml, total coliforms, *E. coli*, *staphylococcus*, *salmonella*, yeasts and moulds were not recorded.

During the storage period of pasteurized milk pH was 6.63, 6.50 and 6.36 after 2nd, 4th and 6th days storage period, respectively. Acidity was 0.17%, 0.18% and

0.19% after 2nd, 4th and 6th days storage period, respectively. Density gave 1.03%, 1.03% and 1.03 after 2nd, 4th and 6th days storage period, respectively. Fat was 3.00%, 2.80% and 2.76% after 2nd, 4th and 6th storage days period, respectively. Protein recorded 2.96%, 2.83 and 2.63 after 2nd, 4th and 6th days storage period, respectively. Lactose recorded 4.10%, 4.00% and 3.90 after 2nd, 4th and 6th days storage period, respectively. Solids not fat was 9.30, 8.61 and 8.51 after 2nd, 4th and 6th days storage period, respectively. Finally, total soluble solids gave 13.00%, 12.90% and 12.80% after 2nd, 4th and 6th days storage period, respectively.

Total bacterial count after the 6th day was 8.6×10^5 cfu/ml, 4th day was 6.3×10^4 cfu/ml and 2nd day was 4.7×10^3 cfu/ml. The highest level of coliforms was (16.6 and 9.00 MPN/ml) in 6th and 4th, respectively, 2nd day was conformed completely free of this type of bacteria. The highest reading for *E.coli*, 6th day 7.33MPN/ml, and the 2nd day recorded no presence of *E.coli* while 4th day was 2.30MPN/ml. *Staphylococcus* was absent in 2nd day, while the 4th day recorded 4.46×10^2 cfu/ml and 8.0×10^2 cfu/ml was recorded by 6th day. *Salmonella* was not detected during the storage periods. Yeast and moulds were not detected in 2nd day and 4th day while on 6th day gave 4.0×10^2 cfu/ml.

الملخص

صممت هذه الدراسة بغرض تقويم نظام تحليل المخاطر والتحكم في النقاط الحرجة (الهاسب) المتبع في خط إنتاج الحليب المبستر وقد تم تحديد النقاط الحرجة في المخطط التصنيعي للحليب المبستر وهي الحليب الخام والحليب بعد البسترة مباشرة وبعد التعبئة. و أثناء فترة الصلاحية بعد يومين وأربعة وستة أيام ومن ثم تم التحليل الفيزيوكيميائي والميكروبي للعينات المأخوذة.

أظهرت النتائج أن تركيز أيون الهيدروجين للحليب الخام بلغ ٦,٦٣ ، كما بلغت الحموضة ٠,١٧ كحامض لاكتيك والكثافة ٠,٠٣ . كما بلغت نسبة كل من البروتين و الدهن و اللاكتوز و الجوامد اللادھنية و المواد الصلبة الكلية ٣,٠٠ % ، ٣,٥٠ % ، ٤,٤٢ % ، ٩,٩٠ % و ١٣,١٠ % ، علي التوالي.

أظهرت التحاليل الميكروبيولوجية للحليب الخام أن العد الكلي للبكتيريا (خلية/مل) $١٠^٥ \times ٥,٧$ و بكتيريا القولون $١٠^٢ \times ٥$ ، الإكولاي ٧,٠ والإستافيلوكوكس $١٠^٢ \times ٥,٧$ و سجل الحليب الخام خلوا كاملا من السالمونيلا والخمائر والأعفان.

بعد بسترة الحليب مباشرة تم إختبار تركيز أيون الهيدروجين و الحموضة والكثافة والدهن و البروتين و اللاكتوز و الجوامد اللادھنية و المواد الصلبة الكلية وكانت ٦,٦٣ % ، ٠,١٧ ، ١,٠٣٢ % ، ٣,٣٠ % ، ٣,٠٠ % ، ٤,٣٠ % ، ٩,٥٠ % و ١٣,١٠ % ، علي التوالي.

أظهرت التحاليل الميكروبيولوجية بعد البسترة أن العد الكلي للبكتيريا (خلية/مل) هو $١٠^٣ \times ٣,٨$ ، وقد كانت العينات خالية من بكتيريا القولون و الإكولاي و الإستافيلوكوكس و السالمونيلا و الخمائر والأعفان بعد البسترة.

بعد تعبئة الحليب المبستر تم إختبار تركيز أيون الهيدروجين و الحموضة و الكثافة و الدهن و البروتين و اللاكتوز و الجوامد اللادھنية و المواد الصلبة والتي بلغت ٦,٦٠ ، ٠,١٧ ، ١,٠٣١ ، ٣,١٠ ، ٣,٠٠ ، ٤,٣٠ ، ٩,٣٥ و ١٣,١٠ ، علي التوالي. أما الإختبارات الميكروبيولوجية بعد البسترة فكان العد الكلي للبكتيريا (خلية/مل) $١٠^٣ \times ٣,٨$ ، أما بكتيريا القولون و الإكولاي و الإستافيلوكوكس و السالمونيلا و الخمائر والأعفان فلم توجد.

أثناء فترة التخزين للحليب المبستر أظهرت النتائج أن تركيز أيون الهيدروجين ٦,٦٣ ، ٦,٥٠ و ٦,٣٠ بعد يومين و أربعة و ستة أيام خلال الفترات التخزينية ، عل التوالي . وسجلت الحموضة ٠,١٧ ، ٠,١٨ و ٠,١٩ بعد يومين و أربعة و ستة أيام خلال الفترات التخزينية ، عل التوالي . وأيضا سجلت الكثافة ١,٠٣ ، ١,٠٣ ، ١,٠٣ بعد يومين ، أربعة و ستة أيام خلال الفترات التخزينية ، عل التوالي . نسبة الدهن سجلت ٣,٠٠ ،

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CHAPTER ONE

1. INTRODUCTION

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).

Milk is a highly nutritious food, ideal for microbial growth and the fresh milk easily deteriorates to become unsuitable for processing and human consumption (FAO, 2001). The hygienic quality problems of milk 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).

Good quality milk production is the important objective of dairy farms. The term good quality refers to normal composition, freedom from pathogenic organism and toxic substances, acceptable sensory characteristics and high nutritive value. In order to achieve high quality standard products, it is necessary to manage the whole production chain from farm to consumer (Smit, 2005).

The major technological advances in the fluid milk processing industry in the last 25 years include standardization, pasteurization, homogenization and packaging (Griffiths and Goffl, 2006). The spoilage of processed milk is primarily due to bacterial activity, presence and activity of post-pasteurization contaminations and type and activity of pasteurization resistant micro-organisms which are the main limiting factors in extending the shelf life of high temperature short time (HTST) pasteurize milk (Fromm and Boor,2004). In addition other factors which limit the shelf life of refrigerated pasteurized milk include the time and temperature of pasteurization, and storage temperature of milk after pasteurization(Rankin,2002).

The hazard analysis and critical control point (HACCP) system is a preventative measure that assesses hazards, estimates risks and establishes specific control measures that emphasize prevention rather than reliance on end-product. The main potential hazards in most dairy products are microbiological and the dairy industry has increased its efforts for quality and safety assurance through the development and implementation of proactive programmes such as HACCP (El-Hofi *et al.*, 2010).

Forsyth and Hayes,(1998) said that HACCP is an approach to hygienic food production by the prevention of problems. Production process is evaluated for hazards and their relative risks. Monitoring and verification procedures are then established to maintain the production of hygienically acceptable product by controlling the key steps in the production process where the hazards were identified.

The HACCP evaluation process describes the product and its intended use, identifies any potentially hazardous food items subject to microbial contamination and proliferation during food processing or preparation (Norman,1999). HACCP is based on prevention and reduces the reliance on end-product inspection and testing. With the use of the hazard analysis and critical control point (HACCP) systems to address food safety in food processing, good manufacturing practice(GMP) has a part of the very basic requirements that must be in place before an effective HACCP system can be implemented (FAO,1998). The success of the HACCP system mandates educating and training management and production personnel in the importance of their role in manufacturing safe dairy foods. This training should also include information for the control of foodborne hazards related to all stages of the food chain (Tamime ,2009).

Objectives

1. to identify the critical points in the process of pasteurized milk at which the hazards may be introduced into product and therefore should be controlled.
2. to study microbiological and physicochemical characteristics of the product.
3. to evaluate the HACCP system and see its impact on the final product safety.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Milk

2.1.1 Introduction

In the diet of every nation, milk is an indispensable food item and is considered as nature's perfect food for human beings as well as other animals. Mammals secrete milk for the nourishment of their young ones and milk of animals like cattle, buffalo, goat, sheep, camel, yak, llama, mithun, mare etc are being used as food for human beings (Kutty, 2004).

Milk is considered as a nearly complete food since it is a good source for protein, fat and major minerals. Also, milk and milk products are main constituents of the daily diet, especially for vulnerable groups such as infants, school age children and old age (Enb *et al.*, 2009).

2.1.2 Definition

Milk is legally defined as the normal secretions of the mammary gland of mammals. It is a white liquid but it can be slightly yellowish, especially during the summer when the cows are out in the meadow. It is supposed to have a typical clean smell and its consistency is homogeneous (Dhuol and Osman, 2014).

2.1.3 Milk production

Total world production of all kinds of milk amounts to some 670 million tons/year. Relatively little is produced in Africa and Oceania, even though Australia and New Zealand are two of the most important countries for world dairy trade. World milk production has grown at an average rate of 2.3% per year since 2003 (Table 2.1).

Table (2.1): World milk production (million tons)

Region	2003	2004	2005	2006	2007
Africa	32.21	31.96	32.27	33.62	33.40
Americas	146.91	148.76	154.96	160.67	161.28
Asia	193.73	205.55	217.87	229.51	236.93
Europe	217.43	214.76	215.43	214.97	213.43
Oceania	24.49	25.21	24.79	25.65	26.26
Total	614.76	626.24	645.33	664	671.30

Source: FAO (2009).

2.1.4 Composition of milk

Milk is often described as a colloidal suspension, containing emulsified globules of fat, a heterogeneous family of major and minor proteins, the carbohydrate lactose, minerals, vitamins and enzymes. While the classes of constituents are similar for milk from most species, there are considerable inter-species differences, both qualitatively (i.e. the exact nature of constituents) and quantitatively (i.e. the amount of each constituent per liter (Tamime ,2009).

The composition of milk is not constant, but shows a wide variation. In the first place the composition depends on the species of animal(Table 2.2). But also within a species we find big differences between the breeds and between individual animals within a breed. The composition might even change from day to day, depending on feeding and climate. But also during one milking the first milk differs from the last milk drops.

Table (2.2): Composition of milk from different animals

composition	Cow milk	Goats milk	Sheep milk
Water	87.2%	85.8%	81.1%
Total solid	12.8%	14.2%	18.4%
Fat	4.0%	4.9%	6.5%
Protein	3.4%	4.3%	6.7%
Lactose	4.5%	4.3%	4.1%
Ash (minerals)	0.9%	0.9%	0.9%

Source: Pandey and Voskuil (2011).

2.1.5 Milk constituents

2.1.5.1 Water

It exists in continuous phase in which other milk constituents are either dissolved or suspended. Most of the water in milk is found in free form and only a very small portion is in bound form (bound by milk protein, phospholipids) (Kutty, 2004).

2.1.5.2 Fat

The bulk of the fat in milk exists in the form of small globules, called fat globules they have size ranging from 0.1 to 22microns and dispersed as oil in water type emulsion. The surface of each fat globule is coated with an adsorbed layer of material, called fat globular membrane. This membrane consist a phospholipid-protein complex that stabilities fat emulsion by keeping the globules separately (Kutty, 2004).

2.1.5.3 Protein

Cow milk generally contains 30–35 g L protein which is commonly divided into two classes on the basis of the solubility at pH 4.6: the insoluble caseins, which represent 80% of total milk protein, and the soluble whey (or serum) proteins, which represent 20% of total milk protein (Tamime, 2009).

Like any protein milk proteins are also composed of various essential and non-essential amino acids. In milk, protein exists as colloidal suspension (Kutty, 2004).

2.1.5.4 Lactose

It is the sugar seen only in milk and hence called as milk sugar. In milk lactose exists in true solution. Chemically lactose is composed of one molecule each of glucose and galactose. Souring of milk is due to the production of lactic acid from lactose by lactose fermenting bacteria and it is important in the preparation of fermented milk products (Kutty, 2004).

2.1.5.5 Indigenous milk enzymes

Indigenous milk enzymes are found in, or associated with various, casein micelles, milk fat globule membrane, milk serum or somatic cells and may originate from blood, somatic cells, the milk fat globule membrane (MFGM) or the cell cytoplasm. These milk enzymes can be used as indices of animal health or thermal history the milk, they can result in quality deterioration or induce desirable changes in milk and dairy products or they may also offer protective effects. Important indigenous milk enzymes, e.g. plasmin, lipoprotein lipase, alkaline phosphatase and lactoperoxidase (Tamime, 2009).

2.1.6 Physicochemical properties of milk

2.1.6.1 Physical state

In milk lactose and portion of mineral salts are found in true solution, protein and remainder of minerals in colloidal suspension and fat as emulsion. Water is the continuous phase in which the above constituents are either dissolved or suspended (Kutty, 2004).

2.1.6.2 pH

The pH of normal milk from a healthy cow is in the range 6.6–6.7. Milk pH is affected by temperature, generally decreasing with increasing temperature, due to changes in dissociation of ionisable groups (Tamime, 2009).

2.1.6.3 Density

The density of cow's milk normally varies between 1.028 and 1.038 g/cm³ depending on the milk composition (TPPS, 2003).

2.1.6.4 Titratable acidity

According to Harding (1999) the natural acidity of normal milk is less than 0.16% lactic acid or equivalent.

Elmagli and Elzubier (2006) found that the mean Titratable acidity of pasteurized milk was 0.14 to 0.86 %.

2.1.6.5 Taste and odor

Normal freshly drawn milk tastes slightly sweet to most people and has a characteristic although not pronounced odor. The odor disappears when milk is allowed to stand a few hours or following cooling and aeration where this practice is followed immediately after milking. It has been shown that the pleasant flavor of milk may be correlated with high lactose and relatively low chloride content (Eckles and Macy, 2002).

2.1.6.6 Boiling point of milk

It is slightly higher than that of water and is a round 100.17°C or 212.3F (Kutty 2004).

2.1.6.7 Color

Milk ranges in color from bluish-white to golden-yellow, depending upon the breed of animal, the kind of feed, and the amount of fat and solids present. In large quantities milk appears entirely opaque while in thin layers it is slightly transparent (Eckles and Macy, 2002).

2.1.7 Milk microbiology

2.1.7.1 Microorganisms in milk

Milk is sterile at secretion in the udder but it is contaminated by bacteria even before it leaves the udder. Milk provides a favorable environment for the growth of microorganism (yeast, moulds and bacteria) particularly at temperature above 16°C; further infection of the milk by microorganism can

take place during milking, handling, storage and other pre-processing activities(ELferran , 2007).

Human microbial pathogens that can be found in raw milk include *Listeria monocytogenes*, *Salmonella* spp. and *Campylobacter jejuni* .In addition to their significance for public health, a very good microbial quality of raw milk is also important to prevent production losses and to achieve an optimal shelf life of dairy products. For example, spore formers of butyric acid bacteria in raw milk are responsible for defects in semi-hard cheeses, and the contamination of raw milk with spores of *Bacillus cereus* limits the shelf life of pasteurized dairy products (Tamime, 2009).

2.1.8 Nutritional value of milk

Milk is very tasteful and is an excellent source of high quality protein that can be digested easily. Milk also contains lots of important vitamins and minerals. In many countries milk and milk products provide 5 – 10 % of the total calories of the daily human diet. It represents one of the best natural sources of essential amino acids for human nutrition.

Moreover, milk is an outstanding source of calcium and a good source of phosphorus. As these 2 elements play an essential role in building the bones and teeth in the body, it is clear that milk should be included in the diet of humans in all their stages of life. In fact milk is the most important source of calcium in the diet of almost all people. These nutritional attributes have made milk a mainstay in the diet, particularly of growing children.

The nutritional as well as the economic value of milk is directly associated with its solids content. The higher the solids content the better its nutritional value and the more of a milk product can be made out of it. For example, cheese yields are directly related to the protein and in particular to the casein content of milk (Pandey and Voskuil, 2011).

2.1.9 Processing of milk

Processing steps should be initiated immediately after milking so that near original quality of milk can be preserved. Milk intended for household or local usage purposes can be processed at these places itself immediately after its arrival. However milk sold to large scale collection –distribution system is usually processed at special processing centers called dairy plants. Here milk from different sources or collection channels are pooled together and collectively processed in large scale processing units(Kutty, 2004).

Various processes involved starting from the reception of milk in the dairy plant till the time of its storage for distribution to consumers include:

2.1.9.1 Filtration /clarification

It is process of removing visible foreign particles which have gained entry into the milk. This can be hair, milk clots, insects, dung particles, soil or dust particles and so on(Kutty, 2004).

2.1.9.2 Standardization

This is the process of adjusting the fat/solid not fat (SNF) content of milk to certain pre-determined level. Adjustment can be either raising or lowering the fat/SNF content and is done adding skim milk (fat removed milk) or cream (fat rich portion) as required (Kutty, 2004).

2.1.9.3 Pasteurization/sterilization

It is process of making the milk free from pathogenic organisms through heating to sufficient temperature for required time (Kutty, 2004).

2.1.9.4 Homogenization

It is process of breaking the fat globules present in the milk into smaller ones by forcing the milk through homogenizer. Purpose of this is to ensure uniform suspension of fat globules throughout the milk and thus to prevent the formation of cream layer (accumulation of fat globules at the top layer of milk) (Kutty, 2004).

2.1.9.5 Bottling/ packaging

Bottling /Packaging are done in suitable containers, considering the convenience of storage and distribution (Kutty, 2004).

2.2 Pasteurization

The term pasteurization has been derived from name of scientist (Louis Pasteur), who invented this technique of processing (Kutty, 2004).

One of the most important heat treatment transactions in dairy plants are pasteurization and sterilization. There are general bases for pasteurization can be summarized in the following two points: the first is health and the eradication of pathological microorganisms in milk, pathogenic organisms and the elimination of 95 to 99% of the number of bacteria present in the milk, as well as the process of pasteurization causes total elimination of yeast and moulds gets through exposure of milk for different temperatures for specified time periods.

The second point is increasing milk storage time as free of the microbes is increases the storage and safety of microbial damage (AL-Hilphy and Ali, 2013).

The International Dairy federation defines pasteurization as a process applied to milk product with the objective of minimizing possible health hazard arising from pathogenic microorganism associated with milk, by heat treatment which is consistent with minimal chemical ,physical and organoleptic change to the product (Harding, 1999).

2.2.1 Methods of pasteurization

Different methods are used depending upon how the process is carried out as well as the temperature-time combination employed. Commonly adopted methods according to kutty(2004) include:

1. high temperature –short time pasteurization (HTST) process
2. batch /holding pasteurization
3. In bottle pasteurization

2.2.2 Factors affecting pasteurized milk quality

2.2.2.1 Hygienic quality of raw milk

Under optimal processing and storage condition, high temperature short time thermal pasteurization is able to extend the shelf life of milk to around three weeks, depending on the initial microbiological quality of the raw milk (Abd Elrahman,2006).

2.2.2.2 Heat treatment and processing

Qstlie *et al* (2005) claimed that the growth of the probiotic strains in UHT milk varied considerably according to incubation temperature where all strains except *bifidobacterium animalis* BB12 showed the most rapid increase and the highest viable cell number in milk incubated at 37°C, than 30°C and 45°C, attaining viable cell number of log 8.65-9.21cfu/ml after 12-24 hrs incubation. In addition to, depending on the probiotic strains, pH decreased from 6.7 to 4.1-5.1 and 3.8-4.5 after 48 hrs of incubation at 30°C, 37°C and 45C, respectively.

2.2.2.3 Packing

Zygoura *et al* (2004) mentioned that no statistically significant differences in mesophilic as well as psychrotrophic counts of milk were recorded for milk samples in all packing materials for a given sampling day during the entire 17-days storage period. Taste panel studies showed that the rate of milk flavors deterioration of pasteurized milk packaged in standard (A) milk and juice(B and D) boards were faster ($p<0.05$) than milk packaged in barrier (C and E) and foil (F) boards(Abd Elrahman,2006).

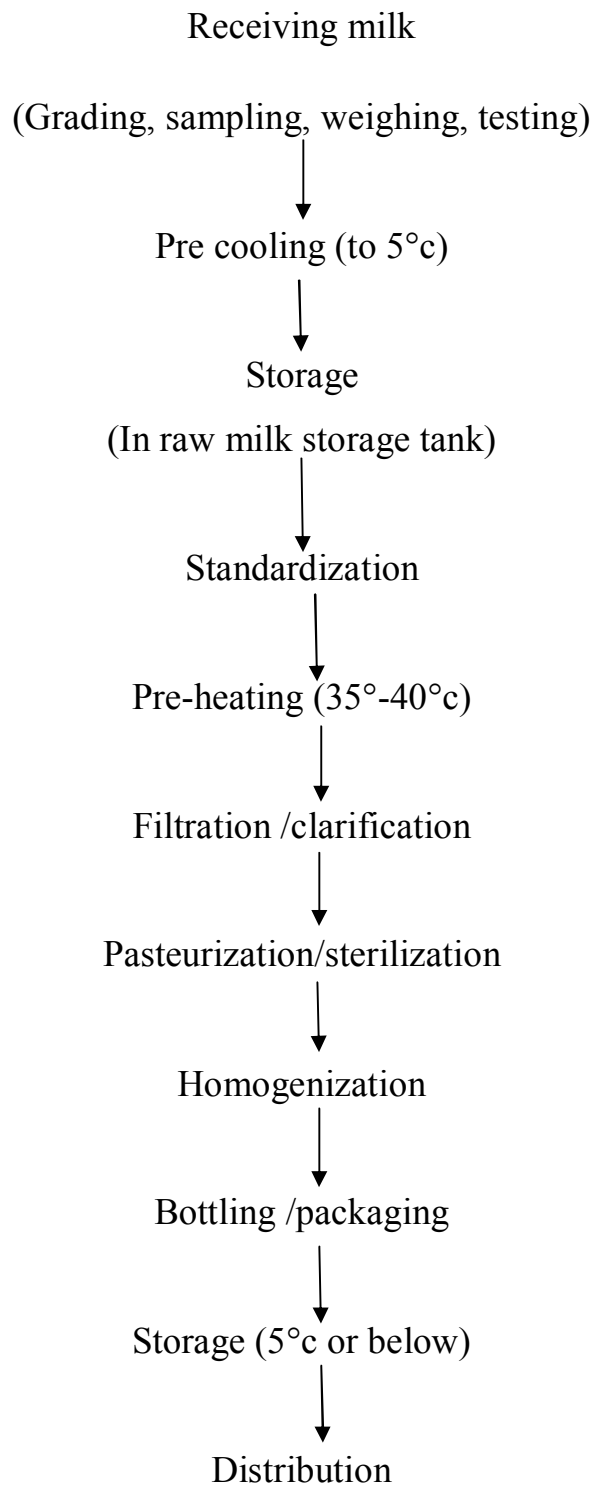


Figure 1: processing of milk

Source: Kutty (2004)

2.2.2.4 Cleaning and sanitation of plant and equipments

Murphy and Boor (2000) stated that the milk residue left on equipment contact surfaces supports growth of variety of microorganisms. Moreover, they reported that cleaning and sanitizing procedures that leave residual soil on equipment could dramatically increase the numbers and influence the types of microbes grow on milk contact surfaces. They mentioned that heat resistant and/or thermotolerant bacteria can persist in low numbers on equipment surfaces that routinely cleaned with hot water.

Gruetmacher and Bradley (1999) found that sequential analysis of fluid milk processing system indicated filling machine and pasteurizer were significant source of post pasteurization contamination. They added that the pasteurizer could be a source of contamination when inadequately cleaned or maintained, also the filling machine was significant source of contamination, however proper cleaning followed by sanitizing with chlorine significantly increase milk shelf life (Abd Elrahman, 2006).

2.2.2.5 Storage condition

The storage conditions of pasteurized milk affect bacterial growth rate, moreover analysis of variance revealed that the storage periods, batches and location played significant roles ($P < 0.001$) in the bacterial growth rate (Elmagli, 2004). He added that fat%, protein%, total solids%, ash%, and lactose% were significantly ($P < 0.001$) affected by storage condition (Abd Elrahman, 2006).

2.3 HACCP

2.3.1 Background

The Hazard Analysis Critical Control Point (HACCP) concept was developed in the early 1970s as a system to assure food safety. The basic principles underlying the concept were not new, but its introduction signaled a shift in emphasis from end-product testing to preventive control of critical aspects of the food chain from “farm to fork”. HACCP is based on the recognition that

manufacturers are responsible for determining the critical aspects of producing safe foods. It helps food manufacturers to improve the efficiency of control by providing a disciplined, systematic approach to the procedures for assuring food safety (Van Schothorst , 2004).

The system grew out of a need to provide safe food for National Aeronautics and Space Administration (NASA), including the elimination of pathogens, toxins, and foreign objects from food and beverages. The application of HACCP was pioneered, during the sixties, by the Pillsbury Company with the cooperation of NASA, Natick Laboratories of the US Army, and the US Air Force Space Laboratory Project Group. Robert Muller from Pillsbury Company was the inventor of the HACCP standards used by the food industry (Sozen and Hecer, 2013).

The acronym HACCP is one which evokes ‘food safety’. Originally developed to ensure microbiological safety of foodstuffs, HACCP has been broadened to include chemical and physical hazards in foods.

The HACCP system for managing food safety concerns grew from two major developments. The first breakthrough was associated with W.E. Deming, whose theories of quality management are widely regarded as a major factor in turning around the quality of Japanese products in the 1950s. Deming and others developed Total Quality Management (TQM) systems, which emphasized a total systems approach to manufacturing that could improve quality while lowering costs. The second breakthrough was the HACCP proposal by the Pillsbury Company, NASA and the US Army laboratories. This was based on the failure, mode and effect analysis (FMEA) as used by engineers in construction designs.

The HACCP concept was introduced in the United States in 1971 at the Conference of Food Protection where it was ‘recommended for widespread use’ (Arvanitoyannis *et al.*, 2009).

2.3.2 Benefits of HACCP:

Many commercial processes involve multiple stages from raw material production or acquisition through to the final product. A properly completed and implemented HACCP study identifies and controls the factors directly affecting the safety of a product. This allows the food producer to target technical resources efficiently. Identifying and monitoring CCPs are a more cost-effective and a more reliable method of assuring safety than the traditional inspection and end-product testing. The records and documentation provide excellent evidence that "all reasonable precautions" were taken and "due diligence" was exercised in order to prevent problems, evidence which may be necessary in case of legal action.

A HACCP study will not result, in all cases, in the elimination of all hazards but will assist in determining how best to minimize the remaining hazards. It is then up to the management to use that information correctly. Moreover, HACCP can improve the relationship between food producers and food inspectors. In the past, conflicts have arisen, often over trivial matters, which have taken their attention away from more important issues. If control procedures follow clearly established rules, inspectors can have greater confidence in food producers. In addition, the availability of data collected throughout the process and over time greatly facilitates the task of the inspectors by providing them with a more complete and accurate picture of the total operation than they would be able to obtain from a single inspection (Van Schothorst, 2004).

2.3.3 Advantages of HACCP:

HACCP offers a number of advantages over current systems according to Llano (2011) and these are :

- i. Focuses on identifying and preventing hazards from contaminating foods.
- ii. Permits more efficient and effective government oversight, primarily because the record keeping allows investigators to see how well a firm is

complying with food safety laws over a period rather than how well it is doing on a given day.

iii. Places responsibility for ensuring food safety appropriately on the food manufacturer or distributor.

iv. Helps food companies compete more effectively in the world market.

v. Reduces barriers to international trade.

Also the advantages of HACCP according to Whitehead and Orris (1995) include;

i. The HACCP system is capable of accommodating change, such as advances in equipment design, processing procedures or technological developments.

ii. Provides a more effective use of resources and more timely response to food safety problems.

iii. The HACCP system is compatible with the implementation of quality management systems, such as the International Organization for Standardization's ISO 9000 series (Afoakwa *et al.*, 2013).

2.3.4 Pre-requisite programmers

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).

2.3.4.1 Good agricultural practices

Primary 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).

2.3.4.2 Good manufacturing practices

2.3.4.2.1 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).

2.3.4.2.2 Control of operation

Effective control measures 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).

2.3.4.2.3 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).

2.3.4.2.4 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).

2.3.4.2.5 Transportation

The method of transportation should be such that measures are taken to prevent any contamination or deterioration of the commodity (FAO and IAEA, 2001).

2.3.4.2.6 Training

All 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).

2.3.4.2.7 Product information and consumer awareness

The end product 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).

2.3.5 Developing a HACCP plan

2.3.5.1 Assemble the HACCP team

The first task in developing a HACCP plan is to assemble a HACCP team consisting of individuals who have specific knowledge and expertise appropriate to the product and process. It is the team's responsibility to develop the HACCP plan. The team should be multi disciplinary and include individuals from areas such as engineering, production, sanitation, quality assurance, and food microbiology. The team should also include local personnel who are involved in the operation as they are more familiar with the variability and limitations of the operation. In addition, this fosters a sense of ownership among those who must implement the plan. The HACCP team may need assistance from outside experts who are knowledgeable in the potential biological, chemical and/or physical hazards associated with the product and the process.

However, a plan which is developed totally by outside sources may be erroneous, incomplete, and lacking in support at the local level.

Due to the technical nature of the information required for hazard analysis, it is recommended that experts who are knowledgeable in the food process should either participate in or verify the completeness of the hazard analysis and the HACCP plan. Such individuals should have the knowledge and experience to correctly: (a) conduct a hazard analysis; (b) identify potential

hazards, (c) identify hazards which must be controlled; (d) recommend controls, critical limits, and procedures for monitoring and verification; (e) recommend appropriate corrective actions when a deviation occurs; (f) recommend research related to the HACCP plan if important information is not known; and (g) validate the HACCP plan (NACMCF, 1997).

2.3.5.2 Describe the food and its distribution

A full description of the product should be drawn up, including relevant safety information such as: composition, physical/chemical data (including aw, pH, etc.), microbial/static treatments (heat treatment, freezing, brining, smoking, etc.), packaging, durability and storage conditions and method of distribution. (WHO, 2008).

To describe your product, you might ask the following questions about the product: (USDA, 1997).

1. Common name?

For example, a cooked sausage could be called franks/hot dogs/wieners.

2. How is it to be used?

Categories might include: Ready-to-eat, to be heated prior to consumption, or for further processing.

3. The type of package?

For example, is it modified atmosphere packaging?

4. Length of shelf life?

In the cooked sausage example, the length of shelf life might be 30 to 50 days for modified atmospheric packaging.

5. Where will it be sold?

For example, will it be sold to wholesale, retail or institutions?

6. Labeling instructions?

“Keep Refrigerated” would be a common labeling instruction for meat and poultry products.

7. How is the product(s) distributed?

For instance, should the product be kept refrigerated at or below 40°F?

8. Who is the consumer and how will the product be used by the consumer?

2.2.5.3 Intended use description

The intended use should be based on the expected uses of the product by the end user or consumer. In specific cases, vulnerable groups of the population, e.g. institutional feeding, may have to be considered. (Teera Sootabuta, 2007). Dairy products are often intended for specific dietary use. Target consumers may be athletes who need extra proteins; members of such a group are not particularly susceptible to pathogens. However, babies, patients, the very old and in the case of *Listeria*, pregnant women may be more prone to acquiring a food borne disease. The product use instructions for these consumer categories should receive particular attention. (Van Schothorst and Kleiss, 1994).

2.3.5.4 Flow diagram development

The purpose of a flow diagram is to provide a clear, simple outline of the steps involved in the process. The scope of the flow diagram must cover all the steps in the process which are directly under the control of the establishment. In addition, the flow diagram can include steps in the food chain which are before and after the processing that occurs in the establishment. The flow diagram need not be as complex as engineering drawings (NACMCF, 1997).

The diagram should not be so complex that it is difficult to follow and understand. The diagram must be complete from the beginning of your process to the end (USDA, 1997).

Many dairy processing lines have simple flow diagrams: milk reception; cooling; standardization; pasteurization; followed by fermentation, or evaporation and drying, or holding and freezing, or sterilization. These processes are followed by packing; hygienic precautions vary at this step

(from aseptic conditions to completely ‘open’ filling) (Van Schothorst and Kleiss, 1994).

2.3.5.5 Verify the flow diagram

The HACCP team should perform an on-site review of the operation to verify the accuracy and completeness of the flow diagram. Modifications should be made to the flow diagram as necessary and documented. (NACMCF, 1997).

After these five preliminary tasks have been completed, the seven principles of HACCP are applied.

2.3.6 HACCP principles

2.3.6.1 Conduct a hazard analysis (principle 1)

Hazard is defined as a chemical, biological, or physical agent in, or a condition of, food with the potential to cause an adverse health effect, while hazard analysis is the process of collecting and evaluating information on hazards and conditions leading to their presence to decide which are significant for food safety and should therefore be addressed in the HACCP plan. Chemical hazards include residues of pesticides and veterinary drugs, certain non-GRAS (generally recognized as safe) additives and preservatives, toxic metals, and chemicals from cleaning. Biological hazards include disease-causing microorganisms such as bacteria, viruses, parasites and fungi, and also certain plants and fish that carry toxins. Physical hazards include dirt, hair, broken glass and crockery, nails, staples, metal fragments or bits of packaging materials that accidentally enter food (WHO, 2008).

The objectives of the hazard analysis and the identification of control measures for each hazard are to:

- identify all hazards reasonably expected to occur and their associated control measures at each process step.
- identify any required modifications to a product or process to provide a greater food safety assurance.

- provide a basis for determining the process critical control points. (NZFSA, 2003).

2.3.6.2 Determine critical control points (CCPs) (Principle 2)

A critical control point is defined as a step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level. The potential hazards that are reasonably likely to cause illness or injury in the absence of their control must be addressed in determining CCPs (NACMCF, 1997).

Each step in the commodity flow diagram, within the scope of the HACCP study, should be taken in turn and the relevance of each identified hazard should be considered. It is also important to remember the stated scope of the HACCP analysis at this stage. The team must determine whether the hazard can occur at this step, and if so whether control measures exist. If the hazard can be controlled adequately, and is not best controlled at another step, and is essential for food safety, then this step is a CCP for the specified hazard (FAO and IAEA, 2001). A decision tree can be used to determine CCPs (figure 2).

2.3.6.3 Establish critical limits (principle 3)

Critical limits are defined as criteria that separate acceptability from unacceptability. A critical limit represents the boundaries that are used to judge whether an operation is producing safe products (FAO, 1998)

Critical limits should not be confused with operational limits, which are established for reasons other than food safety. Critical limits are parameters, which may be established as control measures and include Tamime(2009):

- Temperature
- Time
- Water activity (a_w)
- pH
- Titratable acidity
- Safe or tolerance levels of drug residues.

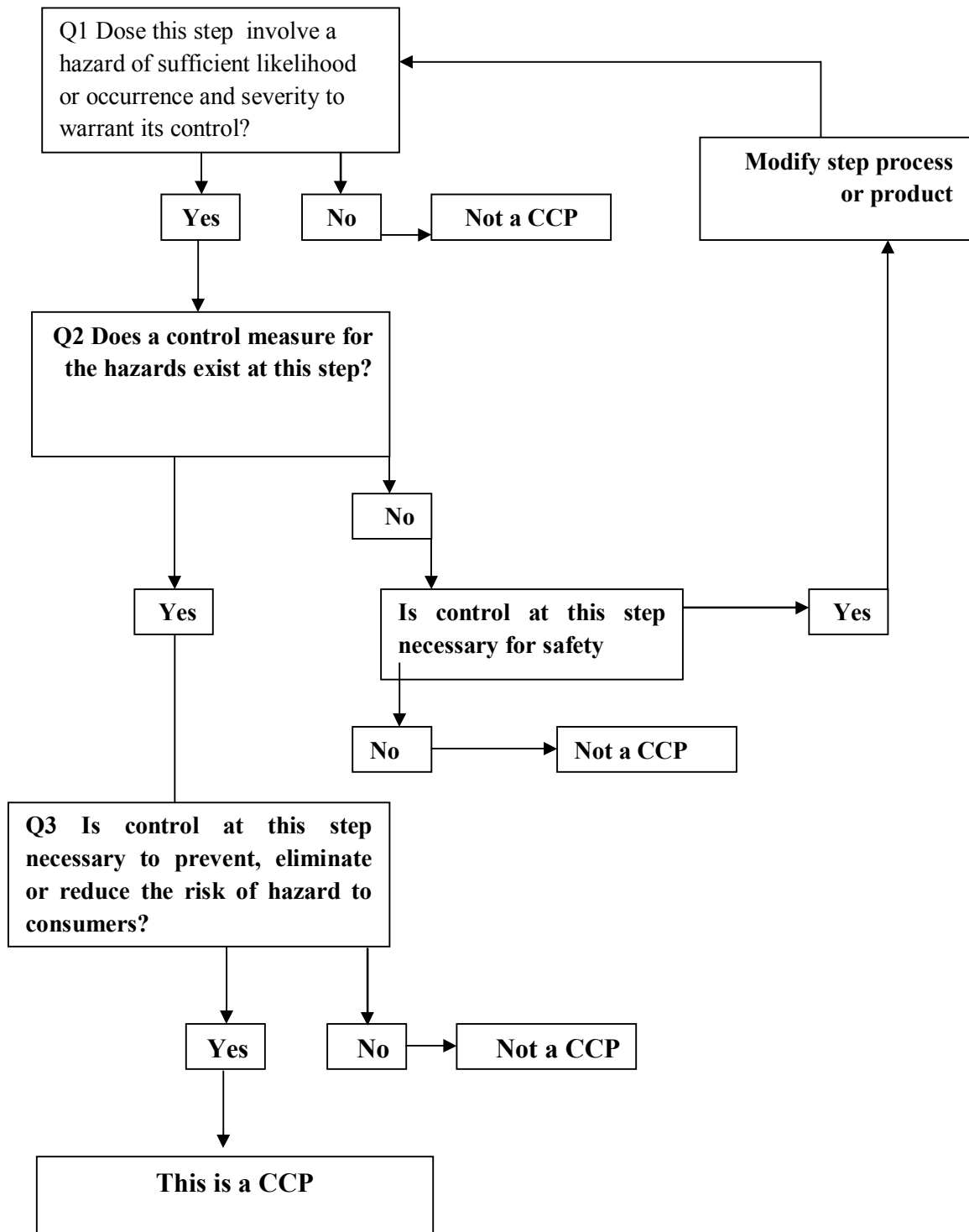


Figure 2: Example of HACCP decision tree.

Source: WHO (2008).

2.3.6.4 Establish monitoring procedures (principle 4)

Monitoring is a planned sequence of observations or measurements to assess whether a CCP is under control and to produce an accurate record for future use in verification. Monitoring serves three main purposes. First, monitoring is essential to food safety management in that it facilitates tracking of the operation. If monitoring indicates that there is a trend towards loss of control, then action can be taken to bring the process back into control before a deviation from a critical limit occurs. Second, monitoring is used to determine when there is loss of control and a deviation occurs at a CCP, i.e., exceeding or not meeting a critical limit. When a deviation occurs, an appropriate corrective action must be taken. Third, it provides written documentation for use in verification. (NACMCF, 1997).

According to NZFSA (2003) monitoring procedures should provide information on:

- Who will undertake the monitoring (this person must be trained and have appropriate responsibility to initiate corrective action or a computer with appropriate recording and software controls).
- frequency of the monitoring including statistically valid sampling regimes.
- What will be monitored.
- Where monitoring will occur.
- How critical limits will be monitored.

To ensure monitoring is effective and compliant, the following points should be implemented:

- Monitoring procedures should provide real time measurements or short-term feedback and should not rely on lengthy test methods for results e.g. microbiological assessments requiring extended incubation times are not practical if product has to be held pending a result at the CCP.

- Monitoring equipment e.g. thermometers, clocks, scales, pH meters, water activity meters etc. should be properly selected to record data within an appropriate range and be calibrated to a recognized standard.
- Monitoring records must be kept and all monitoring activities recorded.

2.3.6.5 Establish corrective actions (principle 5)

The Codex Hazard Analysis and Critical Control Point (HACCP) system and guidelines for its application defines corrective action as "any action to be taken when the results of monitoring at the CCP indicate a loss of control".

Loss of control is considered as a deviation from a critical limit for a CCP. Deviation procedures are a predetermined and documented set of actions to be implemented when a deviation occurs. All deviations must be controlled by taking action(s) to control the noncompliant product and to correct the cause of non-compliance (FAO, 1998).

An important purpose of corrective actions is to prevent foods which may be hazardous from reaching consumers. Where there is a deviation from established critical limits, corrective actions are necessary. Therefore, corrective actions should include the following elements:

- (a) Determine and correct the cause of non-compliance.
- (b) Determine the disposition of non-compliant product.
- (c) Record the corrective actions that have been taken.

Specific corrective actions should be developed in advance for each CCP and included in the HACCP plan. As a minimum, the HACCP plan should specify what is done when a deviation occurs, who is responsible for implementing the corrective actions, and that a record will be developed and maintained of the actions taken. Individuals who have a thorough understanding of the process, product and HACCP plan should be assigned the responsibility for oversight of corrective actions (NACMCF, 1997).

2.3.6.6 Establish verification procedures (principle 6)

Verification is defined as those activities, other than monitoring, that determine the validity of the HACCP plan and that the system is operating according to the plan.

Another important aspect of verification is the initial validation of the HACCP plan to determine that the plan is scientifically and technically sound, that all hazards have been identified and that if the HACCP plan is properly implemented these hazards will be effectively controlled. As stated by NACMCF(1997).

Information needed to validate the HACCP plan often include:

- (1) Expert advice and scientific studies.
- (2) In-plant observations, measurements, and evaluations.

Verification activities that can be used to determine if the HACCP system is working correctly according to WHO (2008) include:

1. Review of the HACCP system and its records.
2. Review of deviations and product dispositions.
3. Confirmation that CCPs are kept under control.
4. Auditing methods, procedures and tests.
5. Random sampling and analysis.
6. System validation (ensuring development of a documented system that meets all Codex requirements and updating the system when changes are made in processes, steps or materials used in production).

2.3.6.7 Establish record-keeping and documentation procedures (principle 7)

Efficient and accurate record keeping is essential to the application of a HACCP system. HACCP procedures should be documented. Documentation and record keeping should be appropriate to the nature and size of the operation and sufficient to assist the business to verify that the HACCP controls are in place and being maintained.

Documentation examples according to CAC(2003) are:

- Hazard analysis.
- CCP determination.
- Critical limit determination. Record examples are:
 - CCP monitoring activities.
 - Deviations and associated corrective actions.
 - Verification procedures performed.
 - Modifications to the HACCP plan

All HACCP procedures must be documented. According to Australian Government (2005). The records kept for the HACCP Plan should include:

1. Summary of the Hazard Analysis

2. HACCP Plan including:

- A description of the food, including ingredients, packaging, storage and distribution
- A verified flow diagram
- HACCP plan summary table with information on:
 - Critical Steps.
 - Potential Hazards.
 - Critical Control Points.
 - Monitoring Procedures.
 - Critical Limits.
 - Corrective Actions .
 - Verification Procedures.

3. Support documentation such as validation records and planned verification activities .

4. Records that are generated during the operation of the plan e.g. monitoring records.

2.3.7 HACCP in dairy industry

The dairy industry presents a unique and complex problem for the implementation of HACCP. The starting point is raw milk collected from a living animal with all the hazards associated with such working conditions; however, the problem then widens as a result of subsequent treatment. In some cases the milk may be pasteurized or sterilized or subjected to ultra-high temperature (UHT) treatments, each of which brings a different combination of challenges to the food scientist.

In other cases, the milk may be used in its raw state thereby giving rise to different challenges associated with the micro flora and bacterial contaminants found in the milk. Further to this, the use of milk in various modified forms, cheese, cream, butter, yoghurt etc. results in yet further processing and a range of different scenarios for the food technologist implementing HACCP (Arvanitoyannis *et al.*,2009).

2.3.8 Application of HACCP system in pasteurized milk

The steps used to apply the HACCP system in pasteurized milk production line as stated by Kassem *et al.*(2002) were as follows:

- The support of senior management of the company for food safety and HACCP application was sought and obtained.
- A team was formed which included: production manager, production engineer, consultant of food hygiene and sanitation, consultant of food microbiology and a technician from the laboratory.
- Products were described in terms of ingredients, processing, packaging, storage and distribution.
- Each step in the process was outlined in sequence in the flow diagram from raw material through processing, packaging and storage.
- In order to identify the hazards the following actions were undertaken:
- Observing operations: Each product preparation process was observed for:
 - Receipt of raw material, storage, heat treatment, cooling and packaging.

- Personal hygiene, education, health, cleanliness, habits, premises, equipment, floors, walls and ventilation (working conditions).
- Measuring operation: Time and temperature applied during the production and storage of milk was measured and recorded on flow diagram.
- The critical control points (CCP) decision tree was used to determine whether a step was CCP for the identified hazard.

2.3.9 Maintenance of the HACCP system

After an HACCP system has been developed and implemented, it must be maintained effectively on a continuous basis. This means that the monitoring procedures, the corrective action procedures (when required), the verification activities, and the record-keeping at each CCP, and for all the HACCP plans in the HACCP system, must operate continuously, and in exactly the manner as they were initially developed and implemented. Any change in any of these activities should only take place after the HACCP coordinator has been informed and has approved the change. For any significant change to the existing HACCP plan activities, the HACCP team should evaluate the change using the same guidelines and principles (*Steps 1 to 12*) that were used in the development of the HACCP system (Alli,2004).

HACCP system audits should review the actual practices and application of any procedures written in the HACCP Plan. According to NZFSA (2003) HACCP system audits may include on-site observations to cover e.g.:

- Introduction of a new raw material.
- Changes to the formulation, processing or packing methods and/or system.
- A change to the intended product use.
- ensuring product description and process flow diagrams continue to be accurate.
- monitoring required by the HACCP Plan at the CCPs is performed.
- ensuring processes are operating within established critical limits.

- Where monitoring has indicated a deviation from critical limits, affected product has been controlled, and corrective actions have been followed.
- seeing that records are filled out accurately.

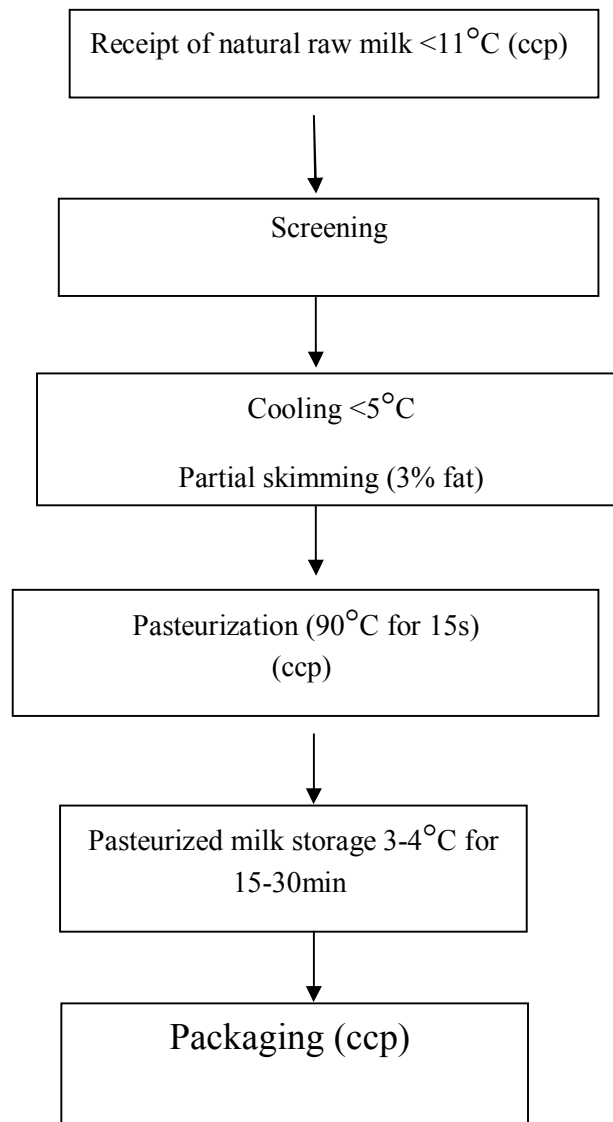


Figure 3: Process flow diagram of pasteurized milk
Source: Kassem *et al.*(2002)

Table(2.3) Summary of the HACCP control charts of milk

Process step	CCP	Hazard	Preventive measure	Critical limits	Monitoring procedure	Frequency	Corrective action
1-receiving raw milk	1.1 1.2 1.3 1.4 1.5	High microbial load Cross contamination High acidity Dust and straw Environmental contamination	Receive at <10 °C GMP	Receiving milk at<10°C and PH>6.10	Temperature and PH measurement	At every receiving	Reject received milk if contamination is evident
4-heat treatment (pasteurization or sterilization)	4.1	Microbial survival	Time and Temperature control	Pasteurization at 90-95°C for 15 second	Time and temperature measurement	At ever heat treatment	Correct time and temperature repasteurize milk
5-pateurized and sterilized milk storage	5.1 5.2	Spores germination in pasteurized milk Cross contamination	Time and Temperature control GMP	Storage at 3-5°C	Time and temperature measurement	At every storage	Corrective time and temperature
6-Packaging	6.1	Cross contamination	GMP	Sealed packages	Visual inspection	At every packaging	Discard if contamination is evident

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Source and processing of milk samples

A total of 27 milk samples were collected from FAABY factory in River Nile State -Sudan. FAABY factory has its own dairy farms and use their own milk for processing. The raw milk was brought from the farm through a cooled bulk tank, transferred into the holding tank, then milk was pasteurized at 72°C for 15 seconds using HTST plate heat exchanger pasteurizer. The pasteurized milk was packaged into Polyethylene terephthalate. Also 27 of the pasteurized milk samples of FAABY factory were collected from market in Khartoum State-Sudan.

The samples were collected at three different levels in different batches of processing as follows:

- 1-Fresh milk samples: 9 samples were collected from raw fresh milk supplied to the factory in clean and sterile bottles.
- 2- After pasteurization samples: 9 samples from the milk tanks were collected immediately after pasteurization in clean and sterile bottles.
- 3- After packaging samples: 9 samples were collected from pasteurized milk after packaging in clean and sterile bottles
- 4- The shelf life tests were carried on market samples: 27 samples of FAABY pasteurized milk were collected from market after 2 days, 4 days and 6 days they had been stored at refrigerator temperature prior to analysis.

3.2 Chemicals and reagents

- HCl (E. Merck, D-6 100 Darmstad, F. R. Germany).
- Sulfuric acid (H₂SO₄ Conc., density 1.815 and 1.86g/ml/ml) (BDH).
- Boric acid (Analar) .
- 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).

3.3 Physicochemical analysis

3.3.1 Fat content

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

Ten ml 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.

3.3.2 Protein content

The protein content was determined by Kjeldahl method according to AOAC (1990).

In a Kjeldahl flask 10 ml of milk were placed. Two Kjeldahl catalyst tablets (each tablet contains 1gm Na₂SO₄ and the equivalent of 0.1 gm Hg) were added to the flask. Twenty five milliliters of concentrated sulfuric acid (density of 1.86gm/ml at 20°C) were added to flask. The mixture was then digested on a heater until a clean solution was obtained (3hours). The flask were removed and left to cool.

The digested sample was poured in 100 ml volumetric flask, and diluted to 100ml with distilled water. Five milliliters were taken and neutralized using 10ml of 40%NaOH. The distillate was received in a conical flask containing 25 ml of 2% boric acid plus 3 drops of indicator (bromocresol green plus methyl red).the distillation was continued until the volume in the flask was 75 ml. the flask was then removed from the distillatory.

The distillate was then titrated against 0.1N HCL until the end point was obtained (red color). Protein content was calculated as follows:

$$\text{Nitrogen (\%)} = \frac{T \times 0.1 \times 20 \times 0.014 \times 100}{\text{Weight of sample}}$$

$$\text{Protein (\%)} = \text{Nitrogen (\%)} \times 6.38$$

Where:

T = Titration figure

0.1 = Normality of HCL

0.014 = Atomic wt. of N1/1000

20 = Dilution

3.3.3 Lactose content

The lactose content was determined by Anthrone Method (Richards,1959) one ml milk was pipette in a 500 millilitres volumetric flask and diluted to 500 millilitres with distilled water. The sample was mixed well then, 0.5 milliliters was transferred in a boiling test tube (in duplicate) the samples were placed in an ice bath, and shaken while adding 10 ml of ice cold anthrone reagent. The tubes contents were mixed and then placed in boiling water bath for 6 min, then transferred back to the ice bath for 30 min. the optical density of the colored solution was then read at 625nm. A blank consisting of distilled water 0.5 milliliters and anthrone reagent and standard containing 100mg/ml of lactose and anthrone reagent were included in each batch of analysis.

The percentage of lactose was then calculated using the following formula:

$$\text{Lactose content} = \frac{\text{O.D of sample} - \text{O.D of blank}}{\text{O.D of standarad-O.D of blank}} \times 4.75 = \text{g/1000ml}$$

OD: optical density

3.3.4 Total soluble solids (TSS)

Total soluble solids (TSS) of the milk were determined at room temperature using digital refractometer with degree Brix° scale 0-100 according to AOAC (1990).

3.3.5 Titratable acidity

Titrate acidity was determined according to AOAC (1990). Ten milliliters 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 milliliter of 0.1N NaOH sodium hydroxide = 0.009gm of lactic acid).

3.3.6 Density

The samples were shaken, then put it in tube at 20°C , the lactometer was put and turn it and set to stabilize and read the level.

3.3.7 pH

The pH of milk was determined according to AOAC (1990). The pH meter was first connected to the power, switched on and left for about 15 minutes to warm up. The needle switched to the neutral position. The electrodes were rinsed with distilled water and wiped then immersed in buffer solution of pH 4 and hence the pH of samples were measured.

3.4 Microbiological analysis

The microbiological analysis of milk was determined as follows:

3.4.1 Preparation and sterilization of glass ware

All glassware used were soaked overnight in tap water then washed in running tap water and allowed to dry. Pipettes were plugged with cotton wool and put in canister; also Petri dishes were placed in Petri dishes cans. Sterilization was done in an oven at 160°C for 2 hours.

3.4.2 Plate count agar (PCA) medium

Plate count agar was prepared by suspending 23,5gm of plate count agar base (M091) in 1000ml distilled water, and boiled to dissolve the ingredients completely, and sterilized at 121°C for 15 min.

2.4.3 McConkey broth medium

McConkey broth was prepared by suspending 40gm of Scharlau McConkey broth in 1000 distilled water and distributed in test tubes, then sterilized in an autoclave at 121°C for 15 min.

3.4.4 Brilliant green bile broth medium

Brilliant green bile broth was prepared by suspending 40gm of Oxoid limited, London, ES19HF in 1000ml distilled water, well mixed distributed in fermentation tubes and sterilized in an autoclave at 121°C for 15min.

3.4.5 Potato dextrose agar medium

Potato dextrose agar was prepared by dissolving 39 gm in 1000 ml distilled water, boiled to dissolve the ingredients completely, sterilized in an autoclave at 121°C for 15 min, cooled to 50°C and poured into sterile Petri dishes.

3.4.6 Staphylococcus medium No.110 (oxoid)

Staphylococcus medium No.110 was prepared by dissolving 105 gm in 1000 ml distilled water, steamed to dissolve completely, sterilized in an autoclave at 121°C for 15min.

3.4.7 Bismuth sulphite agar medium

Bismuth sulphite agar was prepared by dissolving 52gm of dehydrate Bismuth sulphite agar were suspended in 1000 ml distilled water, steamed to dissolve completely, sterilized by boiling in water bath at 100°C for 10 min.

3.4.8 Preparation of sample dilution

10 ml of milk was added to warm 90 ml of distilled water and blended by the stomacher for two minutes. One ml of the bag contents was pipette into separate tubes containing 9 ml of peptone water; the liquids were then mixed carefully by aspirating 10 times with a sterile pipette. With the same pipette

one ml was transferred to another dilution tube containing 9 ml of peptone water, and mixed with fresh pipette.

Serial dilutions of 10^{-1} to 10^{-10} were obtained by repeating the steps of mixing and transferring.

3.4.9 Total bacterial viable count method

One ml of the suitable dilution was transferred aseptically into sterile petri dishes immediately and 15 ml of the melted agar medium that cooled to 45°C were poured into petri dishes. Aliquots were mixed with agar medium and allowed to solidify. When medium was solidified, the dishes were inverted and incubated at 37°C for 48hr. plates were examined and the colonies as colony forming unites per ml (cfu/ml) (Harrigan,1998).

3.4.10 Coliform count method

It was carried out by using the Most Probable Number(MPN) technique. 1ml of each of the three first dilution (10^{-1} , 10^{-2} , 10^{-3}) was inoculated in triplicates of McConkey broth test tubes containing Durham tubes. The tubes were incubated at 37°C for 48 hours. Test tubes with sufficient gases to fill the concave of the Durham tube is recorded as positive presumptive tube.

From every tube showing positive result a tube of brilliant green 2%bile broth was inoculated by using sterile loop. The tubes were incubated at 37°C for 48 hours then the tubes showing positive and negative result were record. The most probable number of total coliform was found out by using the most probable number(MPN) table(Harrigan, 1998) .

3.4.11 *Staphylococcus* count method

One ml of the suitable dilution was transferred aseptically into sterile Petri dishes immediately and 15 ml of staphylococcus medium No.110 was added. Aliquots were mixed with agar medium and allowed to solidify. Plates were then incubated at 37°C for 48 hours and count was expressed as Colony Forming Units (CFU) per gram (Harrigan, 1998) .

3.4.12 Yeast and moulds count method

A mount of 0.1 ml from each sample dilution was pipetted into sterile Petri dish containing 15 ml solidified potato dextrose agar (PDA). The Petri dishes were incubated at 28°C for 72 hours. All colonies were counted by using a colony counter. The number of yeast and moulds were computed per gram by multiplying the reciprocal of the dilution used (Harrigan, 1998).

3.3.13 Presence of *Salmonella* method

Ten ml of the sample were added to conical flask containing 100 ml of sterile nutrient broth and incubated at 37°C for 24 hours. A loopful of 24 hours incubated nutrient broth was transferred aseptically into sterilized selenite cystine broth and incubated at 37°C for 24hours. A loopful of 24hours inoculums was streak-plated on bismuth sulphite agar surface and incubated at 37°C for 24-72 hours. Black metallic sheen discrete colonies indicated the precence of *Salmonella*(Harrigan, 1998) .

3.5 Statistical analysis

Mean \pm standard deviation were tested using One Factor Analysis of Variance according to vision 16 MINITAB statistical software for windows (2006).

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Identification of possible hazards and corresponding control measures:

4.1.1 Raw milk:

This stage is a CCP₁ because the reception test stands for an acceptance test. The exposure of milk to high temperature during transportation may favor the growth of pathogens and the production of heat resistance toxins. Raw milk can contain pathogenic bacteria, such as *salmonella spp*, *mycobacterium bovis*, *Brucella*, *Campylobacter* and *Listeria monocytogenes* (Skovgaard, 1990).

The means of fat, protein, lactose, total solid and solids not fat (SNF) content were 3.53%, 3.00%, 4.32%, 13.10% and 9.90%, respectively in raw milk samples (Table 4.1, Appendix 5). The composition of raw milk in the present study was compared favorably with the composition of milk in Northern Europe, which contained fat of 4.3%, total protein of 3.4%, lactose of 4.65%, ash of 0.73%, TS of 13.3% and SNF of 9.0% (Invensys, 2002). The present study shows that raw milk composition was good when we compared the composition with the limits of Sudanese Standards (SSMO, 2011) which reported for minimum cow milk fat of 3.44%.

The present study revealed that the pH of raw milk was 6.63 (Table 4.1, appendix 5), this was almost in agreement with the range required by the (SSMO, 2011) for raw milk which was 6.59-6.87.

The present study estimated acidity of raw milk for 0.17% as lactic acid (Table 4.1, appendix 5), which was lower than what was reported by Salman and Elnasri (2011) who reported acidity of less than 0.20% in raw milk. It was higher than that obtained by Abd Elrahman (2006) who reported raw milk acidity of 0.145 % lactic acid, but in agreement with the range required by SSMO (2011) for raw milk which was 0.14-0.19%. Salman and Hagar (2013) reported that a high acidity implies a high lactic acid content which, in turn, implies a high bacterial count in the milk.

The present study revealed that the density of raw milk is 1.031 ± 0.001 (Table 4.1, appendix 5). This was almost in agreement with the range required by the SSMO (2011) for raw milk which was 1.027-1.031.

The microbiological quality of raw milk used in processing pasteurized milk show that total bacterial count (TBC) of 5.7×10^5 cfu/ml (Table 4.2, appendix 1), this was almost in agreement with the range required by the SSMO (2011) for raw milk which was 7.5×10^5 cfu/ml, the acceptable limit of TBC of the European Union of raw milk was reported to be 1×10^4 cfu/ml. The findings in this study were comparable to that of Mariana (2001), who reported a range of 4×10^5 cfu/ml.

High bacterial count is expected under tropical conditions such as the Sudan due to the fact that high temperature enhances growth and multiplication of bacteria (Salman and Hagar, 2013).

Coliform in milk is one of the best indices for judging sanitation (Douglas, 2003). This study revealed that the coliform count is 5×10^2 MPN/ml (Table 4.2 appendix 1). This range is higher than that reported by APHA (1985) which is less than 10^2 MPN/ml.

The higher coliform count in raw milk may be due to the unsatisfactory milking practices in the farm from which the milk was collected. It was lower than that obtained by Salman and Hamad (2011) (9×10^3 MPN/ml).

Several workers isolated *E. coli* from milk and stated that it might cause a potential risk particularly for children (Padhye and Doyle, 1991). However, in this study *E. coli* was 70 MPN/ml (Table 4.2, appendix 1), comparable to what was reported by Salman and Hamad (2011) who reported about 32% of the raw bulk milk were *E. coli* positive in Khartoum State.

Table 4.1: Chemical and physical properties of raw milk

Parameter	Value (%)
PH	6.63 ± 0.05
Acidity as lactic acid	0.17 ± 0.05
Density	1.031 ± 0.01
Fat	3.50 ± 0.30
Protein	3.00±0.00
Lactose	4.42±0.02
SNF	9.90±0.01
T. S	13.10±0.20

Table 4.2: Microbiological characteristics of raw milk

Test	Count(cfu/ml)
Total bacterial count	$5.7 \times 10^5 \pm 4.8 \times 10^5$
Total coliform	$5 \times 10^2 \pm 6 \times 10^2$
<i>E.coli</i>	$70 \pm 1.1 \times 10^2$
<i>Staphylococcus aureus</i>	$5.7 \times 10^2 \pm 3 \times 10^2$
<i>Salmonella spp.</i>	-ve
Yeast and moulds	-ve

The present study revealed that the number of *staphylococcus aureus* of raw milk was 5.7×10^2 cfu/ml (Table 4.2, appendix 1), this result is lower than that reported by Salman and Hagar (2013) who found 1×10^4 cfu/ml. This was almost in agreement with that recommended by Bruce (2003) not to exceed 10^3 .

The samples of raw milk in this study yielded negative result for presence of *salmonella*. However the presence of *salmonella* in products is an indication that the plants system for controlling contamination is not working (Tompkin, 1994). Also this study revealed that samples of raw milk yielded negative result for presence of yeasts and moulds.

4.1.2 Immediately pasteurized milk

This stage is a CCP₂ because cannot eliminate any existing hazard. To prevent these hazards, the control of time and temperature and the application of the rules of good manufacture practice (GMP) are needed.

The present study revealed that the pH of pasteurized milk was 6.63 (Table 4.3, appendix 2). This was almost in agreement with that reported by Hoolasi (2005) who stated that, the pH of pasteurized milk is 6.5.

Acidity reported in this study was 0.17% lactic acid (Table 4.3, appendix 5), which is in accordance with SSMO (2007). It was higher than that obtained by Samia, *et al.* (2009) who showed that the mean acidity of pasteurized milk was 0.143 lactic acid in Sudan.

In pasteurized milk the total bacterial count 3.8×10^3 cfu/ml (table 4.4, appendix 2), this was almost in agreement with the range required by SSMO (2007) for pasteurized milk which should not increase over 5×10^4 cfu/ml during shelf life, and FDA (1997) who reported that the bacterial load should not exceed 20,000 cfu/ml for pasteurized milk. This result is lower than was reported by the European Union which was 3×10^4 (Salman and Hagar, 2013).

The result of this study showed that, no growth of coliform in the samples (Table 4.4, appendix 2). The results are in agreement with SSMO (2007) for pasteurized milk which was free from coliform bacteria. Also agree with PMO

(2001) what reported the total bacterial standards for grade A pasteurized milk should be < 10 coliform/ml.

After pasteurization there was no growth of *E.coli* in the samples obtained from factory (Table 4.4, appendix 2). This result was in agreement with that reported by SSMO (2007) for pasteurized milk of *E.coli* which was zero.

The results of this study showed that, no growth of staphylococcus in samples (Table4.4, appendix 2). The results are in disagreement with Laszlo (2003) who reported count of more than 10^3 cfu/ml. While Lílian, et al. (2011) found that 30% of the samples were contaminated with *Staphylococcus aureus*.

After pasteurization there was no growth of salmonella and yeasts and moulds in the samples obtained from factory (Table4.4, appendix 2). The results were in agreement with SSMO (2007) for pasteurized milk which was free from all pathogenic microorganisms.

4.1.3 Packaging

This stage is CCP₃. The aerobic count of packaged pasteurized milk before HACCP was 4.0×10^4 cfu/g and the other tested microorganisms ranged from <10 to none. The potential for contamination at this stage makes it a CCP (Kassem *et al.*, 2002).

Results of TBC for packaged pasteurized milk obtained during this study was 3.8×10^3 cfu/ml (Table4.6, appendix 3). This result is higher than that reported by Kassem *et al.* (2002) for the aerobic count which was 4.5×10^2 cfu/ml after application of HACCP.

This necessitate the application of HACCP in processing of pasteurized milk in this factor

Table 4.3: Chemical and physical properties of immediately pasteurized milk

Parameter	Value (%)
PH	6.63±0.05
Acidity as lactic acid	0.17±0.05
Density	1.032±0.005
Fat	3.30±0.17
Protein	3.00±0.00
Lactose	4.30±0.05
SNF	9.50±0.01
T. S	13.10±0.2

Table 4.4: Microbiological characteristics of immediately pasteurized milk

Test	Count(cfu/ml)
Total bacterial count	$3.8 \times 10^3 \pm 2.8 \times 10$
Total coliform	0.00 ±0.00
<i>E.coli</i>	0.00 ±0.00
<i>Staphylococcus aureus</i>	0.00±0.00
<i>Salmonella spp.</i>	-ve
Yeast and moulds	-ve

This study revealed that the coliforms , *staphylococcus aureus* and *E.coli*, *salmonella* and yeasts and moulds are absent (Table4.6, appendix 3). This results agrees with Kassem *et al.* (2002) after application of HACCP.

The lower bacterial counts might be due to quality of raw milk, good manufacturing practice and efficient storage conditions.

The present study revealed proper pasteurization due to reduction of microorganisms, which agreed with Dumalisile *et al.* (2005).

4.1.4 Inspection of pasteurized milk during the shelf life

The composition of pasteurized milk of fat, protein, lactose, total solids and solid not fat (Table4.7, appendix 6) slightly decreased throughout the storage period. This may be due to the increasing growth of bacteria (Table4.8 appendix 4).

The titratable acidity for pasteurized milk tended to increase slightly throughout the storage period (Table4.7, appendix 6). This result agrees with that reported by Elmagli and Elzubeir (2006) who found that the acidity was significantly affected ($p < 0.001$) by storage conditions and batches. This was due to the fermentation of lactose, which was converted to lactic acid (Abd Elrahman, 2006).

Higher acidity reported here may be due to lack of cooling facilities during transportation or improper storage (Salman and Hagar, 2013).

During storage period (6 days) the pH changed from 6.63 to 6.36 (Table4.7, appendix 6). This is due to high acidity.

The present study revealed that the TBC (Table4.8, appendix 4) at day 4 conform with the limits (9×10^4 cells/ml) cited by Salman and Hagar (2013) from SSMO (2007).

Table 4.5: Chemical and physical properties of pasteurized milk after packaging

Parameter	Value (%)
pH	6.60±0.00
Acidity as lactic acid	0.17±0.05
Density	1.031±0.005
Fat	3.10±0.15
Protein	3.00±0.001
Lactose	4.30±0.02
SNF	9.35±0.01
T. S	13.10±0.2

Table4.6: Microbiological characteristics of pasteurized milk after packaging

Test	Count(cfu/ml)
Total bacterial count	$3.8 \times 10^3 \pm 2.08 \times 10$
Total coliform	0.00 ±0.00
<i>E.coli</i>	0.00±0.00
<i>Staphylococcus aureus</i>	0.00
<i>Salmonella spp.</i>	-ve
Yeast and moulds	-ve

The present study revealed that the coliforms at day4 is 9.0 MPN/ml (Table4.8, appendix 4). This agreed with PMO (2001)who reported that the standards for grade A pasteurized milk should be < 10 coliform/ ml.

This study showed that the *E.coli* at day2 was zero and day4 2.3MPN/ml(Table4.8, appendix4). This is higher than what was reported by SSMO (2007) for pasteurized milk of *E.coli* which was zero.

Staphylococcus in this study at day4 was 4.46×10^2 and increased to 8.0×10^2 at day6 (Table4.8, appendix 4). Several studies showed a wide range of bacterial count, Aggad *et al.* (2010) found that 20% of the samples had a count of more than 10cfu/ml.

In this study the samples showed no growth of *salmonella* during storage period. This agrees with SSMO (2007) for pasteurized milk which was free from all pathogenic microorganisms.

Table 4.7: Chemical and physical properties during storage period of pasteurized milk

Parameter	Day 2(%)	Day 4(%)	Day 6(%)
pH	6.63±0.57	6.50±0.00	6.36±0.57
Acidity as lactic acid	0.17±0.00	0.18±0.05	0.19±0.00
Density	1.027±0.005	1.031±0.002	1.034±0.001
Fat	3.00±0.00	2.80±0.00	2.76±0.05
Protein	2.96±0.57	2.83±0.05	2.63±0.05
Lactose	4.10±0.01	4.00±0.05	3.90±0.01
SNF	9.30±0.05	8.61±0.02	8.53±0.05
T.S	13.00±0.02	12.90±0.00	12.80±0.30

Table 4.8: Microbiological characteristics during storage period of pasteurized milk

Test	Day 2	Day 4	Day 6
Total bacterial count	$4.7 \times 10^3 \pm 2.5 \times 10^2$	$6.3 \times 10^4 \pm 5.03 \times 10^3$	$8.6 \times 10^5 \pm 9.07 \times 10^5$
Total coliform	0.00±0.00	9.00±2.00	16.6±2.08
<i>E.coli</i>	0.00±0.00	2.30±2.08	7.33±1.52
<i>Staphylococcus</i>	0.00±0.00	$4.46 \times 10^2 \pm 1.52 \times 10^2$	$8.0 \times 10^2 \pm 10^2$
Salmonella	-ve	-ve	-ve
Yeasts and moulds	-ve	-ve	$4.0 \times 10^2 \pm 10^2$

CHAPTER FIVE

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

1. From the result and evaluation with the data reported in the literature, it is clear that application of HACCP system can improve the microbiological quality of pasteurized milk by control of critical points.
2. High quality pasteurized milk produced by FAABY contain low bacterial counts.
3. Values of chemical contents are within the standard limits.
4. The pasteurization operation plays an important role in the survival and destruction of different bacterial contamination.
5. The shelf life for pasteurized milk not exceed 4 days.

5.2 Recommendations

From this research it can be recommended that:

1. Education and training of the workers and handlers of milk is a must.
2. Application of HACCP system throughout the chain of pasteurized milk process should be encouraged.
3. Improvement of storage and marketing conditions of dairy products are required.
4. To avoid the spoilage of the product , the manufacturer should apply the CIP (clean in place) after each production.
5. Further studies are needed to confirm the HACCP system in pasteurized milk production in Sudan.

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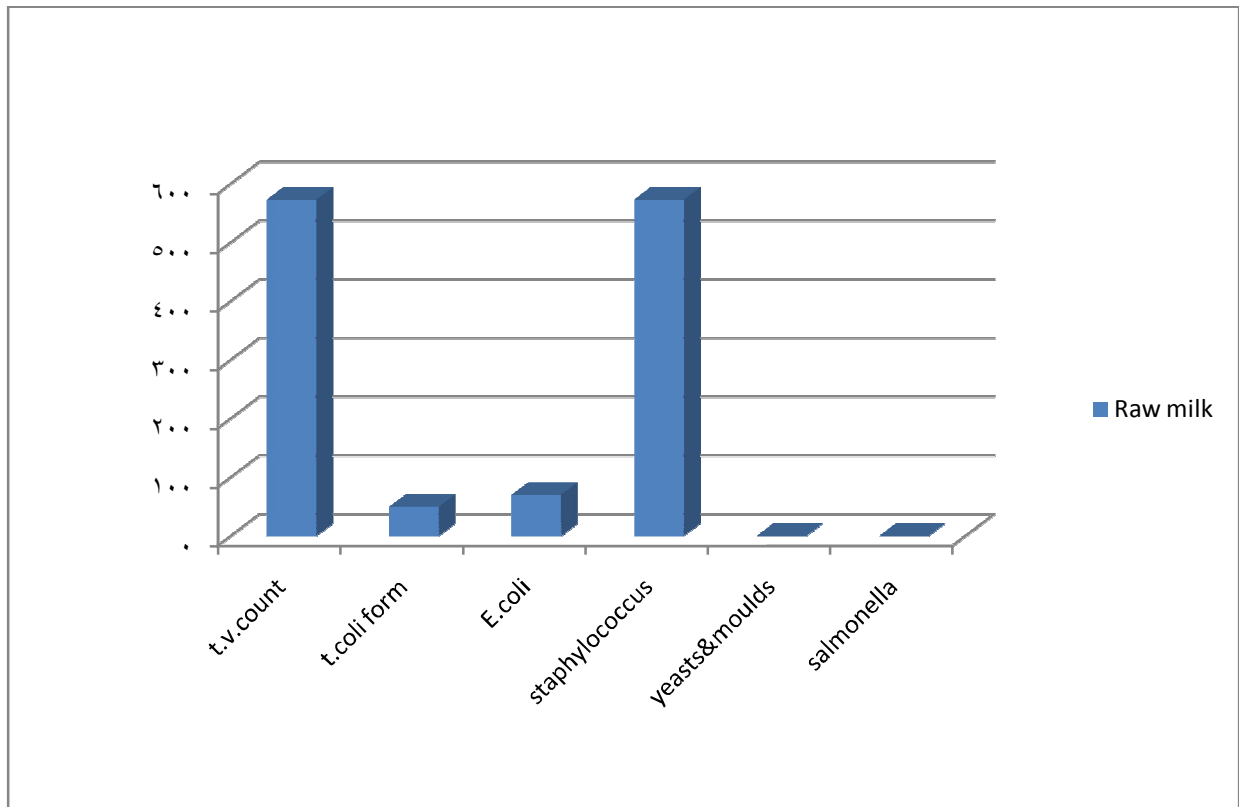
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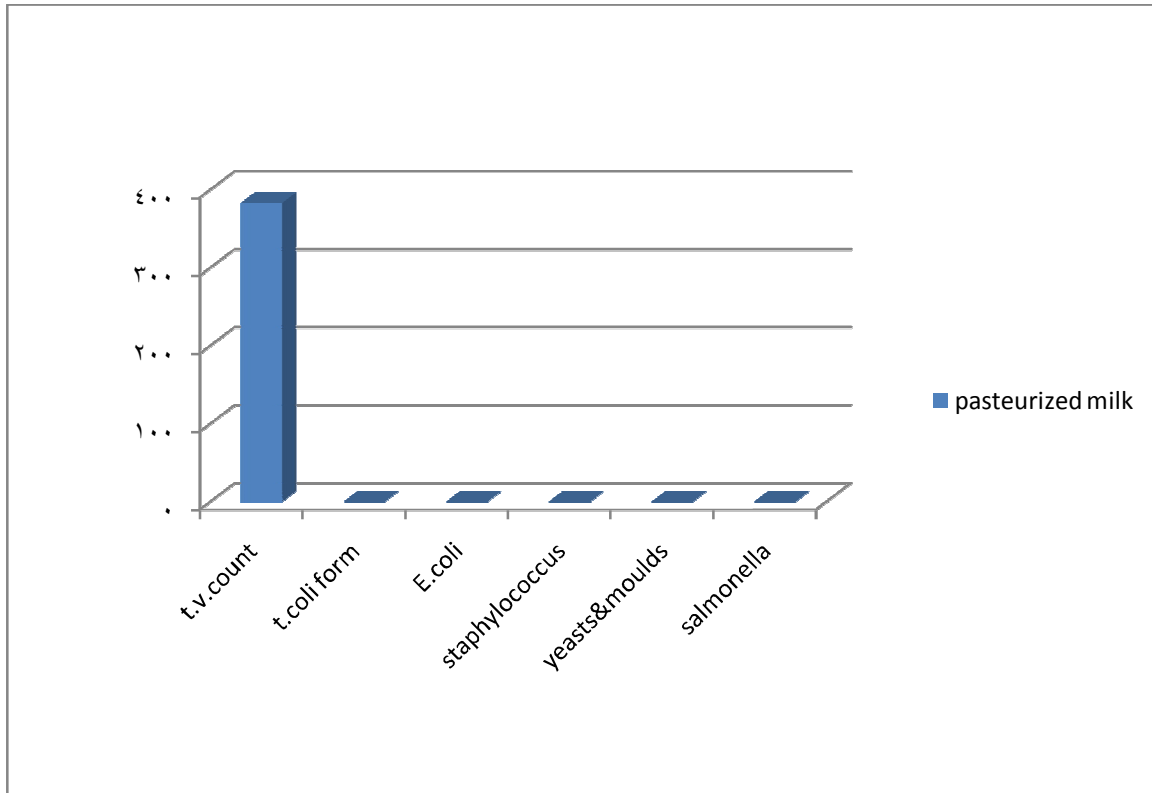
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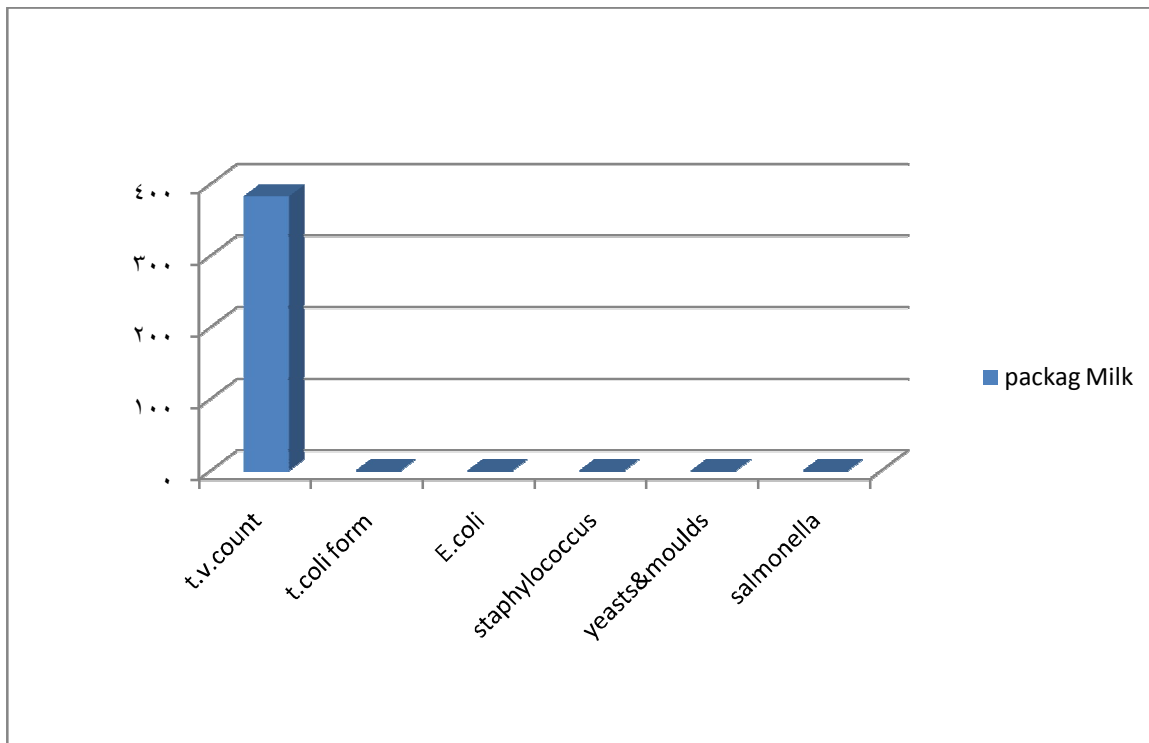
APPENDICES



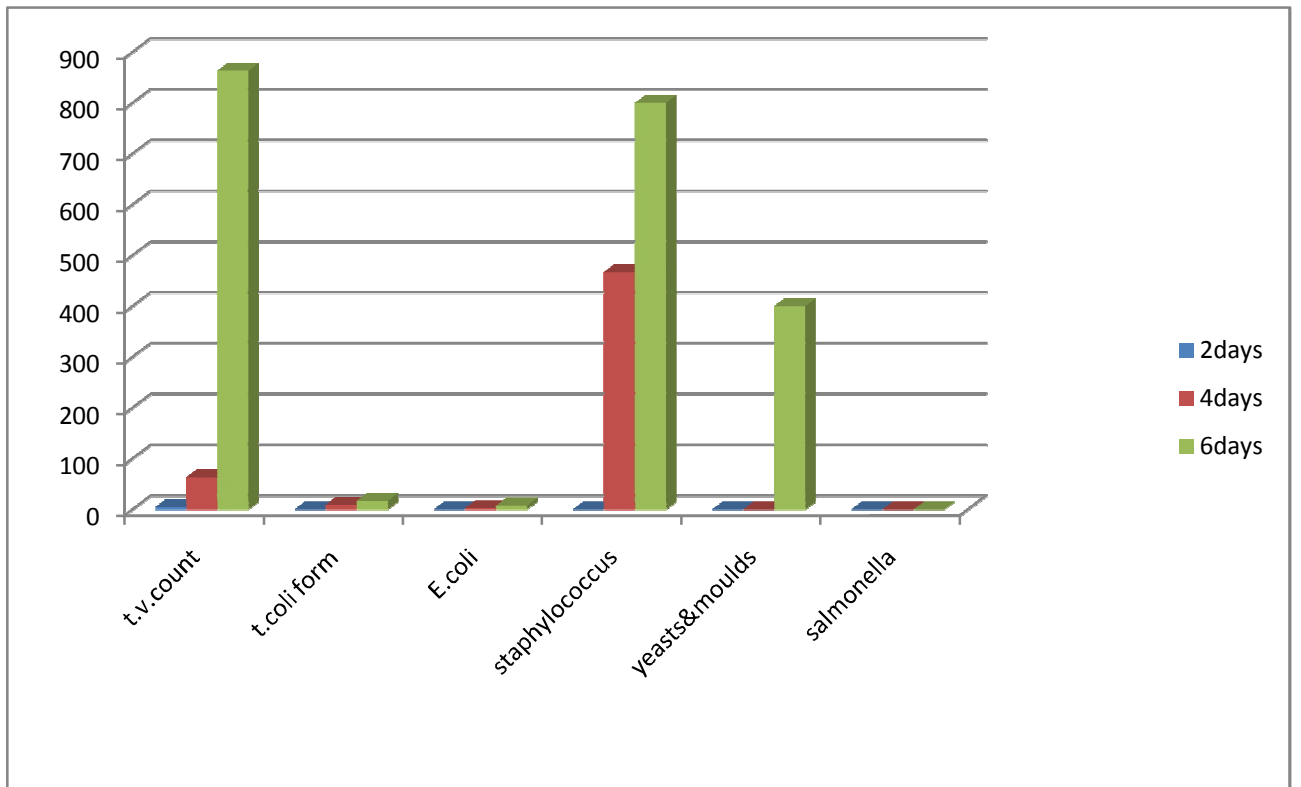
Appendix 1: Microbiological analysis of raw milk



Appendix 2: Microbiological analysis of immediately pasteurized milk

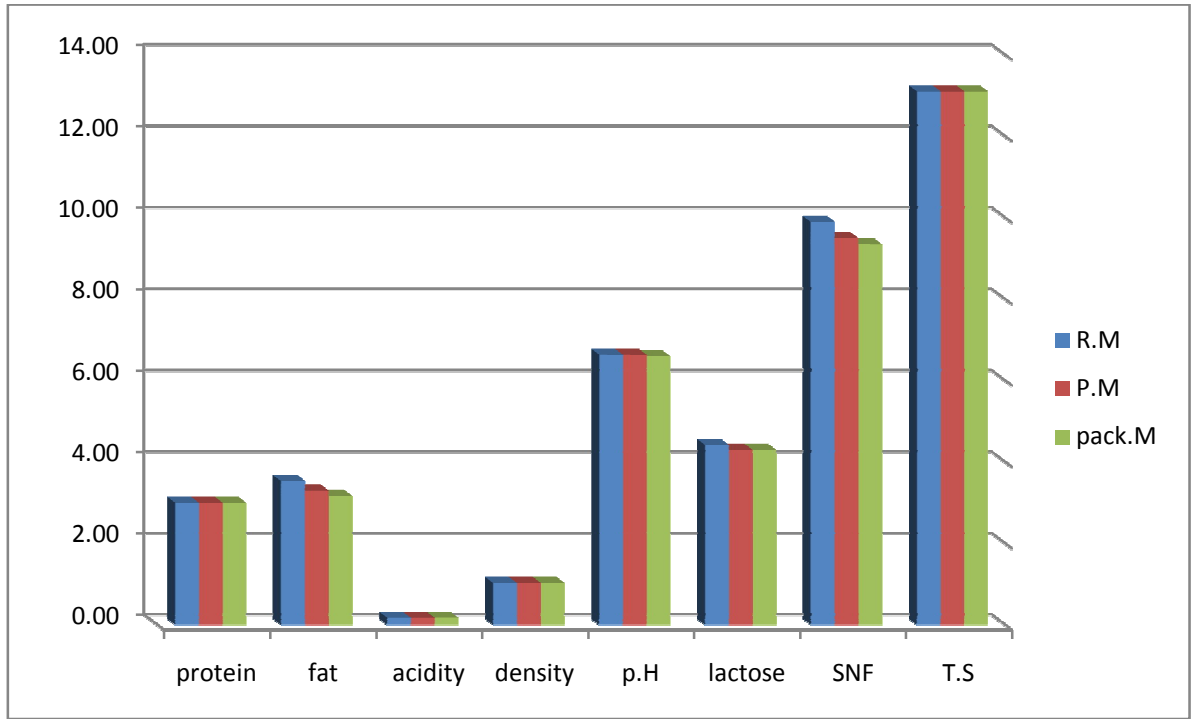


Appendix 3: Microbiological analysis of packed milk



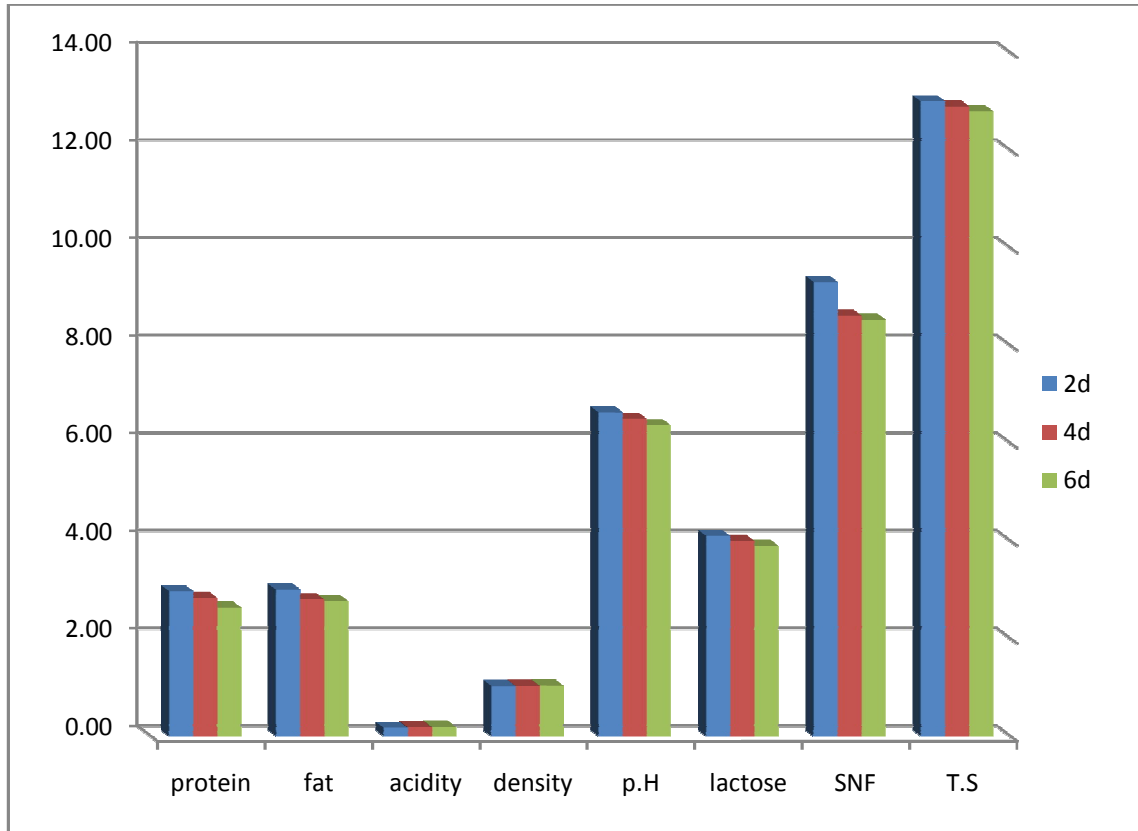
*T. bacterial (cfu/ml*10³) *staphylococcus at 2nd and 6th day (cfu/ml *10) and yeasts &moulds *10

Appendix 4 : Microbiological analysis during storage



*R.M= Raw milk *P.M = pasteurized milk *pack.M = packed milk

Appendix 5: Physicochemical analysis of raw milk, pasteurized milk and package milk



d= day

Appendix 6: Physicochemical analysis during storage