بسم الله الرحمن الرحيم

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Evaluation of HACCP System Implementation in Commercial Stirred Yoghurt and Awareness of Milk Producers on Good Production Practice in Khartoum State Farms

تقييم تطبيق نظام(الهاسب) في الزبادي التجاري المخلوط و وعي منتجي الألبان لممارسة الإنتاج الجيد في ولاية الخرطوم

Thesis Submetment in fulfillment of the requirement PhD. of food quality control

By

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الآية

قال تعالى:

(وَكَذَلِكَ أَوْحَيْنَا إِلَيْكَ رُوحاً مِّنْ أَمْرِنَا مَا كُنتَ تَدْرِي مَا الْكِتَابُ وَلَا الْإِيمَانُ وَلَكِن جَعَلْنَاهُ نُوراً نَّهْدِي بِهِ مَنْ نَّشَاء مِنْ عِبَادِنَا وَإِنَّكَ لَتَهْدِي إِلَى صِرَاطٍ مُّسْتَقِيمٍ)

صدق الله العظيم

سورة الشورى: ٥٢

DEDICATION

To

The sole of my beloved father

To my kindly lovely mother

To my helpful supportive wife love and my children

I dedicate this work...

ACKNOWLEDGEMENT

Thanks to Allah for health, assistance and patience that he has given me to complete this work

I would like to express detest gratitude and thanks for my supervisor Prof. Mohammed Abdel salam

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المستخلص

تمت دراسة تقييم نظام تحليل المخاطر وضبط النقاط الحرجة للزبادي المخلوط وأثر وعي ومعرفة منتجي الحليب لممارسة إنتاج الحليب الجيد (GPP) و المضادات الحيوية التي تعطي لا بقار الحليب (AMR) في مزارع ولاية الخرطوم . تم جمع المعلومات عشوائيا من 30 مزرعة ألبان بإستخدام الاستبيان في محلية شرق النيل بولاية الخرطوم – السودان. وجد أن نسبه 33% من المنتجين تتراوح أعمارهم بين 60 – 46 سنة ، واللذين يملكون قطيع من الابقار بين 25 –15بقرة يمثلون حوالي 43.3%.وأظهرت النتائج عدم وجود مساحه كافية بين الابقار داخل المزرعة 86.67%، كما لوحظ أن 60% من المزارع يزورها الطبيب البيطري فقط 4 مرات في العام عند الضرورة، و 40% يتلقون شرح من البيطري عن سلامة الاغذية و ممارسة الانتاج الجيد للألبان وأن معظمهم لم يتلقوا أي كورس تدريبي عن ذلك وهم يمثلون نسبة 93.3%، كذلك الكثير من المنتجين 40% لايعتقدون ان هناك إرتباط بين المضادات الحيوية التي تعطي للابقار بغرض العلاج والانسان.

أظهرت التحاليل الفيزيوكيميائية نتائج إيجابية للزبادي المخلوط بعد تطبيق نظام تحليل المخاطر و ضبط النقاط الحرجة بالمنشأة. حيث سجل رقم الحموضة 6.67% عند مرحلة إستلام الحليب، 4.63% في تنك التخزين ، 4.67%بمرحلة التحضين و 4.64%عندما كان المنتج جاهزا للتوزيع.وتأثر رقم الحموضة تأثيرا معنويا عند التسويق حيث اعطي4.30،4.21 و 4.20% في ثلاث أماكن تسويقية للمنتج. كما تم إختبار الحموضة المعايرة، محتوي الدهن، الكثافة النوعية، المواد الصلبه اللادهنية SNF والمواد الصلبه TSعند مرحلة الاستلام حيث سجلت 0.15%، 4.1%، 1.03%، 9.3%، 13.4% و لاوجود للمضادات الحيوية. ويعتبر قياس الحرارة مهم جدا حيث كانت 42 م° في تنك التخزين و 12.6 م° للمنتج

النهائي عند التوزيع، بينما كانت 17.4 و 19.1م° في أماكن التسويق. كانت اللزوجة عند مرحلة التوزيع (66.33cp) وسجلت (52.2cp، 42.7 و 33.8) في ثلاث أماكن تسويقية.

أظهرت التحاليل المايكرو بيولوجية أن العدد الكلي للبكتريا كان 1.62×10^5 عند مرحلة استلام الحليب و6.5×10^1 في تنك التخزين، كذلك لم يتم عزل أي من بكتريا الكوليفورم والفطريات والخمائر في جميع مراحل تصنيع الزبادي المخلوط باستثناء ظهور اعداد قليلة (عند المستوي المسموح به عالميا)من بكتريا الكوليفورم عند إستلام الحليب 3.66×10^4. ولتحقيق مبادئي الجودة الشاملة وتطبيق نظام تحليل المخاطر قيمت هذة الدراسة وعي ومعرفة صغار منتجي الالبان لممارسة الإنتاج الجيد وكيفية التعامل مع المضادات الحيوية التي تعطي لأبقار الحليب.

Abstract

This study was conducted to assess small urban dairy farmers' milking hygiene practices and awareness of cattle and milk-borne zoo noses in Khartoum, Sudan. Data were collected from a total of 30 randomly selected dairy farmers using structured questionnaire. The results of the study showed that all respondents were 46 to 60 years 33.3%, herd size 43.33% from 15 to 25. The results showed there was no species of animal than other cattle on farm 86.67%, and high frequency of veterinarian visits to the farm($P \leq 0.05$), then showed 60% less than 4 times/ year. The data also obtained 40% were sometimes discussing good production practicing (GPp) and food safety with the veterinarian ($P \leq 0.05$).The results showed 93.3% previously don't taken a education course and 76.5% didn't taken learn more about GPp and food safety. The producers who were don't think that about antimicrobial resistance (AMR) and making it harder to treat sick animals ($P \leq 0.05$). While others 40% producers don't think that about AMR in humans is linked to antimicrobial use in food animals were 13.3%.

The study was to evaluate the effect implement a HACCP program in a commercial stirred yoghurt and awareness of milk producers on good production practice (GPp) and antimicrobial resistance (AMR)in Khartoum state farms. The physicochemical analysis were showed a positive impact on the raw milk quality after the implementation of HACCP. The pH-values during manufacturing stages of stirred yoghurt was obtained 6.67 in stage receiving raw milk, the stage of buffer tank showed 4.63, followed by incubation 4.67 and distribution stage of stirred yoghurt showed4.64, as well as the pH value of stirred yoghurt products showed 4.30, 4.21and 4.20 where in different three area of markets, then showed significantly decrease ($P \leq 0.05$) comparing with final product factory. Also the titratable acidity, fat content, Specific density, Solid Not Fat, Total solid (TS) and

Antibiotic were investigated during manufacturing stages of stirred yoghurt at receiving raw milk, then were showed 0.15%, 4.1%, 1.03, 9.3%, 13.4 % and -ve respectively. The temperature during manufacturing stages of stirred yoghurt were showed 42℃ ± 0.67 in buffer tank and 12.06 ℃ ± 1.35 max 18 in distributions stage, while the temperature of stirred yoghurt products showed 19.1℃, 17.4℃ and 12.06 ℃ where in different three area of markets($P \leq 0.05$). The viscosity showed 66.33cp in distributions stage of factory and recorded (52.2cp, 42.7cp, 33.8cp) where in different three area of markets($P \leq 0.05$).

Microbiological analysis showed that the total number of bacteria was 1.62×10^5 of stirred yoghurt at the stage of receiving milk 6.5×10^1 in the tank storage, as well as, were not isolate any of the bacteria coliform, fungi and yeasts in all stages of the manufacturing except for the emergence small numbers of coliform bacteria when receiving milk 3.66×10^4 ($P \leq 0.05$). To achieve the Total Quality that embrace the results also were showed milk producers awareness and knowledge of good production (GPp) of in their farms.

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Introduction

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 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 (Scott and Stevenson, 2006; Musaj *et al*.,2009).

The food borne diseases continue to be one of the biggest problems for public hearth 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 infection, of whom 15% undergo hospitalization (Bijo and Malaj,2008). Food borne disease can be classified as either infectious or intoxicatious (Taylor, 2004; Musaj *et al*.,2009). Also in Sudan diseases with food origin stated to become a serious problem, even though very often are unknown. The main reason for this point is missing information about food safety and hygiene. In Sudan, as in the other countries in development, food safety is not priority yet.

Scott and Stevenson, (2006). Stated that, risk related to the production of food products can be reduced to an acceptable level or eliminated through the application of HACCP methodology.

Dairy products such as yoghurts, cheeses and ice creams contain nutrients such as proteins, vitamins and minerals. Consumption of dairy products been associated with decreased risk of osteoporosis, hypertension, colon cancer, obesity and insulin resistance syndrome (IRS). The main dietary source of calcium and vitamin D are dairy products (Weaver, 2003).

El Zubeir and Ahmed,(2007) who were assessment of chemical and microbiological quality of stirred yoghurt in Khartoum State, Sudan and concluded

that the overall picture of stirred yoghurt quality evaluation needs emphasis on quality control during processing and storage. Also standardization of milk for yoghurt manufacture should be observed to meet legal standards and adjustment of yoghurt mix should approach the standard of the yoghurt package label. The first aim of this study was to implement a HACCP program in a commercial stirred yoghurt factory and then to evaluate the program during certain critical stages of the manufacturing process. The second objective was to study the effect awareness and knowledge of milk producers on good production (GPp) and antimicrobial resistance AMR in Khartoum State farms.

Chapter one

Literature review

1.1 Background

Yoghurt is an acidified, coagulated product obtained from milk by fermentation with lactic acid producing bacteria. Of all cultured milk products, yoghurt is the most well known and most popular worldwide (Early, 1998). The culinary art of yoghurt making originated thousands of years ago. It is likely, however, that the origin of yoghurt was the Middle East, and the evolution of this fermented product through the ages can be attributed to the culinary skills of the nomadic people living in that part of the world. Although modern large-scale production is designed to handle thousands of litres per day, using highly sophisticated technology with mechanisation and automatisation, the basic principles underlying the manufacturing process, have altered little with time (Tamime and Robinson, 1999). In recent years it became increasingly important, for various reasons, to manage and control all the elements of food manufacturing processes. One approach in this regard, that is well established and implemented world-wide is the HACCP system (Tamime *et al*., 2002).

Flavour, texture and aroma of yoghurt vary dependent upon country of origin (as well as other factors including raw material formulation and manufacture process). In some areas, yoghurt is produced in the form of a highly viscous liquid, whereas in other countries it takes the form of a softer gel. Yoghurt is also produced in a drinking form and can be frozen or blended with other ingredients to create, for example, mousse type products, sorbet, yoghurt ice-cream or other forms of dairy dessert (Early, 1998).

The initial popularity of yoghurt in Western Europe owed much to the work of the Russian bacteriologist and 1908 Nobel Prize Laureate, Metchnikoff, who at the turn of the century studied the bacteria used to produce yoghurt. The attributed the

good health and longevity of Balkan peasants to the effects of certain bacteria in the yoghurt they consumed. He postulated the theory that prolongation of life would follow ingestion of a lactic acid bacterium named as Bulgarian bacillus. The presence of this organism in yoghurt was supposed to inhibit the growth of putrefactive organisms in the intestine. The *Bulgarian bacillus* is, in fact, *Thermo bacterium bulgaricum*, later designated as *Lactobacillus bulgaricus* (currently known as *Lactobacillus delbrueck*ii subsp. *bulgaricus*) (Tamime and Robinson, 1999).

Yoghurt is a very nutritious food and its continued consumption in the Western World owes much to the development of its health food image (Early, 1990). Consumption of yoghurt is highest in countries around the Mediterranean, in Asia and in Central Europe (Bylund, 1995).

The methods of production of yoghurt have, in essence, changed little over the years and although there have been some refinements, especially in relation to lactic acid bacteria, that bring about fermentation, the essential steps in the process are still the same, namely:

- raising the level of total solids in the processed milk to around 14 – 16 g / 100 g;
- heating the milk, ideally by some method that allows the milk to be held at high temperature for a period of 5 – 30 min; the precise time will depend on the temperature selected;
- inoculating the milk with a bacterial culture in which *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* are the dominant organisms;
- incubating the inoculated milk, in bulk or retail units, under conditions that promote the formation of a smooth viscous coagulum and the desired aromatic flavour/aroma;

- cooling and, if desired, further processing, e.g. the admixture of fruit and other ingredients, pasteurisation or concentration; an
- packaging for distribution to the customer under chilled conditions. At present there are many different types of yoghurt produced worldwide, and Tamime and Deeth (1980) have proposed a scheme of classification that separates all types of yoghurt into four categories based on the physical characteristic of the product. However, these products and in particular yoghurt are subdivided into different groupings based on the following aspects (Tamime and Robinson, 1999):
- legal standards (i.e. existing or proposed) to classify the product on the basis of chemical composition or fat content (full, semi-skimmed/medium or skimmed/low fat);
- physical nature of the product, i.e. set, stirred or fluid/drinking; the latter is considered stirred yoghurt of low viscosity;
- flavours (plain/natural, fruit or flavoured; the latter two types are normally sweetened); and
- Post-fermentation processing (vitamin addition or heat treatment).

Variations in milk composition, irregular behaviour of the starter organisms, faulty regulation of the incubation temperature, along with a number of other process variables, can all give rise to an end product that is deficient in respect of overall quality, and only a thorough understanding of the fermentation can provide an operative with foresight to reduce risk of product failure (Tamime and Robinson, 1999).

1.2 Process control and management tools

1.2.1 Process control

Process control can be defined as the management of all elements of a process that control the legality, safety, contractual, and commercial requirements of the product. The scope is, therefore, from farm to consumer and embraces raw materials, formulation, bacteriocidal or bacteriostatic treatments, plant and equipment hygiene, personnel practices and hygiene, packaging, distribution conditions, and consumer use (Jervis, 2002).

Historically, process requirements have evolved on a basis of need to respond to incidents of product failure and changing marketing criteria. Pasteurisation of drinking milk was, for example, introduced in the 1930's to address public health risks associated with changing patterns of milk distribution in cities. Global incidents of outbreaks of milk-borne disease in humans with "traditional" pathogens, the reality of various new emerging pathogens and the increased importance of bio-security have resulted, for example, in stricter control of plant and environmental hygiene, enhancement of process control to minimise risks and to review current process control parameters. In parallel with the emergence of public health failures has been the trend towards novel and efficient processes, changes in formulations to reduce manufacturing costs per unit of product by increased through-put on high capital plant, and the use of cheaper ingredients more likely to be obtained on a global basis. There has also been an ongoing trend towards healthier foods - for example, lower fat, less salt, and the elimination of preservatives. These factors, together with the commercial demand for longer shelf - life to accommodate consumer shopping patterns and reduce distribution costs, can have a significant effect on the microbiological stability of products. Clearly, process control requirements need to be constantly reviewed and amended to

accommodate change, and this should be done using a disciplined and documented approach that is amenable to constant review (Jervis, 2002).

Proper attention to such a broad scope requires a disciplined and documented approach. It is widely accepted in the food and dairy industry that the required disciplined approach is best provided by the HACCP procedure applied as an integral element of total quality management (TQM) principles, which include good manufacturing practice (GMP), good hygiene practice (GHP), and document control (e.g., ISO 9000 Quality Systems). HACCP is an internationally accepted hazard management tool that can be applied to all stages of food manufacture from farm to consumer.

1.2.2 Total quality management

Total quality management schemes address the approach that a manufacturing organisation needs to take to ensure product quality. They aim to involve every member of the organisation in the achievement of management objectives to produce safe, wholesome food, enhance customer satisfaction and confidence, and identify means of ongoing improvement. The fundamental requirements of the TQM approach are communication at all levels, so that process and product requirements can be translated from the corporate quality statement to the operatives running the process. TQM schemes embracing HACCP and document control form an important framework within which quality requirements can be communicated effectively and in a way that can be demonstrated and audited. The overall approach is summarised in (Jervis, 2002).

1.2.3 Risk analysis

Risk analysis is a structured and formalized approach to quantifying risk and setting levels to which casual agents should be controlled to assure safety. Risk analysis has three components: risk assessment, risk management, and risk communication. Microbiological risk analysis protocols are being addressed

internationally and at national levels, and they are becoming a key element in determining the level of consumer protection (Jervis, 2002). HACCP, correctly integrated into a total quality management scheme is normally the preferred risk management tool.

1.2.4 HACCP

The HACCP system offers a structured approach to the control of hazards in food processing and, properly applied, identifies areas of concern and appropriate control measures before product failure is experienced. The application of HACCP is systematic because structured hazard analysis and implementation are provided. The process is also logical in that each processor understands its own operation and is able to assess controlling the specific process optimally (Jervis, 2002).

1.3 The hazard analysis of critical control point system

The origins of HACCP are traced to the 1960's and the United States of America when the Pillsbury Company, the United States Army Laboratories at Natick, and the National Aeronautics and Space Administration collaborated to develop the system as a means of managing safe food production for manned space flights. The outcome was the HACCP concept, which has been adopted and developed to its current status as the food safety management tool recommended by the Codex Alimentarius Commission to advise on consumer protection under Sanitary and Phytosanitary Measures (1998) agreed at the Uruguay round of GATT negotiations. As such, HACCP is a reference point in international trade disputes, and it is increasingly enshrined in national legislation. The HACCP procedure is generally targeted at food safety management (pathogenic microorganisms and their toxins), but, as an approach in the context of broader quality management, it can be effectively applied to microbiological spoilage, foreign-body contaminations or pesticide contamination. It is preferable to conduct a HACCP program with a narrow scope (a single pathogen or possibly pathogens)

rather than attempt to cover an extended list of hazard areas when documentation will become complex. However, an experienced team might choose to cover the whole spectrum of hazard areas, depending on (a) the resources available to produce and maintain a composite HACCP plan and (b) the way in which it is to be incorporated into the local quality plan and quality system (Jervis, 2002).

1.3.1 Principles of HACCP

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

1. any potential hazards associated with yoghurt production from the collection of raw materials through to manufacture and distribution must be identified and an assessment made of:

- The likelihood that a given hazard will arise; and
- The preventative measures that are necessary to reduce any inherent risks.

2. the precise points in the above sequence that can be controlled in order to eliminate a hazard or minimise the risk of occurrence must also be identified. If failure to control a particular hazard is a risk to public health, then the step in the process is regarded as a critical control point (CCP); if no major risk is involved,

the step may be identified as a control point (CP). For example, the filling machine is a CCP, because contamination with a pathogen could present a direct risk to the consumer, whereas the failure to empty a waste bin in the same area could be treated as a CP because, however undesirable with respect to the growth of potential spoilage organisms, the failure is not likely to result in a consumer health problem. Similarly, it is important that a manufacturer has control over the chemical composition of yoghurt and the details on the label, but again such points need only be graded as CPs.

3. there must be an established set of targets which must be achieved in order for a Section to claim control over a CCP/CP, e.g. total colony counts on product contact surfaces (CCP) or the viscosity of stirred yoghurt with agreed tolerances (CP).

4. a monitoring system must be established to record the particular facets of production that are under control.

5.if the monitoring procedure indicates that a CCP/CP is not under control, then an agreed program of corrective action must be capable of immediate implementation.

6. there must be procedures for verification that the HACCP system is working throughout the factory, e.g. the introduction of supplementary checks to ensure that the principal components of the system are operating to the required standard.

7. a system of documentation must be in place that records accurately the details of all operations, e.g. times/temperatures and microbiological parameters, but also the responsibilities of the individual operators associated with that specific section of the process. In any HACCP system it is vital that the different stages, within each principle, be considered in order and that the required information and conclusion be completed for each stage before moving on to the next. HACCP is designed as a structured approach, and the proper sequencing of activities is crucial to obtain an

effective output. The seven HACCP principles and fourteen sequential stages are outlined in detail by (Jervis, 2002) .

1.3.2 Benefits of HACCP

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

· HACCP has the potential to identify all hazards in the manufacturing process so that controls can be established to assure food safety/quality.

· HACCP is a systematic approach relevant to all stages of food processing covering agriculture and horticultural practices, harvesting, processing, product distribution, and customer practices. HACCP is the preferred risk management tool in total quality management.

· HACCP focuses technical resources on critical parts of the process and provides a cost effective control of food-borne hazards.

· HACCP facilitates the move from retrospective end-product testing to a preventative quality assurance approach enabling the manufacturer to get it right the first time and reduce reject waste.

· HACCP recognized and promoted by international bodies (such as the Codex Alimentarius Commission) as the system of choice for ensuring food safety and is becoming enshrined in national legislation. Proactive application in the food industry will facilitate compliance with developing legislation and demonstrates a diligent approach to food safety (Jervis, 2002).

1.3.3 Application of HACCP

This section addresses in detail what needs to be done at each of the HACCP stages, and it refers to generic flow diagrams and HACCP plan records that have been produced in order to illustrate the points made. It is essential that each HACCP study be based on the specific process and product details, and generic

plans should never be adopted as a shortcut to save time and resources. The different sequential stages are as follows (SABS, 1999; Jervis,2002).

Stage 1: Define terms of reference Terms of reference should clearly define the scope of the intended HACCP study and address the following points:

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

Stage 2: Select a HACCP team, it is important that senior management in the company be made aware of the resources necessary to carry out an effective HACCP study (personal time, appropriate meeting room, secretarial support, and the need to consult outside resources for information) and are committed to providing these resources. The time required to complete the study will depend on the complexity of the process and the terms of reference agreed as Stage 1. If resources cannot be assured to meet the study defined in Stage 1, then the study should not be progressed. HACCP requires a multidisciplinary approach, and the HACCP team should include the following skills:

- A quality assurance/quality control specialist who understands the hazards and risks for the product and process under study. Depending on the study terms of reference, this might involve a microbiologist or chemist; and, if this resource is not available in-company, consultation with an eternal resource might be necessary to obtain information relating to microbiological risk and hazards;
- Aproduction specialist to contribute details of what actually happens on the production line throughout all shift patterns
- An engineer to provide information on (a) the operating characteristics of the process equipment under study and (b) the hygienic design of equipment and buildings; and
- Others co-opted onto the team as necessary. These might include specialist equipment operators, hygiene manager, ingredient and packaging buyers, and distribution managers. It might also be appropriate to consider co-opting specialist technicians from companies to which various scheduled maintenance and calibration functions are contracted (e.g., temperature measurement equipment, pasteurizer plate and jacketed silo integrity, clean in place systems). An individual experienced in HACCP should be nominated as chairman to be responsible for managing the study. The chairman should have received training in the principles of HACCP and be experienced in HACCP team work. While HACCP team members will be selected for their specialist knowledge, it is important that they will also have a working knowledge of the HACCP procedure so that they can contribute effectively to the study. Team members may need some training before commencement of the study, and this can be provided either internally by the HACCP team or externally.

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

Stage 3 : Describe of the product under study should be fully described. This stage often tends to be inadequately covered, but diligent attention to detail here is crucial to the identification of hazards. The product description should be considered against the following headings and recorded as HACCP study notes:

Composition. All factors that might influence the preservative characteristics of the food should be recorded. Basic compositional data should be noted including that on solids/moisture levels, fat levels, type of preservative, if used, etc. Compositional data should also be recorded for any additives used, particularly where these are supplied as fresh, hydrated materials.

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

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

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

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

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

Stage 4: Identify intended use

The consumer target group for the product should be noted. This will range from suitable for all consumer groups.

Stage 5: Construct a flow diagram

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

- the sequence of all process steps within the scope or the study including rework/recycle loops;
- interaction of services (e.g., cooling water, air, compressed air, clean-in-place systems);
- temperature/time history for all raw materials, intermediate products, and final product within the scope of the study, together with microbiological and analytical data with appropriate floor plans and equipment; equipment design with particular attention to ease of cleaning and presence of void

spaces that might accumulate contamination; and personnel and hygiene disciplines.

Stage 6: On-site confirmation of flow diagram

The flow diagram produced at Stage 5 should be confirmed, on site, by the HACCP team. Points to be confirmed are that any effect of shift patterns and weekend working are included on the flow diagram, together with circumstances of any reclaim or rework activity that might be introduced from time to time. If the HACCP study is being applied to a proposed new process line/product, flow diagram confirmation will not be possible. In this case the HACCP plan can be completed, but it must be subject to review as the line/product is finalised.

Stage 7: List all potential hazards associated with each process step, conduct a hazard analysis, and consider and measures to control the identified hazards. This is the final stage in HACCP Principle 1 (Conduct a hazard analysis), and it should be emphasised that no attempt should be made to pre-empt HACCP Principle 2 without considering the critical control points. Stage 7 consists of three parts: listing hazards, conducting a hazard analysis and identifying control measures.

List all potential hazards. The flow diagram (Stage 5) and the product description (Stages 3 and 4), should be used to list all potential hazards relevant to the terms of reference of the study (Stage1). This activity should involve all disciplines in the HACCP team (QA/QC, production, engineering) in a "brainstorming" session that identifies all actual and potential hazards. It is important that the following areas are considered:

- hazards in raw materials;
- hazards introduced during the process (cross-contamination, factory environment, equipment design, equipment cleaning, and introduction by process air or personnel)
- hazards that survive the process steps; and

- the microbiological stability of the product during distribution and in the home. In these considerations, the intrinsic factors of the product (e.g., pH, structure, preservatives, temperature) will be important from the point of view of both (a) the lethal effect of a heating or other process and (b) the way in which the potential for pathogen multiplication might occur before consumption. It is emphasised here that all potential hazards should be listed. This requirement should not be undermined by the concept of Prerequisite Programs that is being developed by Codex Alimentarius and actively applied in some cases. Hazard analysis. The process of collecting and evaluating information on hazards and conditions leading to their presence and to decide which are significant within the scope of exercise should be addressed in the HACCP plan. The objective of Stage 7 is to consider all of the potential hazards identified and identify those that need to be eliminated or reduced to an acceptable level if food, meeting the established Food Safety Requirements (or any other objective set out in terms of reference), is to be produced. To a large extent expert judgement and opinion will be involved and, if the necessary expertise is not available in house, external experts may need to be consulted or co-opted to the HACCP team hazard analysis should consider the following points:
- The consequence of the target micro-organism(s) or toxins being present at harmful levels in the final product at the point of consumption.
- The likelihood of the target micro-organism(s) or toxins being present at harmful levels in the final product at the point of consumption. Conclusions for this and the previous point might be based on previous company or industry experience, on epidemiological data, or on a microbiological risk assessment output.

- The survival and/or multiplication of target micro-organism(s) in the product or the potential for production of the toxin that will persist to the point of consumption at significant (toxic) levels.
- The hurdle effect (the synergistic preservative effect of two or more inhibitory factors) is relevant to these assessments. It should be noted, however, that unless the conclusions with respect to the ability of a formulation to inhibit the growth of, or eliminate, the target micro-organism is definitive, it might be necessary to carry out "spiking trials" to validate the formulation.
- The numbers of consumers potentially exposed and their vulnerability.
- Any relevant food safety objectives or manufacturer's food safety requirements. The data from microbiological risk assessments, in the context of risk analysis, will be useful in the hazard analysis stage of HACCP. In the absence of a formal risk analysis output, hazard analysis in HACCP will be made on quantitative data with appropriate expert input and/or reference to external data sources.

Identification of control measures. For each of the hazards concluded to be significant in the hazard analysis, the HACCP team should identify control measures that will eliminate the hazard or reduce it to an acceptable level. There may be more than one control measure required to control a hazard. In other cases, one control measure at a single point can control more than one hazard (e.g., pasteurisation eliminates all vegetative pathogens and spoilage micro-organisms).

One control measure can be relevant to several process steps where a hazard is repeated (e.g., application of CIP cleaning or environmental cleaning to control recontamination). Where no control measure can be identified to control a hazard, redesign or modification of the process or product formulation may need to be considered. A final point to note is that in identifying control measures in a

HACCP study on an established product and process, the team should not restrict consideration to measures already in place but should be prepared to propose other control measures that might be appropriate.

Stage 8: Determine CCPs (Principle 2)

The objective of Stage 8 (Principle 2) is to systematically assess the hazards and related control measures identified in step 7 by considering each process step (as recorded in the flow diagram) in turn and reaching a conclusion on its "CCP" status before moving on to the next process step- that is, to identify process steps at which control can be applied and which are essential to prevent or eliminate a hazard or reduce it to an acceptable level.

Stage 9: Establish critical limits for each CCP (Principle 3)

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

Stage 10: Establish a monitoring system for a CCP (Principle 4)

Monitoring involves a planned sequence of observations or measurements against critical limits to assess whether a CCP is under control. Ideally, monitoring should identify a trend toward a critical limit maximum or minimum so that corrective action can be taken before the process is out of control and, in any event, should aim to identify violation of critical limits as soon as possible to minimize the amount of embargoed/rejected product. Monitoring can be on-line with automated corrective action (e.g., flow diversion systems on pasteurisers), or they can be off-line when corrective action might involve the rejection of any product

implicated. Physical and chemical measurements are preferred to microbiological testing because they can be completed rapidly and often be indicative of conditions that control the microbiology of the product (e.g., phosphatise test on pasteurised milk).

Stage 11: Establish a corrective action plan (Principle 5)

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

Stage 12: Verification (Principle 6)

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

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

- a review of validation records and, if appropriate, the application of more searching tests at selected CCPs to confirm the efficacy of the control measure.

Stage 13: Establish documentation and record keeping (Principle 7)

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

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

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

Procedures cover the following:

- training for hygiene and operation;
- personnel hygiene and sickness reporting;
- On-site food services; Answer each question in sequence at each process step for each identified hazard.
- use of protective clothing;

- inspection and maintenance of equipment, manufacturing services (water, compressed air, drainage), and the building/site;
- raw materials/ingredients - specification/audit/sourcing;
- waste disposal; and
- Cleaning - equipment/environment; CIP /manual.

In all cases the procedures should state clearly what should be done, how equipment or materials should be used, and by whom and how defects should be recorded, remedial action initiated, and action signed-off when completed. As with guidelines, current procedures should be reviewed in an HACCP study and modified, if necessary, on the basis of the hazard analysis.

Work instructions give detailed instruction to employees as to what has to be done at each process step. This will include, as appropriate, equipment manufacturer's instructions, product recipe (ingredient quantities, process times and temperatures, routing of intermediate product and final product through the factory), and action to be taken in abnormal circumstances.

Monitoring record sheets should be prepared to support, as necessary, work instructions, preferably with critical limits shown, and instructions on how to complete them and action to be taken if critical limits are challenged (process adjustment and/or notify management). The work instructions should be generated directly from the HACCP plan, and the monitoring record sheet gives the detail that would otherwise complicate HACCP documents. Furthermore, there should be a clearly defined mechanism by which abnormal results are notified on an exception reporting system that calls for a traceable record of corrective or remedial action taken and the outcome of these actions, signed off at a designated management level.

HACCP study notes. While the HACCP plan should be issued as a controlled document as part of site quality systems, it is important that the background notes

made during the HACCP study be kept as a file for reference in HACCP review or trouble-shooting exercises. As a minimum, these notes should include the following:

- product description notes (Stage 3);
- basis for decisions taken in Stage 7 (Hazard analysis);
- a note of any "judgment" decision taken at Stage 8 (Determination of CCPs), together with data referred to and/or external expert advice source;
- recommended verification schedule (Stage 12);
- notes on any verification exercise undertaken (Stage 12);
- a schedule of other quality system documents that are derived from/support the HACCP plan; and
- Data derived from HACCP reviews.

Stage 14: Review of HACCP plans

The review of a HACCP plan evaluates any changes in process, product, or manufacturing site against the current HACCP plan to determine whether new hazards have been introduced that are not covered by existing control measures at critical control points or control points. HACCP study notes will afford a valuable background to the review process. If new hazards that are not adequately controlled are identified, the HACCP plan should be amended accordingly and notes of the review should be added to HACCP study notes. HACCP plan reviews should be triggered under the following circumstances:

- by routine schedule at a frequency determined by the HACCP team based on risk;
- change in product formulation;
- change in process;
- change in raw materials;

- change in consumer use/longer shelf life assigned;
- evidence of health or spoilage risk in the market place;
- emergence of “new” food- borne pathogens;
- change to factory layout and environment
- modification to process equipment;
- changes in packaging, storage, and distribution;
- change in cleaning and sanitation program;
- change in staff levels and responsibilities; and
- verification findings.

1.4Yoghurt

Yoghurt is a milk product obtained by the fermentation of milk by the action of symbiotic cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* and resulting in reduction of pH with coagulation. These starter micro-organisms are normally viable, active and abundant in the product to the date of minimum durability. If the product is heat-treated after fermentation the requirement for viable micro-organisms does not apply (FAO/WHO, 2002).

Yoghurt is the best known of all fermented milk products and the most popular almost all over the world. Yoghurt was first manufactured in South Africa in the mid – 1950’s (Clover, 1999) in Durban. Pietermaritzburg and Mayfair (NCD), Johannesburg, it was initially produced in half pint glass bottles and the inoculated yoghurt milk incubated in water at the desired temperature and after fermentation, cooled with chilled water (Hall, 2004). Since then, and especially during the last two decades, the production and consumption of yoghurt is steadily growing in South Africa. The annual consumption of yoghurt in 2002 / 2003 amounts to nearly 67 million litres, which represents an increase of 10%, compared to the consumption during 2001/ 2002 (Coetzee, 2004a). The demand for yoghurt is also

illustrated by the fact that nearly 45 000 litres of yoghurt has been imported between January and October 2003 (Coetzee, 2004b). It is estimated that the market share for yoghurt in South Africa is R1003 million annually.

1.4.1 Yoghurt types

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

b. Stirred yoghurt: Yoghurt is first made in a large container and then spooned or otherwise dispensed into secondary serving containers. The consistency of the "set" is broken and the texture is less firm than set yoghurt. This is the most popular form of commercial yoghurt.

c. Drinking sweet yoghurt: Stirred yoghurt to which additional milk and flavours are mixed in. Fruit or fruit syrups are added to taste. Milk is added and mixed to achieve the desired thickness. The shelf life of this product is 4–10 days, since the pH is raised by fresh milk addition. Some whey separation will occur and is natural (Ramesh and Charles, 2008).

d. Fruit yoghurt: Fruit, fruit syrups, or pie filling can be added to the yoghurt. They are placed on top, on bottom, or stirred into the yoghurt (Robinson and Tamime, 1986).

e. Yoghurt cheese: It is a fresh cheese made by draining overnight by separating the whey. The flavour is similar to that of a sour cream with the texture of a soft cream cheese. A litter of yoghurt will yield approximately 500 mL of cheese. Yoghurt cheese has a shelf life of approximately 7–14 days when wrapped and placed in the refrigerator and kept at less than 4°C (Keceli *et al*., 1999).

f. Frozen yoghurt: After manufacturing yoghurt, it is frozen by batch or continuous freezers.

g. Dried yoghurt: Yoghurt is sun dried for longer preservation.

1.4.2 The manufacture of yoghurt

Fermentation is one of the oldest procedures for transferring raw materials of plant or animal origin into products with extended shelf-life, and it is assumed that the fermentation of milk dates back approximately 10 000 years (Stanley, 1998 and Smit, 2003). The term 'fermented milk' or 'cultured milk' refers to products such as yoghurt, sour milk, cultured buttermilk and sour cream, which are usually made from cows' milk by pure lactic acid fermentation. Additionally, some products are made from milk from other species such as ewes, goats or mares, and combined fermentation (by, e.g., lactic acid bacteria and yeasts) results in products known as kefir or koumiss. Yoghurt represents the most popular fermented milk product worldwide and originates from countries around the Balkan and the Eastern Mediterranean Sea (Staff, 1998; Walstra *et al*., 1999).

Generally Smit (2003) stated that yoghurt is manufactured from preheated milk, with fat and dry matter content varying with respect to region and legislation, either in the plain form or with added material such as fruits or fruit premixes, sugar, cereals, or additives such as gelling agents, flavourings or colourants. Legislation and codex regulations differ widely around the world; in the one or other country, the use of additives is prohibited, or the presence of a certain number of viable starter bacteria in yoghurt is required (e.g., 107 bacteria per gram in the USA; Mistry, 2001).

Consumption statistics for fermented milks show highest per capita consumptions throughout Europe and a continuous growth in nearly all major markets. Exceptions are countries with an already existing high consumption level, such as the Netherlands and Iceland. Generally speaking, cultured or fermented milk products are made by inoculation of milk with a specific combination of microorganisms, which are able to convert lactose into lactic acid. Milk is a complex fluid with highly amounts of proteins and minerals which, as it is

intended to nourish young mammals, varies in composition according to the species' needs (Smit, 2003). Especially the major part of the milk proteins, the casein, which occurs in conjunction with calcium phosphate in the form of colloidal particles 100–500 nm in diameter and of MW approximately 108 is of great importance for the functional behaviour of the final acidified product. The colloidal calcium phosphate (CCP) plays an important role in maintaining the integrity of the casein micelles, which are in dynamic equilibrium with their surroundings. Therefore, a lot of structural research has been undertaken to explain the mechanisms of the stability of casein micelles and their sub-units, irrespective of whether or not these are present in the form of sub-micelles (Schmidt and Both, 1982; Walstra, 1990; Rollema, 1992; Visser, 1992; Holt, 1993; Horne, 1998; Walstra *et al*., 1999).

During fermentation of yoghurt, the milk sugar in the base milk is partially converted into lactic acid by the action of various enzymes, originating from the growth of thermophilic lactic acid bacteria. This causes a sufficient decrease in the pH, resulting in a dissociation of the CCP, a destabilisation of the casein micelles and even some liberation of individual casein molecules, accompanied by reaching a maximum in voluminosity (Dalgleish and Law, 1988; Lucey and Singh, 1998). Below a pH of 5.5 the casein micelles begin to swell and, as almost all CCP is dissociated, start to precipitate.

This precipitation leads to a sufficient decrease in the voluminosity of casein micelles (van Hooydonk *et al*., 1986) and to the formation of clusters and chains that link together to form a gel, composed of a continuous three-dimensional network with the milk serum containing whey proteins, lactose and salts entrapped as liquid phase (the amount of whey proteins depends on heat treatment; see below). Electron microscopy shows the particulate character of acidified milk gels

with empty spaces or pores in the network where the serum was entrapped (Kalab, 1979, 1993; McManus *et al*., 1993).

The classical yoghurt starter culture is a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, with a cocci–rods ratio of usually 1:1 (Hassan and Frank, 2001; Hutkins, 2001). These organisms grow in a protocooperative relationship, resulting in rapid acidification by stimulating each other. Depending on type and activity of the starter cultures, other metabolites such as carbon dioxide, acetic acid, diacetyl, acetaldehyde, large molecular weight exopolysaccharides or several other compounds are produced besides lactic acid, resulting in the characteristic properties of the products regarding flavour, texture and aroma. Since *Streptococcus thermophilus* is weakly proteolytic its growth is stimulated by the rods, which liberate free amino acids and small peptides from casein. The *cocci* in turn encourage the growth of *Lactobacillus delbrueckii* ssp. bulgaricus by producing formic acid and carbon dioxide (Matalon and Sandine, 1986; Rajagopal and Sandine, 1990). Nowadays, microorganisms such as Bifidobacterium spp. And Lactobacillus acidophilus is often added for therapeutic purposes (Yucuchi *et al*. 1992; Mistry, 2001).

Generally base on the accumulating knowledge from well-defined, randomised and placebo-controlled studies, health-promoting effects of some strains used for yoghurt fermentation become more and more evident. Because of their slow acid production, these bacteria are usually used in combination with classical yoghurt starters, resulting in so-called ‘yoghurt-like products’; depending on local legislation, this distinction might be of great importance (Marshall and Tamime, 1997; Hassan an Frank 2001).

Lactic acid bacteria that produce high molecular weight extracellular polysaccharides (EPS) are now commonly used in the yoghurt industry to improve

product texture, partly replacing the addition of stabilisers and gelling agents, by enhancing yoghurt viscosity, independent of the fat content.

1.4.2.1 Manufacturing methods vary considerably

for example, depend on the country, the type of product manufactured, the raw materials used and the product formulation. However, a number of common principles are generally applied (Staff, 1998):

· The total solids content of the base milk is increased to enhance the water holding capacity of the product.

· A heat treatment of the base milk, usually >80ºC for some time, is applied to achieve a proper denaturation of the whey proteins, also increasing the water binding capacity.

· Inoculation with a specific starter culture and subsequent incubation with a time-temperature profile depending on the properties of the starter, and on technical requirements.

· Cooling and addition of appropriate ingredients (fruit premixes, flavours).

· Packaging and chilled storage.

Yoghurt types are usually distinguished according to their physical state in the retail container, which results from differences in the manufacturing process. Apart from set yoghurt and stirred yoghurt, with production figures varying from country to country, there is a generally increasing demand for yoghurt drinks consisting of yoghurt mixed with skimmed milk, whey or water, and of yoghurts with increased shelf-life such as frozen or the rmised yoghurt (Smit, 2003).

1.4.2.2 Incubation of set yoghurt

Takes place in retail containers (plastic cups or glasses of different sizes) until the required pH (around 4.4–4.7) is reached, leading to an undisturbed gel. The viscoelastic gel network consists of aggregated spherical casein particles forming a continuous structure and enclosing fat globules and serum. From a structural point

of view, yoghurt belongs to particulate gels with disordered structures (Walstra *et al*., 1999). Stirred yoghurt is incubated in large fermentation vessels; the formed gel is then gently stirred to obtain a smooth and viscous, but still pourable, product, and finally packed. By breaking up the gel, a highly viscous, non-Newtonian liquid is formed and showed a strongly shear-rate and time dependent flow behaviour (Tamime and Deeth, 1980).

Drinking yoghurt is produced from low solid milk on the basis of the stirred manufacture process, or regular stirred yoghurt is diluted to some extent. Increased shelf-life of yoghurt may be achieved either by freezing or by thermisation of the fermented product. Whereas the thermisation process is designed to reduce the number of potential spoilage microorganisms and, therefore, results in a partial inactivation of the starter culture, the freezing procedure, provided that appropriate methods are applied, leaves the culture bacteria viable. In frozen yoghurt, higher amounts of sugar and stabilisers are required to maintain the air bubble structure during the freezing process (Tamime and Deeth, 1980).

1.4.3 Quality control in yoghurt manufacture

The quality of any food product can be defined against a wide range of criteria, including, for example the chemical, physical, microbiological and nutritional characteristics, or simply in relation to its overall appeal to potential consumers. As a result, quality has to be judged by a range of tests with varying degrees of objectivity, and yet all of them can be useful in ensuring that a product:

- is safe for human consumption with respect to both chemical or microbial Contamination;
- conforms to any regulations enshrined in law, or advisory/statutory requirements laid down by public health or other local authorities/agencies;
- is capable of achieving a specified shelf life without spoilage;

- Has as high an organoleptic standard as can be achieved within the existing constraints of manufacture or marketing.

An examination of some of these points implies, naturally enough, a critical laboratory assessment of the retail product, but it is essential to bear in mind that the end product can only be as sound as the raw materials from which it is made and, in hygienic terms, as "clean" as the plant in which it was manufactured. This breadth of potential for conflict means that quality control must be regarded as an all-embracing concept and, furthermore, one that demands constant attention. Thus, enthusiasm in response to a crisis is of little value in maintaining standards and the successful companies are those that rate quality appraisal as a high priority. Even small firms with minimal facilities can achieve a great deal by maintaining records of simple features like incubation times, product acidity and so on, and even though the services of a consultant may be required for more specialised examinations, the value of routine monitoring should never be underestimated. Indeed, routine has become the lynchpin of successful manufacture and is enshrined in two compatible and, to some extent, overlapping concepts – good manufacturing practice (GMP) and the hazard appraisal (analysis) critical control points (HACCP) system. Specifically for yoghurt, there are codes of practice that may or not be observed according to views of the producer (MAFF, 1975;DTF, 1983). In all European Union (EU) countries, labelling is covered by Council Directive 79/112 (EU, 1979) and most producing regions will have similar patterns of legislation (Pappas, 1988; Anon., 1998; Glaeser, 1992).

Assuming that, in theory at least, neither the product nor the packaging contravenes any of these Regulations, and then the manufacturer must be able to demonstrate that compliance with the Regulations is being achieved in actual practice. The key word is, of course, demonstrate, for while it is anticipated that any manufacturer can produce a faulty batch of produce, what the same

manufacturer must be able to show is that the fault arose despite due diligence being shown by all concerned. It was this blanket responsibility that gave rise to the HACCP concept, and the basic principles of the system are now widely accepted as the basis for responsible operation of a factory.

1.4.4 HACCP in yoghurt products

The system was applied based on the applicable laws, regulations and other standards. The manager of the dairy HACCP began to see it as a working tool, by creating conditions for program implementation. Initially, the HACCP team made a balance to determine the scope of the enterprise and the aims towards successful implementation of the program. The program was led in the preparation of HACCP for the production and all measures of risk control were separately documented. In this study the receipt of fresh milk and pasteurization were identified as critical control points. For the verification of the program, microbiological, physical and chemical analyses were conducted periodically. The samples were taken in different phases of the process of production of yoghurt and were analyzed before and after the implementation of the HACCP program. The implementation of HACCP in a microbiological aspect has had an influence of the fresh milk as raw material. The results were also affected by the successful implementation of GMP, GHP. The program positively influenced the microbiological quality in the assessment of the final product. The implementation of the system resulted in the decrease of complaints from the customers in terms of quality of safety of the product.

1.4.4.1 Principles of HACCP of yoghurt

In theory, the only way of ensuring that every carton of yoghurt from a given production line is safe, from a chemical or microbiological standpoint, is to test every carton. Clearly, such a suggestion is totally ludicrous, so that instead, a representative group of cartons is withdrawn against a sampling plan appropriate

for the product and the history of the plant. However, whilst this approach is essential to confirm that preset standards of hygiene are being met and that potential contaminants are at a low level or absent, the procedure can never prevent some spoiled cartons from reaching the consumer. Consequently, the emphasis within quality assurance has turned to the avoidance of problems, a concept that forms the basis of HACCP.

The HACCP system aims to identify specific hazards that, if they arose, could adversely affect the safety of a food and to put in place a procedure that will either prevent a hazard arising or will be able to control the situation in a manner that reduces the risk to the consumer (Vazquez, 1988; Pierson and Corlett, 1992; Corlett, 1992;WHO, 1993; Asperger, 1994; Mortimore and Wallace, 1994; IDF, 1994a; van Schothorst and Kleiss, 1994; Loken, 1995; FAO, 1995; Anon., 1997a, 1998a). In particular, the system identifies seven aspects of production that merit constant attention and these aspects are enshrined in seven principles:

• First – any potential hazards associated with yoghurt production from the growth/collection of raw materials through to manufacture and distribution must be identified and an assessment made of: (a) the likelihood that a given hazard will arise, and (b) the preventative measures that are necessary to reduce any inherent risks.

• Second – the precise points in the above sequence that can be controlled in order to eliminate a hazard or minimise the risk of occurrence must also be identified. If failure to control a particular hazard is a risk to public health, then the step in the process is regarded as a critical control point (CCP); if no major risk is involved, the step may be identified as a control point (CP). For example, the filling machine is a CCP, because contamination with a pathogen could present a direct risk to the consumer, whereas the failure to empty a waste bin in the same area could be treated as a CP because, however undesirable with respect to the growth of

potential spoilage organisms, the failure is not likely to result in a consumer health problem. Similarly, it is important that a manufacture has control over the chemical composition of a yoghurt and the details on the label, but again such points need only be graded as CPs.

• Third – there must be established set of targets which must be achieved in order for a Section to claim control over a CCP/CP, e.g. total colony counts on product contact surfaces (CCP) or the viscosity of stirred yoghurt with agreed tolerances (CP).

• Fourth – a monitoring system must be established to record that particular facets of production are under control.

• Fifth – if the monitoring procedure indicates that a CCP/CP is not under control, then an agreed programme of corrective action must be capable of immediate implementation.

• Sixth – there must be procedures for verification that the HACCP system is working throughout the factory, e.g. the introduction of supplementary checks to ensure that the principal components of the system are operating to the required standard.

• Seventh – a system of documentation must be in place that records accurately the details of all operations, e.g. times/temperatures and microbiological parameters, but also the responsibilities of the individual operators associated with that specific section of the process.

At first glance, this approach may appear daunting but, if each stage in a manufacturing process is identified and considered as a separate entity, then isolating the areas of risk can bring considerable benefits to a manufacturer. For example, retailers have confidence in a company that has proper control over its manufacturing procedures and, for this reason, the introduction of HACCP is fast becoming essential of operation in the commercial world. It is important, however,

that no two production plants are ever identical, and hence the personnel responsible for routine examinations must exercise their discretion as to which tests are both desirable and feasible in a given situation (Cullor, 1997; Gardner, 1997).

Although the systems employed to monitor the quality of yoghurt fall within the HACCP umbrella, each aspect of production has, by its very nature, to be assessed in a different way, and hence it is appropriate to deal with the separate facets of quality on an individual basis. It is relevant in this context that, although quality control is a broad concept, hygiene is inevitably a dominant feature and excellent accounts of the principles and practice of microbiological quality control in the dairy industry have been published by Lück and Gavron 1990 and IDF (1992e); anyone likely to be concerned with the hygienic aspects of production would be well advised to consult these works.

1.4.5 Stirred yoghurt

After fermentation of yoghurt in large vats, the gel is broken by stirring, thus forming a viscous non-Newtonian liquid, which is strongly shear-rate thinning.
Defining the stirring regime is a crucial process which induces considerable changes in the rheological properties of the final product. At a given shear rate, the apparent viscosity of stirred yoghurt depends on the firmness of the gel before stirring, giving higher viscosity with higher firmness. Additionally, higher gel firmness allows more vigorous stirring, consequently leading to smoother products which do not become too thin. Higher firmness of the gel in the vat also lowers the risk of syneresis, which might lead to less viscous and
more lumpy products.

There are no generally accepted rules for the layout of the time–temperature profile during stirring and cooling, and the applied procedures vary from manufacturer to manufacturer. However, it is generally accepted that the stirred

product needs some time after stirring to rebuild some structure. Typically, after reaching a particular pH, the product may be slowly stirred in the fermentation vat to achieve a homogeneous temperature distribution during cooling. Upon reaching 22–24ºC, the product may then be pumped to the filling and packaging unit, where relatively high shearing forces are applied. During the subsequent cooling process of the packed product, a desired increase in viscosity will be achieved.

2.4.5.1 Quality of stirred yoghurt in Sudan

El Zubeir and Ahmed,(2007) stated that acidification of milk by fermentation is one of the oldest methods of preserving milk and there are different methods of carrying out this fermentation in various parts of the world, which resulted in a wide range of fermented milk products, including kumiss, kefir, acidophilus milk and yoghurt (Thapa, 2000). Under normal dairy processing industry, selected lactic starter cultures are used to ferment milk during preparation of variety of cultured dairy products (Tamime and Robinson, 1999). Yoghurt is a semi–solid fermented milk product which originated centuries ago in Bulgaria, its popularity has grown and is currently consumed in most parts of the world (Tamime and Deeth, 1980). Yoghurt is one of the most unique, yet universal dairy products (Ebenezer and Vedamuth, 1991). To make a good quality product, raw milk used must be of low bacterial count, free from antibiotics, sanitizing chemicals, mastitis milk and colostrum and the milk also should be free from contamination by bacteriophages (Thapa, 2000). Yoghurt is highly nutritious and easily digestible diet due to the predigested nutrients by bacterial starters, it is perishable in view of its unused lactose content (Durga *et al*.,1986). Yoghurt is produced with a mixed culture of S. *salivarius sub* –spp. *thermophilus* and *L. delbrueckii*, sub – spp. *bulgaricus* in a 1:1 ratio (Kosikowski, 1982).

Musa, (1997) examined yoghurt prepared from fresh cow's milk and reported 3.2%, 4.5% and 19.39% for fat, protein and total solids, respectively. Uraltas and

Nazli, (1998) when studied Turkish fruit yoghurt, found that dry matter content ranged from 22.2 to 23.5% and values for fat ranged from 2.2 to 2.8% and SNF values ranged from 19.4 to 23.5%. Agaoglu *et al*. (1997) found that the average dry matter, fat, protein and mineral were 18.15%, 1.2%, 4.08% and 0.94%, respectively. Changes in the physical, chemical, and microbiological structure of yoghurt determine the storage and shelf life of the product (Sofu and Ekinci, 2007). Moreover Salvador and Fiszman, (2004) reported that studies of changes in these quality characteristics during storage would enable producers to predict the shelf life of the product more accurately.

1.4.5.2 HACCP of stirred yoghurt

Yang, (2009) stated that HACCP refers to a sort of preventive management system guaranteeing food security and sanitation.

Chapter Two

Materials and Methods

2.1Materials

All samples (40) of stirred yoghurt selection from Capo Company – Sudan durin manufacturing stages of stirred yoghurt receiving raw milk, buffer tank, incubatior distribution and others samples (40) from markets.

2.2Methods

2.2.1Yoghurt manufacturing process

The main processing steps involved in these two types of stirred yoghurt manufacture include the receiving raw milk, homogenization, milk heat treatment, incubation/ fermentation, cooling, and storage.

2.2.2Physicochemical analysis

2.2.2.1 Fat content

Fat content determined by Gerber method according to (Bradley *et al*., 1992) .Ten ml of sulphuric acid (density 1.815 gm / ml at 20°C) were poured in to clean dry Gerber tubes, then 5 grams of stirred yoghurt were added, followed by the addition of 1ml amyl alcohol and 5ml distilled water at 20 °C the content of the tube were thoroughly mixed till no white particles were seen. The tubes were then centrifuged at 1110 revelations per mint (rpm) for 5min. The tubes were transferred to a water bath at 65 °C for 3 min. after which the fat content was immediately read.

2.2.2.2 Solids-not-fat

Solids-not-fat was determined by conducting total solids and fat analyses. fat percentage was subtracted from the percentage of total solids to obtain the percentage of solids-not-fat.

2.2.2.3 Total solids content

The total solid content of fenugreek milk products samples were determined according to AOAC (1990). Two grams of yoghurt samples were placed in a clean dried flat bottomed aluminum dish. The dishes were heated on a steam bath for 10-15 min. and transferred to an air-oven for 3 hr. at 103±2°C. The dishes were placed into desiccators to cool and weighed. Heating, cooling and weighing were repeat several times until the difference between weighting was less than 0.5 gm. The total solid content was calculated as follows:

$$\text{Total solids \%} = \frac{\text{Weight of sample after drying} \times 100}{\text{Weight of sample before drying}}$$

2.2.2.4 pH values

The pH value determined by using digital pH meter model A00567 H. Germany.

2.2.2.5 Titratable acidity

Titratable acidity was determination according to Bradley *et al*., (1992) as followed: nine ml of the sample were measured in beaker and mixed gently. The phenolphthalein indicator (0.5 ml) was added and the mixture was titrated with 0.1 N Na OH until the color was changed to pink which lasted for 30second. The titratable acidity was then calculated from the following equation:

$$\text{Titratable acidity (\%)} = \frac{\text{ml NaOH} \times \text{N NaOH} \times 0.009}{\text{Weight of Sample}}$$

(1 ml 0f 0.1 NaOH = 0.009 gm of lactic acid).

2.2.2.6 Viscosity measurements

The viscosities of the stirred yoghurt were monitored as a function of shear rate (0.29-231 s-1) with a Bohlin VOR Rheometer (Bohlin Rheologi AB, Sweden)

using a concentric cylinder geometry.

A small volume of the sample was placed in the cup and the bob was lowered to position and a slight excess of the sample was allowed to cover the top of the bob. Measurements were started 5 min after placing the sample in the cup, to allow for temperature equilibration (Euston and Hirst, 2000).

2.2.2.7 Density measurement

The density of the yoghurt samples were measured using the specific gravity method as described by Smith (1980).

2.2.2.8 Temperature measurement

The temperature of the yoghurt samples were measured using the thermometer

2.2.3 Microbiological analysis

2.2.3.1 Sterilization of equipment and media

Flasks, test tubes, pipette and Petri dishes were sterilization by hot air oven at 160 °C for 60 mints. The media were prepared as described by the manufacture and brought to boiling before sterilization by autoclaving at 121 °C for 15 mints. The media were the to cool at 45 - 46 °C before purring into Petri dishes (Singleton, 1992).

2.2.3.2 Microbiological media

The preparation of the media was used according to (Singleton, 1992).

2.2.3.2.1 Standard Plate Count (SPC)

Standard plate count agar was used; at pH 7±0. 2.

- Casein enzyme 5g.
- Yeast extracts 2.50g.
- Dextrose 1g, Agar1g.
- Distilled water1000g.

- Hydrolyste 2.50g.

2.2.3.2.2 Mac Conkey Agar

Mac Conkey Agar was used; At pH 7±0.2.

- Pancreatic digest of gelatin17g
- Peptic digest of animal tissue1.5g
- Casein enzymatic hydrolyste1.5g
- Lactose10g
- Bile salt1.5g
- Sodium chloride5g
- Neutral red0.03g
- Crystals violet 0.001g
- Agar15g

2.2.3.2.3 Yeast Extract Agar

Yeast extract agar was used 23g of yeast extract agar in 1000ml and pH 7±0.2.

2.2.3.3 Plating Enumeration and Counting of Bacteria

2.2.3.3.1 Preparation of sample dilution

Representative sample of low fat ice cream (1g) was diluted (1:10) with stirred distilled water, diluted serially (10^{-1} to 10^{-6}) and one ml from each of the selected dilution after thoroughly mixing were carefully transferred into petri dishes using sterile pipette.

2.2.3.4 Enumeration of total bacteria

The method described by Houghtby *et al*.(1992), each dilution 1ml samples aseptically transferred in to sterile Petri dishes in duplicate, following by adding 10 - 12 ml of standard plate count agar at 45- 46 c˚. The Petri dishes were converted and mixed by gentle rotation to allowed to solidified. The plate were inverted and incubation at 37 c˚ for 48 hours. The developed colonies were counted using

colony counter, plates 25- 250 or less than 25 colonies were selected. The average number of colonies in each dilution was multiplied the reciprocal of the diluation factor and record as colony forming units/g.

2.2.3.5 Enumeration of Coli form Bacteria

The method described by Christen *et al*. (1992) was used. From each dilution 1ml sample were aseptically transferred in to sterile Petri dishes followed was by adding 10 - 15ml Mac Conky Agar media at 44s- 46 c˚. The content was allawed to solidify (10 - 15mints) on leveled surface. Then additional 3 - 4 ml Mac Conkey Agar were added to each petri dishes as on overlay to completely cover the surface of the solidified medium to inhibit surface colony formation. The plate were then inverted and incubated at 37 c˚ for 48 hours. The number of dark red colonies measuring ≥ 0.5 mm diameter on (15 - 150 cfu/gm) plate were counted and resulted were recorded as follows coli form count: number of colonies × factor of dilution = cfu / ml.

2.2.3.6 Enumeration of yeast and mould

The method described by Frank, *et al*.(1992) was used. One milliliter from each dilution was carefully transferred into Petri dished using sterile pipettes, and then added 10- 12 ml of yeast extract agar into plates. It was mixed by gentle rotation and incubated at 25 c˚ for days. The developed colonies were counted using colony counter and plate counting 15 - 150 colonies in each dilution were multiplied by the reciprocal of the dilution factor and recorded as per gram.

2.2.4Antibiotic

For extraction of the antibiotic residues of milk samples, methods specific to liquid chromatography developed by Tyczkowska *et al*.(1989) were used. Accordingly, it was extracted 1 ml from each milk sample and placed in centrifuge tubes. In order to precipitate the proteins in milk 1 ml of acetonitrile - methanol,

deionized water (40:20:20) then mixture was added on it. After stirring with hand thoroughly then centrifuged at 3000 rpm for 10 min and the portion remaining on supernatant after proteins were precipitated was used for analysis.

2.2.5 Statistical analysis

All obtained data were statistically analyzed by using SPSS program, 2002. Mean ± standard deviation was occurred. Also, differences of correlation coefficients were statistically significant at P. values <0.05, <0.01 and <0.001.

Chapter Three

Results

Table 1. Showed the pH-values during manufacturing stages of stirred yoghurt. The highest pH-value was obtained 6.67 in stage receiving raw milk, the stage of buffer tank showed pH-values 4.63, followed by incubation 4.67 and distribution stage of stirred yoghurt showed 4.64 pH-value. The pH value were significantly ($P \leq 0.05$) affected by the manufacturing stages of stirred yoghurt.

In table 1. Showed the titratable acidity during manufacturing stages of stirred yoghurt. The highest titratable acidity was obtained (0.15 %) in stage receiving raw milk, The titratable acidity were significantly ($P \leq 0.05$) affected by the manufacturing stages of stirred yoghurt.

The fat content during manufacturing stages of stirred yoghurt. The highest fat content was obtained (4.1%) in stage receiving raw milk. The fat content were significantly ($P \leq 0.05$) affected by the manufacturing stages of stirred yoghurt. Table 1. showed the specific density during manufacturing stages of stirred yoghurt. The highest specific density was obtained (1.03%) in stage receiving raw milk. The specific density were significantly ($P \leq 0.05$) affected by the manufacturing stages of stirred yoghurt.

The solid not fat during manufacturing stages of stirred yoghurt. The highest solid not fat was obtained (9.3%) in stage receiving raw milk. The solid not fat were significantly ($P \leq 0.05$) affected by the manufacturing stages of stirred yoghurt.Table 1. Showed the total solid (TS) during manufacturing stages of stirred yoghurt. The highest (TS) was obtained (13.4%) in stage receiving raw milk. The (TS) were significantly ($P \leq 0.05$) affected by the manufacturing stages of stirred yoghurt.

The antibiotic during manufacturing stages of stirred yoghurt. The highest antibiotic was showed (-ve) in stage receiving raw milk, The antibiotic were significantly (P≤0.05) affected by the manufacturing stages of stirred yoghurt.

Showed the viscosity during manufacturing stages of stirred yoghurt. The highest viscosity was showed (66.33cp) in stage distributions of yoghurt. The viscosity were significantly (P≤0.05) affected by the manufacturing stages of stirred yoghurt. Showed the temperature during manufacturing stages of stirred yoghurt. The highest temperature was showed (42℃) in stage buffer tank of milk. also the temperature were recorded (12.06 ℃) in stage distribution of stirred yoghurt (P≤0.05).

Table 1. Physicochemical analysis of stirred yoghurt in factory

Item	Manufacturing stages of stirred yoghurt			
	Receiving raw milk	**Buffer tank**	**Incubation**	**Distributions**
pH value	6.67± 0.05	4.63± 0.05	4.67± 0.05	4.46± 0.13 max 4.3
Titratable acidity(%)	0.15± 0.003			
Fat(%)	4.1± 0.19			
Specific	1.03± 0.001			
Solid Not Fat	9.3± 0.17			
Total solid (TS)	13.4± 0.3			
Antibiotic	-ve			
Viscosity				66.33 ± 4.1 max 50
Temperature		42^{a} ± 0.67		12.06^{b} ± 1.35 max 18

Mean± SD. having different superscript letters on rows are significantly different ($P \leq 0.05$).

As well as the pH value of stirred yoghurt products in different of markets (table 2). The highest pH value was showed 4.46 in factory, followed by 4.30, 4.21 where in the first and second area of market respectively, while the lowest pH value were recorded 4.20 in third area of market (P≤0.05). The highest viscosity was showed 66.33cp in factory, followed by 52.2cp, 42.7cp where in the first and second area of market respectively, while the lowest viscosity were recorded 33.8cp in third area of market (P≤0.05). But the temperature was 19.1℃ in second area of market, followed by 17.4℃ where in others area of market, while the lowest temperature were recorded 12.06 ℃ (table 2)in factory (P≤0.05).

Table 2. Physicochemical analysis of stirred yoghurt in markets

Item	In factory	Areas of markets		
		A	B	C
Temperature	12.06^{c}± 1.3	17.4^{b}± 0.17	19.1^{a}± 0.05	17.4^{b}± 0. 3
Viscosity	66.33^{a} ± 4.1	52.2^{b} ± 1.99	42.7^{c} ± 2.96	33.8^{d} ± 1.85
pH value	4.46^{a} ± 0.13	4.3^{b} ± 0.12	4.21^{c} ± 0.01	4.2^{c} ± 0.06

Mean± SD. having different superscript letters on rows are significantly different (P≤0.05).

Table 3 showed the total variable count (TVC) during manufacturing stages of stirred yoghurt. The highest TVC showed 1.62×10^5 in stage receiving raw milk, also the TVC were recorded 6.5×10^1 in stage of buffer tank of milk of stirred yoghurt ($P \leq 0.05$).

Table 3. Microbiological analysis (TVC) of stirred yoghurt in factory

Manufacturing stages of stirred yoghurt		
	TVC	**Max.**
Receiving raw milk	$1.62 \times 10^5 \pm 2.8 \times 10^5$	10×10^5
Buffer tank	$6.5 \times 10^1 \pm 2.8 \times 10^1$	10×10^1
Incubation and Distributions	----------	

Mean± SD. having different superscript letters on rows are significantly different ($P \leq 0.05$).

As shown in Table 4 the *Coliform* was 3.66×10^4 in stage receiving raw milk, but Nill in both stages of incubation and distribution of stirred yoghurt ($P\leq 0.05$). Table 4 showed the mould and yeast during manufacturing stages of stirred yoghurt in factory. The mould and yeast were Nill in both stage incubation and distribution (Table 4) of stirred yoghurt ($P\leq 0.05$).

Table 4. Microbiological analysis (Isolation) of stirred yoghurt in factory

Manufacturing stages of stirred yoghurt	**Isolation**		
	Coli form	**Yeast**	**Mould**
Receiving raw milk	$3.66\times 10^4 \pm 9 \times 10^5$ max 10×10^4		
Buffer tank	Nil		
Incubation and Distributions	Nil	Nil	Nil

Mean± SD. having different superscript letters on rows are significantly different ($P\leq 0.05$).

Table 5 showed the negative results for stirred yoghurt from microbial consummation ($P \leq 0.05$).

Table 5. Microbiological analysis (Isolation) of stirred yoghurt in markets

Areas of markets	Isolation		
	Coli form	Yeast	Mould
In factory	Nil	Nil	Nil
A	Nil	Nil	Nil
B	Nil	Nil	Nil
C	Nil	Nil	Nil

The results showed the highest percentage from producers of age group 46to 60 years 10 (33.3%), whilst the lowest percentage in age group less than < 30 years 3 (10%). While the data showed significant effect relationship between the gender and producers (Table 6). Moreover the results showed the highest value from producers were males 100%, While the females producers were zero (Nil). Then the data showed significant effect relationship between the gender and producers (Table 6). While the results showed the highest percentage from producers were herd size 15 to 25 was 43.33%, whilst the lowest percentage was less than < 30 13.33% (Table 8). The results showed in(Table 6) there was no species of animal than other cattle on farm 86.67%. While the females producers were 13.33%. ($P \leq 0.05$).

The result showed high frequency of veterinarian visits to the farm($P \leq 0.05$), then showed 60% less than 4 times/ year, followed by26.66% from 5 to 8 times/ year, while the lowest veterinarian visits were recorded 13.33% over than 8 times/ year (Table 6). Also Table 6 showed the high significantly effected of producers frequency who were discussing good production practicing (GPp) and food safety with the veterinarian ($P \leq 0.05$). The data obtained was 40%, followed by 20% were , while the lowest answer was recorded 3.3%. Then table 6 showed 93.3% of respondent didn't taking a continuing education course. While others producers were taken course or seminar 6.7%. ($P \leq 0.05$). Table 6. About 76.5% didn't taking learn more about GPp and food safety. While others producers was recorded 23.5% as shown in (Table 6). In table 6. the data was obtained 56.5% preferred by "Feed or product salesman", followed by " veterinarian" 16.5%, while the lowest was learn that by" Courses or seminars" recorded 3.3%. However the producers 13.3% didn't think that antimicrobial resistance (AMR) is making it harder to treat sick animals ($P \leq 0.05$) (Table 6).

While others producers were thinking that AMR is making it harder to treat sick animals were recorded 86.7% (Table 6). Finally the results in (Table 6) showed 40% didn't think that about humans linking to antimicrobial use in food animals. While 60% producers were thinking that.

Table 6. Respondents' farm characteristics towards food safety characteristic.

Item	Age group				Total
Producer age	< 30	30-45	46-60	>60	
Count	3	8	10	9	30
Percentage(%)	10%	26.6%	33.3%	30%	100
Item	Gender				Total
Producer	Male		Female		
Count	30		0		30
Percentage(%)	100%		zero		100
Item	Herd size				Total
Herd size	< 15	15-25	25-30	>30	
Count	8	13	5	4	30
Percentage(%)	26.66%	43.33%	16.66%	13.33%	100
Item	Animal species				Total
	No		Yes		
Count	26		4		30
Percentage (%)	86.67%		13.33		100
Item	Veterinarian visits/year				Total
Frequency	< 4 times	5 – 8 times	>8 times		
Count	18	8	4		30
Percentage(%)	60%	26.66%	13.33%		100
Item	discussing GPp and food safety with the veterinarian				Total
Frequency	Never	Rarely	Sometimes	Often	Always	
Count	2	6	12	9	1	30
Percentage(%)	6.67	20	40	30	3.3	100
Item	Education course about GPp				Total
	No		Yes		
Count	28		2		30
Percentage(%)	93.3		6.7		100
Item	Learn more about GP p				Total
	No		Yes		
Count	23		7		30
Percentage(%)	76.5		23.5		100
Item	discussing GP p and food safety with the veterinarian						Total
Frequency	Veterinarian	Feed or product salesman	Farm newspapers	Newsletters	Courses or seminars	Internet or email	
Count	5	17	3	4	1	0	30
Percentage(%)	16.5	56.5	10	13.3	3.3	0	100
Item	AMR about to treat sick animals			Total
	No		Yes	
Count	4		26	30
Percentage(%)	13.3		86.7	100
Item	AMR in humans is linked to antimicrobial			Total
	No		Yes	
Count	12		18	30
Percentage(%)	40		60	100

Most of producer (40%) were always sick cattle in an area separate from healthy cattle ($P \leq 0.05$), followed by 20% sometimes doing that, while others never doing that were recorded 10% (table 7). Also the data in (table.7) obtained 46.67% they always use disposable treatment equipment or clean and disinfect the equipment after each use, followed by 33.3% were rarely doing that, while the lowest were sometimes doing that recorded 10%.

the result showed high significantly effected of producer respondents' reported to use special places and procedures for disposal of needles, gloves, bottles, etc.. Moreover the data obtained 53.3% never doing that, followed by 16.67% they sometimes doing that, while others rarely doing that were recorded 10%.

As well as the result in (table 7) obtained 23.3% they rarely to ensure appropriate drug withdrawal times are met before milking and/or shipping cattle, while 13.3% they rarely doing that, likewise the result in (table 6) showed 53.3% of producer didn't keeping production records on the farm. while others rarely doing that were recorded 3.33%.

Table 7. Respondents' reported use of good production practice

Practice	Responses in each category								
	Never	Rarely	Sometimes	Often	Always	Total	Chi	df	P. value
Isolate sick cattle in an area separate from healthy cattle	3	6	6	3	12	30	9.1	4	0.061
%	10	20	20	10	40	100			
Use disposable treatment equipment or clean and disinfect the equipment after each use	2	10	3	1	14	30	21.67	4	0.00
%	6.67	33.3	10	3.3	46.67	100			
Use special places and procedures for disposal of needles, gloves, bottles, etc.	16	3	5	2	4	30	21.67	4	0.00
%	53.3	10	16.67	6.67	13.3	100			
Use animal health products according to label instructions	14	10	4	1	1	30	22.33	4	0.00
%	46.67	33.3	13.3	3.33	3.33	100			
Ensure appropriate drug withdrawal times are met before milking and/or shipping cattle	4	7	9	4	6	30	3	4	0.56
%	13.3	23.3	30	13.3	20	100			
Keep production records on the farm	16	1	7	4	2	30	24.3	4	0.00
%	53.3	3.33	23.3	13.33	6.67	100			
Keep records of diseases on the farm	13	2	8	4	3	30	13.66	4	0.008
%	43.3	6.67	26.67	13.33	10	100			

Use restricted access signs or locked gates/doors to control entry to the farm	10	7	6	5	2	30	5.66	4	0.22
%	33.33	23.33	20	16.67	6.67	100			
Ensure visitors wash their hands before and after farm entry	24	1	3	2	0	30	48.6	4	0.00
%	80	3.33	10	6.67	0	100			
Ensure visitors wear protective clothing and boots	21	1	3	3	2	30	47.3	4	0.00
%	70	3.33	10	10	6.67	100			
Ensure farm employees wear protective clothing and boots	18	2	5	2	3	30	31	4	0.00
%	60	6.67	16.67	6.67	10	100			
Ensure farm employees frequently wash their hands	16	3	5	2	4	30	21.22	4	0.00
%	53.33	10	16.67	6.67	13.3	100			

Chapter four

Discussion

4.2.1 Physicochemical properties of stirred yoghurt in factory

4.2.1.1 pH value

Starter cultures, and incubation temperatures on changing pH of yoghurt during fermentation and finished yoghurt were investigated. The results determined that dry matter fortification does not influence pH progression. In addition, incubation temperature and heat treatment affect pH development and treatments of starter culture during incubation time on pH is variable (De Brabandere and Baerdemaeker, 1999).

Hakimi *et al*. (2014) found that, five factors at two levels are selected for application of DOE to homemade yoghurt production process. The list of process factors together with their levels which are used for experiments summarized in (Table 1). In addition, it was decided to perform experiments in order to determine significant process factors and interactions between them to pH level of homemade yoghurt after fermentation as a response. According to studies, consumers prefer to use yoghurt with moderate acidity (4.2 to 4.6) (Chandan *et al*., 2006). This acceptable range is considered for finished or cooled yoghurt; also, cooling yoghurt after fermentation of the milk influence to reduce the pH of fermented milk about 2 degrees. Therefore, the range of optimal pH of fermented milk after fermenting and before cooling stage, is designated to be 4.4 to 4.6 as an optimal target for responses in DOE.

There should be no agitation during incubation. The yoghurt curd or “coagulum” begins to form as more lactic acid is produced as the iso-electric point of casein (pH 4.6 – 4.7) is approached. A “solidity” of the gel will begin to be seen at approximately pH 5.6 (Hakimi *et al*.,2014).

Although there was a statistically significant difference between the before and after HACCP values for pH and temperature of the raw milk, all the test results complied with the standards/specifications for pH (6.60 - 6.75) and temperature (< 6°C) (Hakimi *et al*.,2014).

Mean and standard deviation of the percentage butterfat, freezing point, pH, in raw milk before and after the implementation of HACCP showed that B:6.757, A:6.731 Probability level (under null hypothesis) (P< 0.001).

4.2.1.2 Titratable acidity

Titratable acidity in all samples increased progressively during storage period (Galal *et al*., 2004 and Guoda *et al*. 2004), it refers to an increase in lactic acid by starter culture.

4.2.1.3 Fat content

Hoolasi, (2005) showed that, the standardization of milk refers to the standardization of fat and solid-non-fat content (SNF). Bovine milk fat content varies from 3.2%–4.2% w/w. The fat content of the milk is adjusted to range from <0.5%, for skim milk, to 1.5%–2%, for semi-fat milk, to 3.5% for full fat milk. As far as yoghurt is concerned, the fat content ranges from 0.1%–10% according to consumer demands.

In practice, to achieve the designed fat level, either the addition of skim milk or milk fat or the separation of fat from milk via centrifuge and mixing milk fat with skimmed milk is carried out (Tamime and Robisons, 2007). The standardization process is of paramount importance, because the fat content of the milk influences the yoghurt characteristics; increasing the fat content of milk results in an increase in the consistency and viscosity of yoghurt (Shaker *et al*.,2000; Walstra *et al*.,2006[b]). Also, the milk fat content affects the maximum rate of pH decrease and pH lag phase during yoghurt fermentation (Soukoulis *et al*.,2007).

Mean and standard deviation of the percentage fat content, in raw milk before and after the implementation of HACCP showed that % Butterfat B: 3.982, A: 3.761 Probability level (under null hypothesis) ($P< 0.001$).

4.2.1.4 Specific density

Gemechu *et al*.,(2015) stated that, the means specific gravity, of milk samples were 1.030 ± 0.000cp. The specific gravity of normal milk ranges from 1.027 – 1.035 g per ml with a mean value of 1.032 g per ml (Tamime, 2009).

In the current study, the result of milk samples collected from four sources falls within the ranges of Tamime (2009) finding. According to O'Connor (1993), the higher value of specific gravity (1.035) indicates skimming off fat whereas the lower value than normal value of specific gravity of milk (1.020) is indicative of addition of water. Similar on-farm result of specific gravity of 1.030 was reported by Zelalem and Ledin (2001).

Furthermore, adulteration of milk with water that was usually done in order to increase the quantity of milk lowers milk's specific gravity while addition of solids such as flour or sugar into milk and removing the butterfat increases the specific gravity of milk beyond 1.035 (O'Connor, 1995; Omore *et al*., 2005).

4.2.1.5 Solid Not Fat

Hoolasi, (2005) showed that, the term of standardization is also applied to the SNF content of the milk. The SNF components of milk mainly consist of lactose, protein and minerals; SNF content of milk varies from 11% to 14% of the total weight of the milk while the SNF of yoghurt ranges from 9% to 16%. The SNF content of milk used for yoghurt manufacture is altered, in some cases, by producers in order to attain the desired characteristics of the coagulum; the higher the SNF level, the higher the resulting yoghurt's viscosity and firmness. The addition of native milk components is permitted to yoghurt and fermented milk products in some countries. It is quite common in yoghurt manufacturing to fortify

the milk mixture with milk powder (skimmed or full fat), whey protein concentrates or casein powder, to achieve the desired SNF content and subsequently an increase in firmness and cohesiveness (Walstra, *et al*., 2006[b]).

It must be noted that the fat and SNF content of milk has an impact on the fermentation process. In particular, the interaction of milk SNF content and fermentation temperature has a significant effect on the duration of the fermentation process; an increase of SNF increases the duration of the fermentation process (Kristo *et al*.,2003).

SNF content of milk from dairy cooperative milk collection centers averaged 8.90± 0.00%. This value is greater than the finding reported by Teklemichael (2012) for milk obtained from dairy farms (8.75 ± 0.301%) in Dire Dawa town.

According to European Union quality standards for unprocessed whole milk, solids-not fat content should not be less than 8.5% (Tamime, 2009). Accordingly, the average SNF content (8.59%) observed for four milk samples were within the recommended standards.

Gemechu *et al*.,(2015) found that, the average SNF content of milk samples obtained less than the findings of Bille *et al*. (2009); Janštová *et al*. (2010) and Fikrineh *et al*. (2012) who reported higher value of 8.7, 8.96 and 9.10%, respectively from raw cow's milk samples.

Debebe (2010) also reported the minimum (8.3 ± 0.36%) and maximum (8.7 ± 0.36%) SNF content of raw cow's milk obtained from street-vendors and milk producers in and around Addis Ababa, respectively. The difference observed in SNF content of milk could be due to difference in the feeding practices, season, milking method and lactation period exerted (Suman *et al*., 1998).

4.2.1.6 Total solid (TS)

The average total solids content of milk samples from dairy cooperative milk collection centers was 13.40 ± 0.06%. This value is greater than the

findings of Bille *et al*. (2009), Mirzadeh *et al*.(2010) and Teklemichael (2012) who have reported TS of 12.33%, 12.57%and 12.580%, respectively.

Total solids content of milk collected from hotels, small shops and small scale milk producers averaged between12.90 ± 0.21%, 12.67 ± 0.07%, and 12.50 ± 0.00%, respectively (Gemechu *et al*., 2015).

European Union established quality standards for total solids content of cow milk not to be less than 12.5% (FAO/WHO, 2007). Therefore, the average total solid content (12.87%) of milk samples in the present study was within the recommended standards. Different values for total solid content of raw milk samples have been reported by different scholars. The variation could be due to difference in breed, feeding and management practices which have important effects on milk composition and quality (O‟Connor,1995).

4.2.1.7 Antibiotic

New York State milk standards are based on those defined in the FDA Pasteurized Milk Ordinance (PMO, 2009), Cornell University (1998) and Wehr and Frank (2004) stated that, the no positive test on drug residue detection maximum limit not to exceed 10/mL.

4.2.1.8 Viscosity

Intermediate storage should be as short as possible since physical changes take place that can affect final yoghurt quality. The product may release whey that is difficult to re-incorporate, resulting in loss of yield. Viscosity and body will develop that will largely be lost when the yoghurt is disturbed again. The ability of the yoghurt to bind whey will be reduced by cold disturbance (Early, 1998).

4.2.1.9 Temperature

The fermentation process is the most important stage of yoghurt manufacture. During this stage *Thermophilus* (ST) is the only species in the *Streptococcus* genus that is used in dairy starter cultures. ST is Gram positive and usually considered

thermophilic, however, as the optimum temperature for its growth is 35–53 ° C. *bulgaricus* (LB) is rod-shaped, Gram-positive, anaerobic bacteria and its optimum growth temperature is 40 – 44 °C. LB can produce very high amounts of lactic acid by metabolizing lactose (Walstra *et al*.,2006[a] ; Vedamuthu, 2006).

Due to proteolysis caused by starter culture, amino acids (mainly proline and glycine) are released into the yoghurt, temperatures even during storage at 4 °C (Vedamuthu, 2006).

Fluctuating temperatures during distribution can adversely affect the coagulum stability reducing viscosity and encouraging syneresis.

Any significant fluctuation in temperature may also result in the continuation of fermentation by starter culture micro-organisms, which will affect quality in an adverse manner (i.e. over acidification, increase in syneresis). Ideally a temperature of 8 - 10°C is optimal, depending upon storage time. After heat treatment the milk is required to be cooled to a suitable temperature prior to inoculation. In most cases this will be carried out in the regenerative section of the plate heat exchanger. Yoghurt, manufactured in a batch tank or churn, can simply be allowed to cool via cold water jackets or tank (effectively in a water bath) (Early, 1998).

The inoculation temperature for short set method will approximate to 42ºC. This temperature can be lowered if an extended incubation period is required (approximately 30 – 32ºC).

Allowances need to be made for incubation tank wall temperature, cold starter addition and latent heat effects and, therefore, the actual cooling temperature as measured on exiting cooling (regeneration) section, is likely to be 1 – 2ºC higher than required, dependent upon volume, agitation system, distance travelled, etc. For short set incubation it is critical to achieve an accurate inoculation temperature since too high a temperature can inhibit and ultimately kill starter culture micro-

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

Hoolasi, (2005) showed that, the mean and standard deviation of the percentage butterfat, freezing point, temperature, in raw milk before and after the implementation of HACCP showed that B: 3.717 + 0.697. A: 3.318 + 1.791 with probability level (under null hypothesis) ($P< 0.001$).

Raw milk undergoes, in the dairy industry, centrifugal clarification to remove somatic cells and any other solid impurities (Tamime and Robisons, 2007).

Afterwards, a mild heating process, known as thermalization, is performed at temperature range 60–69 °C for 20–30 s, aiming at the killing of many vegetative microorganisms,this process causes almost no other irreversible change in the milk (Walstra *et al*.,2006[a]). After thermalization, milk is cooled <5 °C or inoculated with lactic acid bacteria or other microfloras to control the growth of the psychrotrophic bacteria (Tamime and Robisons, 2007).

4.2.2 Microbiological analysis of stirred yoghurt in factory

4.2.2.1 Total variable count (TVC)

New York State milk standards are based on those defined in the FDA Pasteurized Milk Ordinance (PMO, 2009), Cornell University (1998) and Wehr and Frank (2004) showed the total bacteria maximum limit 30,000/mL

Microbiologically, there was a positive impact on the raw milk quality after the implementation of HACCP. The maximum total plate count before HACCP implementation was, for example, 200 000 cfu/ml which was still within the legal specification and after HACCP implementation it was 85 000 cfu/ml. The standard deviation for the psychrotrophic bacterial counts was much smaller after the implementation of HACCP (Hoolasi, 2005). Stricter controls in terms of the GMPs, GLPs and CCPs have resulted in this marked improvement. Mean and standard deviation of the percentage butterfat, freezing point, Total plate count

(log 10), in raw milk before and after the implementation of HACCP showed that B: 3.560, A: 3.220, with probability level.

During incubation, the starter culture growth results in an increase in the system's microbial content from 10^8 to 10^{10} CFU g−1. That my be due to the fermentation product by two live bacterial strains of *Streptococcus salivarius subsp*. *thermophilus and Lactobacillus delbrueckii subsp. bulgaricus* in abundance. However, yoghurt starter cultures may include other microorganisms as well, like *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus lactis*, *Lactobacillus jugurti*, *Lactobacillus helveticus*, *Bifidobacterium longum*, *Bifidobacterium bifidus* and *Bifidobacterium infantis*. *Streptococcus thermophilus subsp*. t*hermophilus* (ST) is the only species in the *streptococcus* genus that is used in dairy starter cultures (Walstra *et al*.,2006[a] ; Vedamuthu, 2006).

4.2.2.2 Coliform

Mean and standard deviation of the coliform count (log 10) of various samples, during process manufacture, before and after the implementation of HACCP. Obtained 0.00 ± 0.00 was Probability level (under null hypothesis) NS. New York State milk standards are based on those defined in the FDA Pasteurized Milk Ordinance (PMO, 2009), Cornell University (1998) and Wehr and Frank (2004) showed the coliform maximum limit not to exceed 10/mL.

Coliform in milk is one of the best indices for judging sanitation (Douglas, 2003). 76% and 32% of raw milk samples in factory A and B were in the range of 1×103-9×104 respectively. This range is higher than that reported by less than 102 (American Public Health Association ,1985).The higher Coliform count in raw milk used in factory B may be due to the unsatisfactory milking practices in the farm from which the milk was collected. A similar count of 9×103 cfu /ml in Khartoum state was reported (Salman and Hamadm, 2011; Reena *et al*., 2003).

In Jordan a higher range of 2.5×104-1.4×106 was reported. In this study lower TBC value was obtained for pasteurized milk compared to what reported by Shaltout *et al*. (2003) who showed a range of 6.5×105 to 6.5×1014 cfu. The official limits set by the SSMO for pasteurized milk for Coliform bacteria was 1x5 -1x102 cfu/ml. The result of this study showed that, 80%of the samples from factory A and 40% from factory B conform with this limits. The results were in agreement with Pasteurized milk ordinance PMO, (2001) who suggested a range of less than 102 cfu/ml. In Algeria Aggad *et al*.(2010) stated that (31.5 %) and (6.5 %) samples of milk from the two sources of samples at sales point were not in compliance with the acceptability threshold fixed at 10 cfu/ml. Coliform bacteria counts of pasteurized milk showed lower numbers than these reported by (Elmagli and El Zubeir, 2006). The lower Coliform counts might be due to hygienic quality of raw milk, proper pasteurization process, good packaging and good storage conditions. This agreed with Pasteurized milk ordinance PMO, (2001). who reported that the total bacterial standards for grade A pasteurized milk should be < 10 Coliform/ ml.

4.2.2.3 Mould and yeast

Mean and standard deviation of the yeast and mould count (log 10) of various samples, during process manufacture, before and after the implementation of HACCP. Obtained 0.00 ± 0.00 was Probability level (under null hypothesis) NS (Hoolasi, 2005).

4.2.3 Respondents' farm characteristics and attitudes towards food safe characteristic.

Young *et al*. (2010) who studied a survey of canadian dairy producers: knowledge and attitudes towards food safety and the canadian quality milk program use of good production practices and stated that, approximately producers

age were 15.0% less than 30 years, followed 42.3% there are ranged between 30 to 45% and similarly with age 60 to 46 39.7%.

knowledge and attitudes towards food safety and the canadian quality milk program and reported use of good production practices and stated that,86.8% producers were males and 13.2%were female (Young *et al*. 2010).

In general survey of canadian dairy producers: knowledge and attitudes towards food safety and the Canadian quality milk program and reported use of good production practices and stated that, approximately showed herd size were 24.8% less than 36 years, followed 32.8 size were ranged between size 36 to 50 and similarly with age 60 to 46 39.7% (Young *et al*., 2010).

In this survey, it was noticed that many farms owners were used traditional treatment like fire for mastitis and other diseases. El Zubeir and Mahala (2011) reported that most of the farms applied drugs without veterinary instructions or inspections. Also from the survey it was noticed that in most of the farms, diseases control and management were not satisfactory.

Young *et al*. (2010) stated that, most producers considered veterinarians to be the most knowledgeable and favourable source of new information about OFFS, indicating that veterinarians should have an important role in future continuing education of producers. On the other hand, consumers and government stakeholders were seen as less knowledgeable about OFFS in dairy production, and several (n=71) commented that consumer knowledge and awareness of food safety should be increased. Future efforts should be made to enhance communication and knowledge exchange between producers, consumers and the government.

In general survey of canadian dairy producers: knowledge and attitudes towards food safety and the canadian quality milk program and reported use of good production practices and stated that, approximately showed producers were

No completed a course in dairy health management 66.9, while others were completed a course 33.1 (Young *et al*. 2010).

In general survey of Canadian dairy producers: knowledge and attitudes towards food safety and the Canadian quality milk program and reported use of good production practices and stated that, approximately showed producers were No completed a course in food safety73.7%, while others were completed a course 26.3% Young *et al*. (2010).

The producers would like to receive food safety information in the future via: education courses 63.8% followed by websites 61.6%, veterinarian 26.9%, newsletters 42.2% and newspaper/magazines 49.8%, while others were completed a course 97.5%, while others producers think that AMR is making it harder to treat sick animals were recorded 86.7% (Young *et al*. 2010).

In general antibiotics are used on many farms to treat mastitis infections. Cows under antibiotic treatment for mastitis infections may have antibiotic residues in their milk, therefore, milk from treated cows is either discarded or collected into a separate tank. Milk containing antibiotic residues is not used for human consumption. The legal standard, as defined by the FDA, requires that milk contain no detectable antibiotics when analyzed using approved test methods (Grade A Pasteurized Milk Ordinance, 2007).

Makovec and Ruegg (2003); Tikofsky *et al*. (2003) stated that, 84.4% of producers reported that they do not (selected 1 or 2) add antibiotics to feed or milk replacer, over 80% of producers reported that they treat all clinical cases of mastitis. Although research has shown that the prevalence of AMR in mastitis-causing bacteria such as Staphylococcus is low on dairy farms in the USA, the extensive use of antimicrobials for disease prophylaxis and treatment of mastitis could contribute to the selection of antimicrobial- resistant bacteria in dairy cattle (De Francesco *et al*.,2004; Pol and Ruegg, 2007). Producers and veterinarians are

encouraged to use antimicrobials judiciously to minimize AMR selection pressure on bacteria in dairy cattle.

Producers were highly concerned about the impacts of AMR in their industry, which also corresponds to previous research (Vanbaale *et al*.,2003; Raymond *et al*.,2006,). In addition, most producers correctly identified that *Salmonella* and *E. coli* can be transmitted through beef or milk to humans and that BVD cannot be transmitted in this way. However, awareness of Brucella should be improved, as younger producers may not be familiar with this pathogen since its eradication from canadian cattle. Knowledge of Cryptosporidium should also be improved, as roughly 70% of producers were unsure if it can be transmitted from dairy cattle to humans through beef or milk and 60% were not concerned that it could cause consumers to become ill (Young *et al*. 2010).

3.2.4 Respondents' reported use of GPP

Azeze and Tera, (2015) stated that, the hygienic handling practice of the milk with respect to quality has received a great concern around the world. This is especially true in developing countries where production of milk and various milk products usually takes place under unsanitary conditions and poor production practices. It was also reported that dairy production has a great contribution in improving human nutrition, particularly women and children (Ahmed *et al* ., 2004).

Production of milk for consumers requires good hygienic practices such as clean milking utensils, washing milker's hands, washing the udder and use of individual towels during milking and handling, before delivery to consumers or processors (Getachew, 2003).

Getachew, (2003) found that, the production of milk of good hygienic quality for consumers requires good hygienic practices, such as clean milking utensils, washing milker's hands, cleaning udder, and use of individual towels during milking and handling, before delivery to consumers or processors. Results of the

present findings revealed that majority (96.3%) of the farmers practiced hygienic milking, such as washing hand, milk containers and udder before milking. In the present study majority (85.19%) of the farmers used warm water for washing udder. Consistent with this study, Shewangizaw and Adisu (2014) reported that 93 and 77% of the farmers in Wolayta Sodo, Ethiopia washed hand and udder before milking, respectively.

Depiazzi and Bell (2002) reported that pre- milking udder preparation and teat sanitation plays important part in the microbial load of milk, infection with mastitis, and environmental contamination of raw milk during milking.

In others study, most (85.19%) of the farmers used warm water and detergents to wash hand, milk handling containers and udder before milking. The respondents also reported that they wash their milk containers before and after use. The study observed that there was no a practice of medical examination of farm workers, particularly milkers for the reason of preventing the contamination of milk with diseases carried by man (e.g. typhoid, typhus and tuberculosis), which are the most common diseases in this study area. In addition, most of the dairy farm works had no proper farm cloths, boots, and hair cover (Belay and Janssens, 2015).

Conclusions

A HACCP program was successfully implemented in a large commercial stirred yoghurt plant within a one year period. The following conclusions based on the presence of antibiotics and foreign material in raw milk, effective pasteurization and homogenization, as well as maintaining the correct fermentation temperature, were identified as critical control points.

The implementation procedures and actions to ensure that the CCPs and activities are in line with the requirements of the HACCP system are crucial.

Physicochemical there was a positive impact on the raw milk quality after the implementation of HACCP. This was due to stricter controls in terms of GMPs, GLPs , GHPs and CCPs.

The pH value of stirred yoghurt products showed (4.30, 4.21and 4.20) where in different three area of markets, then showed significantly decrease ($P \leq 0.05$) comparing with final product factory.

Moreover the temperature was importance during manufacturing stages of stirred yoghurt were showed (42℃ ± 0.67) in buffer tank and (12.06 ℃ ± 1.35 max 18) in distributions stage, while the temperature of stirred yoghurt products showed (19.1℃, 17.4℃ and 12.06 ℃) where in different three area of markets($P \leq 0.05$). The viscosity showed normal in distributions stage of factory and recorded where in different three area of markets ($P \leq 0.05$).

The presence of microbiological parameters of stirred yoghurt were showed the highest total variable count (TVC) in stage receiving raw milk and buffer tank ($P \leq 0.05$).

However the isolation of Coli form showed (3.66×10^4) in stage receiving raw milk, and recorded (Nil) in buffer tank, incubation, distributions and others different area of markets. So that yeast and molds also were showed (Nil) in all

samples of raw milk and stirred yoghurt during manufacturing and distribution yoghurt in markets ($P \leq 0.05$).

To achieve the total quality that embrace the results also were showed milk producers awareness and knowledge of good production (GPp) of in their farms.

Indicators of efficient and effective implementation of the HACCP system include the trend(s) in the customer complaints, the nature of the customer complaints, credits passed, legal liabilities due to alleged claims of unsafe products, in- process deviations, finished goods compliance with specifications (defect levels and defect rates).

Recommendations

- Commitment and direct involvement from the most senior levels in the company as well as from the plant's management is crucial to ensure the success of any initiative, such as HACCP. Successful implementation of the HACCP based approach requires the synergistic interaction between all role players in the HACCP team.
- The criteria used for the selection of the HACCP team leader are very important. Adequate HACCP training, knowledge of the product (s) and the manufacturing process are also some of the key requirements of the team leader the person should also have leadership abilities, defined responsibilities, and authority. Ongoing education, training and motivation of all personnel on HACCP principles are essential and good production practice especially on milk producers.
- The HACCP team members who identify the CCPs, should comprise of the necessary skills in the relevant fields. Non-managerial personnel (e.g., shop floor personnel) could contribute significantly to the project as these personnel may have a better understanding of the process, limitations, problems and practical concerns. Depending upon the level of the maturity

and the scope of the HACCP system in a dairy company, and the nature of the products, backward and / or forward integration of the HACCP system can be done.

- In the case of a stirred yoghurt manufacturer, the quality of raw milk can be improved by the implementation of HACCP at farm level (milk suppliers to the company) – this is an example of backward integration of the HACCP system.

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Appendixes

Questionnaire

1/ Respondents' farm characteristics and attitudes towards food safe

Characteristic

Producer age

< 30

30-45

46-60

>60

Gender

Male

Female

Herd size

< 15

15-25

25-30

>30

Animal species other than cattle on farm

No

Yes

Frequency of veterinarian visits to the farm

≤ 4 times/year

5-8 times/year

> 8 times/year

Frequency of discussing GPp and food safety with the veterinarian

Never

Rarely

Sometimes

Often

Always

Have previously taken a continuing education course or seminar about GPp and food safety

No

Yes

Want to learn more about GPp and food safety

No

Yes

Preferred ways to learn more about GPp and food safety:

Veterinarian

Feed or product salesman

Farm newspapers

Newsletters

Courses or seminars

Internet or email

Think that AMR is making it harder to treat sick animals

No

Yes

Think that AMR in humans is linked to antimicrobial use in food animals

No

Yes

GPP = Good Production Practice
AMR= Antimicrobial Resistance

2/ Respondents' reported use of GPP

Practice		Responses in each category				
		Never	**Rarely**	**Sometimes**	**Often**	**Always**
Isolate sick cattle in an area separate from healthy cattle						
Use disposable treatment equipment or clean and disinfect the equipment after each use						
Use special places and procedures for disposal of needles, gloves, bottles, etc.						
Use animal health products according to label instructions						
Ensure appropriate drug withdrawal times are met before milking and/or shipping cattle						
Keep production records on the farm						
Keep records of diseases on						

the farm						
Use restricted access signs or locked gates/doors to control entry to the farm						
Ensure visitors wash their hands before and after farm entry						
Ensure visitors wear protective clothing and boots						
Ensure visitors wear protective clothing and boots						
Ensure farm employees wear protective clothing and boots						
Ensure farm employees frequently wash their hands						