ADDIS ABABA UNIVERSITY INSTITUTE OF TECHNOLOGY

SCHOOL OF GRADUATE STUDIES

CHEMICAL ENGINEERING DEPARTMENT

EVALUATION OF GHEE PROCESSING METHODS ON SHELF STABILITY AND YIELD

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A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Food Engineering

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List of Abbreviations

ASLT	Accelerated Shelf Life Test
a_w	Water activity
CBM	Creamery Butter Method
CI	Confidence Interval
CSA	Central Statistical Authority of Ethiopia
CV	Coefficient of Variation
DCM	Direct Cream Method
E_A	Activation Energy
EBM	Ethiopian Butter Method
EBM_a	Ethiopian Butter Method with additives
EBM_{wa}	Ethiopian Butter Method without additives
E_h	Redox Potential
FAO	Food and Agriculture Organization
FER	Fat to Energy Ratio
FFAs	Free Fatty Acids
IDF	International Dairy Federation
IS	Indian Standards
LAB	Lactic Acid Bacteria
masl	Meters above sea level
mEq	Milliequivalents
mg	Milligram
ml	Milliliter
ppm	parts per million
PV	Peroxide Value
RH	Relative humidity
SD	Standard Deviation
WHO	World Health Organization

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Abstract

The aim of this study is to evaluate three different Ghee processing methods, namely Direct Cream Method (DCM), Creamery Butter Method (CBM) and modified Ethiopian Butter Method (EBM). Yield and shelf stability were major criterion for comparison. Shelf stability of samples was estimated by using the accelerated shelf-life testing method. The Arrhenius kinetic model was used using a successive two step ordinary linear least squares fit. Samples were stored at 45 and 70 ^{o}C in dark place for 26 days. Then peroxide values, free fatty acid values and sensory qualities were measured and used to monitor deterioration. Furthermore, the effect of two traditional additives, Lippia adoensis and Aframomum corrorima, on the shelf stability of EBM Ghee was analyzed. The Ghee samples made by EBMwa (Ethiopian Butter Method without additives) and CBM had lowest (0.16±0.03mEq/Kg)and highest (1.29 ± 0.06mEq/Kg) peroxide value after 26 days storage at 45 ^{o}C. The Ghee samples made by EBMa (Ethiopian Butter Method with additives) and CBM had lowest (0.46±0.04mEq/Kg)and highest (6.11±0.29mEq/Kg) peroxide value after 26 days storage at 70 ^{o}C. DCM Ghee exhibited the maximum shelf life (7.5 month) followed by EBMa Ghee samples (3.45 months) and then CBM Ghee (2.7 months). On the other hand, yield was highest for CBM Ghee (91%) followed by EBMwa Ghee (83%), EBMa Ghee (80%), and DCM Ghee (73%). To conclude, it is advisable to process Ghee by Direct Cream Method. The addition of the aforementioned additives had positive effect in retarding oxidation process, which resulted in less formation of peroxides in EBMa (0.46±0.04mEq/Kg) in comparison to EBMwa (1.04±0.09mEq/Kg) after 26 days of storage at 70 ^{o}C. Finally, a preliminary techno-feasibility analysis for DCM and/or EBM$_a$ processing unit was also carried out.

Keywords: *Accelerated Shelf Life Testing; CBM; DCM; EBM; EBM$_a$; EBM$_{wa}$; Peroxide Value; Shelf Stability; Yield.*

CHAPTER ONE

1. Introduction

1.1.Background

Milk contains large amounts of essential nutrients and has rightly been recognized as nature's single most complete food. It provides more essential nutrients in significant amounts than any other single food. Milk is defined as the secretion of the mammary glands of mammals, its primary natural function being nutrition of the young. Milk of some animals, especially cows, buffaloes, goats and sheep, is also used for human consumption, either as such or in the form of a range of dairy products. (Walstra, *et al.*, 2006)

Milk fat or butter fat is the second largest component of milk and is of major commercial value. It serves nutritionally as an energy source and supplies essential fatty acids. Rural producers make butter and Ghee from the fat fraction of milk. Ghee has excellent storage stability. Where Ghee is not made, butter is occasionally spiced and heated to preserve it (O'Mahony, 1988).

Milk in Ethiopia comes from four main sources; local Zebu cattle, crossbreed cattle (mainly with Friesian and Holstein), pure or nearly pure breed Friesian and Holstein cattle. In some regions, milk is also derived from sheep and goats (Gonfa, *et al.*, 2001).

Ethiopia is believed to have the largest livestock population in Africa. A report by Central Statistical Authority of Ethiopia 10 years ago (CSA, 2000) indicates that total livestock population was estimated to be 57.31 million in which the share of cattle was 33 .08 million, which is 57.72%. The number of dairy cows was estimated to be about 10.36 million of which 6.48 million are milking cow. Recent survey for year 2009/2010 (CSA, 2011), shows that the total cattle only population in Ethiopia is around 53.4 million. Out of this total cattle population, female cattle constitute about 55.2 percent and the remaining 44.8 percent are male cattle. 99.26 percent of the total cattle in the country are local breeds. Milking cows are estimated to be around 10.7 million heads.

Clearly, the number of livestock population has been increasing. There is a significant increase in number of both cattle and milking cows. The estimate of total cow milk production for the rural sedentary areas of the country during the reference period (November 11, 2009 to November 10, 2010) was about 4.06 billion liters. The average lactation period

per cow during the reference period at country level was estimated to be about *six* months, and average milk yield per cow per day was about 1.85 liters (CSA, 2011). These figures above, point out that Ethiopia has a great potential in the dairy industry.

However, while there is several milk processing plants in Africa, much of the milk produced by rural small holders is processed on-farm using traditional technology. Farmers in Ethiopian highlands produce sour milk, butter and cottage cheese for sale, and similar products are made in the rangelands (O'Mahony, 1988). Of the total annual milk production in 2009/10 (CSA, 2010),85 percent was used for household consumption, 7 percent was sold, only 0.3 percent was used for wages in kind and the rest 7.7 percent was used for other purposes (could be for the production of butter, cheese, and the likes).

The typical Ethiopian highland farm produces a small surplus of milk for sale. Farmers close to main roads with in 120 km of Addis Ababa have no marketing problems; they can sell their milk directly to consumers or to traders, as well as to the Addis Ababa dairy industry through an established milk collection system. Elsewhere in Ethiopia, farmers near towns generally have a ready outlet for their liquid milk. However most farmers live a long way from major roads and have poor access to such markets, and these farmers have to rely on products which have more storage stability than fresh milk (O'Mahony & Bekele, 1985).

A common justification to the poor performance of the Ethiopian dairy sector is related to the fasting practices of national consumers (Ahmed, *et al.*, 2003). The calendar of the Orthodox Church involves four prolonged fasting periods per year, preceding major Christian festivities, and two fasting days every week (Wednesday and Friday), for a total of 180–250 days of fasting per year. During fasting days Orthodox Christians abstain from consuming products of animal origin, including milk and dairy products. Since milk is highly perishable and produced on a daily basis, fasting-induced fluctuations in consumer demand pose significant problems to the commercialization of dairy products within Ethiopia. Dairy trade in Ethiopia is constrained also by incipient urbanization and widespread poverty, which result in thin national markets and a production system dominated by a myriad of smallholder farmers producing milk mainly for home consumption. (Francesconi, *et al.*, 2010)

The major fermented milk products produced in Ethiopia by smallholder farmers by traditional methods are "*Ergo*" (fermented sour milk), "*Ititu*" (fermented milk curd), "*Qibe*" (traditional butter), "*NeterQibe*" (traditional Ghee), "*Ayib*" (cottage cheese), "*Arerra*" (sour defatted milk), and "*Aguat*" (whey) (Gonfa, *et al.*, 2001). **Error! Reference source not**

found. Error! Reference source not found. shows flow diagram for processing major fermented milk products produced in Ethiopia.

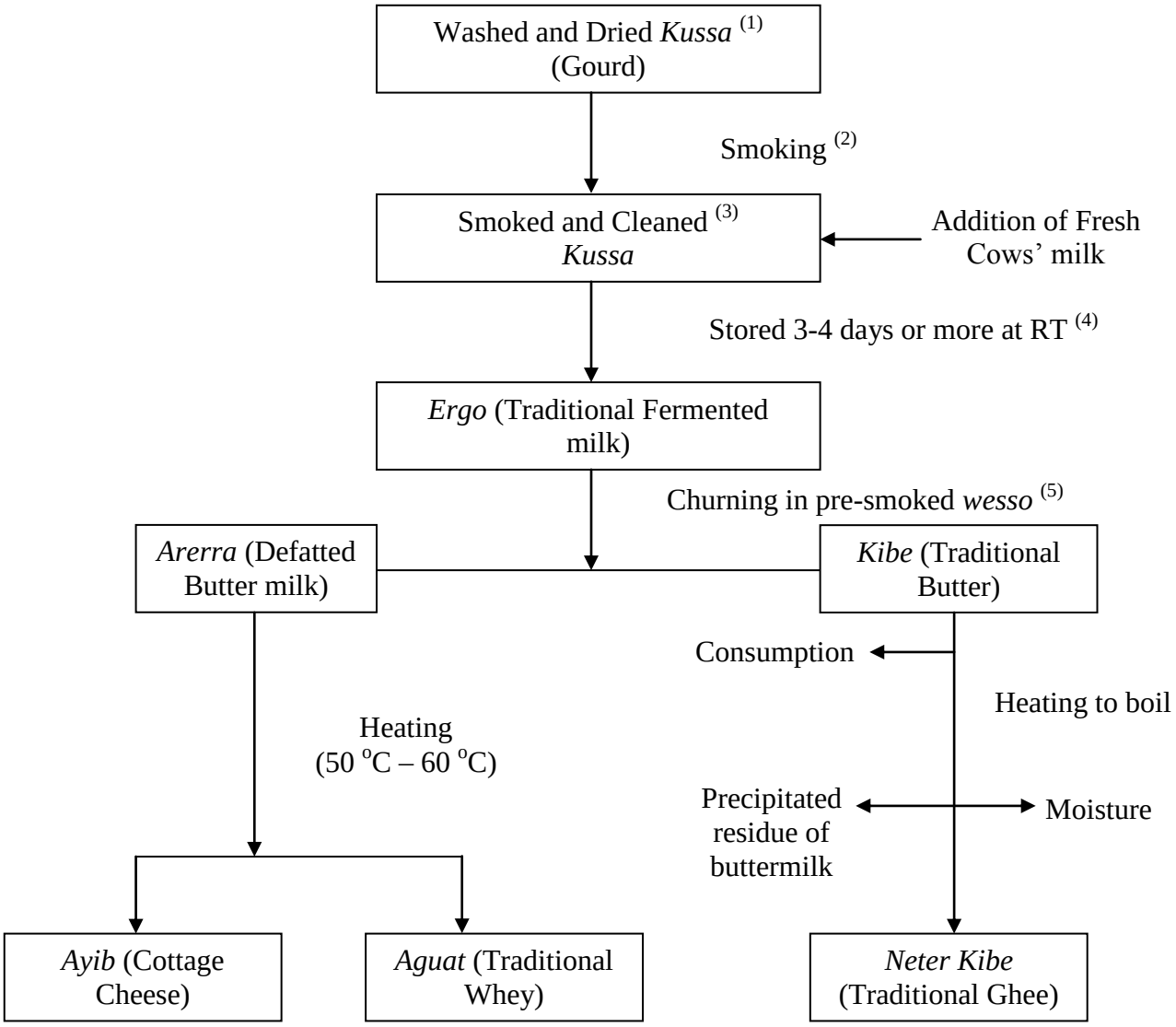


Figure 1: Flow scheme for processing of various traditional fermented milk products. (1)Kussa is a traditional storage utensil made up of calabash, clay pot or hollowed wood. (2)Smoking is done using burned stems of selected plants. (3)Cleaning is done using plant fiber materials commonly known as *foxso*. (4)RT – room temperature (5)*Wesso* is a traditional utensil, which may be either a calabash, larger clay pot, hollowed wood or animal skin for the purpose of churning. (Gonfa, Foster, & Holzapfel, 2001, pp. 173–186)

Ghee is the most widely used milk product in the Indian sub-continent. Ghee is also gaining popularity in Australia, Arabian countries, the United States, the United Kingdom, Belgium, New Zealand, Netherlands and many other African and Asian countries (Illingworth, *et al.*, 2009).

Products similar to Ghee have been available in other parts of the world probably since equally ancient time and known as "*Samna*" in Egypt (Abou-Dhonia & El-Agamy, 1993), "*Meshho*" in ancient Assyrian empire (2400 BC to 612 BC) (Abdalla, 1994), "*Samin*" in

Sudan (Hamid, 1993), "*Maslee*" or "*Samn*" in Middle East, "*Rogan*" in Iran (Urbach & Gordon, 1994), and "*Samuli*" in Uganda (Sserunjogi, *et al.*, 1998). In Ethiopia Ghee is known by the name "*Neter Qibe*", which stands for clarified butter.

Ghee can be defined as a pure clarified fat exclusively obtained from milk, cream or butter, by means of processes involving application of heat at atmospheric pressure, which results in the almost total removal of moisture and solid nonfat and which gives the product a characteristic flavor and physical structure and texture (Illingworth, *et al.*, 2009).

Some ambiguity in the definition of Ghee occurs mainly due to regional differences and preferences for the product, commonly used for culinary purposes but also for particular social functions and therapeutic purposes (Sserunjogi, *et al.*, 1998).

According to Codex Alimentarius (FAO/WHO, 1997, 2006) Ghee is defined as a product exclusively obtained from milk, cream or butter by means of processes, which result in almost total removal of water and non-fat solids, with an especially developed flavor and physical structure.

Generally, there are four methods for the production of Ghee; (Illingworth, *et al.*, 2009)

- The Indigenous Milk Butter Method
- The Direct Cream Method
- The Cream Butter Method
- The Pre-Stratification Method

This paper focuses on comparing these processing methods using yield and shelf stability as the major comparison criteria. For the indigenous milk butter method, traditional Ethiopian Ghee processing method was evaluated with some modification. Pre-stratification method was not included in this study because it is not being used and outdated.

1.2.Statement of the Problem

Fat recovery is an important factor determining the efficiency and profitability of smallholder dairy enterprises in the Ethiopian highlands (Gonfa, *et al.*, 2001). In Ethiopia, traditional butter processing is associated with "losses" of up to 12% due to low rates of butterfat recovery (ILRI, 2004). These loses should be avoided.

Members of the Ethiopian Coptic church abstain from milk and animal products for approximately 150 days per year. Hence, there are periods when nearly all the milk produced must be converted into butter and cottage cheese; which have poor storage stability and are usually sold in markets nearby. The excess milk during these periods should be converted into a more shelf stable dairy product, such as Ghee.

In addition, market demand for milk fat is high during Easter and other festivals but low during the fasting periods prescribed by the Coptic Church. Processing and storing milk fat product in good quality from surplus milk is important to insure distribution of milk fat at affordable price to the society.

According to WHO/FAO dietary recommendations for combating chronic diseases, share of fat in total energy intake – the fat to energy ratio (FER)should be in the 15% to 30%range.Total fat energy of at least 20% is consistent with good health (Irz, *et al.*, 2003). Assessment of Ethiopians daily fat consumption with respect to the WHO/FAO norms, using food balance sheet data contained in the FAOSTAT Nutrition database, was attempted and reported in FAO/WHO Dietary Recommendations report, 2003. Accordingly, for Ethiopia, calories intake was1887 kcal/person/day; fat intake was19.5grams/person/day, and Fat to Energy Ratio (Fat as % of kcal) was 9.3%.The result shows that fat consumption per person is much lower than the recommended range for good health. This reality indicates the probability of certain nutrients deficiency.

The existing unhygienic milk fat products distribution system; lack of consistency in milk fat products quality; loss of milk fat due to improper handling of milk; presence of non-standardized milk fat products without proper package and label in market ,affecting consumer health; are the major problems associated with milk fat production in Ethiopia.

1.3. Objectives

General Objectives

- To assess the effect of different Ghee processing methods on shelf stability and yield

Specific Objectives

- ✓ Determine processing parameters to obtain optimum recovery of Ghee
- ✓ Determine optimum processing parameters for maximum stability of Ghee
- ✓ To determine the physical and chemical characteristics of Ghee produced by different methods
- ✓ To predict shelf life of Ghee and evaluate thermal shelf stability of Ghee produced by different methods
- ✓ To study the effect of traditionally added preservatives(*Lippia adoensis* and *Aframomum corrorima*) on thermal shelf stability of Ghee
- ✓ To study techno-economic feasibility of selected Ghee processing method

1.4. Significance of the Study

This study focuses on evaluating three Ghee production methods; namely the Direct Cream Method (DCM), the Creamery Butter Method (CBM), and Ethiopian Butter Method (EBM). The thesis will contribute as a preliminary feasibility study for designing a processing plant for the production of Ghee. Moreover, the estimated expected product shelf life associated with each processing method will be useful for comparison.

Gonfa, *et al.* (2001) mentioned that no information is available on the time – temperature relationships related to spoilage of "*Neter Qibe*", pH of the final product or its microbial spectrum. In this study, the time – temperature relationship during deterioration of Ghee for afore mentioned processing methods is studied.

CHAPTER TWO

2. Literature Review

2.1.Milk Fat Products

The codex Alimentarius standard for milk fat products (FAO/WHO, 1997, 2006) states that Anhydrous Milk Fat, Milk Fat, Anhydrous Butter oil, Butter oil and Ghee are fatty products derived exclusively from milk and/or products obtained from milk by means of processes that result in almost total removal of water and nonfat solids.

The principle behind the manufacture of milk fat products is the removal of water and water soluble components of milk to leave only pure fat or oil (Anonymous, 2003).

2.1.1. Butter

Butter is made by separating the fat in milk and contains more than 80% fat with a small amount of water dispersed through the fat as tiny droplets in the form of discontinuous phase. Butter has a pale yellow/cream color and a smooth consistency (Fellows, 2008). Butter is an emulsion of water in oil and has the following approximate composition. Fat 80%, moisture 16%, salt 2%, and milk solid non-fat 2% (O'Mahony, 1988). Butter can be made from either whole milk or cream. However, it is more efficient to make butter form cream, than from whole milk (O'Mahony, 1988). Flavor, shelf life, consistency, color and homogeneity, yield, and by-products are the most important specific requirements that must be considered for butter product manufacture (Walstra, *et al.*, 2006).

Butter making is essentially the controlled destabilization of the oil in water emulsion of cream, selective concentration of the lipid components by removal of the aqueous buttermilk fraction, and subsequent formation of a stable water-in-oil emulsion (Walstra, *et al.*, 2006).

During churning the fat globule membrane substances spreads out over the surface of the air bubbles, partly denuding the globules of their protective layer. Thus a liquid portion of the fat exudes from the globules and partly or entirely covers the globule, rendering hydrophobic. In this condition, the globules tend to stick to the air bubbles. The free fat destabilizes the foam, causing it to collapse. During churning about one half of the stabilizing material is liberated into the buttermilk. The partly destabilized globules clinging to the air bubbles thus collect in clusters cemented together by free fat. These clusters appear as butter grains (O'Mahony, 1988).

Factors influencing churning time and recovery of butter fat as butter are: (O'Mahony, 1988)

- Milk acidity: milk containing at least 0.6% lactic acid is easier to churn. Acidity higher than 0.6% does not significantly influence churning time or fat recovery
- Temperature: At low temperatures churning time is long; butter grain formation can take five hours or longer. As churning temperature increases churning time decreases. This becomes marked at temperatures above 20 ^{o}C, but as little as 60% of the butterfat may be recovered as butter at 26 ^{o}C. Sour milk is normally churned at between 15 – 26 ^{o}C depending on environmental temperatures.
- Degree of agitation: Increasing agitation reduces churning time. However, the process is temperature dependent and churning at temperatures above 20 ^{o}C results in short churning times with poor recovery of fat. The optimum churning temperature is between 17 and 19 ^{o}C.
- Extent of filling the churn: churn should be filled one third to half of its volumetric capacity. Filling to more than half the volumetric capacity increases churning time considerably but does not reduce fat recovery.

Churning efficiency is measured in terms of the time required to produce butter granules and by the loss of fat in the buttermilk. Efficiency is influenced markedly by churning temperature and the acidity of the milk or cream (O'Mahony, 1988).

The temperature of the cream during churning is of great importance. If too cool, butter formation is delayed and the grain is small and difficult to handle. If the temperature is too high, the yield of butter will be low, because a large proportion of the fat will remain in the butter milk, and the butter will be spongy and of poor quality. Cream should be churned at 10–12 ^{o}C in the hot season and at 14 – 17 ^{o}C in the cold season (O'Mahony, 1988).

In well-made salted butter the surviving micro flora will be tolerant of low temperature and relatively high salt concentrations, allowing micrococci to predominate. Environmental contaminants are likely to be inhibited and may slowly die out if the moisture is finely dispersed. Where the storage environment has a high humidity, particularly if a permeable packaging such as vegetable parchment is used, then psychrotrophic molds may grow on the surface of the butter (Robinson, 2002).

Unsalted sweet cream butter is the most susceptible to microbial contaminants because there is only the fine dispersion of the aqueous phase to inhibit growth. In ripened butters the lactic acid bacteria and reduced pH will inhibit many contaminants, including *Pseudomonas spp*.,

but will allow yeasts to grow if free moisture is available. As with all fresh products contamination of wash water with lipolytic psychrotrophs will present a risk to product quality (Robinson, 2002).

Control of shelf life of butter is multi factorial. Raw material quality is especially important because the droplets of aqueous phase entrained in the fat phase have the potential to support bacterial growth. Consequently, heat treatment of raw milk must be efficient and levels of heat stable extracellular enzyme must be low. The psychrotrophic count in the raw milk should not exceed $5x10^6$ cfu ml^{-1}. After heat treatment, the total bacterial count in the cream should be less than 10^3cfu ml^{-1} (Smit, 2003).

Furthermore, dispersion of the water droplets within the butter must be maintained. Coalescence of droplets to form free water offers the potential for rapid spoilage even when contamination is slight. Even under optimum production conditions the shelf life of butter is limited at room temperature. Butter is best stored at -25 ^oC and sweet cream, salted butter keeps satisfactorily for several years. Oxidation is an important feature of butter during storage. Shelf life can be usefully prolonged by exclusion of oxygen during packaging and during storage (Smit, 2003).

2.1.2. Traditional Ethiopian Butter

"*Qibe*", the traditional Ethiopian butter, is produced by churning *"Ergo"* in traditional utensils with a volume of 20 – 25 liters. Milk for churning is accumulated over several days in the utensil and allowed to sour in to "*Ergo*". Traditionally the processing of 20 – 25 liters of "*Ergo*" needs one to four hours of churning time and about one kg of butter is produced (Ashenafi, 2006).

The moisture content of traditional "*Qibe*" ranges from 20% to 43% (Gonfa, *et al.*, 2001). Ashenafi has reported that "*Qibe*" has 17.2% moisture, 1.3% protein, 81.2% fat, 0.1% carbohydrate, 0.2% ash, 0.024% calcium and 0.0015% iron (Ashenafi, 2006). Spoilage when stored at room temperature for a long time is probably mainly by putrefactive microorganisms (Gonfa, *et al.*, 2001).

"*Qibe*" is always made from soured milk ("*Ergo*"); cream is not used (O'Mahony & Bekele, 1985). "*Ergo*" is a traditional, spontaneously fermented milk product, which has some resemblance to yoghurt. It is thick, smooth and of uniform appearance and usually has a white milk color when prepared carefully. The product is semisolid and has a pleasant odor and taste. Depending on temperature it can be stored for 15 to 20 days (Gonfa, *et al.*, 2001).

Unhygienic milk production and processing, and absence of starter cultures result in "*Ergo*" with variable characteristics and short shelf life and higher spoilage risks due to the wide variety of microorganisms in the product (Gonfa, *et al.*, 2001).

Starter cultures can basically be classified as: (Y.H.Hui, *et al.*, 2004)

- Single-strain starter: a single well defined strain with known technological properties,
- Multiple-strain starter: 2 -6 well defined strains with known technological properties,
- Mixed-strain starter: an unknown number of undefined strains.

Single strain starter cultures are primarily used for yeasts and molds in production of beer and wine, and Lactic Acid Bacteria (LAB) for the production of a few dairy products, sausages, and sauerkraut. Multiple starter cultures are used for dairy products, sourdough, sausages and wine. Mixed undefined bacterial starter cultures, also called traditional starters are primarily used in the dairy industry and in sourdough production. (Y.H.Hui, *et al.*, 2004)

In most urban homes, no attempt is made to control the fermentation. Raw milk is left either at ambient temperatures or kept in a warmer place to ferment. In rural areas, particularly among the pastoralists, raw milk is usually kept in a well-smoked container and milk from a previous fermentation serves as source of inoculum. Lactic acid bacteria also become established on the inner walls of the container and serves as starter culture (Ashenafi, 2006).

Lactic acid bacteria are gram-positive rods or cocci, non-spore formers, catalase negative, obligatory fermentative, micro-aerophilic, usually non-motile bacteria having extensive growth requirement. They produce mainly lactic acid from glucose fermentation (Y.H.Hui, *et al.*, 2004).

The lactic acid bacteria in "*Ergo*" included *Streptococcus thermophilus, Streptococcus acidominus, Enterococcus faecalis*var *liquefaciens, Streptococcus bovis, Streptococcus mitis, Streptococcus agalactiae, Lactococcus cermoris, Leuconostoc dextranicm, Leuconostoc lactis, Lactobacillus xylosus* and *Lactobacillus lactis.* Other bacteria belonged to micrococcus species and coliforms. Yeast and mold were isolated in fairly high numbers after 96 hours fermentation in glass bottles (Gonfa, *et al.*, 2001).

In most cases "*Ergo*" was formed at 24 hours and the average aerobic mesophilic count was greater than 10^9cfu/ml. Coliform counts, which were less than10^4cfu/ml at initiation of fermentation, reached 10^6cfu/ml within 12 hours but this count decrease markedly thereafter. Lactic acid bacteria had initial counts of less than $10^4 – 10^6$cfu/ml, and maximum counts of $10^7 – 10^9$ were attained at 24 hours. Yeasts were at undetectable levels initially but reached

counts 10^5cfu/ml at 24 hours. The drop in pH was gradual until 12 hours and fell sharply thereafter with most samples reaching values of 4.3 or below. Average initial titratable acidity increased from 0.16% to 0.88% at *"Ergo"* formation. The lactic micro flora during the fermentation was dominated in most cases by cocco-bacillus shaped *Lactobacilli* (Ashenafi, 2006).

The lacto cocci were the most dominant group throughout the fermentation reaching up to 10^9cfu/ml at the end of fermentation. The lacto cocci produced L-lactic acid and were identified as *Lactococcus garvieae* and *Lactococcus lactis* subsp. *Lactis*. The lactobacilli produced D-lactic acid and belonged to one species, but the strain appeared to be different form other species of the genus *Lactobacillus*. The aerobic mesophilic bacteria also had similar counts and the yeast population increased to 10^5cfu/ml at 24 hours. Fairly high numbers of micrococci, spore formers and coliforms are detected during the first 14 – 16 hours of fermentation (Ashenafi, 2006).

Despite the general assumption that the low pH in *"Ergo"* controls the proliferation of undesirable microorganisms, the danger of listeriosis or salmonellosis from fresh "*Ergo*" must not be underestimated. (Ashenafi, 2006). As *"Ergo"* is preferably consumed soon after fermentation, 24 hours, traditional fermentation of "*Ergo*" would not guarantee that *E.coli O157:H7* can be controlled; therefore, *"Ergo"* can be a potential health hazard if prepared from milk contaminated with *E.coli O157:H7*. (Tsegaye & Ashenafi, 2005) Post fermentation inoculation of *E.coli O157:H7* in *"Ergo"* at an initial level of $\log_{10}$ 3 cfu/ml, resulted in complete elimination of test organisms at 6 hours at ambient temperature storage. (Tsegaye & Ashenafi, 2005)"*Ergo*" cannot be defined in terms of microbiological or biochemical properties. It does not have definite temperature and duration of incubation. Fermentation is carried out at ambient temperatures and precipitation of the casein is usually the sign of completion of fermentation (Ashenafi, 2006).

For production of "*Qibe*" the curd in "*Ergo*" is broken by agitation before churning starts. Agitation of churn is carried out by rocking the churn placed on the ground forwards and backwards, or by suspending it from a tripod or doorpost or shaking it on a person's lap. This process results in the formation of fat granules that will coalesce in to larger grains towards the end of the churning time. Final rotating of the churn on its base would lump the fat grains together in to "*Qibe*" which is then skimmed off. The "*Qibe*" is kneaded in cold water to remove any residual buttermilk (Ashenafi, 2006). The churn may have previously been smoked with *Olea Africana*. Besides imparting a distinct flavor to the butter, this practice has

a bacterio static effect, and may reduce processing time by heating the churn (O'Mahony & Bekele, 1985).

O'Mahony & Bekele (1985), in the survey of traditional butter production, observed four different methods of agitation,

- The churn is placed on the floor, on a soft pad of material such as sheepskin or straw, tilted at an angel of 75 ^{o}C to the horizontal and rocked back and forth.
- The churn is hung on a tripod or doorpost and swung to and fro.
- The churn is rocked on the lap
- The churn is shaken with both hands.

The latter three methods are used only with bottle gourds, and only when fewer than 10 liters of milk are churned (O'Mahony & Bekele, 1985).

The break point (the point when butter starts to form) can be detected by a change in the sound of the milk and also by inserting a straw into the churn through the vent; if there are small butter grains adhering to the surface of the straw, the break point has been reached. After churning for a few minutes more, the straw is again inserted through the vent. If the straw is clean this indicates that the butter granules have coalesced into larger grains. The churn is then rotated on its base; the grains that collect in the center form lumps of butter, which are skimmed off. The butter is then kneaded in cold water and washed to remove visible residual buttermilk. In rural markets butter is sold by volume, the weight of which can vary considerably. In the Addis Ababa market butter is sold by weight (O'Mahony & Bekele, 1985).

2.1.3. Anhydrous Milk Fat

The manufacture of Anhydrous Milk Fat is widely practiced in virtually all countries that have dairying as a major industry, particularly in the countries of European Union, notably Belgium and the Netherlands, and in both Australia and New Zealand (Illingworth, *et al.*, 2009).

According to Ethiopian standards ES 3465: 2009, which is identical to Codex Alimentarius (Codex, 2007), anhydrous milk fat, anhydrous butter oil and butter oil are fatty products delivered exclusively from milk and (or) products obtained from milk by means of processes which result in almost total removal of water and non-fat solids. O' Mahony (1988) describes

butter oil or anhydrous milk fat as a refined product made by centrifuging melted butter or by separating milk fat form high fat cream.

Anhydrous milk fat lacks the characteristic flavor, aroma and texture that are otherwise required in Ghee, although the chemical composition is almost identical to that of Ghee (Illingworth, *et al.*, 2009).

Illingworth, *et al.*, (2009) described anhydrous milk fat processing steps as follows. Milk containing 3 – 4 g fat per 100g is concentrated to 40 g fat per 100g by removal of the skimmed milk, and then further concentrated to about 75 g fat per 100g by separation and removal of the serum phase, the high fat emulsion can be directly inverted to release the fat. Further concentration, to remove the remaining milk solid nonfat and polishing of the fat using separators, produce 99.5 g fat per 100g butter oil that can be dehydrated, resulting in Anhydrous milk fat. Alternatively 40 g fat per 100g cream can be churned to butter (80 – 82 g per 100 g), a process that also involves inverting the cream emulsion, removing butter milk in the process similar to the direct from cream process.

Both salted and unsalted sweet cream butters together with lactic butter can be used as starting materials, either from fresh production or more commonly using butters that have been stored frozen for some time (Illingworth, *et al.*, 2009).

An important application of anhydrous milk fat is production of recombined liquid milk. Low-heat- or medium-heat skim milk powder is mixed with water and anhydrous milk fat. Anhydrous milk fat can also be used in production of blends, low-fat dairy spreads and blended spreads; and an obvious application for low-melting fractions of anhydrous milk fat is in butter and dairy fat spreads in order to make these products more spreadable at low temperatures. Considerable quantities of anhydrous milk fat are used for production of ice cream in which it contributes not only to the flavor of the product, but also to a smooth, full, rich and creamy mouth-feel. One of the advantages of using anhydrous milk fat instead of cream or butter is that it is easier to handle in an automatic process line for preparing the ice cream mix. Anhydrous milk fat is also used as an important ingredient in a variety of confectionery products despite its relatively high cost compared with most other fats and oils. (Mortensen, 2011)

2.1.4. Ghee

Codex Alimentarius defines Ghee as a product exclusively obtained from milk, cream or butter by means of processes, which result in almost total removal of water and non-fat solids,

with an especially developed flavor and physical structure (Codex, 2007). Ghee is primarily distinguished from butter oil by its flavor characteristic (Illingworth, *et al.*, 2009).

Ghee can be defined as a pure clarified fat exclusively obtained from milk, cream or butter, by means of processes involving application of heat at atmospheric pressure, which results in the almost total removal of moisture and solid nonfat and which gives the product a characteristic flavor and physical structure and texture (Illingworth, *et al.*, 2009).

The International Dairy Federation (IDF) defined Ghee as a product exclusively obtained from milk, cream or butter form various animal species by means of process, which results in the almost total removal of moisture, and solids-not-fat and which gives the product a particular physical structure. The standard specifies Ghee to have 96% minimum milk fat, 0.3% maximum moisture, 0.3% maximum free fatty acids expressed as oleic acid, and a peroxide value less than 1.0 (Sserunjogi, *et al.*, 1998).

Chemically, Ghee is a complex lipid of glycerides (usually mixed), free fatty acids, phospholipids, sterols, sterol esters, fat-soluble vitamins, carbonyls, hydrocarbons, carotenoids (only in Ghee derived from cow milk), small amounts of charred casein and traces of calcium, phosphorus, iron, etc. It contains not more than 0.3% moisture. Glycerides constitute about 98% of the total material. Of the remaining constituents of about 2%, sterols (mostly cholesterol) occur to the extent of about 0.5%. Ghee has a melting range of 28 to 44 ^{o}C. The Saponification number is not less than 220. Ghee is not highly unsaturated, as is evident from its iodine number of from 26 to38. The fatty acid profile of glycerides of Ghee is very complex and still not completely elucidated (Ganguli & Jain, 1972). General composition requirement for Ghee and other milk fat products is shown in Table 1 below.

The component fatty acids in Ghee range from C_4 to C_{22}. Cow Ghee Contains approximately 72 µg of carotenoids per 100 g, which gives it a distinctive yellow color. Various FFA including C_6, C_8, C_{10}, C_{12}, $C_{14:1}$, C_{15}, C_{16}, C_{18}, and $C_{18:2}$ were reported to occur in Ghee. The short chain FFA, C_2 to C_4, have not been identified in cow or buffalo Ghee. The absence of these short-chain FFA in Ghee is possibly due to volatilization during the heat clarification of butter/cream (Sserunjogi, *et al.*, 1998).

Table 1: Requirements of anhydrous milk fat, anhydrous butter oil, milk fat, butter oil and Ghee (ES, Milk fat products - Specification, 2009, p. 2)

Characteristics	Milk fat	Butter oil	Anhydrous butter oil	Anhydrous milk fat	Ghee	Test method
Milk fat, min,% by mass	99.6	99.6	99.8	99.8	99.6	ES 3476
Moisture, max,% by mass	-	-	0.1	0.1	-	ES ISO 5536
Acidity, max, % by mass as oleic acid	0.4	0.4	0.3	0.3	0.4	ES ISO 1740
Peroxide value, max, milliequivalent of oxygen 1kg fat	0.6	0.6	0.3	0.3	0.6	ES ISO 3976
Copper, max, ppm	0.05	0.05	0.05	0.05	0.05	ES ISO 5738
Iron, max, ppm	0.2	0.2	0.2	0.2	0.2	ES ISO 6732

Ghee is susceptible to deterioration from exposure to light, air and metal ions (Illingworth, Patil, & Tamime, 2009).Ghee is preserved by a combination of heat, which destroys enzymes and contaminating microorganisms, and by removing water from the oil to prevent microorganisms growing during storage. It has a long shelf life if it is stored in a cool place, using airtight, lightproof and moisture-proof containers to slow down the development of rancidity. (Fellows, 2008).

Ghee is a more convenient product than butter in the tropics because it keeps better under warm conditions. It has low moisture and milk solid non-fat contents, which inhibits bacterial growth (O'Mahony, 1988). The storage stability of Ghee is attributed to the low moisture content and the high content of phospholipids (ca. 400 mg/Kg). The low acidity of the Ghee and the presence of natural antioxidants are also believed to contribute to the extension of its shelf life (Sserunjogi, *et al.*, 1998).

Ghee is primarily used for cooking and frying and as dressing or toppings for various foods. It is also used in the manufacture of snacks and sweets often mixed with vegetables, cereals, fruits, and nuts. In some parts of the world, Ghee is considered as a sacred product and is used in religious rites. (Mortensen, 2011)

Ghee contributes significantly towards nourishment of people of all age groups. It is a good source of fat-soluble vitamins (A, D, E and K) and essential fatty acids. Ghee contains about 0.2–0.4% cholesterol. Consumption of Ghee and other fat rich dairy products makes appreciable contribution to cholesterol intake. Furthermore, some cholesterol oxidation products have been reported as cytotoxic, atherogenic, mutagenic and carcinogenic. Recent wave against cholesterol-containing foods is damaging the image and market growth of Ghee and other dairy products. (Kumar, *et al.*, 2010)

2.1.5. Traditional Ethiopian Ghee

Traditional Ghee "*Neter Qibe*" is a more convenient product than "*Qibe*" in the tropics, because of its better shelf life under warm conditions. "*Neter Qibe*" has an attractive appearance, a grainy texture and a light yellow color. At room temperature it is semi-solid. It has a pleasant odor and good taste. Its good keeping quality allows storage for more than a year without any change (Gonfa, *et al.*, 2001).

During production of "*Neter Qibe*", heating of the melted butter is continued until bubbling ceases. Fresh leaves of "*O. hadiense*", "*O.basilicum*", mashed *Allium sativum* (garlic), *Zigeber officinale* (ginger) and other herbs may be added during processing. The "*Neter Qibe*" is decanted into another container leaving the curd material in the pan (Gonfa, *et al.*, 2001).

2.2. Ghee Processing Technology

Currently, Ghee is produced by a variety of methods, from cows' or buffaloes' milk. Cream can be obtained by centrifugal separation, possibly followed by starter addition and fermentation; butter is also used for Ghee manufacture. Heating to remove water can be done in various apparatuses, and solids are often removed by filtration (Walstra, *et al.*, 2006).

Ghee may be produced through heat clarification of cream or via conversion into butter, followed by heat desiccation. There are four methods for the production of Ghee: (Illingworth, *et al.*, 2009)

- ✓ The indigenous milk butter method
- ✓ The direct cream method
- ✓ The cream butter method
- ✓ The pre-stratification method

A general flow diagram for the four processing methods is shown in **Error! Reference source not found. Error! Reference source not found.**.

The creamery butter and direct cream methods are more suitable for commercial operations because less fat is lost. Direct cream method is reportedly most economical for preparing Ghee and the product has better keeping quality. In the pre-stratification method, advantages such as economy in fuel consumption and production of Ghee with low acidity and comparatively longer shelf life have been claimed. However, this method has not been adopted by industry. With industrial interest, the creamery butter and direct cream methods are increasing (Ganguli & Jain, 1972).

In addition to the above four methods a continuous Ghee processing method was developed to overcome the problems of low heat transfer coefficients, limitations of the scale of operation and excessive exposure of the plant operators to the stress of heat and humidity associated with the batch methods. (Sserunjogi, *et al.*, 1998)

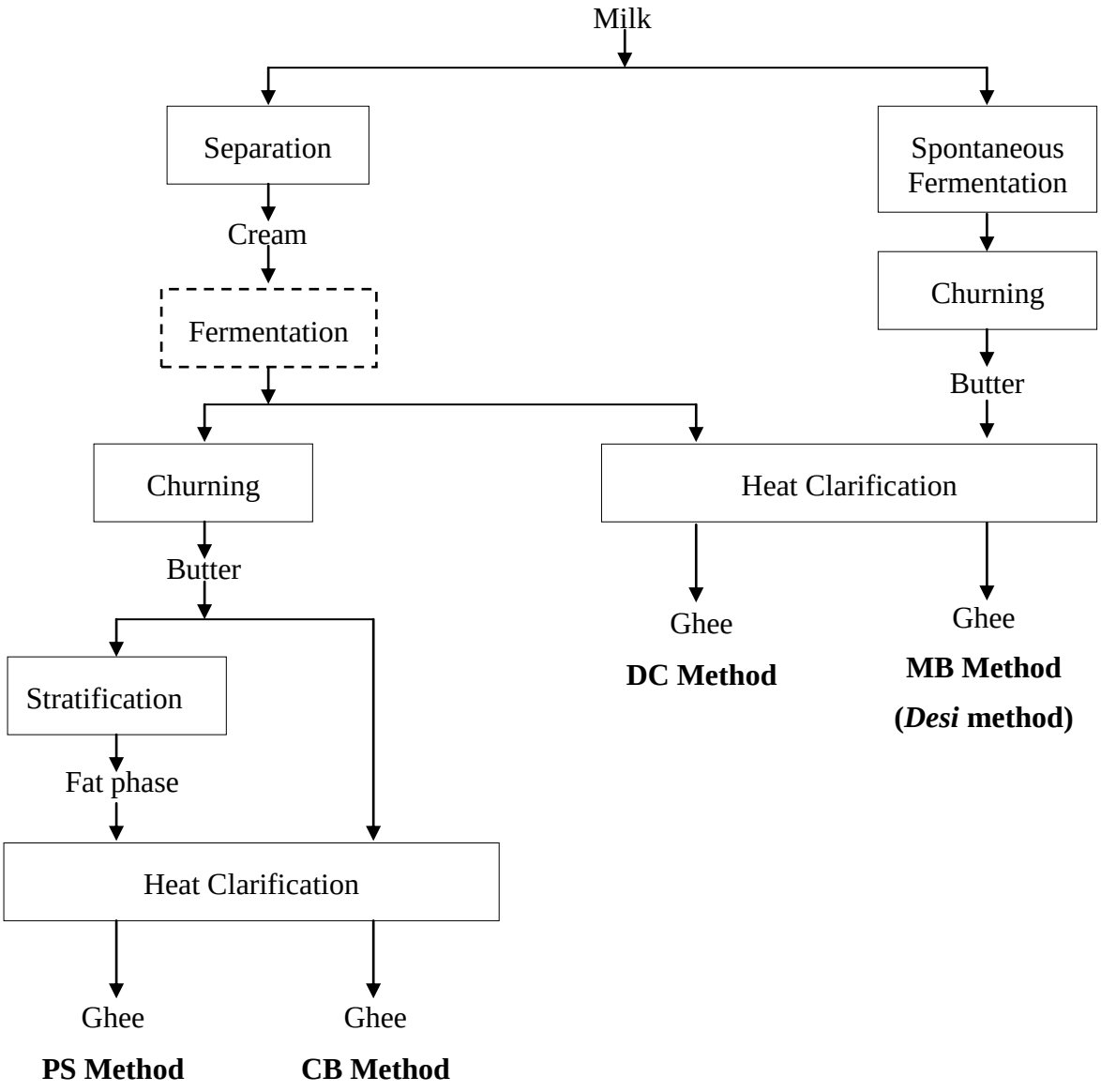


Figure 2: Flow diagram illustrating four methods of Ghee manufacture: milk butter (MB) (desi); cream butter (CB); direct cream (DC); pre-stratification (PS). (Sserunjogi, Abrahamsen, & Narvhus, 1998, p. 679)

The first step of Ghee manufacture involves the preparation of the raw material, that is, whole milk, clotted milk, cream or butter. If the starting material is milk, it is normally allowed to ferment to produce fermented milk before it is churned to produce butter. It is also preferable

to use soured cream; otherwise the resultant Ghee is regarded as flat and tasteless (Illingworth, *et al.*, 2009).

The quality of Ghee depends on milk, cream, or butter, methods of preparation, temperature of clarification, storage conditions, and type of animal feed. These factors in turn will determine the physicochemical characteristics of Ghee (Ganguli & Jain, 1972).

Milk composition is affected by genetic and environmental factors: (O'Mahony, 1988)

- Genetic: milk composition varies considerably among breeds of dairy cattle. Jersey and Guernsey breeds give milk of higher fat and protein content than shorthorns and Friesians. Zebu cows can give milk containing up to 7% fat. The potential fat content of milk from and individual cow is determined genetically, as are protein and lactose levels. Thus, selective breeding can be used to upgrade milk quality.
- Environmental: Interval between milking, stage of lactation, age, feeding regime, disease and completeness of milking affect composition.

In milk, the fat is enclosed within a membrane that consists of proteins, phospholipids and cholesterol. This membrane protects the fat against attack by bacteria that can lead to both lipolysis and oxidation, and maintains the fat in stable oil in water emulsion. To recover the fat, it is necessary to rupture the membrane and invert the emulsion (Illingworth, *et al.*, 2009).

To convert butter or cream to Ghee, heat is applied at controlled temperatures at the various stages of processing. Initially, the temperature is gradually raised to about the boiling point of water while stirring to control frothing. In the second stage, most of the free water evaporates which requires a considerable amount of heat. As most of the water evaporates the rate of heating is controlled and maintained at about 103 ^{o}C to prevent the charring of solid not fat so as not to develop bitter flavors and/or a brown color. Overheating could drive off desirable volatile flavor materials and also impair the formation of suitable grains up on cooling (Sserunjogi, *et al.*, 1998).

A lower heating temperature improves the color, but decreases the keeping quality of the Ghee obtained due to its greater residual moisture content. A higher temperature, on the other hand, tends to reduce the vitamin A content (with acid butter) and darken the color, but increases the keeping quality of the finished product (Illingworth, *et al.*, 2009).

Overheating could drive off desirable volatile flavor materials and also impair the formation of suitable grains upon cooling. The impairment of crystal formation appears to be associated with the possible volatilization of some short-chain free fatty acids, which changes the normal

composition of the fat in Ghee. Finally, the temperature is raised to between 105 and 118 ^{o}C with constant agitation in order to remove the water bound to the solid non-fat and to develop the characteristic flavor. In general, a temperature range of 110 – 120 ^{o}C is preferred (Illingworth, *et al.*, 2009).

Flavor is greatly influenced by the fermentation of the cream or butter and the heating process. Carbonyls, lactones and free fatty acids are reported to be the key Ghee flavoring compounds (Sserunjogi, *et al.*, 1998). Much of the typical flavor, in Ghee, comes from the burned milk solid non-fat remaining in the product (O'Mahony, 1988). The most important factor controlling the intensity of flavor in Ghee is the temperature of clarification. The acidity of the cream or butter also affects the flavor of Ghee (Ganguli & Jain, 1972). The flavor of Ghee has been reported to be superior when the average free fatty acid content is well above 0.3%. It is preferable to use soured cream, otherwise the resultant Ghee is regarded as flat and tasteless (Sserunjogi, *et al.*, 1998).

Ghee undergoes physic-chemical changes, dependent primarily on the temperature of storage. Crystallization occurs with the formation of solid, semi-solid and liquid layers. Ghee (cow and buffalo) kept either in a metal or glass container at 20 ^{0}C or below, solidifies uniformly with fine crystal. Above 20 ^{0}C and below 30 ^{0}C, solidification is a loose structure. Ghee stored at high temperature is susceptible to oxidative deterioration, rancidity, and off flavor. Shelf life of Ghee is also dependent on the method of preparation (Ganguli & Jain, 1972).

Granularity is considered to be an important criterion of quality and even purity. The granular form of Ghee is primarily due to a certain content of glycerides of higher melting saturated fatty acids, especially Palmitic and Stearic (Sukumar, 1990). The impairment of crystal formation appears to be associated with the possible volatilization of some short-chain FFA, which changes the normal composition of the fat in Ghee (Sserunjogi, *et al.*, 1998). Heating Ghee to 60 – 100 ^{o}C, followed by rapid cooling, yields small grains in Ghee; however, if the above Ghee is held for crystallization at a temperature about 1^{o}C above the melting point of Ghee (for cow Ghee 29 ^{o}C) a large number of big grains result (Sukumar, 1990). Cold storage of Ghee should be avoided, since it leads to a loss of granularity and the development of a waxy consistency in the stored product (Sukumar, 1990).

2.3.Shelf Stability Analysis

2.3.1. Shelf Life

Shelf life is the length of time that a product may be stored without deterioration. The Institute of Food Science and Technology have defined shelf-life as "the period of time during which the food product will remain safe; be certain to retain desired sensory, chemical, physical and microbiological characteristics; comply with any label declaration of nutritional data." (Wilbey, 1997)

The ultimate shelf life of a product will be determined by its deterioration on storage. The main mechanisms of deterioration are: microbiological, structural, biochemical and chemical. (Wilbey, 1997)

Many factors can influence shelf life, and can be categorized into intrinsic and extrinsic factors. Intrinsic factors are the properties of the final product. Intrinsic factors are influenced by such variables as raw material type and quality, and product formulation and structure. They include the following: (Kilcast & Subramaniam, 2000)

- ✓ Water activity (a_w) (available water)
- ✓ pH value and total acidity; type of acid
- ✓ Redox potential (E_h)
- ✓ Available oxygen
- ✓ Nutrients
- ✓ Natural micro flora and surviving microbiological counts
- ✓ Natural biochemistry of the product formulation (enzymes, chemical reactants)
- ✓ Use of preservatives in product formulation (e.g. salt)

Extrinsic factors are those factors the final product encounters as it moves through the food chain. They include the following: (Kilcast & Subramaniam, 2000)

- ✓ Time–temperature profile during processing; pressure in the headspace
- ✓ Temperature control during storage and distribution
- ✓ Relative humidity (RH) during processing, storage and distribution
- ✓ Exposure to light (UV and IR) during processing, storage and distribution
- ✓ Environmental microbial counts during processing, storage and distribution
- ✓ Composition of atmosphere within packaging
- ✓ Subsequent heat treatment (e.g. reheating or cooking before consumption)
- ✓ Consumer handling

All these factors can operate in an interactive and often unpredictable way; and the possibility of interactions must be investigated. A particularly useful type of interaction occurs when factors such as reduced temperature, mild heat treatment, antioxidant action and controlled atmosphere packaging operate in concert to restrict microbial growth, the so-called 'hurdle

effect'. This way of combining factors that, individually, are unable to prevent microbial growth but, in combination, provide a series of hurdles, which do so, allows manufacturers to use milder processing techniques, which retain more of a product's sensory and nutritional properties (Kilcast & Subramaniam, 2000).

The shelf life of products can be extended by the use of processing treatments which kill the microorganisms (e.g. heat, radiation) or through the control of microbial growth by controlling temperature (chilling and freezing), reducing the a_w (drying and pickling) and by the addition of preservatives (Kilcast & Subramaniam, 2000).

There are a range of points in the food chain where manufacturers can influence the mix of intrinsic and extrinsic factors, which affect shelf-life. These include: (Kilcast & Subramaniam, 2000)

- ✓ Raw material selection and quality
- ✓ Product formulation and assembly
- ✓ The processing environment
- ✓ Processing and preservation techniques
- ✓ Packaging
- ✓ Storage and distribution
- ✓ Consumer handling

Designing a shelf life test is a synthetic approach that requires sufficient understanding of all food-related disciplines, namely food engineering, food chemistry, food microbiology, analytical chemistry, physical chemistry, polymer science, and food regulations (Taoukis, *et al.*, 1997)

A major goal for the food scientist is the prediction of the change in quality of a particular food as a function of both time and environmental condition. In order to make useful predictions about shelf life, there search scientist needs information regarding (Labuza, 1984)

- The potential major modes for loss of quality of the product,
- The factors which control the initial quality or nutritional value during manufacture,
- The environmental conditions the food will be exposed to including temperature, relative humidity and light,
- Whether it is packaged in a semi-permeable container, and, if so, the permeability of that film to oxygen, water vapor, and light, and
- The kinetics of the reactions leading to loss of quality or nutritional value as a function of the reaction phase conditions in the food and the external environment

Most foods contain some amount of unsaturated fatty acids in the form of triglycerides and as part of membrane phospholipids. The reaction between the unsaturated fat and oxygen is a complex, free radical mechanism and results in the production of low molecular weight volatiles such as aldehydes, ketones, acids and alcohols. Some of these, such as hexanal, impart an off odor to the food making it unacceptable even at a very low extent of reaction. (Labuza, 1984)

Other intermediate products of the free radicals themselves can react with:

- Proteins, causing loss of solubility and biological value
- Fat soluble pigments, such as carotenoids, causing color bleaching, and
- Vitamins such as A, C, and E, causing loss of their efficacy.

In addition, the radicals and peroxides can interact leading to the production of high molecular weight polymers, which can darken the product and may have toxicological properties. Lipid oxidation probably is the major form of deterioration of most dry foods as well as some frozen foods. (Labuza, 1984)

In many cases it is impossible to identify the exact chemical reaction leading to loss of quality. In these cases many researchers have made sensory evaluations of the food quality using trained or untrained panelists o evaluate the food and assign it some numerical value. This value then becomes the "chemical species", and change in this value becomes the mode of deterioration. If some minimum allowed value is chosen, then the same approach that is used to study reaction rates can be applied to predict the sensory quality shelf life. (Labuza, 1984)

2.3.2. Lipid Deterioration

The term rancidity refers to the off odors and flavors resulting from lipolysis (hydrolytic rancidity) or lipid oxidation (oxidative rancidity) (O'Keefe & Pike, 2010). In hydrolytic rancidity fatty acids are broken off from the glycerol molecules by lipase enzymes produced by milk bacteria. The resulting free fatty acids are volatile and contribute significantly to the flavor of the product (O'Mahony, 1988). Lipolysis is the hydrolysis of fatty acids from the glyceride molecule. Because of their volatility, hydrolysis of short-chain fatty acids can result in off odors. Fatty acids shorter than C_{12} (lauric acid) can produce off-odors in foods. Free C_{12} is often associated with a soapy taste but no aroma. FFAs longer than C_{12} do not cause significant impairment in taste or odor (O'Keefe & Pike, 2010).

Oxidative rancidity occurs when fatty acids are oxidized. In milk products it causes tallowy flavors (O'Mahony, 1988). Lipid oxidation (also called autoxidation) as it occurs in bulk fats and oils proceeds via a self-sustaining free radical mechanism that produces hydro peroxides (initial or primary products) that undergo scission to form various secondary products including aldehydes, ketones, organic acids, and hydrocarbons (final or secondary products) (O'Keefe & Pike, 2010). Oxidative or hydrolytic decomposition of lipids can lead to serious problems in bulk oil in a storage tank, in the continuous phase of triacylglycerols in butter, in milk fat globules, in the phosphor-lipid bi-layer in cell membranes, in tissue lipids in lean fish, in skin fat in chicken, and in frying oil or fried food (Taub & Singh, 1998).

2.3.2.1. Hydrolytic Rancidity

The majority of natural lipids consist of fatty acids attached to glycerol through carboxylic ester bonds. Hydrolysis of the ester bonds, catalyzed by acid, alkali, heat, moisture, or lipolytic enzymes, will result in the liberation of free fatty acids (Taub & Singh, 1998).

Lipolytic enzymes usually act at oil–water interfaces. Most have specificity for positions on the triacylglycerols molecules. For example, pancreatic lipase is specific for certain positions. Such enzymes may be present naturally in food or in constituents mixed with the food; some could be associated with microbial contamination. Temperature, moisture, and pH are among the factors that control lipase activity (Taub & Singh, 1998).

Off-flavors resulting from hydrolytic rancidity (such as soapy taste in milk or coconut oil) are more likely to occur in fats containing relatively short chain fatty acids (i.e., C4-10). The potential for lipolysis in milk, however, is minimized due to the structure of the milk emulsion, which limits physical contact between the triacylglycerols substrates residing in fat globules and the lipase enzyme in the skim milk. Agitation during processing, storage, or transportation can disrupt the native milk structure and promote enzyme–substrate interaction. Although heat inactivates the lipolytic microorganisms, the lipases produced by them can survive normal pasteurization temperatures (Taub & Singh, 1998).

In general, several strategies can be followed to protect lipids from hydrolysis; foremost among these is the selection of high-quality raw materials. Inactivating lipolytic enzymes by heat and avoiding storage conditions that promote growth of contaminating microorganisms of high lipolytic activity are also effective strategies (Taub & Singh, 1998)

2.3.2.2. Oxidative Rancidity

Lipid oxidation is a major cause of food quality deterioration, and has been a challenge for manufacturers and food scientists alike. Lipids are susceptible to oxidative processes in the presence of catalytic systems such as light, heat, enzymes, metals, metalloproteins, and microorganisms, giving rise to the development of off-flavors and loss of essential amino acids, fat-soluble vitamins, and other bio actives. Lipids may undergo autoxidation, photo-oxidation, thermal oxidation, and enzymatic oxidation under different conditions, most of which involve some type of free radical or oxygen species. (Shahidi & Zhong, 2005)

Autoxidation is the most common process leading to oxidative deterioration and is defined as the spontaneous reaction of atmospheric oxygen with lipids. The process can be accelerated at higher temperatures, such as those experienced during deep fat frying, which is called thermal oxidation, with increases in free fatty acid and polar matter contents, foaming, color, and viscosity. Unsaturated fatty acids are generally the reactants affected by such reactions, whether they are present as free fatty acids, triacylglycerols (as well as diacylglycerols or monoacylglycerols), or phospholipids. It has been accepted that both autoxidation and thermal oxidation of unsaturated fatty acids occurs via a free radical chain reaction that proceeds through three steps of initiation, propagation, and termination (Shahidi & Zhong, 2005).

Oxidation processes begin virtually as soon as the membranes around the milk fat globules in milk are ruptured, allowing air to come into contact with the fat (Illingworth, *et al.*, 2009). In general, lipid oxidation proceeds via a typical self-propagating free radical mechanism where oxygen attack occurs mainly at positions adjacent to the double bonds. The chain reaction is preceded by an initiation step in which free radicals are produced by some catalytic means; e.g., metal catalysis, exposure to light or high energy radiation, singlet oxygen, or decomposition of added peroxides (Taub & Singh, 1998).Although saturated fatty acids can react with oxygen, the susceptibility of lipids to oxidation is significantly greater with higher unsaturation in the fatty acid chains. Fatty acids containing one or more of the non conjugated pentadiene systems, –CH=CH–CH_2–CH=CH–, are especially sensitive. (Taub & Singh, 1998)

As oxidation normally proceeds very slowly at the initial stage, the time to reach a sudden increase in oxidation rate is referred to as the induction period. Lipid hydro peroxides have been identified as primary products of autoxidation; decomposition of hydro peroxides yields aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds, known as secondary oxidation products. These compounds, together with free radicals,

constitute the bases for measurement of oxidative deterioration of food lipids. (Shahidi & Zhong, 2005).

2.3.3. Factors Affecting Lipid Oxidation

Rate of oxidation is affected by a number of factors such as nature of the substrate, Free Fatty Acids, Oxygen Concentration, Temperature, Water Content, Physical Condition, Pro-oxidants, and Antioxidants (Taub & Singh, 1998).

In general, FFA oxidizes faster than their glycerol esters. The presence in commercial oils of relatively large amounts of FFA can enhance the pick up of trace metals from equipment or storage tanks and thereby increase the rate of oxidation (Taub & Singh, 1998). FFA is undesirable in milk fat products because the shorter-chain homologues are primarily responsible for the rancid flavor typified by butyric acid. The short chain FFA has been reported also to catalyze the fat oxidation reaction. (Sserunjogi, *et al.*, 1998) However, FFA contributes to the normal flavor of Ghee and their level has been closely related to the flavor quality.

At very low oxygen pressure, the rate of oxidation is approximately proportional to oxygen pressure. If the supply of oxygen is unlimited, the rate of oxidation is independent of oxygen pressure. The availability of oxygen clearly plays a critical role in determining competitive oxidative pathways, (e.g., per oxidation vs. polymerization). For temperature, rates of reaction increase with increase in temperature. However, as the temperature increases, the increase in rate with increasing oxygen concentration becomes less evident, because oxygen becomes less soluble (Taub & Singh, 1998).

In dried food with very low moisture content (aw <0.1), oxidation proceeds very rapidly. Increase in aw to about 0.3 retards lipid oxidation, reportedly by reducing metal catalysis, quenching free radicals, promoting non-enzymatic browning, and/or impeding oxygen accessibility. At higher aw (0.55–0.85), the rate of oxidation increases again, presumably due to increased mobilization of the catalysts present. In addition, the rate of oxidation increases in proportion to the surface area of the lipid exposed to air (Taub & Singh, 1998).

Pro oxidants such as enzymes can catalytically promote lipid oxidation. Transition metal ions, particularly copper and iron, are also major catalysts for oxidation. At very low concentrations, <0.1 ppm, they can decrease the induction period and increase the rate of oxidation. Such metal ions either in free and bound forms occur naturally in plant and animal tissues, membranes, and enzymes. They are also introduced into food by contact with metallic

equipment used in processing or storage. These are also substances, occurring naturally or synthesized, that can delay the onset or slow the rate of oxidation. The main antioxidants used in food are mono or polyhydric phenols with ring substitutions. They either inhibit the formation of free radicals in the initiation step or interrupt the propagation of the free radical chain reaction. For maximum efficiency, primary antioxidants are used in combination with other phenolic antioxidants, or with metal-sequestering agents (Taub & Singh, 1998).

2.3.4. Measuring Lipid Deterioration

Lipid oxidation is a complex process involving numerous reactions that give rise to numerous chemical and physical changes. These reactions may occur stepwise, simultaneously, and/or competitively. The rates of these reactions, and the nature and fate of the products they form, are influenced by many variables. Consequently, no single test can possibly measure all oxidative events, and no single test can be universally applicable to all foods, all conditions of processing, or all stages of the oxidative process. Obviously, a more reliable evaluation can be obtained by using a combination of tests (Taub & Singh, 1998). The available methods to monitor lipid oxidation in foods can be classified into five groups based on what they measure: the absorption of oxygen, the loss of initial substrates, the formation of free radicals, and the formation of primary and secondary oxidation products (Shahidi & Zhong, 2005).

A number of physical and chemical tests, including instrumental analyses, have been employed in laboratories and the industry for measurement of various lipid oxidation parameters. These include the weight-gain and headspace oxygen uptake method for oxygen absorption; chromatographic analysis for changes in reactants; iodometric titration, ferric ion complexes, and Fourier transform infrared (FTIR) method for peroxide value; spectrometry for conjugated dienes and trienes, 2-thiobarbituric acid (TBA) value, p-anisidine value (p-AnV), and carbonyl value; Rancimat and Oxidative Stability Instrument (OSI) method for oil stability index; and electron spin resonance (ESR) spectrometric assay for free-radical type and concentration. Other techniques based on different principles, such as differential scanning calorimetry (DSC) and nuclear magnetic resonance (NMR), have also been used for measuring lipid oxidation. In addition, sensory tests provide subjective or objective evaluation of oxidative deterioration, depending on certain details (Shahidi & Zhong, 2005).

2.3.4.1. Measurement of Reactant Change

Lipid oxidation can be assessed by quantitatively measuring the loss of initial substrates. In foods containing fats or oils, unsaturated fatty acids are the main reactants whose composition

changes significantly during oxidation. Changes in fatty acid composition provide an indirect measure of the extent of lipid oxidation (Shahidi & Zhong, 2005).

Measures of fat acidity normally reflect the amount of fatty acids hydrolyzed from triacylglycerols. FFA is the percentage by weight of a specified fatty acid (e.g., percent oleic acid). Acid value (AV) is defined as the mg of KOH necessary to neutralize the free acids present in 1 g of fat or oil. In samples containing no acids other than fatty acids, FFA and acid value may be converted from one to the other using Equation 1 below. Acid value conversion factors for lauric and Palmitic are 2.81 and 2.19, respectively (O'Keefe & Pike, 2010).

Equation 1

$$\%FFA(as\ oleic) * 1.99 = Acid\ Value$$

If the fatty acids liberated are volatile, FFA or acid value may be a measure of hydrolytic rancidity (O'Keefe & Pike, 2010).

However, measuring fatty acid is an insensitive way of assessing oxidative deterioration. For comparison through calculation, oxidation of 0.4% polyunsaturated fatty acids to mono hydro peroxides would represent a change of 16 meq oxygen/kg oil in peroxide value, whereas a change of less than 1.0 meq oxygen/kg oil could readily be detected by measuring peroxide value. Additionally, the application of this method is limited because of its inability to serve as an indicator of oxidation of more saturated lipids. Nevertheless, its usefulness for measuring oxidation of highly unsaturated oils cannot be underestimated (Shahidi & Zhong, 2005).

2.3.4.2. Measurement of Primary Products of Oxidation

Lipid oxidation involves the continuous formation of hydro peroxides as primary oxidation products that may break down to a variety of nonvolatile and volatile secondary products. The formation rate of hydro peroxides outweighs their rate of decomposition during the initial stage of oxidation, and this becomes reversed at later stages. Analytical methods for measuring hydro peroxides in fats and oils can be classified as those determining the total amount of hydro peroxides and those based on chromatographic techniques giving detailed information on the structure and the amount of specific hydro peroxides present in a certain oil sample (Shahidi & Zhong, 2005).

The PV represents the total hydro peroxide content and is one of the most common quality indicators of fats and oils during production and storage (Shahidi & Zhong, 2005). Peroxides

are primary intermediates of lipid autoxidation. Standard methods for their measurement are based on their ability to liberate iodine from potassium iodide or to oxidize ferrous to ferric ions (Taub & Singh, 1998). Peroxide value is defined as the mill equivalents (meq) of peroxide per kilogram of sample. It is a redox titrimetric determination. The assumption is made that the compounds reacting under the conditions of the test are peroxides or similar products of lipid oxidation (O'Keefe & Pike, 2010). To measure peroxide the sample is dissolved in solvent, treated with potassium iodide, and the iodine liberated by the peroxides present in rancid fat or oil is titrated with sodium thiosulfate solution. Often the number of mill moles of peroxide oxygen is reported, and the result is then half that of peroxide value (S.Ranganna, 1986).

Although PV is useful for monitoring oxidation at the early stages, it can be misleading because its accuracy may vary with the procedure used and the treatment history of the food product. As the oxidation progresses, PV reaches a peak and then declines (Taub & Singh, 1998). A low value may represent either the beginning of oxidation or advanced oxidation, which can be distinguished by measuring peroxide value over time or by using a procedure that measures secondary products of oxidation (O'Keefe & Pike, 2010). If the food is subjected to elevated temperatures (e.g., as in frying), peroxides will decompose and PV will decline. Accordingly, a low PV does not always mean a low level of oxidation (Taub & Singh, 1998).

For determination in foodstuffs, a disadvantage of this method is the 5 g fat or oil sample size required; it is difficult to obtain sufficient quantities from foods low in fat. This method is empirical and any modifications may change results. Despite its drawbacks, peroxide value is one of the most common tests of lipid oxidation. High-quality, freshly deodorized fats and oils will have a peroxide value of zero. Peroxide values >20correspond to very poor quality fats and oils, which normally would have significant off flavors (O'Keefe & Pike, 2010).

A number of methods have been developed for determination of PV, among which the iodometric titration, ferric ion complex measurement spectrophotometry, and infrared spectroscopy are most frequently used (Shahidi & Zhong, 2005).

Iodometric titration assay, which is based on the oxidation of the iodide ion by hydro peroxides (ROOH), is the basis of current standard methods for determination of PV. In this method, a saturated solution of potassium iodide is added to oil samples to react with hydro peroxides. The liberated iodine is then titrated with a standardized solution of sodium thiosulfate and starch as an endpoint indicator. The PV is obtained by calculation and reported

as mill equivalents of oxygen per kilogram of sample (meq/kg) (Shahidi & Zhong, 2005). Reaction mechanism is shown in Equation 2 and Equation 3below. (O'Keefe & Pike, 2010, p. 251)

Equation 2

$$ROOH + K^+I^- \xrightarrow{H^+,\quad heat} I_2 + ROH + \underset{(excess)}{K^+OH^-}$$

Equation 3

$$I_2 + \underset{(blue)}{Starch} + 2NaS_2O_3 \rightarrow Na_2S_4O_6 + \underset{(colorless)}{Starch} + 2NaI$$

Although iodometric titration is the most common method for measurement of PV, the method suffers from several disadvantages. The procedure is time-consuming and labor intensive. The assay includes six steps: accurate weighing of the sample, dissolution of lipids in chloroform, acidification with acetic acid, addition of potassium iodide, incubation for exactly 5 minutes, and titration with sodium thiosulfate. This technique requires a large amount of sample and generates a significant amount of waste. Furthermore, possible absorption of iodine across unsaturated bonds and oxidation of iodide by dissolved oxygen are among potential drawbacks of this method. Besides, lack of sensitivity, possible interferences, and difficulties in determining the titration endpoint are also the main limitations. To overcome these drawbacks, novel methods based on the same reaction have been developed, in which some other techniques are adopted as modification of the classical iodometric assay (Shahidi & Zhong, 2005).

2.3.4.3. Sensory Evaluation

Sensory evaluation is a scientific discipline used to evoke measure, analyze and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch and hearing (Stone & Sidel, 2004). For the food industry, the detection of oxidative off-flavors by taste or smell is the main method of deciding when a lipid-containing food is no longer fit for consumption (Shahidi & Zhong, 2005).

Many of the lipid oxidation products have distinctive flavor characteristics that contribute to the overall perception of objectionable changes in an oxidized food. However, depending on the type of food and the stage and conditions of oxidation, such off-flavors vary not only in their intensity but also in their character. Terms such as rancid, stale, beany, metallic, cardboard, green, fishy, etc., are often used to describe the state of lipid oxidation in foods.

The testing of oxidative off-flavors can be conducted by trained taste panels using special score forms (Taub & Singh, 1998).

Many researchers have conducted sensory evaluation of lipid oxidation. However, as a subjective method, the reproducibility of sensory analysis is generally considered worse than that of chemical or instrumental methods. More recently, use of an electronic nose to monitor the formation of volatile compounds associated with off-flavors from lipid oxidation has been proposed to supplement information from human sensory panels (Shahidi & Zhong, 2005).

2.3.5. Accelerated Shelf Life Testing for Oxidative Rancidity in Foods

The following steps outline the Accelerated Shelf Life Testing procedures: (Taoukis, *et al.*, 1997)

- Evaluate the microbiological safety factors for the proposed food product and process.
- Determine which biological and physico-chemical reactions will significantly affect shelf life and hence can be used as quality loss indices
- Select the package to be used for the shelf life test
- Define the test's storage temperatures
- From the desired shelf life at expected storage and handling temperatures, and based on available information on the most likely Q_{10}, calculate testing time at each selected temperature. If no information is available on the expected Q_{10} value, minimum three testing temperatures should be used.
- Decide the type and frequency of tests to be conducted at each temperature. At each storage condition, at least six data points are required to minimize statistical errors; otherwise, the statistical confidence in the obtained shelf life value is significantly reduced.
- Plot the data as it is collected to determine the reaction order and to decide whether test frequency should be altered.
- From each test storage condition, determine reaction order and rate, make the appropriate Arrhenius plot, and predict the shelf life at the desired actual storage condition.

The accelerated tests, Schaal oven test, active oxygen method (AOM), and oxygen absorption, are available to predict the resistance of oil to oxidative rancidity. In the Schaal oven test samples are stored at about 65°C and periodically tested organoleptically or by measuring PV (Taub & Singh, 1998).The oven test, also known as the cabinet or Schaal test, was introduced

about fifteen years ago. The biscuit and cracker industry, to provide a relative rating for the stability of the various shortenings offered, developed it. Thus, it made possible the selection of those shortenings, which indicated the greatest resistance to rancidity development in the baked products as they moved through the regular channels of distribution to the consumer. (Joyner & Mclntyre, 1938)

It is possible to estimate the oven test using peroxide formation as the index. To do this a curve must be plotted showing the course of peroxide formation during the oven test. Any one kind of fat, which has been subjected to the same processing conditions, will nearly always develop organoleptic rancidity at a fairly uniform peroxide concentration. The length of time required to reach this concentration is determined by the rate of peroxide formation, which in turn is governed by the stability of the particular sample. (Joyner & Mclntyre, 1938)

Changes in color can be used to advantage in determining the end point. As the end of the induction period is approached the color gradually darkens and rancidity soon becomes evident; if the test is carried beyond this stage to extreme rancidity the color will generally become lighter and this change occurs quickly, frequently overnight. Thus, the darkening in color may serve to indicate rancidity is approaching.(Joyner & Mclntyre, 1938)

Other accelerated stability tests have been developed which are more rapid in their action upon the sample under examination. Most of these methods are based upon either oxygen absorption or peroxide formation (Joyner & Mclntyre, 1938). The active oxygen method (AOM) involves maintaining the sample at 97.8°C while air is continuously bubbled through it at a constant rate and then determining the time required to obtain a specific PV. In Oxygen absorption the amount of oxygen absorbed by the sample is determined by the time required to produce a specific pressure decline in a closed chamber under specific oxidizing conditions.(Taub & Singh, 1998)The equipment used and the evaluations of the data obtained are naturally more complicated. The simplicity of the oven test determination probably explains why it is today still one of the most widely used methods for evaluating the stability of oils and fats. (Joyner & Mclntyre, 1938)

Basically, four parameters are manipulated in ASLT procedures to speed up the oxidation and development of rancidity in foods or oils. They are (Ragnarsson & Labuza, 1977)

- Temperature (Normal range: 60 – 140 ^{o}C)
- Oxygen pressure (Normal range: 3 – 165 psi)
- Added metals (Normal range: 25 – 100 ppm)
- Reactant contact

A shelf life loss kinetic model is characteristic not only of the studied food but equally important to the set of environmental conditions of the experiment. These conditions can determine the reaction rates and have to be defined and monitored during kinetic experiments. From environmental factors namely temperature, relative humidity, total and partial pressure of different gases, light and mechanical stress, the factor most often considered and studied is temperature (Taoukis, *et al.*, 1997). Increased temperature is the most common and effective means of accelerating the oxidation. The rate of the reaction is exponentially related to the temperature; thus shelf life should decrease logarithmically with increasing temperature. (Ragnarsson & Labuza, 1977)

The most prevalent and widely used model in reasoning temperature effect on reactions is the Arrhenius equation (Equation 4) (Taoukis, *et al.*, 1997).

Equation 4

$$k = k_A \exp\left(\frac{-E_A}{RT}\right)$$

Where;

- K_A = Arrhenius equation constant,
- E_A = Activation energy

The key to the application of kinetics to prediction of quality loss is selection of the major mode of deterioration, measurement of some quality factor related to this mode, and then application of mathematical models to make the needed predictions. Two main sources of error associated with these predictions are the inability to identify some quality index related to the major mode of deterioration and the analytical errors involved in when it is measured. (Labuza, 1984)

Oya O¨ zkanli and Ahmet Kaya showed how Arrhenius kinetic model can be used to predict shelf life of butter oil from sheep milk. In order to estimate the Arrhenius parameters, a two step ordinary linear least squares fit was applied to each set of experimental data. The increase in peroxide concentration with time was applied to the zero, half, first and second order models. Then the error mean squares were compared and the model with the least error mean square was selected to calculate rate constant at each temperature (zkanli & Kaya, 2007).

If data is collected during a storage period for some mode of deterioration or chemical reaction, the data can be used to make projection about longer storage periods or other storage conditions such as higher or lower temperature. (Labuza, 1984)

For example, for reaction shown in Equation 5 below

Equation 5

$$aA + bB \underset{k_b}{\overset{k_f}{\rightleftarrows}} cC + dD$$

A reacts with *B*, with the forward rate constant k_f, to form two products, *C* and *D*, which can also back react with a rate constant of k_b. The lowercase letters indicate that more than one molecule of each component is participating. The standard equation defining the rate of loss of the reactants or gain of any component is: (Labuza, 1984)

Equation 6

$$\frac{-dA}{dt} = \frac{-dB}{dt} = \frac{+dC}{dt} = \frac{+dD}{dt} = k_f[A]^a[B]^b - k_b[C]^c[D]^d$$

To solve the Equation 6, which predicts the change in concentration of any component with time, one needs to know the concentrations of each component as a function of time. Even if concentration is known, still there is only one equation with six unknowns (k_f, k_b, a, b, c, d). Thus the situation is technically impossible to solve. (Labuza, 1984)

To get around this problem basically one needs to choose conditions such that either the forward or backward reaction predominates and/or the concentrations of several species such as B are so high that their change in concentration with time is negligible. Thus, if $k_b <<< k_f$and[B] is very large, the loss in [A] or gain in either [C] or [D] is

Equation 7

$$\frac{-dA}{dt} = k_f'[A]^a$$

Where k_f' is the Pseudo forward rate constant. Obviously this form of the equation ignores the true mechanism of the reaction but is useful from a practical standpoint. A food system is very complex. Assuming that, for a given mode of deterioration, the above assumptions hold, one can simply write for the rate of loss of a desirable quality factor [A]or gain of an undesirable quality factor [B] using Equation 8 and Equation 9, respectively (Labuza, 1984).

Equation 8

$$\frac{-d[A]}{dt} = k[A]^n$$

Equation 9

$$\frac{+d[B]}{dt} = k[B]^n$$

Where: k is the pseudo rate constant, n is the order of the reaction, and [A] or [B] are the quality factors measured.

The above forms of the equations are used only for curve fitting of the data and in fact may have nothing to do with the true mechanistic steps in the degradation. The major caveats in using these equations are that the above assumptions must hold (i.e., the back reaction is negligible, and the concentrations of other species are not limiting) and that all reaction phase condition; (pH, water activity, temperature, redox potential, concentration of all other species) must remain constant. Basically this means that the pseudo rate constant k is particular to a given food system. (Labuza, 1984)

There are two general ways to approach shelf life testing. The most common method is to select some single abuse condition such as high temperature, analyze the product two or three times during some specified storage period, and then extrapolate the results to normal storage conditions by using some "fudge factor," perhaps gained by experience with similar foods. The better approach is to assume that certain principles of chemical kinetics apply with respect to temperature acceleration, e.g. the Arrhenius relationship, and utilize kinetic design to produce a more accurate prediction. (Labuza, 1984)

The most common method to estimate Arrhenius parameters is the classic successive two steps ordinary linear least squares fit. The first step is the regression of the quality function vs. time, at each temperature, to estimate the rate constant k, and the initial concentration A_o. The estimation of A_o avoids bias in the determination, and provides an additional criterion of the adequacy of the model to describe the experimental data. A significant discrepancy between the estimated and experimental A_o suggests that a problem exists. The problem may be due to an inadequate kinetic model, large experimental error, insufficient number of data, etc. The second step is regression of ln k vs. 1/T to obtain the Arrhenius parameters k and E_A/R (Taoukis, *et al.*, 1997).

In practical terms it means that if values of K are available at different temperatures and ln k is plotted against the reciprocal absolute temperature (1/T), a straight line is obtained with a slope of $-E_A/R$ (Taoukis, *et al.*, 1997).

Equation 10

$$\ln k = lnk_A - \frac{E_A}{R} * \frac{1}{T}$$

If the rate constants k_2 and k_1 at two temperatures T_2 and T_1 are known, the Arrhenius parameters can be calculated by the equation: (Taoukis, *et al.*, 1997).

Equation 11

$$E_A = \ln\left(\frac{k_2}{k_1}\right) * \frac{RT_1T_2}{T_2 - T_1}$$

Equation 12

$$k_A = k_1^{\left(\frac{T_1}{T_1 - T_2}\right)} * k_2^{\left(\frac{T_2}{T_2 - T_1}\right)}$$

In practice, since there is experimental error involved in the determination of the values of k, calculations of E_A from only two points will give a substantial error (Taoukis, *et al.*, 1997).

2.4. Design of Experiments and Evaluation of Analytical Data

2.4.1. Design of Experiments

To design an experiment means to choose the optimal experiment design to be used simultaneously for varying all the analyzed factors. (Lazic´, 2004) Designing of experiment generally comprises six basic steps to solve a problem. The first is recognition of and statement of the problem. Second step is choosing factors, levels and ranges, and also selection of the response variable. Choice of experimental design comes third. Performing the experiment according to the chosen design, statistically analyzing the data, and putting conclusion and recommendations accordingly are the 4^{th}, 5^{th} and 6^{th} step, respectively. (Montgomery, 2001)

Randomization implies the allocation of experimental material and the order in which the individual runs or trials of the experiment to be performed are randomly determined. It helps in avoiding the introduction of a systematic bias into the experimental results. Blocking is a design technique used to improve the precision with which comparisons among the factors of interest are made. Often blocking is used to reduce or eliminate the variability transmitted from nuisance factors; that is, factors that may influence the experimental response but which are not of direct interest to the experimenter. (Montgomery, 2001)

Accuracy refers to how close a particular measure is to the true or correct value. Precision is a measure of how reproducible or how close replicate measurements become. To increase accuracy and precision, as well as to evaluate these parameters, the analysis of a sample is usually performed (repeated) several times. Because it is difficult to know which value is closest to the true value, the mean (or average) is determined using all the values obtained and result of the mean is reported. But, an indication of how repeatable the tests were or how close results were to the true value is necessary. (Smith, 2010)

Replication is repetition of the basic experiment. It reflects sources of variability both between runs and within runs. Replication is used to obtain an estimate of the experimental error, thus help in determining whether observed differences in the data are really statistically different. In addition, it helps to obtain a more precise estimate of the effect of a factor, if sample mean is to be used. (Montgomery, 2001)

The most commonly used statistical evaluation of the precision of analytical data is the standard deviation. (Smith, 2010)

One has to remember that it is very difficult, if not impossible, and very time consuming to analyze the entire food product. Assuming all parts of the food product were evaluated (which could be an infinite amount of assays) standard deviation can be calculated by the formula as proposed by (Smith, 2010)

Equation 13

$$\sigma = \sqrt{\frac{\sum(X_i-\mu)^2}{n}}.$$

Where,

- σ=standard deviation
- X_i = individual sample values
- μ= true mean
- n = total population of samples

For a large number of samples (Smith, 2010)

Equation 14

$$SD = \sqrt{\frac{\sum(X_i-\bar{X})^2}{n}}.$$

Where,

- SD=standard deviation
- X_i = individual sample values
- X= mean
- n = total population of samples

If the number of replicate determination is small (about 30 or less) the "n" is replaced by n–1 term (Smith, 2010); $SD = \sqrt{\frac{\sum(X_i-\bar{X})^2}{n-1}}$

One easy way to interpret standard deviation is to calculate the coefficient of variation (CV), also known as relative standard deviation. (Smith, 2010); $CV = \frac{SD}{\bar{X}} * 100\%$

Small CV indicates high level of precision or reproducibility of the replicates. As a rule, a CV below 5% is considered acceptable. (Smith, 2010)

Many populations that exist in nature are said to have a normal distribution. In a population with normal distribution 68% of hose value would be within ±1 standard deviation form the mean, 95% would be within ±2 standard deviation, and 99.7% would be within ±3 standard deviation. Another way of understanding the normal distribution curve is to realize that the probability of finding the true mean is within certain confidence intervals as defined by the standard deviation. (Smith, 2010)

For large number of samples, we can determine the confidence limit or interval around the mean using the statistical parameter called Z-value. (Z-value is obtained from statistical tables for certain degree of confidence) (Smith, 2010)

Equation 15

$$\text{Confidence Interval (CI)} = \overline{X} \pm \text{Z value} * \frac{SD}{\sqrt{n}}$$

For small numbers of samples, the confidence interval should be calculated using statistical t-tables. (t-value should be obtained from tables based on the degrees of freedom and the desired level of confidence) (Smith, 2010)

Equation 16

$$\text{Confidence Interval (CI)} = \overline{X} \pm \text{t value} * \frac{SD}{\sqrt{n}}$$

2.4.2. Sources of Errors

There are several sources of error, which can be classified as systematic error (determinate), random error (indeterminate), and gross error or blunders. Systematic or determinate error produces results that consistently deviate from the expected value in one direction or the other. It often involves inaccurate instruments or measuring devices. Sometimes impure chemicals or the analytical method itself are the cause. We can overcome systematic errors by proper calibration of instruments, running blank determinations, or using a different analytical method. (Smith, 2010)

Random or indeterminate errors are always present in any analytical measurement. This type of error is due to our natural limitations in measuring a particular system. These errors fluctuate in a random fashion and are essentially unavoidable. Reading an analytical balance, judging the end point change in a titration, and using pipette are examples of random errors. Blunders are easy to eliminate, since they are so obvious. The experimental data are usually scattered, and the results are not close to an expected value. Error is a result of using wrong reagent or instrument of sloppy technique. (Smith, 2010)

CHAPTER THREE

3. Material and Methods

3.1. Source of Materials and Sample Preparation

3.1.1. Study Area and Source of Materials

Product development was carried out at Ethiopian Institute of Agricultural Research Holetta Research Center; altitude, 2400 masl; annual rainfall, 1100 mm; average minimum and maximum temperatures, 6^{o}C and 24^{o}C. Raw milk, 4% fat content, was collected from the research center's dairy farm. Shelf stability study was conducted in Food Engineering Laboratory of Chemical Engineering Department, Addis Ababa Institute of Technology; Elevation, 2355 meter; annual rainfall, 1180 mm; average minimum and maximum temperatures, 11 ^{o}C and 23 ^{o}C.

3.2. Processing Methods

3.2.1. Ghee Production by Direct Cream Method

25-liter raw milk was collected in three stainless steel containers. Raising the temperature to 63±1 ^{o}C and holding for 30 minutes pasteurized the milk. Then the pasteurized milk in three separate containers was mixed into a bigger stainless steel container to facilitate mixing and rapid cooling. Samples for milk fat content, moisture content, and titratable acidity analysis were taken. When the temperature reached to 42±1^{o}C, the milk was separated in to cream and skim milk using an electric motor centrifugal milk separator. Samples from cream and skim milk were taken for analysis.

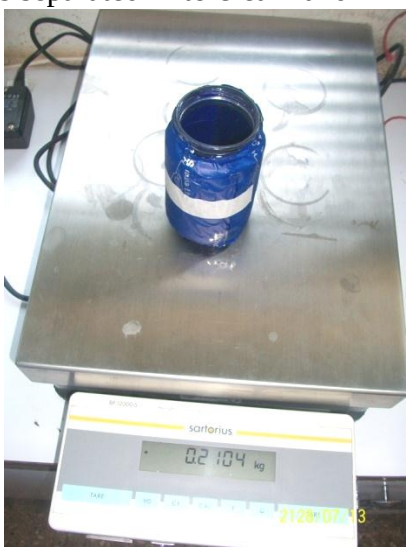


Figure 3: A light insulated glass package for Ghee packaging being weighed

For the production of Ghee, applying heat directly evaporated moisture from the cream. The cream was continuously stirred during heating to avoid film formation at the bottom of the container. When the color of Ghee residue changed from light yellow to light brown, heating was stopped and the Ghee was allowed to slowly cool to 79±1 ^{o}C. During this holding time, all Ghee residues settled at the bottom of container. The Ghee was separated from the Ghee residue by slowly decanting the Ghee off the container through a double layer muslin cloth into a light

insulated glass package. The glass package was made translucent by wrapping using a carbon paper. (Figure 3) Weight of Ghee package and weight of muslin cloth, before and after filling, were measured. The package was filled up to top and closed tightly to prevent oxidation. The product was stored at room temperature until further analysis. Composition analysis results are summarized in Table 3 on page 51.

3.2.2. Ghee Production by Creamery Butter Method

34-liter raw milk was collected in four stainless steel containers. Raising the temperature to 63±1 ^{o}C and holding for 30 minutes pasteurized the milk. Then milk in the containers was mixed into a bigger stainless steel container to facilitate rapid cooling and also mixing. Samples for milk fat content, moisture content, and titratable acidity analysis were taken. When the temperature reached 42±1 ^{o}C, milk was separated in to cream and skim milk using an electric motor centrifugal milk separator. Samples from cream and skim milk were taken for analysis.

Starter propagation was carried out one day earlier by adding one gram probiotic cultures in to 100 ml sterilized milk. Direct vat set (DVS) type probioticABT-2 yoghurt cultures manufactured by Chr. Hansen were used. These freeze-dried cultures contain *Lactobacillus delbrueckii* ssp. *Bulgaricus* and blends of probiotics with *Streptococcus thermophilus*. The microbes were incubated at 26±1^{o}C for 24 hours. 59.8224 g propagated probiotic culture at pH 4.17 and temperature 26±1 ^{o}C was inoculated into 2.9921 kg pasteurized and cooled cream. The acidity change during fermentation was recorded by taking samples at certain time interval; shown in Table 5 on page 54.

Skim milk addition was required to reach the minimum filling level of the churn.3.1708 Kg (three liter) skim milk of 0.46±0.01% titratable acidity (as lactic) at pH 5.61 and 20±1^{o}C having a fat content of 0.12% was mixed with 2.9921 Kg cream having a titratable acidity of 0.36±0.04 (as lactic) at pH 4.43 and 20±1 ^{o}C. Thus, cream was churned at pH 5.02, 0.41% titratable acidity and temperature of 20±1 ^{o}C.

At the eighth minute, small butter granules were observed and foaming decreased. After 13 minutes churning first round collection of butter was carried out. Second round churning was carried out for another 13 minutes and butter was collected. A third round churning was carried out for 10 minutes but yield was small. The collected butter was washed several times by water at 12±1^{o}C, by hand. Samples were taken from the butter, after melting and mixing, for moisture content, solid not fat and titratable acidity analysis

Applying direct heat evaporated moisture from the butter. Continuous stirring was necessary during heating to avoid film formation at the bottom of the container. Heating was stopped when color of Ghee residue changed from light yellow to light brown. After cooling to 80±1 ^{o}C, the Ghee was separated from the Ghee residue by slowly decanting the Ghee off the container and passing through a double layer muslin cloth into a light insulated glass package.

Weight of Ghee package and weight of muslin cloth before and after filling were measured. The package was filled up to top and closed tightly to prevent oxidation. The product was stored at room temperature until further analysis. Composition analysis results are summarized in Table 4 on page 54.

3.2.3. Ghee Production by Ethiopian Butter Method

35-liter raw milk was collected in four stainless steel containers. The milk was pasteurized by raising the temperature to 63±1^{o}C and holding for 30 minutes; and then cooled to room temperature rapidly by immersing the containers in chilled water. Samples for milk fat content, moisture content, and titratable acidity analysis were taken from each container.

Spontaneous fermentation by naturally occurring microbes in milk was used for acidification of milk during production of traditional Ethiopian butter. Inoculum was prepared by allowing one liter sterilized milk to naturally ferment at room temperature (17±1 ^{o}C) for 48 hours.2% (wt/wt) sample from the fermented milk at pH 4.70 and temperature 23±1^{o}C was inoculated into each four containers containing pasteurized milk. To study the effect of temperature on acidity change during this fermentation, each batch was stored at different temperature and acidity change was continuously monitored; as shown in Table 7 page 58. Batch one was incubated at 26±1 ^{o}C, Batch two and three at 18±1 ^{o}C, and Batch four at 35±1 ^{o}C. However, acidity change in Batch four was rapid. To monitor the change more closely, Batch three was transferred in to incubator at 35±1 ^{o}C.

At the same time, the effect of acidity and churning temperature on overall butter yield during churning was also studied. When pH of fermentation batch two reached to 4.45, the batch was cooled and churned at two different temperatures, 15±1 ^{o}C and 20±1 ^{o}C. A constant filling level of five-liter volume was used for each churning. Fermentation of batch three was stopped at pH 5.1 by rapid cooling, and then churned at two different temperatures, 12±1 ^{o}C and 19±1 ^{o}C. Filling level was five liter, for each churning. Change during churning was continuously monitored by opening the churn every four minutes and observing. Weight of butter yield, time taken for butter formation and butter milk fat content were measured and

presented in Table 9 on page 64. Each butter products obtained from churning milk at different pH temperature combination were melted and samples were taken for analysis of moisture content, solid non-fat and titratable acidity.

Moisture was removed from butter by applying direct heat. To analyze the effect of traditionally added additives on shelf stability, two batches of Ethiopian Ghee product were prepared.

Butter products from milk fermentation batch two were mixed and used for production of Ethiopian Ghee batch one. The two most common traditional additives, *Lippia adoensis* and *Aframomum corrorima*, were added at 2.5% (wt/wt); i.e. 5±0.1 gram. *Aframomum corrorima* was added when butter temperature passed 60 ^{o}C and *Lippia adoensis* was added at the last moment when temperature of butter was above 100 ^{o}C. When the color of Ghee residue changed from light yellow to light brown, heating was stopped and the Ghee was allowed to slowly cool down to 79±1 ^{o}C. During this time all Ghee residue settled at the bottom of the container. The Ghee was separated from the Ghee residue by decanting the Ghee off the container through a double layer muslin cloth into a light insulated glass package.

Butter products from milk fermentation batch three were mixed and used for production of Ethiopian Ghee batch two. No additive was added to this batch. When the color of Ghee residue changed from light yellow to light brown, heating was stopped and the Ghee was allowed to slowly cool down to 79±1 ^{o}C. The Ghee was separated from the Ghee residue by decanting the Ghee off the container through a double layer muslin cloth into a light insulated glass package.

The products were stored at room temperature until further analysis. Composition analysis results are summarized in Table 6 on page 57.

3.3. Analysis Methods

3.3.1. Moisture Content

Predried crucibles were labeled and weighed accurately. 5±0.5 gram of sample was placed in the crucible and weighed accurately. Then sample was placed in a forced draft oven at 110 ^{o}C over night. Dried sample was cooled in a desiccator for 15 minutes and weighed. (Bradley, 2010)

Percentage moisture was calculated (wt/wt) as:

Equation 17

$$\%\ moisture\ ({}^{wt}/_{wt}) = \frac{Wt\ of\ H_2O\ in\ sample}{Wt\ of\ wet\ sample} * 100$$

Equation 18

$$\%\ moisture({}^{wt}/_{wt}) = \frac{(Wt\ of\ wet\ sample + pan) - (Wt\ of\ dried\ sample + pan)}{(Wt\ of\ wet\ sample + pan) - (Wt\ of\ pan)} * 100$$

3.3.2. Fat Content

3.3.2.1. Milk

First 10 ml 90 % sulphuric acid was pipetted in to butyrometer caliberated to read 0 – 8% fat conent. Then 11 mlmilk was added gently in to the butyrometer. One ml amyl alcohol was added. The neck of the butyrometer was cleaned with dry cloth and closed tightly using a clean, dry stopper. The butyrometer was shaked and invereted several times until all the buttermilk has been absorbed by the acid. The butyrometer was placed in a water bath at 65 ^{o}C for five minutes and then centrifuged for four minutes at 1100 rpm. Again it was placed back in to the water bath, after five minutes fat content was read. (O'Connor, 1995)

3.3.2.2. Cream

Sample was warmed at50 ^{o}C and mixed throughly. Five gram of cream was added in to butyrometer caliberated to read 0 – 40% fat content. Six ml warm distilled water, 10 ml 90% sulfuric acid and one ml amyl alcohol were added successively and carefully. The neck of the butyrometer was cleaned with a dry cloth and stoppered tightly using a clean, dry stopper. The butyrometer was shaked by inverting it several times until all the cream has been absorbed by the acid. The butyrometer was then placed in a water bath at 65 ^{o}C for five minutes, centrifuged for four minutes at 1100 rpm and again placed in the water bath for five minutes. Finally, fat percentage was read. (O'Connor, 1995)

3.3.2.3. Butter milk and Skim milk

10 ml 90% sulphuric acid was pipetted in to butyrometer caliberated to read 0 – 4% and graduated at 0.1% level. Then 11 ml buttermilk was added gently in to the butyrometer. one ml amyl alcohol was added. The neck of the butyrometer was cleaned with dry cloth and closed tightly using a clean, dry stopper. The butyrometer was shaked and invereted several times until all the buttermilk has been absorbed by the acid. Then it was placed in a water bath

at 65 ^{o}C for five minutes and then centrifuged for four minutes at 1100 rpm. Again it was placed back in to the water bath for two minutes. Fat content was read. Readings obtained were corrected as follows: (O'Connor, 1995)

Table 2: Correction factor for percentage read on butyrometer during fat content determination of skim and/or butter milk.

Percentage read on the butyrometer	Correction
< 0.10%	Add 0.05%
0.10 to 0.25%	Add 0.02%
>0.25%	No correction required

3.3.2.4. Butter and Ghee Residue:

For butter and Ghee residue fat content was calculated by subtracting moisture content percentage and solid non-fat percentage from100%. Moisture content and solid non-fat content percentage were calculated according to procedure defined in section 3.3.1 above and section 3.3.3.2 below, respectively.

Equation 19

$$w_f = 100\% - (w_m + w_s)\%$$

Where; $W_f$is the fat content, in percent by mass, of the test sample used; W_m is the moisture content, in percent by mass, of the test sample obtained; W_S is the non-fat solids content, in percent by mass, of the same test sample. (ES, 2009)

3.3.3. Solid Non Fat

3.3.3.1. Milk, Cream and Skim milk

Solid non fat in milk, cream and skim milk were calculated by subtracting the mass fraction of substances determined by the procedures specified in moisture content and fat content.

Equation 20

$$\%\ Total\ solids\ (^{wt}/_{wt}) = \frac{Wt\ of\ dry\ sample}{Wt\ of\ wet\ sample} * 100$$

Equation 21

$$\%\ Solid\ Non\ Fat\ \left(^{wt}/_{wt}\right) = \%\ Total\ Solids - \%Fat$$

3.3.3.2. Butter and Ghee Residue:

30 ml petroleum ether was added to dried samples remaining from moisture analysis. Beaker and its contents were heated to boil the petroleum ether, taking adequate precautions against fire, in order to prevent any non-fat solids from floating. Then the beaker was cooled to room temperature.

Then 60 ml of petroleum ether was added to the beaker contents and mixed carefully. Then the beaker was placed in a sloping stand until all the non-fat solids have settled on the bottom of the beaker. The petroleum ether was decanted to waste. (There should be no loss of non-fat solids particles during decanting, as this will cause an underestimation of the non-fat solids content)

The petroleum ether extraction step was repeated one more time. Then the beaker and its contents were heated gently between 70 ^{o}C and 80 ^{o}C on a hot plate until all solvent is evaporated and the non-fat solids are thoroughly dried. (Care should be taken to avoid losses due to bursting of non-fat solids.)

The petroleum ether extraction was repeated one more time by adding 40 ml of petroleum ether and breaking up the lumps with a glass rod. The glass rod was rinsed with 20 ml of petroleum ether, adding the rinsing to the beaker. Contents were mixed carefully and allowed to settle by placing the beaker in a sloping stand. Then the petroleum ether was decanted to waste. Finally the beaker and its contents are heated gently between 70 ^{o}C and 80 ^{o}C on a hot plate until all solvent have been removed, cooled to room temperature in a desiccator and weighed to the nearest one mg.

The non-fat solids (W_s) content will be expressed as a percentage by mass (ES, 2009)

Equation 22

$$w_s = \frac{(m_1 - m_0)}{(m_2 - m_0)} * 100\%$$

Where;

- ✓ m_o is the mass, in grams, of the empty beaker
- ✓ m_1 is the mass, in grams, of the beaker + washed and dried non-fat solids
- ✓ m_2 is the mass, in grams, of the sample before washing+ beaker

3.3.4. Titratable Acidity

Titratable acidity deals with measurement of the total acid concentration contained with in a food. (Sadler & Murphy, 2010) Reagent preparation procedure and standardization tables are shown in Appendix.

The titration burette was filled with 0.1M NaOH and the level of NaOH in the burette was adjusted to the top mark. 10 ml sample was added in beaker. Three drops of phenolphthalein was added into the sample. The NaOH was allowed to flow slowly in to the cup containing the sample, while stirring continuously. When a faint but definite pink color that persists for 15 seconds was observed the titration was stopped. The reading of the NaOH in the burette at the lowest point of the meniscus was noted. The following formula was used to calculate % acid as lactic acid. (Sadler & Murphy, 2010)

Equation 23

$$\%\ Acid\left(^{wt}/_{vol}\right) = \frac{Normality\ of\ NaOH\left(^{mEq}/_{ml}\right) * Volume\ of\ NaOH(ml) * Eq\ wt\ of\ predominant\ acid(mg/mEq)}{Volume\ of\ sample\ (ml) * 10}$$

Equation 24

$$\%\ Acid\left(^{wt}/_{vol}\right) = \frac{Molarity\ of\ NaOH\left(^{mol}/_{l}\right) * Volume\ of\ NaOH(ml) * Molecular\ wt\ of\ predominant\ acid(g/mol)}{Volume\ of\ sample\ (ml) * 10}$$

Equation 25

$$\%\ \boldsymbol{Acid\ as\ lactic\ acid}\left(^{\boldsymbol{wt}}/_{\boldsymbol{vol}}\right) = \frac{Molarity\ of\ NaOH\left(^{mol}/_{l}\right) * Volume\ of\ NaOH(ml) * 90.08\ g/mol}{Volume\ of\ sample\ (ml) * 10}$$

3.3.5. Milk Specific Gravity

Milk sample was heated to 40 ^{o}C to get all the fat into a liquid state since crystalline fat has a very different density to liquid fat. After five minutes, the milk was cooled to 20 ^{o}C. Then milk sample was filled sufficiently in a cylinder; lactometer was held by the tip and inserted into the milk. The lactometer was allowed to float freely until it reached equilibrium. Then the reading at the bottom of the meniscus was recorded. Simultaneously, thermometer was inserted and the temperature of the milk was recorded. The following formula was used to calculate the milk specific gravity.

Equation 26

$$specific\ gravity = \frac{Lactometer\ reading}{1000} + 1$$

If the temperature of the milk is between 17 and 24^{o}C, the following correction factors are used to determine Lactometer degree: For every degree above 24 ^{o}C, 0.1 degree was added, to the lactometer reading. (O'Mahony, 1988)

3.3.6. Peroxide Value

Peroxide value was determined by titration method as defined in AOAC 965.33. (AOAC, 2000) Reagent preparation procedure and standardization tables are shown in Appendix.

5.00±0.05 gram Ghee samples were pipetted into 250 ml Erlenmeyer flask.30 ml CH_3COOH–$CHCl_3$solution was added and swirled to dissolve using magnetic stirrer. Then 0.5 ml saturated KI solution was pipetted in and allowed to stand for one minute before adding 30 ml distilled water. 0.5 ml 1% starch indicator solution was added while stirring and slowly titrated using 0.001 M $Na_2S_2O_3$solution until the dark red brown color formed disappeared. The peroxide value was calculated using the following formula

Equation 27

$$Peroxide\ value\ = \frac{S * M * 1000}{Gram\ of\ sample}$$

Where;

- Peroxide value = meq peroxide per kg of sample
- *S* = volume of titrant (ml) for sample
- *M*= molarity of $Na_2S_2O_3$ solution
- 1000 = conversion of units (g/kg)
- *W* = sample mass (g)

3.3.7. Free Fatty Acids

Free fatty acid value was determined by titration method as outlined in AOAC 940.28. (AOAC, 2000) Reagent preparation procedure and standardization tables are shown in Appendix.

7.05±0.05g Ghee samples were accurately weighed into a 250-ml Erlenmeyer flask. 75 ml neutralized ethanol and 0.5 ml phenolphthalein indicator were added. Sample was shaken to dissolve the mixture completely. Then the solution was titrated using the standard 0.1 M

NaOH solution, shaking vigorously until the endpoint is reached. Titration was stopped when a slight pink color that persists for 30 seconds appeared.

The volume of titrant used was recorded and % free fatty acid as oleic acid was calculated using the following formula.

Equation 28

$$\%FFA_s(as\ oleic) = \frac{V * N * 282}{W} * 100\%$$

Where;

- %FFA = percent free fatty acid (g/100 g) expressed as oleic acid
- V = volume of NaOH titrant (ml)
- N = normality of NaOH titrant (mol/1000 ml)
- 282 = MW of oleic acid (g/mol)
- W = sample mass (g)

3.4.Storage Stability Analysis

The Schaal oven test was applied for analyzing shelf stability. Accelerated Shelf Life Testing (ASLT) was used to speed up the oxidation and development of rancidity. From the common four parameters manipulated in ASLT temperature was used for this case. Ghee products obtained by the three different processing methods described above, namely Direct Cream Method Ghee (DCM), Creamery Butter Method Ghee (CBM) and Ethiopian Butter Method Ghee with additive and without additive (EBMa and EBMwa), were evaluated for shelf stability.

For chemical analysis, the four Ghee products obtained from the three different processing methods (DCM, CBM, EBMa, EBMwa) were added into eight clean 500 ml plastic beakers, 72g in each. Four beakers were labeled and put in oven set at 45^{o}C and the remaining four beakers were put in oven set at 70^{o}C.

For sensory analysis, three Ghee products (DCM, CBM, and EBMa) were added into six clean 100 ml plastic beakers, 40 g in each. Three beakers were put in oven set at 45^{o}C and the remaining three beakers were put in oven set at 70^{o}C, after labeling.

Samples were taken out from these beakers periodically and tested organoleptically at laboratory level and chemically by measuring Peroxide Value and Free Fatty Acid Value. The sensory evaluation was conducted to follow up the change in sensory attribute with change in peroxide value.

3.5. Shelf Life Prediction Method

A similar approach as elaborated by scholars Labuza *et.al* (1984), was applied to the experimental data to predict shelf life. The Arrhenius kinetic model was used using successive two steps ordinary linear least squares fit. First, regressions of the quality function (peroxide value) vs. time, at each temperature, were performed to estimate the rate constant *k*, and the initial concentration PV_o. Peroxide concentration change in Ghee with time was applied to zero order, first order and second order models. Model equation with the least root mean square error was chosen and used to estimate *k* and PV_o. Then, regressions of ln *k* vs. 1/T were performed to obtain the Arrhenius parameters k_A and E_A/R.

$$ln\ k = ln\ k_A - \frac{E_A}{R} * \frac{1}{T}$$

Where: k_A= the pre-exponential factor; E_A = the activation energy (kJ/mol); T = oven temperature (^oK); R is the gas constant (8.314 J/mol*^oK), and *k* = estimated rate constant for peroxide formation.

3.6. Experimental Design and Statistical Data Analysis

3.6.1. Chemical Analysis

For chemical analyses, a randomized blocked experimental design containing three factors and two responses was applied. The responses were peroxide value and free fatty acid value. The factors were processing method at four levels (DCM, CBM, EBMa, EBMwa), oven temperature at two levels (45^{o}C, 70^{o}C), and storage time at two levels (week 1, week 2). All analyses were conducted in triplicate. Thus, total number of runs was 4*2*2*3 = 48.Order of experimental run was randomized. However, it was technically difficult to conduct all 24 runs with in one day. Therefore, chemical analyses were conducted in three-day time, 8 run per day. Two remove the variability in PV and FFA value from experimental error blocking was necessary. Thus, storage time was blocked for each level (week 1 and week 2). 24 analyses were conducted within the first there days (blocks), 8 run per block, for the first week, and then again repeated after 20 days storage for second week. In total, 48 peroxide values analysis and 48 free fatty value analyses were conducted. Chemical analysis results are summarized in Table 10 section 4.4.2 below on page 65.

Means Comparisons was performed using one-way analysis of variance (ANOVA) for all processing methods at each storage temperature. Comparisons for each pair was performed using Student's t at α= 0.05,to see whether the actual difference in the peroxide and free fatty

acid value means is greater than the difference that would be significant. Statistical data analysis were carried out by *JMP*® Statistical Discovery Software Version 5; SAS Institute Inc. (2002).

3.6.2. Sensory Analysis

For sensory analysis a composite scoring test was applied as discussed by S. Ranganna. (Ranganna, 1986). Sensory characteristics flavor, odor and color were evaluated. The scale was weighted in order that, flavor accounted for a large part of the total score; odor second largest and color accounted least. The resulting scores were compounded to arrive at a composite score for each panelist. From five chosen non professional panelists only three showed up during test to rate coded samples using the evaluation card shown in Appendices page 110 below. The panelists were trained how to use the weighted scale, and also score the sample by comparing with a good quality Ghee product (the reference). As a reference pure cow Ghee from India market was used. After opening, the reference sample was stored in a refrigerator.

Sensory evaluation was conducted after storing products for three days and twenty days in the ovens. Melted Ghee sample was added in a plastic cup, forming 3 – 4 milliliters thick, from each container in the ovens using pipette. Blind references were prepared similarly using canned cow Ghee from market as a sample. The cups were coded appropriately.

Evaluation was started with visual observation of samples, followed by odor and finally flavor. Each panelist was offered simultaneously two coded samples and a third reference sample. The panelists were asked to score the samples in comparison with the reference, assuming the reference to have a score of 100 out of 100. The order of evaluation between coded samples was randomized. One of the coded samples was a blind reference identical with the reference. Data of the blind reference was used to check the consistency of the panelist's judgment. All units were discarded after evaluation. No failure of criterion was necessary since the study aims only comparing change in acceptability or sensory characteristics with change in peroxide value.

Panelists score data are summarized in Table 11 section 4.4.2 below on page 65.

CHAPTER FOUR

4. Results and Discussion

4.1. Ghee Production by Direct Cream Method

Results of composition analysis of milk, cream, skim milk, butter, buttermilk, Ghee and Ghee residue are summarized in Table 3. Titratable acidity was slightly high. Normally raw milk should have a titratable acidity value of 0.14 – 0.16% (O'Mahony, 1988). The milk fat content was higher than expected. Unpublished data from Holleta Research Center shows that average fat content of milk for September and October 2011 was 4.01% (Calculated from 180 samples collected during September and October). Moisture content of Ghee was 0.25%. According to CODEX STAN minimum fat content of Ghee should be 99.6% (Mortensen, 2011). In this case fat content was 99.7%.

Table 3: Composition analysis summary of raw materials and products for Ghee production by Direct Cream Method

	Moisture Content (%)	Fat Content (%)	Solid Non Fat (%)	Titratable Acidity (%)
Milk	86.74±0.07	4.90±0.14	8.36±0.21	0.18±0.01
Cream	56.79±0.38	41.04±0.21	2.17±0.59	0.12±0.01
Skim milk		0.55±0.07		0.13±0.00
Ghee	0.25±0.16	99.75±0.16	-	
Ghee residue	5.36±0.13	30.59±1.6	64.05±1.47	

All values are triplicate means ± SD at 95% Confidence Interval; except Fat content which is duplicate ± SD

Fat loss during processing

For Ghee Production by Direct Cream Method, there are two unit operations where there could be significant fat loss. One is during centrifugal cream separation and the other is during Ghee residue filtration. Fat loss during cream separation was calculated by measuring fat content in skim milk. Fat content of skim milk was 0.55%, which is significantly high. Normally fat content in skim milk after separation should be 0.04 – 0.05% or lower.

$$Percentage\ loss = \frac{Quantitiy\ of\ fat\ loss\ in\ skimmilk}{Quantitiy\ of\ fat\ in\ milk} * 100$$

Fat loss in skim milk = 118.8 g

Fat in milk = 1.1828 kg

$$\boldsymbol{Percentage\ loss = \frac{118.8\ g}{1182.8\ g} * 100 = 10.04\%}$$

As explained by O'Connor (1995) efficiency of centrifugal cream separation is influenced mainly by four factors; the speed of the bowl, residence time in the bowl, the density

differential between the fat and liquid phase and the size of the fat globules. Speed of the bowl was constant, and temperature of milk during separation was 42±1^{o}C, which is optimum temperature for separation. The high loss of fat in this case was due to operational error in laboratory. Skim milk collected during the first 5 minutes of separation should have been recycled back to increase efficiency. To avoid inclusion of this significant fat loss in Ghee yield calculations, percentage outturn of Ghee was calculated on the basis of cream and fat in cream.

Fat loss during Ghee residue filtration is mainly affected by amount of Ghee residue and type of filtration technique used. In this case since laboratory scale product development was carried out, the amount of Ghee residue obtained is low. Therefore, muslin cloth filtration and hand pressing was applied. At industrial scale processing centrifugal Ghee residue filtration can be applied. The relatively higher Ghee residue yield during DCM Ghee processing contributes to the higher fat loss during residue filtration. As shown in Table 9 on page 64 percentages out-turn of Ghee residue was 7.9%. Fat content of Ghee residue was 30.6%. This high fat content of Ghee residue was expected, since hand pressing was used. But the fat lost with residue can be reduced via the application of centrifugal separators. Ghee yield results are summarized in Table 9 page 64. Percentage out-turn of Ghee on the basis of creamwas29.9%, percentage out-turn of Ghee on the basis of fat in cream was 73.0%.

Shelf life prediction

Peroxide concentration change with time for DCM Ghee was applied to zero order, first order and second order models. According to the fit, root mean square error was minimum for zero order model; both at 45 ^{o}C and 70 ^{o}C. Thus, the model-representing rate of change of peroxide value will be:

$$\frac{+d[PV]}{dt} = k[PV]^0$$
$$[PV] = [PV_0] + kt$$

Where: k = rate constant for peroxide formation (meq$_*$kg $^{-1}{}_*$day^{-1}), t = time (days), PV_o = initial peroxide value estimated (meq/kg) and PV = peroxide value in meq/kg at time t.

k and PV_o values predicted for both temperatures are summarized in Table 8 on page 63. The estimated PV_o for both temperatures was not significantly different form initial experimental values, which suggests model adequacy is satisfactory.

Regressions of ln k vs. 1/T were performed to obtain the Arrhenius parameters k_A and E_A/R.

$$ln\ k = ln\ k_A - \frac{E_A}{R} * \frac{1}{T}$$

Where: k_A= the pre-exponential factor ($meq*kg^{-1}*day^{-1}$); E_A = the activation energy (kJ/mol); T = oven temperature (^oK); R is the gas constant (8.314 J/mol*^oK), and *k* = estimated rate constant for peroxide formation.

lnk was plotted against the reciprocal absolute temperature (1/T) to obtain a straight line with a slope of -E_A/R and intercept *ln* k_A.

The Arrhenius parameters estimated are summarized in Table 8. E_A value is within range (62 – 105 kJ/mol) of data presented by Labuza for lipid oxidation (Labuza, 1984).

Using the estimated E_A value (66.95 KJ/mol) rate constant for peroxide formation and shelf life were calculated at 27 ^oC; assuming the product will be stored at room temperature (27 ^oC), and a peroxide value of 0.6 meq/kg as end of shelf life indicator quality index.

$$k = 0.0026 \frac{meq}{kg\ day}$$

$$Shelf\ life = t = \frac{[PV] - [PV_0]}{k}$$

$$Shelf\ life = \frac{\left[0.6 \frac{meq}{kg}\right] - \left[0.01 \frac{meq}{kg}\right]}{0.0026 \frac{meq}{kg\ day}} = 227\ days = 7.5\ months$$

4.2. Ghee Production by Creamery Butter Method

Results of composition analysis of milk, cream, skim milk, butter, buttermilk, Ghee and Ghee residue are summarized in Table 4. Titratable acidity of milk was higher than the expected range for raw milk; 0.14 – 0.16% (O'Mahony, 1988). Higher milk fat content (4.85%) was observed. Average fat content of milk registered in the research center was 4%. Moisture content of Ghee was 0.12. Fat content of Ghee is 99.88; which is within codex standards limits; minimum fat content allowed is 99.6% (Codex, 2007).

Table 4: Composition analysis summary of raw materials and products for Ghee production by Creamery Butter Method

	Moisture Content (%)	Fat Content (%)	Solid Non Fat (%)	Titratable Acidity (%)
Milk	85.81±0.24	4.85±0.07	9.34±0.31	0.28±0.02
Cream	41.51±1.12	55.30±0.07	3.19±1.19	0.18±0.01
Skim milk		0.12±0.00		0.27±0.02
Creamery Butter	19.37±0.46	77.71±0.92	2.92±0.46	
Butter milk		3.30±0.14		
Ghee	0.12±0.02	99.88±0.02	-	
Ghee residue	2.30±0.09	49.90±5.05	47.80±4.96	

All values are triplicate means ± SD at 95% Confidence Interval; except Fat content which is duplicate mean ± SD

Fat loss during processing

For Ghee production by Creamery Butter Method, fat loss can occur during cream separation, cream churning and Ghee residue filtration. Fat loss during cream separation was calculated by measuring skim milk fat content. Fat content of skim milk was 0.12%.A fat loss of 2.26% was recorded.

$$Percentage\ loss = \frac{Quantitiy\ of\ fat\ loss\ in\ skimmilk}{Quantitiy\ of\ fat\ in\ milk} * 100$$

Fat loss in skim = 38.2989g

Fat in milk =1.6930 kg

$$\boldsymbol{Percentage\ loss = \frac{38.2989\ g}{1693.0\ g} * 100 = 2.26\%}$$

Fat loss during churning of cream for butter production was calculated by measuring butter milk fat content. Factors influencing churning time and recovery of butter fat as butter are milk acidity, temperature, degree of agitation, and extents of churn filling level (O'Mahony, 1988).

The acidity change during fermentation of cream was recorded by taking samples at certain time interval; data shown in Table 5.

Table 5: Cream Fermentation Acidity change summary for the production of creamery Ghee

	Time (hrs)	pH	Temp during analysis (^oC)	Incubation Temperature (^oC)	Titratable Acidity (%)	% Total increase in Acidity
Cream fermentation for creamery butter production	0	5.12	25.0	26±1 ^oC	0.18±0.01	-
	18.3	4.45	27.5	26±1 ^oC	0.34±0.01	88.9%
	22.5	4.43	28.3	26±1 ^oC	0.36±0.04	100%

Titratable Acidity values are triplicate means ± SD at 95% Confidence Interval

During fermentation the cream reached a pH of 4.4 in less than 24 hours. Titratable acidity also increased by twofold. The cream became viscous and, a desirable flavor was developed. The cream was churned at pH 5.02, 0.41% titratable acidity and temperature of 20±1 ^{o}C.

Churning efficiency was measured in terms of the time required to produce butter granules and by the loss of fat in the buttermilk (O'Mahony, 1988). Fat content of buttermilk was 3.3%. Due to the higher churning temperature applied churning time was low, only 8 minutes. Churning time is the time interval between the start of churning and the visible formation of butter grains (O'Mahony & Bekele, 1985).

Fat loss in butter milk = 45.329 g

Fat in milk = 1.6930 kg

$$\boldsymbol{Percentage\ loss} = \frac{\mathbf{45.329}\ \boldsymbol{g}}{\mathbf{1693.0}\ \boldsymbol{g}} * \mathbf{100} = \mathbf{2.68}\%$$

2.68% fat loss during churning is considered high. Increasing acidity and lowering the churning temperature by 3 – 5 ^{o}C can reduce this loss. However, reducing churning temperature will increase over all churning time.

As described earlier, fat loss during Ghee residue filtration is mainly affected by amount of Ghee residue and type of filtration technique used. In this case since laboratory scale product development was carried out, the amount of Ghee residue obtained is very low. Therefore, muslin clothes filtration and hand pressing was applied. At industrial scale processing centrifugal Ghee residue filtration can be applied.

CBM Ghee processing yields a lower amount of Ghee residue, since most of the solid non-fats have already been removed with the skim milk and buttermilk. Therefore, during residue filtration fat loss was not as high as in the case of DCM processed Ghee. As shown in Table 9 percentages out-turn of Ghee residue was only 2.5%. Fat content of Ghee residue was 49.9%. This high fat content of Ghee residue was expected, since hand pressing was used. But the overall fat lost with residue is low because amount of residue is small; and can be further reduced via the application of centrifugal separators. Ghee yield results are summarized in Table 9 page 64. Percentage out-turn of Ghee on the basis of butter was 70.9%, percentage out-turn of Ghee on the basis of fat in butter was 91.3%.

Shelf life prediction

Peroxide concentration change with time for CBM Ghee was applied to zero order, first order and second order models. According to the fit, root mean square error was minimum for zero

order models; both at 45 ^{o}C and 70 ^{o}C. Thus, the model-representing rate of change of peroxide value will be:

$$\frac{+d[PV]}{dt} = k[PV]^0$$
$$[PV] = [PV_0] + kt$$

Where: k = rate constant for peroxide formation ($meq{*}kg^{-1}{*}day^{-1}$), t = time (days), PV_o = initial peroxide value estimated (meq/kg) and PV = peroxide value in meq/kg at time t.

k and PV_o values predicted for both temperatures are summarized in Table 8 on 63. The estimated PV_o for both temperatures is not significantly different form initial experimental values, which suggests model adequacy is satisfactory.

Regressions of ln k vs. 1/T were performed to obtain the Arrhenius parameters k_A and E_A/R.

$$ln\ k = ln\ k_A - \frac{E_A}{R} * \frac{1}{T}$$

Where: k_A= the pre-exponential factor ($meq{*}kg^{-1}{*}day^{-1}$); E_A = the activation energy (kJ/mol); T = oven temperature (^{o}K); R is the gas constant (8.314 J/mol*^{o}K), and k = estimated rate constant for peroxide formation.

lnk was plotted against the reciprocal absolute temperature (1/T) to obtain a straight line with a slope of -E_A/R and intercept *ln* k_A.

The Arrhenius parameters estimated are summarized in Table 8 on page 63 below. E_A value is within range (62 – 105 kJ/mol) of data presented by Labuza for lipid oxidation (Labuza, 1984).

Using the estimated E_A value (69.59 KJ/mol) rate constant for peroxide formation and shelf life were calculated at 27 ^{o}C; assuming the product will be stored at room temperature (27 ^{o}C), and a peroxide value of 0.6 meq/kg as end of shelf life indicator quality index.

$$k = 0.00739 \frac{meq}{kg\ day}$$
$$t = \frac{[PV] - [PV_0]}{k}$$
$$Shelf\ life = t = \frac{\left[0.6 \frac{meq}{kg}\right] - \left[0.01 \frac{meq}{kg}\right]}{0.00739 \frac{meq}{kg\ day}} = 80\ days = 2.7\ months$$

4.3. Ghee Production by Ethiopian Butter Method

Results of composition analysis on milk, cream, skim milk, butter, buttermilk, Ghee and Ghee residue are summarized in Table 6. Titratable acidity of milk was higher than expected range for raw milk; 0.14 – 0.16% (O'Mahony, 1988). Similarly, higher milk fat content was observed. The fat content was 4.45% and 4.25% for batch 2 and batch 3, respectively. Moisture content of Ghee was 0.18% and 0.11%, for Ghee with additives and Ghee without additives, respectively. Fat content was 99.82% and 99.89%, for Ghee with additives and Ghee without additives, respectively.

Table 6: Composition analysis summary of raw materials and products for Ghee production by Traditional Ethiopian Method

		Fermentation Batch 2				Fermentation Batch 3			
		Moisture Content (%)	Fat Content (%)	Solid Non Fat (%)	Titratable Acidity (%)	Moisture Content (%)	Fat Content (%)	Solid Non Fat (%)	Titratable Acidity (%)
Milk		87.01±0.68	4.45±0.07	8.54±0.75	0.23±0.01	87.12±0.08	4.25±0.07	8.63±0.15	0.24±0.02
Traditional Ethiopian butter	Churn Batch 1	18.02±4.00	80.9±6.66	1.08±2.66	-	-	-	-	-
	Churn Batch 2	24.36±0.95	72.31±1.28	3.33±0.33	-	-	-	-	-
	Churn Batch 3	-	-	-	-	15.52±0.75	79.77±2.4	4.71±1.65	-
	Churn Batch 4	-	-	-	-	23.91±5.93	72.70±6.14	3.39±0.21	-
Butter milk	Churn Batch 1	-	0.35±0.07	-	-	-	-	-	-
	Churn Batch 2	-	0.45±0.07	-	-	-	-	-	-
	Churn Batch 3	-	-	-	-	-	1.60±0.42	-	-
	Churn Batch 4	-	-	-	-	-	1.10±0.00	-	-
Ghee with additives		0.18±0.09	99.82±0.09	-	-	-	-	-	-
Ghee residue from Ghee with additives		1.98±0.12	60.63±2.37	37.39±2.25	-	-	-	-	-
Ghee without additives		-	-	-	-	0.11±0.03	99.89±0.03	-	-

All values are triplicate means ± SD at 95% Confidence Interval; except Fat content which is duplicate mean ± SD

Acidity change during fermentation of milk for Ghee production by Modified Traditional Ethiopian Method

The production of "*Neter Qibe*" (Ethiopian Ghee) involves milk fermentation by microbes naturally occurring in raw milk. As explained by M. Ashenafi (2006), to standardize the fermentation step, isolation of bacteria from fermentation of milk in various ecological zones of the country, identification and combination of the cultures and testing them in a controlled fermentation on pasteurized milk is necessary. Then those combinations having favorable processing and organoleptic quality can be used for large-scale production.

However, performing the above mentioned microbial isolation studies would be out of the scope of this thesis. But then again Ethiopian Ghee processing involves milk fermentation by microorganisms naturally occurring in raw milk; and to increase efficiency understanding favorable growth condition is important. Thus, effect of incubation temperature on acidity change during fermentation of milk was observed. To study the effect of temperature change on acidity, four different batches were stored at different temperature and acidity changes were monitored; presented in Table 7.

Table 7: Milk Fermentation Acidity change summary for the production of traditional Ethiopian Ghee

		Time (hrs)	pH	Temp during analysis (^{o}C)	Incubation Temperature (^{o}C)	Titratable Acidity (%)	% Total increase in Acidity
Milk fermentation for traditional butter production	Fermentation Batch 1	0	6.30	25.1	26±1 ^{o}C	0.26±0.02	-
		18.7	5.96	22.7	26±1 ^{o}C	0.33±0.01	26.9%
		20.8	5.66	25.7	26±1 ^{o}C	0.37±0.05	42.3%
		24.6	5.22	28.1	26±1 ^{o}C	0.55±0.01	111.5%
		43.7	4.56	16.4	26±1 ^{o}C	0.85±0.01	226.9%
	Fermentation Batch 2	0	6.53	26.2	18±1 ^{o}C	0.23±0.01	-
		18.6	6.40	20.8	18±1 ^{o}C	0.23±0.08	0%
		21.6	6.32	23.2	18±1 ^{o}C	0.26±0.01	11.5%
		42.8	4.45	30.5	18±1 ^{o}C	0.80±0.03	247.8%
	Fermentation Batch 3	0	6.47	26.5	18±1 ^{o}C	0.24±0.02	-
		18.6	6.38	21.7	35±1 ^{o}C	0.25±0.01	4.2%
		19.8	6.32	23.0	35±1 ^{o}C	0.26±0.00	8.3%
		21.6	6.27	24.6	35±1 ^{o}C	0.20±0.03	-16.7%
		23.5	5.95	25.0	35±1 ^{o}C	0.29±0.03	20.8%
		24.4	6.05	25.6	35±1 ^{o}C	0.32±0.03	33.3%
	Fermentation Batch 4	0	6.56	27.8	35±1 ^{o}C	0.19±0.00	-
		18.6	4.56	28.3	35±1 ^{o}C	0.53±0.02	178.9%

Titratable Acidity values are triplicate means ± SD at 95% Confidence Interval

Milk fermentation batch one, incubated at 26±1 ^{o}C, took less than 30 hours for the acidity to reach 0.6% as lactic acid. Whereas, milk fermentation batch two, incubated at room

temperature (18±1^{o}C), took more than 30 hours for the acidity to reach 0.6% as lactic acid. Reducing temperature slowed down the acidification process.

Gonfa, *et.al.* (2001) discussed that if the temperature during incubation is too high, fermentation will be rapid and over-souring occurs, causing a separation of the liquid and solid phase and gas production, thus leading to deterioration of appearance and texture (Gonfa, *et al.*, 2001). Similar result was observed in milk fermentation batch four, incubated at 35 ^{o}C. It took less than 20 hours for the acidity to reach 0.5% as lactic acid. The pH rapidly dropped to 5.0 in less than 15 hours. This rapid souring resulted in coagulation and complete separation of liquid from solid. As a result, it was difficult to either churn or take sample for analysis from this batch. To see the acidity change more closely, fermentation batch three, first left to ferment at room temperature for about 18 hours was transferred in to an incubator set at 35 ^{o}C. During the 18 hours fermentation at room temperature, the pH and titratable acidity changed only from 6.47 to 6.38 and 0.24% to 0.25%, respectively. However, it took only six hours for the pH and titratable acidity to change from 6.38 to 6.05 and 0.25% to 0.32%, respectively; when transferred in to 35±1^{o}C environment.

Ashenafi (2006) discussed that at lower temperatures, the rate of acid formation was lower but the titratable acidity in the final product was higher. Milk incubated at lower temperatures had a better *"Ergo"* flavor. As the temperature of incubation was raised, the rate of pH drop was faster and the time of coagulation became shorter. Similarly, in this study pH drop was faster at higher temperature.

O'Mahony (1988) points out that milk acidity is one of the factors influencing churning time and recovery of butter fat as butter, and that milk containing at least 0.6% lactic acid is easier to churn. Nonetheless, in this case, when higher incubation temperature (>30 ^{o}C) is used the milk fully coagulated even before reaching a titratable acidity level of 0.6%; making it impossible to churn.

Effect of Churning Temperature and Acidity on Percentage Fat Loss

The effect of acidity and churning temperature on over all churning efficiency were studied. Milk from fermentation batch two and fermentation batch three were used. Milk acidity, temperature, degree of agitation and extent of filling the churn are factors influencing churning time and recovery of butter fat as butter (O'Mahony, 1988). Increasing degree of agitation reduces churning time.

In addition, churn should be filled to between a third and half their volumetric capacity. There should be enough room for air in the churn. As butter making is essentially the controlled destabilization of the oil in water emulsion of milk and because during churning the fat globule membrane substances spreads out over the surface of the air bubbles, partly denuding the globules of their protective layer the presence of air in the churn is necessary. There should be enough air so that partly destabilized fat globules can attach themselves to the air bubbles; and later on collect together to form butter grains.

In this study, to avoid effect of agitation speed and filling level on butter yield, degree of agitation was kept constant for all churning batches; and all batches were filled below half the volumetric capacity of the churn. The difference in filling level between churn batches was insignificant. Results are summarized in Table 9 on page 64.

$$Percentage\ loss = \frac{Quantitiy\ of\ fat\ loss\ in\ buttermilk}{Quantitiy\ of\ fat\ in\ milk} * 100$$

Fat loss increased with increase in pH both at lower and high churning temperatures. The loss was significantly higher when the churning temperature is low. However, fat loss decreased with increase in temperature when churned at lower acidity. At higher acidity, churning temperature did not affect fat loss much. Similar results have been reported by O'Mahony (1988), in which he discussed that increasing acidity will make churning easier, i.e. yield will be higher and churning time lower. It has also been mentioned that yield will be reduced with increasing temperature. O'Mahony (1988) also indicated that even though at lower temperatures yield is higher, grains size will be small and difficult to handle, thus may contribute to loss.

At lower churning temperature, the churning time increased with increasing pH, but at higher churning temperature the churning time decreased when the pH level was increased. At higher acidity, churning time was not much affected by churning temperature. But at lower acidity, churning time decreased significantly with increasing churning temperature.

Fat loss during processing

For Ghee production by modified Ethiopian Butter Method, fat loss can occur during milk churning and Ghee residue filtration processes. Fat loss during churning of milk for butter production was calculated by measuring butter milk fat content. Accordingly, fat loss was highest (36.69%) in churn batch three churned at pH 5.17 equivalent to 0.64±0.01% titratable acidity (as lactic) and temperature 12±1 ^{o}C. Churning time was also the highest in this batch. Increasing the temperature to 19±1 ^{o}C has reduced fat loss to 24.87%; and significantly reduced churning time. The lowest fat loss (7.52%) was registered in churn batch one churned at pH 4.45 equivalent to 0.80±0.03% titratable acidity (as lactic) and temperature 15±1^{o}C. Increasing the temperature to 20±1^{o}C reduced churning time by few minutes but increased the fat loss to 9.62%

Fat loss during Ghee residue filtration is mainly affected by amount of Ghee residue and type of filtration technique used. In this case since laboratory scale product development was carried out, the amount of Ghee residue obtained is very low. Therefore, muslin clothes filtration and hand pressing was applied. At industrial scale processing centrifugal Ghee residue filtration can be applied.

EBM Ghee processing yields a lower amount of Ghee residue when compared to DCM Ghee processing because most of the solid non-fats will be removed with and butter milk. Therefore, during residue filtration fat loss was not as high as in the case of DCM processed Ghee. As shown in Table 9, on page 64, percentages out-turn of Ghee residue was only 3.2%. However, additions of traditional additives increased percentage out-turn of Ghee residue to 11.1%. Fat content of Ghee residue with additives was 60%. In case of Ghee residue without additives, amount of Ghee residue was too small to conduct fat content analysis in triplicate.

Overall, it can be seen that the presence of additives significantly contributes to the reduction of Ghee yield. Ghee percentages out-turn for Ghee with additives and Ghee without additives are summarized in Table 9, page 64 below. For Ghee with additives percentage out-turn on the basis of butter was 61.1%, and percentage out-turn on the basis of fat in butter was 79.8%. For Ghee without additives percentage out-turn on the basis of butter was 63.2%, and percentage out-turn on the basis of fat in butter was 82.9%.

Shelf life prediction for Ghee with additives

Peroxide concentration change with time for EBMa Ghee was applied to zero order, first order and second order models. According to the fit both at 45 ^{o}C and 70 ^{o}C, root mean

square error was minimum for zero order models. Thus, the model-representing rate of change of peroxide value will be:

$$\frac{+d[PV]}{dt} = k[PV]^0$$

$$[PV] = [PV_0] + kt$$

Where: k = rate constant for peroxide formation ($meq_{*}kg^{-1}{}_{*}day^{-1}$), t = time (days), PV_o = initial peroxide value estimated (meq/kg) and PV = peroxide value in meq/kg at time t.

k and PV_o values predicted for both temperatures are summarized in Table 8 on page 63 below. The estimated PV_o for both temperatures is not significantly different form initial experimental values, which suggests model adequacy is satisfactory.

Regressions of ln k vs. 1/T were performed to obtain the Arrhenius parameters k_A and E_A/R.

$$ln\ k = ln\ k_A - \frac{E_A}{R} * \frac{1}{T}$$

Where: k_A= the pre-exponential factor ($meq_{*}kg^{-1}{}_{*}day^{-1}$); E_A = the activation energy (kJ/mol); T = oven temperature (^oK); R is the gas constant (8.314 J/mol*^oK), and k = estimated rate constant for peroxide formation.

lnk was plotted against the reciprocal absolute temperature (1/T) to obtain a straight line with a slope of -E_A/R and intercept *ln* k_A.

The Arrhenius parameters estimated are summarized in Table 8 below. E_A value differs from the expected data range (62 – 105 kJ/mol) presented by Labuza for lipid oxidation (Labuza, 1984).

Using the estimated E_A value (18.15 KJ/mol), rate constant for peroxide formation and shelf life were calculated at 27 ^oC; assuming the product will be stored at room temperature (27 ^oC), and a peroxide value of 0.6 meq/kg as end of shelf life indicator quality index.

$$k = 0.00569 \frac{meq}{kg\ day}$$

$$Shelf\ life = t = \frac{\left[0.6 \frac{meq}{kg}\right] - \left[0.01 \frac{meq}{kg}\right]}{0.00569 \frac{meq}{kg\ day}} = 104\ days = 3.45\ months$$

Table 8: Statistical analysis summary of zero order kinetic parameters for DCM Ghee, CBM Ghee, and EBMa Ghee from peroxide value data

Sample	Parameter	45 °C	70 °C
Direct Cream Method Ghee	PV_o (meq/kg)	0.24	0.33
	k (meq$*$kg $^{-1}*$day^{-1})	0.012	0.076
	R^2	0.711963	0.989136
	$ln\ k_A$	20.90	
	E_A(kJ/mol)	66.95	
Creamery Butter Method Ghee	PV_o (meq/kg)	0.399	0.005
	k (meq$*$kg $^{-1}*$day^{-1})	0.036	0.244
	R^2	0.910281	0.989803
	$ln\ k_A$	22.99	
	E_A(kJ/mol)	69.59	
Ethiopian Butter Method Ghee with additives	PV_o (meq/kg)	0.05	0.12
	k (meq$*$kg $^{-1}*$day^{-1})	0.0086	0.0142
	R^2	0.733942	0.867383
	$ln\ k_A$	2.109	
	E_A(kJ/mol)	18.15	

4.4. Ghee Production Techniques Comparison

4.4.1. Percentage Out-turn of Ghee

Percentage out-turns of Ghee production for the three methods were calculated by the following formulas.

Equation 29

$$Percentage\ outturn\ of\ ghee\ (on\ the\ basis\ of\ cream/butter) = \frac{Quantity\ of\ ghee\ obtained}{Quantity\ of\ cream/butter\ taken} * 100$$

Equation 30

$$Percentage\ outturn\ of\ ghee\ (on\ the\ basis\ of\ fat\ in\ cream/butter) = \frac{Quantity\ of\ ghee\ obtained}{Quantity\ of\ fat\ in\ cream/butter} * 100$$

Equation 31

$$Percentage\ outturn\ of\ ghee\ residue = \frac{Quantity\ of\ ghee\ residue}{Quantityof\ cream/butter\ taken} * 100$$

Results of percentage out-turn are summarized in Table 9 below.

Table 9: Fat loss and Percentage out-turn summary for Ghee production by Direct cream method, Creamery Butter method and Traditional Ethiopian method

	Direct Cream Method	Creamery butter method	Traditional Ethiopian method			
			Churn Batch 1	**Churn Batch 2**	**Churn Batch 3**	**Churn Batch 4**
Milk Weight (kg)	24.1392	34.9078	4.3730	4.7314	4.4426	4.0408
Cream Weight (kg)	2.5440	2.9921	-	-	-	-
Skim milk Weight (kg)	21.5952	31.9157	-	-	-	-
Fat loss during separation (%)	10.04	2.26	-	-	-	-
Butter weight (kg)	-	1.6185	0.1896	0.2275	0.1120	0.1569
Butter milk weight (kg)	-	1.3736	4.1834	4.5039	4.3306	3.8839
Fat loss during churning (%)	-	2.68	7.52	9.62	36.69	24.87
Churning time (min)	-	8	15	12	70	4
Butter/Cream weight used for Ghee (kg)	2.4906	1.4806	0.3786		0.2274	
Ghee Weight (kg)	0.7466	1.0509	0.2314		0.1438	
Ghee residue weight (g)	196.3	37.3	42.2		7.4	
Percentage out-turn of Ghee on the basis of cream/butter (%)	29.9	70.9	61.1		63.2	
Percentage out-turn of Ghee on the basis of fat in cream/butter (%)	73.0	91.3	79.8		82.9	
Percentage out-turn of Ghee residue (%)	7.9	2.5	11.1		3.2	

Percentage outturn was highest for Ghee produced by creamery butter method. The lowest Ghee residue outturn was also registered for this method. Lower Ghee residue means lower fat loss with Ghee residue, thus higher Ghee yield. Second highest Ghee outturn was registered by Ghee prepared by Ethiopian butter method. However, when additives were added in Ghee the residue significantly increased, thus resulted in lower overall yield. The lowest yield was registered by Ghee prepared by direct cream method. If fat loss during cream separation can be minimized, over all yield of direct cream can be comparative with the Ghee form Ethiopian butter method.

4.4.2. Shelf Stability

For shelf stability analysis, peroxide value, free fatty acid value and organoleptic characteristics were followed with time for each Ghee product from the different Ghee processing techniques. ASLT method was applied; and as in all the classical ASLT methods temperature was used as the dominant acceleration factor. Lipid deterioration occurs either due to the off odors and flavors resulting from lipolysis (hydrolytic rancidity) or lipid oxidation (oxidative rancidity) (O'Keefe & Pike, 2010). To monitor the hydrolytic rancidity during ASLT free fatty acid change was monitored. To follow up oxidative rancidity change

peroxide value was monitored. Peroxide value and free fatty acid value change are summarized in Table 10. Table 11 shows a summary of sensory evaluation.

Table 10: Summary of chemical analysis result for an accelerated shelf stability analysis of Ghee made by different processing methods

Processing Method (Storage time)	45 °C		70 °C	
	PV(mEq/Kg)	FFA (%)	PV(mEq/Kg)	FFA (%)
DCM (3 days)	0.27 ± 0.12	0.23 ± 0.07	0.48 ± 0.12	0.18 ± 0.07
DCM (26 days)	0.55 ± 0.10	0.20 ± 0.02	2.22 ± 0.11	0.22 ± 0.02
CBM (3 days)	0.47 ± 0.24	0.25 ± 0.08	0.48 ± 0.42	0.21 ± 0.04
CBM (26 days)	1.29 ± 0.06	0.20 ± 0.02	6.11 ± 0.29	0.17 ± 0.04
EBMa (3 days)	0.01 ± 0.00	0.19 ± 0.03	0.14 ± 0.11	0.18 ± 0.05
EBMa (26 days)	0.27 ± 0.01	0.20 ± 0.05	0.46 ± 0.04	0.17 ± 0.00
EBMwa (3 days)	0.14 ± 0.11	0.19 ± 0.03	0.07 ± 0.11	0.19 ± 0.03
EBMwa (26 days)	0.16 ± 0.03	0.16 ± 0.05	1.04 ± 0.09	0.17 ± 0.04

Table 11: Summary of sensory analysis result for an accelerated shelf stability analysis of Ghee made by different processing methods

Score of sample from Oven 1 (45°C)				
Processing Method (Storage time)	Color	Odor	Flavor	Total
DCM (3 days)	8.90 ± 0.36	25.33 ± 4.62	54.33 ± 3.05	88.57 ± 8.03
DCM (26 days)	7.67 ± 1.53	20.00 ± 5.29	43.67 ± 10.26	71.33 ± 17.08
CBM (3 days)	8.33 ± 1.15	25.50 ± 3.90	50.00 ± 8.18	83.83 ± 13.25
CBM (26 days)	7.33 ± 1.15	23.33 ± 7.23	46.67 ± 14.47	77.33 ± 22.86
EBMa (3 days)	6.67 ± 0.58	27.33 ± 2.08	53.00 ± 1.73	87.00 ± 4.39
EBMa (26 days)	6.67 ± 0.58	20.33 ± 5.13	46.67 ± 9.50	73.67 ± 15.21
Score of sample from Oven 2 (70°C)				
Processing Method (Storage time)	Color	Odor	Flavor	Total
DCM (3 days)	9.00 ± 0.5	26.00 ± 4.00	52.67 ± 4.16	87.67 ± 8.66
DCM (26 days)	8.33 ± 1.53	20.33 ± 5.86	44.00 ± 12.29	72.67 ± 19.68
CBM (3 days)	9.67 ± 0.58	28.00 ± 1.00	55.00 ± 2.64	92.67 ± 4.22
CBM (26 days)	3.67 ± 1.53	16.33 ± 7.50	34.00 ± 20.07	54.00 ± 29.11
EBMa (3 days)	7.00 ± 2.00	25.57 ± 5.01	49.33 ± 7.02	81.90 ± 14.03
EBMa (26 days)	5.67 ± 2.08	19.67 ± 8.74	40.00 ± 18.68	65.33 ± 29.50

The keeping quality of Ghee can be affected by many factors, such as method of manufacture, packaging material type, package permeability to oxygen and moisture, presence of antioxidants, light, and others. During this study manufacturing methods are generally being compared, thus, other factors were kept similar, if not constant. This means package used, storage environment, storage time, and oxygen contact surface (i.e filling level) were made similar for all products during the test period.

Means Comparisons was performed using one-way analysis of variance (ANOVA) for all processing methods at each storage temperature. Comparisons for each pair was performed using Student's t at $\alpha = 0.05$,to see whether the actual difference in the peroxide and free fatty acid value means is greater than the difference that would be significant. Comparisons for each week are summarized in Table 12.

Table 12: Summary for One-Way analysis of Variance of Peroxide value and free fatty acid value by processing method

		Processing Method			
		CBM	DCM	EBMa	EBMwa
Week 1	PV at 45 ^{o}C	0.473^{a}	0.270^{ab}	0.073^{b}	0.136^{b}
	FFA at 45 ^{o}C	0.247^{a}	0.233^{a}	0.193^{a}	0.193^{a}
	PV at 70 ^{o}C	0.476^{a}	0.476^{a}	0.136^{a}	0.073^{a}
	FFA at 70 ^{o}C	0.206^{a}	0.176^{a}	0.180^{a}	0.193^{a}
Week 2	PV at 45 ^{o}C	1.293^{a}	0.546^{b}	0.266^{c}	0.163^{c}
	FFA at 45 ^{o}C	0.196^{a}	0.196^{a}	0.196^{a}	0.156^{a}
	PV at 70 ^{o}C	6.110^{a}	2.223^{b}	0.460^{d}	2.223^{c}
	FFA at 70 ^{o}C	0.170^{a}	0.223^{a}	0.170^{a}	0.170^{a}

Values, in raw, not mentioned by same letter are significantly different

As the data and figures indicate, peroxide value increased with time, for all products; especially for those stored at 70 ^{o}C. On the other hand, change in free fatty acid value with time was not statistically significant. Free fatty acid value increased with time only for Ghee from direct cream method. In the other products free fatty acid value showed a decline. Thus it can be said that oxidative rancidity was dominant.

During the first week, peroxide value for CBM stored at 45 ^{o}C was significantly higher than the other processing methods. At 70 ^{o}C peroxide values were not significantly different for all processing methods. However, at the end of week 2 peroxide values became significantly different for each processing method at both temperatures. This shows that some samples performed better under the stressed conditions. For example, from samples stored at 70 ^{o}C CBM scored the highest PV value, followed by DCM, EBMwa, and EBMa, respectively.

Similarly, during second week, for samples stored at 45 ^{o}C peroxide value was higher for CBM. DCM had second largest peroxide value. EBMa and EBMwa scored significantly lower value. The fact that Ghee from EBMa performs better when compared to EBMwa under stressed conditions indicates that the additives could have some positive effect in retarding oxidation or peroxide formation. Free fatty acid value showed no significant difference at both temperatures for all processing methods during the analysis period.

The results show that, after storage at high temperature, highest peroxide value was registered by Ghee from creamery butter method and the lowest value was registered by Ghee form

Ethiopian butter method with additives. Furthermore, sensory analysis result corresponds with peroxide value result. Lowest value was scored by Ghee from creamery butter method. With regard to organoleptic characteristics, Ghee from direct cream method scored the highest value.

Shelf life prediction results show that DCM Ghee has the longest shelf life (7.5 month) followed by EBMa Ghee (3.45 months) and CBM Ghee (2.7 months). Similar results were reported by Singh et.al. (1979) (Singh, *et al.*, 1979) indicating DC Ghee had the longest keeping quality (12 month) followed by desi Ghee (India indigenous Ghee) (9 month) and cream butter (CB) Ghee (4 months).

Storage stability of Ghee is attributed to the low moisture content and the high content of phospholipids. However, the antioxidative properties of phospholipids have been argumentative. Some researchers (Banks, 1991) have elaborated that phospholipids are more prone to oxidation because of their unsaturated nature and that the free radical process is assumed to originate in the phospholipids. Others underline antioxidative properties of phospholipids, suggesting that phospholipids may exhibit antioxidant activity by binding metals, regenerating other antioxidants, and providing a synergism with phenolic antioxidants.(Richardson & Korycka-Dahl, 1988)

Ghee residue could have antioxidant properties due to the presence of tocopherols, phospholipids and products of browning reactions (Sripad, *et al.,* 1996). It has been suggested by some researchers, (Unnikrishnan & Rao, 1977, Berg, 1988), that during heating especially after most of the moisture has been evaporated antioxidants are produced from phospholipids. The heat-modified phospholipids are believed to be absorbed by the fat and hence contribute to the keeping quality of the Ghee. Consequently, the Ghee produced by DCM is said to be more stable than that produced by CBM method because of the longer heat treatment and the higher phospholipids content. Thus, similarly in this case, the better performance by DCM can be attributed to the antioxidant effect of the high amount of residue produced during manufacture.

On the other hand, temperature effect on rate of lipid oxidation is best analyzed in terms of the overall activation energy, E_A for lipid oxidation (Ragnarsson & Labuza, 1977). Ragnarsson & Labuza (1977) discussed that lipid oxidation can be broken down into a number of elementary step reactions where each step has its own activation energy; and the controlling overall activation energy can change as a result of a temperature elevation alone.

Presence of antioxidants, change in oxygen pressure, or presence of certain metals can alter the mechanism and hence the activation energy.

Increasing temperature can result in both physical and chemical changes. Some of these changes may affect reaction mechanism thus cause errors. Water activity will increase with increasing temperature. A new catalytic surface contact may also be formed if there is phase change, such as melting. E_A changes, therefore, could cause erroneous prediction of the shelf life at room temperature on the basis of data collected at a higher temperature; when comparison is done between different fat products. Nonetheless, in this case water activity is very low and temperature was set so that all fat portions are at liquid state. In addition, the probability of a change in the mechanism occurring as a result of an increase in temperature is much greater in complex foods rather than in simple lipid systems. Thus, in this study, for shelf life prediction it has been assumed that E_A, the activation energy, will stay approximately constant.

Higher E_A value is associated with the presence of a primary antioxidant. Antioxidants can lower the rate of the oxidation at least partly by increasing the overall energy of activation (Ragnarsson & Labuza, 1977).E_A values for Creamery Butter Method Ghee, Direct Cream Method Ghee and Ethiopian Butter Method Ghee with additives are 69.59kJ/mol, 66.95kJ/mol and 18.15kJ/mol, respectively. Then again, DCM, which is supposed to have the highest antioxidant content due to the large amount of residue, scored second highest E_A value. Even though CBM had the highest E_A, it performed poorly during the shelf stability study.

The fact that E_A value was higher in CBM could mean higher amount of phospholipids are present. Singh, *et al.* (1979) argues phospholipids had no positive effect on the keeping quality of Ghee. In fact, CBM Ghee, which is known to contain higher amounts of phospholipids, had poor keeping quality. On the other hand, DCM Ghee, which has been reported to contain less phospholipid, had superior keeping quality.

As explained by Pagote and Bhandari (1988), phospholipids possess antioxidant properties; but the antioxidant property of Ghee does not depend on one constituent alone. Other constituents such as amino acids, sulphydryl compounds, free sugars and products of their interaction with protein during heating are also considered to contribute, mainly because of their reducing capacity. Therefore, it can be said DCM and EBMa Ghee products performed better than CBM not only due to the presence of higher amount of phospholipids, but also the higher residue content and presence of other compounds from the residue.

CHAPTER FIVE

5. Techno-feasibility study

5.1.Process Design

5.1.1. Unit operations List, Equipment List and Equipment Layout

Direct Cream Method Unit Operations

- ✓ Milk reception
- ✓ Pasteurization
- ✓ Cream Separation
- ✓ Evaporation
- ✓ Centrifugal separation
- ✓ Packaging
- ✓ Cooling
- ✓ Storage

Modified Ethiopian Butter Method Unit Operations

- ✓ Milk reception
- ✓ Pasteurization
- ✓ Culture preparation
- ✓ Milk ripening and souring
- ✓ Temperature treatment
- ✓ Churning and working
- ✓ Melting and heating
- ✓ Evaporation
- ✓ Centrifugal separation
- ✓ Packaging
- ✓ Cooling
- ✓ Storage

Valve List	
No.	*Description*
V1	Quality Control samplilng line valve
V2	Process line Selection/Control valve
V3	Starter Propagation Vessel filling valve
V4	Butter Milk (byproduct) Collection line valve
V5	Skim Milk (byproduct) Collection line valve
V6	Ghee Boiler filling point control valve
V7	Cold water circulation tank filling line valve
V8	Steam Generator water supply line valve
V9	Pasteurizer steam supply line valve
V10	Ghee Boiler steam supply line valve

Equipment List	
No.	**Description**
1	Raw milk storage tank filling pump (milk)
2	Raw milk storage tank
3	Pasteurizer feed pump (milk)
4	Plate heat exchanger type Pasteurizer
5	Pasteurized milk storage tank
6	Milk Fermenter, starter propagator and cream separator feed pump (milk)
7	Milk Fermentation reactor
8	Fermented Milk cooling tank feed pump (acidified milk)
9	Cooling vessel
10	Churner feed pump (acidified milk)
11	Churner
12	Screw conveyor for butter transportation in to heater
13	Butter heater
14	Ghee Boiler feed pump (melted butter)
15	Starter propagation vessel
16	Milk fermenter feed pump (Activated culture)
17	Butter milk storage vessel
18	Inline centrifugal pump
19	Centrifugal cream separator
20	Cream storage vessel
21	Ghee Boiler feed pump (Cream)
22	Skim milk storage vessel
23	Additives mixer
24	Ghee Boiler
25	Centrifugal Ghee separator/clarifier feed pump (Ghee+Ghee residue)
26	Centrifugal Ghee separator/clarifier
27	Ghee storage tank
28	Ghee packaging unit feed pump (Ghee)
29	Ghee residue and sugar mixer
30	Cooling media (water) storage tank
31	Heating media (water) storage tank
32	Heating media (steam) Generator
33	Inline centrifugal pump
34	Inline centrifugal pump

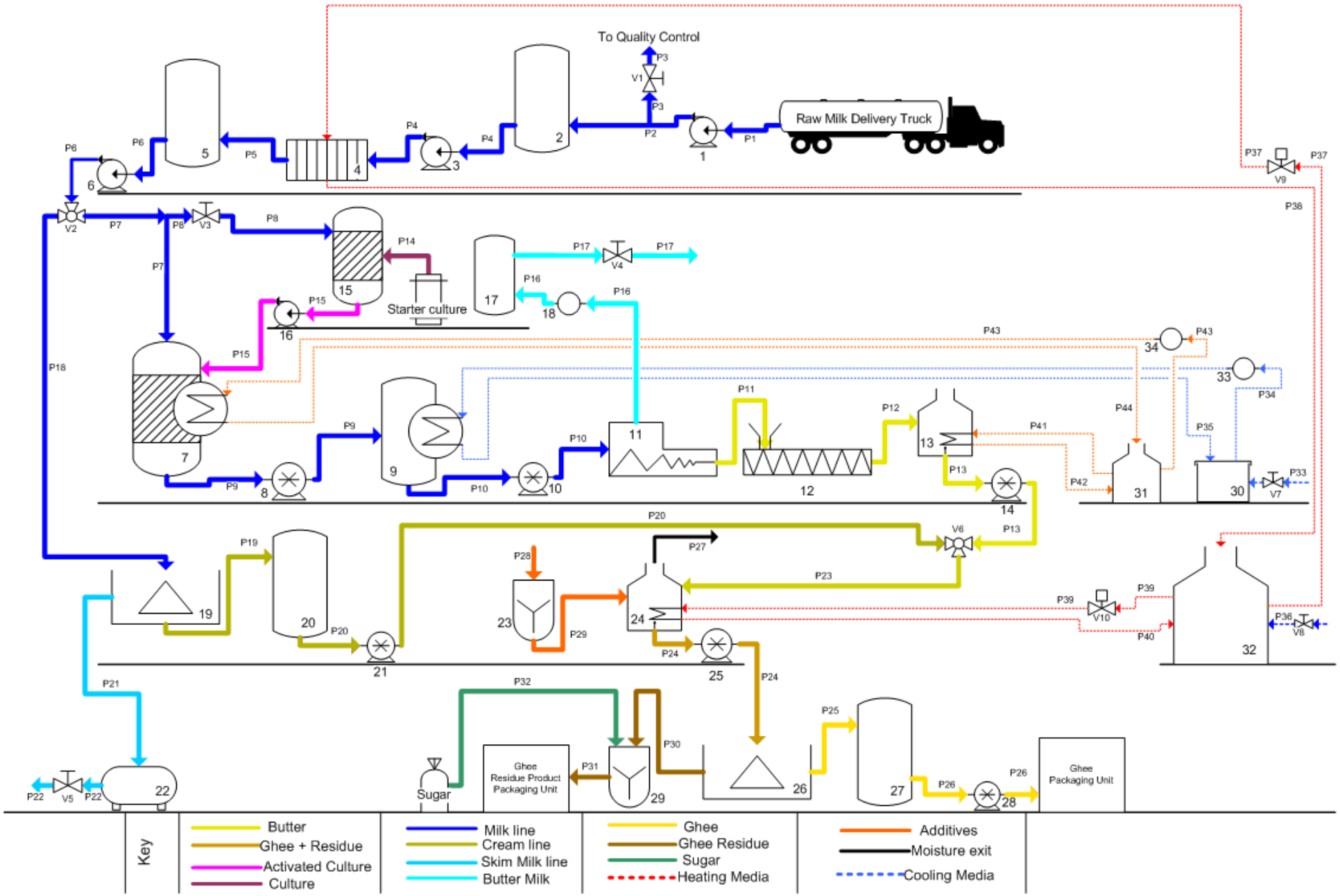


Figure 4: DCM and EBMa Ghee processing unit Equipment layout

5.1.2. Material and Energy Balance

In this design, the factory embraces two processing lines, which can work alternatively depending on the type of product required. The first process line is for Ghee production by modified Ethiopian Butter Method. The second process line is for Ghee production by direct cream method. The overall material and energy balance for major unit operations are outlined below. It should be noted that some unit operations are common for both process line. i.e. process lines share the same equipment. All assumptions are based on data obtained during product development at laboratory level study.

Material Balance:

The general conservation equation for any process system can be written as:

$$\text{Material out} = \text{Material in} + \text{Generation} - \text{Consumption} - \text{Accumulation}$$

Assuming steady state condition and no chemical reaction during processing the balance reduces to:

$$\text{Material out} = \text{Material in}$$

Energy Balance

From first law of thermodynamics

$$Accumulation = Energy\ in - Energy\ out + Generation - Consumption$$

$$\dot{E_A} = \dot{E_{in}} - \dot{E_{out}} + \dot{E_G} - \dot{E_C}$$

Where: $\dot{E_A}$ = Rate of energy accumulation, $\dot{E_{in}}$= Rate of energy at inlet, $\dot{E_{out}}$ = Rate of energy at outlet, $\dot{E_G}$ = Rate of energy generation, and $\dot{E_C}$ = Rate of energy consumption

Assumingsteady state condition and none or insignificant level of heat generation or consumption

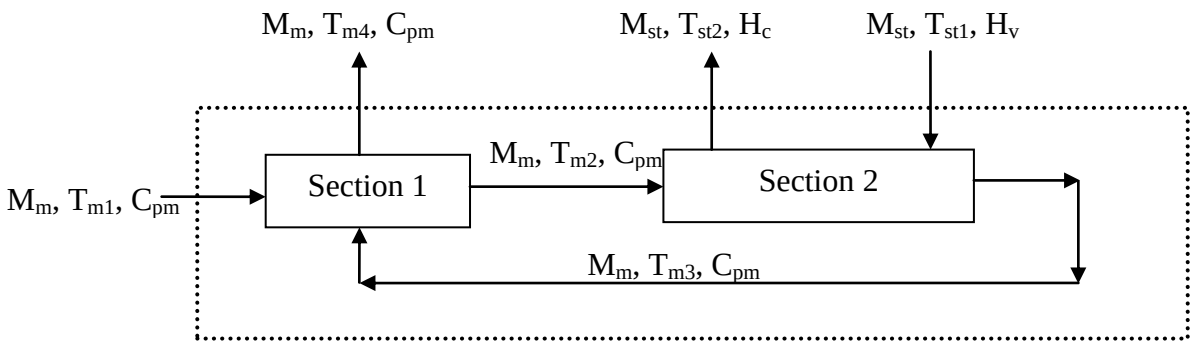
$$0 = \dot{E_{in}} - \dot{E_{out}}$$

$$\dot{E_{in}} = \dot{E_{out}}$$

$$H_{in} + Q_{in} = H_{out} + Q_{out}$$

$$H_{in} - H_{out} = \Delta Q$$

On pasteurizer (common for process line 1 and 2)



Where:

- M_m – mass of milk
- M_{st} – mass of steam
- T_{m1} – Temperature of milk at inlet
- T_{m2} – Temperature of milk at leaving section 1 and entering section 2
- T_{m3} – Temperature of milk at leaving section 2 and entering section 1
- T_{m4} – Temperature of milk at outlet
- T_{st1} – Temperature of steam at inlet
- T_{st2} – Temperature of steam at outlet
- C_{pm}– milk specific heat capacity
- H_v – Vapor steam enthalpy
- H_c – Condensate steam enthalpy

Energy balance

Assumptions

- T_{m1} = 10 ^oC = 283 K
- T_{m3} = 75 ^oC = 348 K
- T_{m4} = 42 ^oC = 315 K
- T_{st1} = T_{st2} = 120 ^oC = 393 K
- Heating process is under constant pressure
- Basis = 656 kg milk

From saturated steam properties table, for steam at 120 ^oC (Singh & Heldman, 2009, p. 794)

- H_v = 2706.3kJ/kg
- H_c = 503.71 kJ/kg

From physical properties of milk table (Walstra, Wouters, & Geurts, 2006, p. 756)

- Specific heat C_p of milk = 3.9kJ/kg K

Mass of steam required for milk heating

$$H_{in} + Q_{in} = H_{out} + Q_{out}$$

$$H_{in} - H_{out} = \Delta Q$$

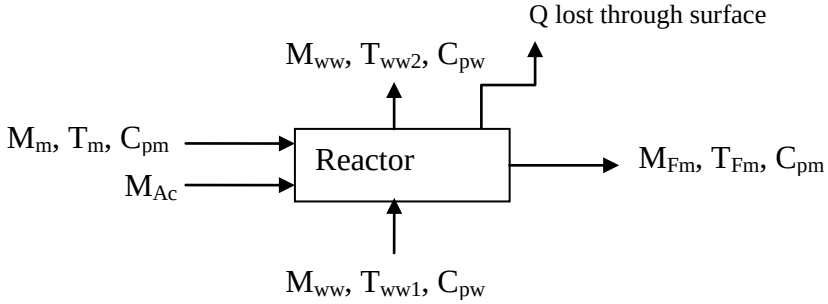
$$M_{st} * H_v - M_{st} * H_c = M_m * C_{pm} * (T_{m4} - T_{m1})$$

$$M_{st} * (2706.3\frac{\text{kJ}}{\text{kg}} - 503.71\frac{\text{kJ}}{\text{kg}}) = 656kg * 3.9\frac{\text{kJ}}{\text{kg} * \text{K}} * (315\ \text{K} - 283K)$$

$$M_{st} * (2202.59\frac{\text{kJ}}{\text{kg}}) = 81{,}868.8 * kJ$$

$$M_{st} = 37.169\ kg$$

On reactor for milk acidification (Process line 1)



Where:

- M_m – mass of milk
- M_{Ac} – mass of activated culture
- M_{Fm} – mass of fermented milk
- T_{ww1} – Temperature of warm water at inlet
- T_{ww2} – Temperature of warm water at outlet
- C_{pm} – milk specific heat capacity
- C_{pw} – water specific heat capacity
- T_m – temperature of milk at inlet
- T_{Fm} – temperature of fermented milk at outlet

Assumptions

- Basis = 637 liter milk = 656 kg
- M_{Ac} = 2% * M_m
- Reactor volume = 700 liter = 0.7 m^3
- Reactor height = 0.5 m
- Radius = 0.67m
- Reactor wall thickness; X = 5 * 10^{-3} m
- Inside temperature = 42 ^oC = 315K
- Outside temperature = room temperature = 25 ^oC = 298 K
- T_{ww1} = 42 ^oC = 315 K
- T_{ww2} = 32 ^oC = 305 K
- T_m = T_{Fm} = 42 ^oC = 315 K

Mass Balance

Over all material balance

$$Mass\ in = Mass\ out$$
$$M_m + M_{Ac} = M_{Fm}$$
$$M_m + 0.02 * M_m = M_{Fm}$$
$$656\ kg + 13.12\ kg = M_{Fm}$$
$$M_{Fm} = 669.12\ kg$$
$$M_{Ac} = 13.12\ kg$$

Energy Balance

Amount of heat loss through surface during fermentation

$$Q = U * A * \Delta T$$
$$\text{Total Surface Area } = 2\pi rh + \pi r^2$$
$$\text{Total Surface Area } = 2\pi * 0.67 * 0.5 + \pi * 0.67^2$$
$$\text{Total Surface Area } = 2\pi * 0.67 * 0.5 + \pi * 0.67^2$$
$$\text{Total Surface Area } = 3.51\ m^2$$

For tank with empty double jacket, no stirrer containing whole milk and in contact with Surrounding air; Coefficient of Total Heat Transfer U = 3 W/(m^2K) (Walstra, Wouters, & Geurts, 2006, p. 760)

$$Q = 3\frac{W}{m^2K} * 3.51\ m^2 * (315\ K - 298\ K) = 179.01\ w$$

Assuming time of fermentation = 60 min

$$Q_{\text{lost in 1 hour}} = 3600\ \text{sec} * 179.01\ w = 644.436\ kJ$$

Mass of warm water required for maintaining reactor temperature

From Physical Properties of Water at the Saturation Pressure table (Singh & Heldman, 2009, p. 792)

- C_{pw} = 4.18kJ/kg °C

$$Q = M_{ww} * C_{pw} * \Delta T$$

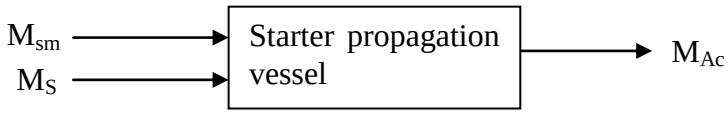
$$Q = M_{ww} * C_{pw} * (T_{ww1} - T_{ww2})$$

$$M_{ww} = \frac{Q}{C_{pw} * (T_{ww1} - T_{ww2})}$$

$$M_{ww} = \frac{644.436 \text{ kJ}}{4.18 \text{ kJ/[kg ° C]} * (42\text{oC} - 32\text{oC})}$$

$$M_{ww} = 15.42\ kg$$

<u>On reactor for starter culture activation (process line 1)</u>



Where;

- M_{sm} – mass of skim milk
- M_S – mass of starter culture
- M_{Ac} – mass of activated culture

Assumptions

- M_S = freeze dried concentrated bulk starter for direct vat inoculation
- M_S = 1% * M_{sm}
- Basis 13.12 kg activated culture

<u>Mass Balance</u>

Over all material balance

$$Mass\ in = Mass\ out$$

$$M_{sm} + M_S = M_{Ac}$$

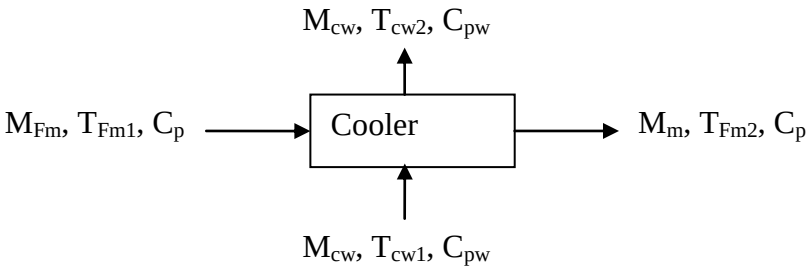
$$M_{sm} + 0.01 * M_{sm} = M_{Ac}$$

$$M_{sm} + 0.01 * M_{sm} = 13.12\ kg$$

$$M_{sm} = 12.99\ kg$$

$$M_S = 0.1299\ kg$$

<u>On cooling unit prior to churning (process line 1)</u>



Where;

- M_{Fm} – mass of fermented milk
- M_{cw} – mass of cooling water
- T_{Fm1} – Temperature of fermented milk at inlet
- T_{Fm2} – Temperature of fermented milk at outlet
- T_{cw1} – Temperature of cooling water at inlet
- T_{cw2} – Temperature of cooling water at outlet
- C_p– milk specific heat capacity
- C_{pw} – water specific heat capacity

Assumptions

- T_{Fm1} = 42 ^oC = 315 K
- T_{Fm2} = 15 ^oC = 288 K
- T_{cw1} = 5 ^oC = 278 K
- T_{cw2} = 35 ^oC = 308 K
- Cooling process is under constant pressure
- Basis = 669.12 kg fermented milk

<u>Energy Balance</u>

Amount of heat to be removed during cooling of fermented milk

From Physical Properties of Water at the Saturation Pressure table (Singh & Heldman, 2009, p. 792)

- C_{pw} = 4.18kJ/kg °C

From physical properties of milk table (Walstra, Wouters, & Geurts, 2006, p. 756)

- Specific heat C_p of milk = 3.9kJ/kg K

$$Q = M_{Fm} * C_p * \Delta T$$

$$Q = M_{Fm} * C_p * (T_{Fm2} - T_{Fm1})$$

$$Q = 669.12kg * 3.9\frac{\text{kJ}}{\text{kg} * \text{K}} * (288\ \text{K} - 315\ \text{K})$$

$$Q = -70{,}458.3\ kJ$$

Mass of cooling water required to remove heat from fermented milk

$$Q_{removed} = M_{cw} * C_{pw} * \Delta T$$

$$Q_{removed} = M_{cw} * C_{pw} * (T_{cw2} - T_{cw1})$$

$$M_{cw} = \frac{Q_{removed}}{C_{pw} * (T_{cw2} - T_{cw1})}$$

$$M_{cw} = \frac{70{,}458.3\ kJ}{4.18\ \text{kJ/[kg °C]} * (35\ \text{oC} - 5\text{oC}\ )}$$

$$M_{cw} = 561.866\ kg$$

Heat transfer area needed to cool fermented milk

$$Q = U * A * \Delta T_{lm}$$

Where;

- Q – Heat transferred per unit time
- A – Area available for flow of heat
- ΔT_{lm} – logarithmic mean temperature difference between cooling water and milk
- U – Overall heat transfer coefficient

From Coefficient of Total Heat Transfer table (Walstra, Wouters, & Geurts, 2006, p. 760)

- U for Double-walled tank, stirrer but no scraper yoghurt coolers that uses water as a cooling media = 290W/(m^2K)

Assuming U remains constant

$$\Delta T_{lm} = \frac{(T_{Fm2} - T_{cw1}) - (T_{Fm1} - T_{cw2})}{\ln \frac{(T_{Fm2} - T_{cw1})}{(T_{Fm1} - T_{cw2})}}$$

$$\Delta T_{lm} = \frac{(288\ \text{K} - 278\ \text{K}) - (315\ \text{K} - 308\ \text{K})}{\ln \frac{10\ K}{7\ K}}$$

$$\Delta T_{lm} = 8.41\ K$$

Assuming total time required for cooling of milk is 1 hour

$$\dot{Q} = \frac{Q}{time} = \frac{70{,}458.3\ kJ}{3600\ sec} = 19.572\ kW$$

$$A = \frac{\dot{Q}}{U * \Delta T_{lm}}$$

$$A = \frac{19571.7\ W}{290\ \frac{\text{W}}{\text{m}^2\text{K}} * 8.41\ K}$$

$$A = 8.025\ m^2$$

<u>On churner (Process line 1)</u>

Where;

- M_{Fm} – mass of fermented milk
- M_{Bm}– mass of butter milk
- M_B– mass of butter

Assumptions

- Fat loss with Butter milk = 0.3%
- Milk fat content = 4.5 %
- Butter fat content = 80%
- Basis = 669.12 kg fermented milk
- Churning temperature = 15 ^oC
- Fermented milk acidity = pH 4.5 and TA 0.8%

Mass Balance

Over all material balance

$$Mass\ in = Mass\ out$$

$$M_{Fm} = M_{Bm} + M_B$$

Equation 32

$$669.12\ kg = M_{Bm} + M_B$$

Component material balance on fat content

$$0.045 * M_{Fm} = 0.003 * M_{Bm} + 0.80 * M_B$$

$$0.045 * 669.12\ kg = 0.003 * M_{Bm} + 0.80 * M_B$$

Equation 33

$$30.1104\ kg = 0.003 * M_{Bm} + 0.80 * M_B$$

Combining Equation 32 and Equation 33

$$30.1104\ kg = 0.003 * (669.12\ kg - M_B) + 0.80 * M_B$$

$$28.1030\ kg = 0.797 * M_B$$

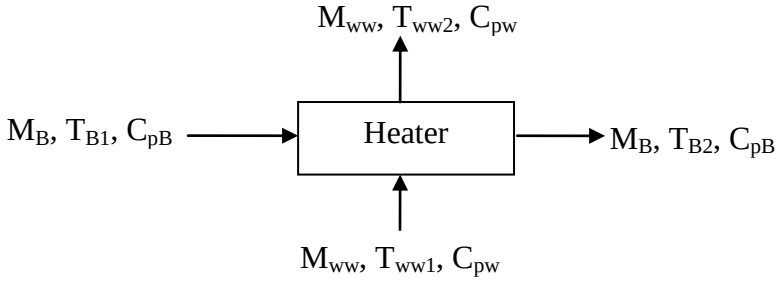
$$M_B = 35.261\ kg$$

Inserting M_B value in Equation 32

$$669.12\ kg = M_{Bm} + 35.261\ kg$$

$$M_{Bm} = 633.859\ kg$$

On Butter heating unit after churning (Process line 1)



Where;

- M_B – mass of Butter
- M_{ww} – mass of warm water
- T_{B1} – Temperature of Butter at inlet
- T_{B2} – Temperature of Butter at outlet

- T_{ww1} – Temperature of warm water at inlet
- T_{ww2} – Temperature of warm water at outlet
- C_{pB} – Butter specific heat capacity
- C_{pw} – water specific heat capacity

Assumptions

- T_{B1} = 15 ^{o}C = 288 K
- T_{B2} = 40 ^{o}C = 313 K
- T_{ww1} = 42 ^{o}C = 315 K
- T_{ww2} = 32 ^{o}C = 305 K
- Heating process is under constant pressure
- Basis = 35.261 kg Butter

Energy Balance

Amount of heat required to melt butter

From physical properties of milk and milk products table (Walstra, Wouters, & Geurts, 2006, p. 756)

- Specific heat C_p of Butter = 2.3kJ/kg K

From Physical Properties of Water at the Saturation Pressure table (Singh & Heldman, 2009, p. 792)

- C_{pw} = 4.18kJ/kg °C

$$Q = M_B * C_{pB} * \Delta T$$

$$Q = M_B ** C_{pB} * (T_{B2} - T_{B1})$$

$$Q = 35.261 \text{ kg} * 2.3 \frac{\text{kJ}}{\text{kg} * \text{K}} * (313 \text{ K} - 288 \text{ K})$$

$$Q = 2{,}027.5\ kJ$$

Mass of warm water required to melt butter

$$Q = M_{ww} * C_{pw} * \Delta T$$

$$Q = M_{ww} * C_{pw} * (T_{ww1} - T_{ww2})$$

$$M_{ww} = \frac{Q}{C_{pw} * (T_{ww1} - T_{ww2})}$$

$$M_{ww} = \frac{2{,}027.5\ kJ}{4.18 \text{ kJ/[kg} ^\circ \text{C]} * (42\text{oC} - 32\text{oC})}$$

$$M_{ww} = 48.5\ kg$$

Heat transfer area needed to heat butter

$$Q = U * A * \Delta T_{lm}$$

Where;

- Q – Heat transferred per unit time
- A – Area available for flow of heat
- ΔT_{lm} – logarithmic mean temperature difference between the steam and Butter
- U – Overall heat transfer coefficient

From approximate design values of overall heat-transfer coefficients table (Peters & Timmerhaus, 1991, pp. 600 - 601)

- U for heaters = 100Btu/(hft^2°F) = 567.83 W/(m^{2}K)

Assuming U remains constant

$$\Delta T_{lm} = \frac{(T_{ww2} - T_{B1}) - (T_{ww1} - T_{B2})}{\ln \frac{(T_{ww2} - T_{B1})}{(T_{ww1} - T_{B2})}}$$

$$\Delta T_{lm} = \frac{(305\ \text{K} - 288\ \text{K}) - (315\ \text{K} - 313\ \text{K})}{\ln \frac{17\ K}{2\ K}}$$

$$\Delta T_{lm} = 7\ K$$

Assuming total time required for melting butter is 20 minutes

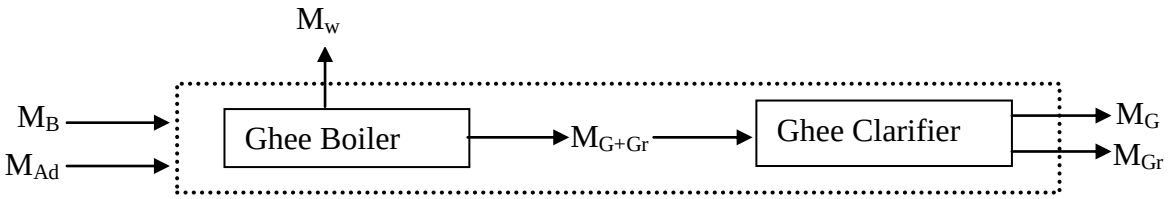
$$\dot{Q} = \frac{Q}{time} = \frac{2{,}027.5\ kJ}{1200\ sec} = 1.689\ kW$$

$$A = \frac{\dot{Q}}{U * \Delta T_{lm}}$$

$$A = \frac{1{,}689.58\ W}{567.83\ \frac{\text{W}}{\text{m}^2\text{K}} * 7\ K}$$

$$A = 0.425\ m^2$$

<u>On Ghee Boiler and Ghee Clarifier (process line 1)</u>



Where;

- M_B – mass of Butter
- M_{Ad} – mass of additives
- M_w – mass of moisture evaporated
- M_G – mass of Ghee
- M_{Gr} – mass of Ghee Residue
- M_{G+Gr} – mass of Ghee and Ghee residue

Assumptions:

- Moisture content of Ghee = 0.2%
- Moisture content of Ghee residue = 5%
- Moisture content of additives = 4 %
- Butter moisture content = 16 %
- Butter solid non fat content = 4 %
- Ghee residue solid non fat content = 35%
- Ghee residue fat content = 60%
- Ghee residue in remaining in Ghee = 0%
- Basis = 35.261 kg Butter
- M_{Ad} = 2.5% * M_B

<u>Mass Balance</u>

Over all material balance

$$Mass\ in = Mass\ out$$

$$M_B + M_{Ad} = M_w + M_G + M_{Gr}$$

$$35.261\ kg + 0.8815\ kg = M_w + M_G + M_{Gr}$$

Equation 34

$$36.1425\ kg = M_w + M_G + M_{Gr}$$

Component material balance on moisture content

$$0.16 * M_B + 0.04 * M_{Ad} = 1 * M_w + 0.002M_G + 0.05M_{Gr}$$

$$0.16 * 35.261\ kg + 0.04 * 0.8815\ kg = 1 * M_w + 0.002M_G + 0.05M_{Gr}$$

Equation 35

$$5.6771\ kg = M_w + 0.002M_G + 0.05M_{Gr}$$

Component material balance on solid non fat

$$0.04 * M_B + 0.96 * M_{Ad} = 0 * M_w + 0 * M_G + 0.35 * M_{Gr}$$

$$0.04 * 35.261\ kg + 0.96 * 0.8815\ kg = 0.35M_{Gr}$$

$$2.2567\ kg = 0.35M_{Gr}$$

$$M_{Gr} = 6.448\ kg$$

Inserting M_{Gr}value in Equation 34 and Equation 35

Equation 36

$$29.6945\ kg = M_w + M_G$$

Equation 37

$$5.3547\ kg = M_w + 0.002M_G$$

Combining Equation 36 and Equation 37

$$5.3547\ kg = 29.6945\ kg - M_G + 0.002M_G$$

$$0.998 * M_G = 24.3398\ kg$$

$$M_G = 24.388\ kg$$

Inserting $M_G$and M_{Gr} value in Equation 34

$$36.1425\ kg = M_w + 24.388\ kg + 6.448\ kg$$

$$36.1425\ kg = M_w + 30.836\ kg$$

$$M_w = 5.3065\ kg$$

<u>On cream separator(process line 2)</u>

Where;

- M_m – mass of milk
- M_{sm} – mass of skim milk
- M_c – mass flow rate of cream

Assumptions

- Fat loss with skim milk = 0.1%
- Milk fat content = 4.5 %
- Cream fat content = 55 %
- Basis = 637 liter milk = 656 kg
- Milk specific gravity = 1.030
- Milk temperature = 42 ^{o}C

Mass Balance

Over all material balance

$$Mass\ in = Mass\ out$$

$$M_m = M_{sm} + M_c$$

Equation 38

$$656\ kg = M_{sm} + M_c$$

Component material balance on fat content

$$0.045 * M_m = 0.001 * M_{sm} + 0.55 * M_c$$

$$0.045 * 656\ kg = 0.001 * M_{sm} + 0.55 * M_c$$

Equation 39

$$29.52\ kg = 0.001 * M_{sm} + 0.55 * M_c$$

Combining Equation 38 and Equation 39

$$29.52\ kg = 0.001 * (656\ kg - M_c) + 0.55 * M_c$$

$$28.864\ kg = 0.549 * M_c$$

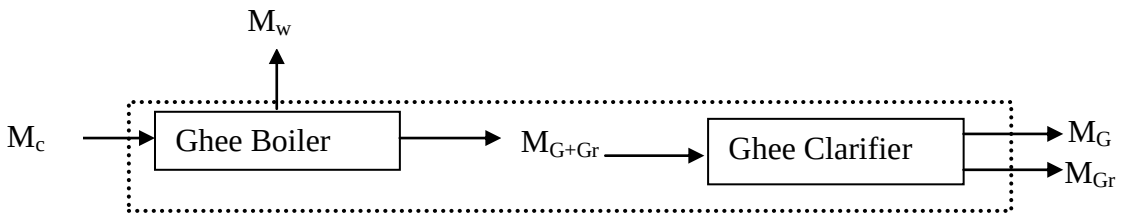
$$M_c = 52.576\ kg$$

Inserting M_C value in Equation 38

$$656\ kg = M_{sm} + 52.576\ kg$$

$$M_{sm} = 603.424\ kg$$

<u>On Ghee Boiler and Ghee Clarifier(process line 2)</u>



Where;

- M_c – mass of cream
- M_w – mass of moisture evaporated
- M_G – mass of Ghee
- M_{Gr} – mass of Ghee Residue
- M_{G+Gr} – mass of Ghee and Ghee residue

Assumptions

- Moisture content of Ghee = 0.2%
- Moisture content of Ghee residue = 5%
- Cream moisture content = 42 %
- Cream solid non fat content = 3 %
- Ghee residue solid non fat content = 75%
- Ghee residue fat content = 20%

- Ghee residue remaining in Ghee = 0%
- Basis = 52.576 kg cream

Mass Balance

Over all material balance

$$Mass\ in = Mass\ out$$

$$M_c = M_w + M_G + M_{Gr}$$

Equation 40

$$52.576\ kg = M_w + M_G + M_{Gr}$$

Component material balance on moisture content

$$0.42 * M_c = 1 * M_w + 0.002M_G + 0.05M_{Gr}$$

$$0.42 * 52.576\ kg = 1 * M_w + 0.002M_G + 0.05M_{Gr}$$

Equation 41

$$22.082\ kg = M_w + 0.002M_G + 0.05M_{Gr}$$

Component material balance on solid non-fat

$$0.03 * M_c = 0 * M_w + 0 * M_G + 0.75M_{Gr}$$

$$0.03 * 52.576\ kg = 0.75M_{Gr}$$

$$M_{Gr} = 2.103\ kg$$

Inserting M_{Gr}value in Equation 40 and Equation 41

Equation 42

$$50.473\ kg = M_w + M_G$$

Equation 43

$$21.977\ kg = M_w + 0.002M_G$$

Combining Equation 42 and Equation 43

$$21.977\ kg = 50.473\ kg - M_G + 0.002M_G$$

$$0.998 * M_G = 28.496\ kg$$

$$M_G = 28.553\ kg$$

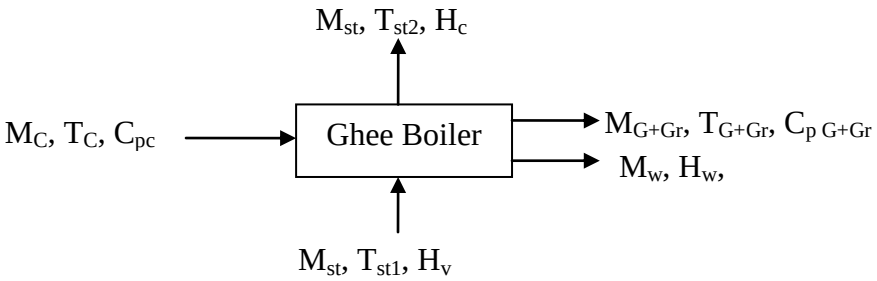
Inserting $M_G$and M_{Gr}value in Equation 40

$$52.576\ kg = M_w + 28.553\ kg + 2.103\ kg$$

$$52.576\ kg = M_w + 30.656\ kg$$

$$M_w = 21.92\ kg$$

Energy Balance on Ghee boiler



Where;

- M_C – mass of cream
- M_{st} – mass of steam
- M_{G+Gr} – mass of Ghee and Ghee residue
- M_w – mass of moisture evaporated
- T_C – Temperature of cream at inlet
- T_{G+Gr} – Temperature of Ghee and Ghee residue at outlet
- T_{st1} – Temperature of steam at inlet
- T_{st2} – Temperature of steam at outlet
- C_{pc} – cream specific heat capacity
- $C_{p\ G+Gr}$ – Ghee and Ghee residue specific heat capacity
- H_w– Vapor steam enthalpy at 100 ^oC
- H_v – Vapor steam enthalpy at 120 ^oC
- H_c – Condensate steam enthalpy at 120 ^oC

Assumptions

- $T_C = 40^oC = 313$ K
- $T_{st1} = T_{st2} = 120^oC = 393$ K
- $T_{G+Gr} = 110^oC = 383$ K
- Heating process is under constant pressure
- Basis = 52.576 kg cream
- $M_w = 21.92$ kg
- $M_{G+Gr} = 30.656$ kg

From physical properties of milk and milk products table (Walstra, Wouters, & Geurts, 2006, p. 756)

- Specific heat of cream,C_{pc}= 3.5kJ/kg K

From Physical Properties of Water at the Saturation Pressure table (Singh & Heldman, 2009, p. 792)

- C_{pw} = 4.18kJ/kg °C

From saturated steam properties table, for steam at 120 ^oC (Singh & Heldman, 2009, p. 794)

- H_v = 2706.3kJ/kg
- H_c = 503.71 kJ/kg

From saturated steam properties table, for evaporated water (steam at 100 ^oC)

- Enthalpy = H_w = 2676.1 kJ/kg

Specific heat of Ghee + Ghee residue calculation

Assumptions

- Moisture content of Ghee and Ghee residue = 0.5%
- Ghee and Ghee residue solid non fat content = 5%
- Ghee and Ghee residue fat content = 94.5%

To calculate specific heat capacity of Ghee + Ghee residue empirical equation proposed by Charm (1978) was used. (Singh & Heldman, 2009, p. 258)

$$C_p = 2.093 * X_{fat} + 1.256 * X_{snf} + 4.187 * X_{moisture}$$

Where; X = mass fraction

$$C_{pG+Gr} = 2.093 * 0.945 + 1.256 * 0.05 + 4.187 * 0.005$$

$$C_{p\,G+Gr} = 2.0616 \frac{\text{kJ}}{\text{kg}}\text{K}$$

Mass of steam required to evaporate moisture from cream

$$H_{in} + Q_{in} = H_{out} + Q_{out}$$

$$(M_{st} * H_v) + \left(M_c * C_{pc} * T_c\right) = (M_{st} * H_c) + (M_w * H_w) + (M_{G+Gr} * C_{pG+GR} * T_{G+Gr})$$

$$M_{st} = \frac{(M_w * H_w) + \left(M_{G+Gr} * C_{pG+GR} * T_{G+Gr}\right) - \left(M_c * C_{pc} * T_c\right)}{H_v - H_c}$$

$$M_{st} = \frac{\left(21.92\ kg * 2676.1 \frac{\text{kJ}}{\text{kg}}\right) + \left(30.656\ \text{kg} * 2.0616 \frac{\text{kJ}}{\text{kgK}} * 383\ \text{K}\right) - \left(52.576\ \text{kg}\ * 3.5 \frac{\text{kJ}}{\text{kgK}} * 313\ \text{K}\right)}{(2706.3 \frac{\text{kJ}}{\text{kg}} - 503.71 \frac{\text{kJ}}{\text{kg}})}$$

$$M_{st} = \frac{25{,}268.86\ kJ}{(2202.59 \frac{\text{kJ}}{\text{kg}})}$$

$$M_{st} = 11.472\ kg$$

Heat transfer area needed evaporate moisture from cream

$$Q = U * A * \Delta T_{lm}$$

Where;

- Q – Heat transferred per unit time
- A – Area available for flow of heat
- ΔT_{lm} – logarithmic mean temperature difference between the steam and Butter
- U – Overall heat transfer coefficient

From approximate design values of overall heat-transfer coefficients table (Peters & Timmerhaus, 1991, pp. 600 - 601)

- U for evaporators = 500Btu/(hft^2°F) = 2839.15 W/(m^{2}K)

Assuming U remains constant

$$\Delta T_{lm} = \frac{(T_{st2} - T_C) - (T_{st1} - T_{G+Gr})}{\ln \frac{(T_{st2} - T_C)}{(T_{st1} - T_{G+Gr})}}$$

$$\Delta T_{lm} = \frac{(393\ \text{K} - 313\ \text{K}) - (393\ \text{K} - 383\ \text{K})}{\ln \frac{80\ K}{10\ K}}$$

$$\Delta T_{lm} = 33.66\ K$$

Assuming total time required for moisture evaporation from cream is 30 minutes.

$$\dot{Q} = \frac{Q}{time} = \frac{25{,}268.86\ kJ}{1800\ sec} = 14.038\ kW$$

$$A = \frac{\dot{Q}}{U * \Delta T_{lm}}$$

$$A = \frac{14{,}038.26\ W}{2839.15\ \frac{\mathrm{W}}{\mathrm{m^2K}} * 33.66\ K} = 0.147 m^2$$

5.2.Equipment Design, Sizing and Selection

It has been assumed that plant daily production capacity is 100 kg Ghee; with four operating cycles each producing 25 kg Ghee. Basis for equipment sizing is one operating cycle.

Sizing of pasteurizer

Assuming the milk to be pasteurized in 4 hour;

$$Pasteurizer\ capacity = \frac{637 liter}{4\ hour} = 159.25 \frac{liter}{hour}$$

Select 160-liter/hour capacity pasteurizer with plate type heat exchanger, stainless steel 310constructions, with pasteurization temperature range 70 ^{o}C – 90 ^{o}C.

Sizing of Fermentation reactor

Volume of reactor needed, assuming the reactor is 75% full

$$Volume\ of\ reactor = \frac{637\ liter}{0.75} = 849.3\ liter = 0.849\ m^3$$

Select 0.85 m^3 jacketed stirred reactor, with approximately 3.5 m^2 jacket area; AISI 304stainless steel construction.

Sizing of Starter propagation vessel

Assuming the reactor is 75% full

$$Volume\ of\ reactor = \frac{13\ liter}{0.75} = 17.3\ liter = 0.017\ m^3$$

Select 0.017 m^3 jacketed reactor; AISI 304 stainless steel construction.

Sizing Pre churning fermented milk cooler

Assuming the vessel is 75% full

$$Volume\ of\ reactor = \frac{689\ liter}{0.75} = 918.7\ liter = 0.918\ m^3$$

Select 0.92 m^3 jacketed vessel; with approximately 8 m^2 jacket area; AISI 304 stainless steel construction.

Sizing Churner

Assuming churner is 66% full

$$Volume\ of\ churner = \frac{689\ liter}{0.66} = 1043.9\ liter = 1.044\ m^3$$

Select 1 m^3AISI 304 stainless steel construction churners.

Sizing of conveyor

To provide Butter heater filling in 10 minutes:

$$conveyor\ capacity = \frac{35\ kg}{10\ minutes} = 3.5\frac{kg}{minute}$$

Select 3.5 kg/min capacity screw conveyor; AISI 304 stainless steel construction

Sizing butter heating unit

Butter density = 950 kg/m^3 and amount of butter to be heated = 35.261 kg

Volume of butter heating vessel, assuming 90% of the vessel will be full

$$Volume = \frac{35.261\ \text{kg}}{950\frac{\text{kg}}{\text{m3}} * 0.9} = 0.0412\ m^3$$

Select 0.04 m^3 jacketed vessel with stirrer, with 0.425 m^2 jacket area, 310 stainless steel construction.

Sizing of Ghee boiler

The Ghee boiler is common for both process lines; in process line one, butter will be used for producing Ghee, while in process line two cream will be used. Since the amount of cream is always larger, the volume of Ghee boiler is calculated taking cream volume as a basis.

Cream density = 1009 kg/m^3 and amount of cream to be heated = 52.576 kg

Volume of Ghee boiler, assuming 50% of the vessel will be full

$$Volume = \frac{52.576\ \text{kg}}{1009\ \frac{\text{kg}}{\text{m3}} * 0.5} = 0.1042\ m^3$$

Select 0.11 m^3 jacketed vessel with stirrer, with 0.147 m^2 jacket area, 310 stainless steel construction.

Sizing of Cream separator

Assuming the milk to be skimmed in 4 hour;

$$Separator\ capacity = \frac{637 liter}{4\ hour} = 159.25\ \frac{liter}{hour}$$

Select 160 liter/hour capacity separator, stainless steel 304 construction.

Sizing storage vessels

Assuming all storage vessels to be 90% full during processing

- Raw milk, pasteurized milk, butter milk and skim milk storage tanks

$$Volume = \frac{637\ liter}{0.90} = 707.7\ liter = 0.707\ m^3$$

Select 0.7 m^3AISI 304 stainless steel construction storage vessel

- Cream storage tank

$$Volume = \frac{52.1\ liter}{0.90} = 57.89\ liter = 0.0579\ m^3$$

Select 0.06 m^3AISI 304 stainless steel construction storage vessel

- Ghee storage tank

$$Volume = \frac{31.17\ liter}{0.90} = 34.63\ liter = 0.0346\ m^3$$

Select 0.04 m^3AISI 304 stainless steel construction storage vessel

Sizing of pumps

Pumps 1, 3, 6, 8, and 10

Assuming the milk to be filled in to vessels with in 10 minutes

$$Pump\ capacity = \frac{637\ liter}{10\ minutes} = \frac{0.637 m^3}{10\ min} = 0.0637\ {}^{m^3}/_{min}$$

Select 0.06 m^3/min AISI 304 stainless steel construction centrifugal and positive displacement pumps

Pumps 14, 21, 25, 28

Assuming the milk to be filled in to vessels with in 10 minutes

$$Pump\ capacity = \frac{51\ liter}{10\ minutes} = \frac{0.051 m^3}{10\ min} = 0.051\ {}^{m^3}/_{min}$$

Select 0.05 m^3/min AISI 304 stainless steel construction centrifugal or positive displacement pumps

Sizing of steam generator

Amount of steam necessary to for pasteurizer and Ghee boiler = 48.641 kg

Total processing time required involving steam energy usage is approximately 4.5 hours

$$steam\ generator\ capacity = \frac{48.641\ kg}{4.5\ hour} = 10.8\ {}^{kg}/_{hour}$$

Select 11 kg/hour capacity 310 stainless steel construction steam generator

Sizing of cold water storage tank

Mass of cooling water required to remove heat from fermented milk = 561.866 kg

Density of water at 10 ^oC = 999.7 kg/m^3, assuming tank to be 80% full

$$Volume = \frac{561.866\ \text{kg}}{999.7 \frac{\text{kg}}{\text{m3}} * 0.8} = 0.702\ m^3$$

Select 0.7 m^3 capacity 302 stainless steel construction storage tank

Sizing of warm water storage tank

Mass of warm water required for butter heating unit and milk reactor = 63.92kg

Density of water at 42 ^oC = 992.2 kg/m^3, assuming tank to be 80% full

$$Volume = \frac{63.92\ \text{kg}}{992.2 \frac{\text{kg}}{\text{m3}} * 0.8} = 0.0805\ m^3$$

Select 0.08 m^3 capacity 302 stainless steel construction storage tank with an electrical heating system.

Packaging unit specification

Assuming 25 – 30 kg Ghee production in one processing cycle, and the product to be aseptically packaged into 0.5 kg cans within 30 min;

$$packaging\ unit\ capacity = \frac{30\ kg\ ghee}{30\ min} = \frac{60\ ghee\ packs}{30\ min} = 2\frac{packs}{min}$$

Select 120 packs (0.5 kg)/hour capacity aseptic filling and canning machine for liquid foods.

Table 13: Purchased equipment size and price summary

Equipment type	Size	Quantity	Unit price ($)	Total Price ($)
Pasteurizer	160 liter/hour	1	500	500
Fermentation reactor	0.85 m^3	1	5,000	5,000
Starter propagation vessel	0.017 m^3	1	1,500	1,500
Cooling tank	0.92 m^3	1	6,000	6,000
Churner	1 m^3	1	2,000	2,000
Conveyor	3.5 kg/min	1	100	100
Butter heating unit	0.04 m^3	1	300	300
Ghee boiler	0.11 m^3	1	550	550
Cream separator	50 liter/hour	4	220	880
Storage vessels	0.7 m^3	4	4,500	18,000
Storage vessel	0.06 m^3	1	4,000	4,000
Storage vessel	0.04 m^3	1	3,500	3,500
Pumps	0.06 m^3/min	5	450	2,250
Pumps	0.05 m^3/min	4	400	1,600
Steam generator	11 kg/hour	1	400	400
Cold water storage tank	0.7 m^3	1	100	100
Warm water storage tank	0.08 m^3	1	60	60
Packaging unit	40-60 bag (0.6liter)/ min	1	6,000	6,000
			Total sum	**52,740**

5.3.Economics

5.3.1. Project Cost Estimation

5.3.1.1. Total Capital Investment

Total Capital Investment = Fixed Capital Investment + Working Capital Investment

For this case, capital investment items are calculated based on delivered equipment cost for fluid processing plant.

$$\text{Fixed Capital Investment} = f(\text{Purchased Equipment Cost})$$

Purchased Equipment Cost = $52,740.00= 930,861.00 Birr

Assuming delivery cost = 10% of Purchased Equipment Cost

Purchased-Delivered equipment cost = **1,023,947.10 Birr**

Table 14: Total capital investment summary

Item	Percentage (%)	Price (Birr)
Direct Cost		
Purchased & Delivered equipment cost	100.00	1,023,947.10
Purchased Equipment Installation	47.00	481,255.14
Instrumentation and Controls	18.00	184,310.48
Piping Installation	66.00	675,805.09
Electrical Installation	11.00	112,634.18
Buildings	18.00	184,310.48
Yard Improvements	10.00	102,394.71
Service Facilities	70.00	716,762.97
Land	6.00	61,436.83
Total Direct Cost	**346.00**	**3,542,856.97**
Indirect Cost		
Engineering and Supervision	33.00	337,902.54
Construction Expenses	41.00	419,818.31
Total Indirect Cost	**74.00**	**757,720.85**
Total Direct and Indirect Cost	**420.00**	**4,300,577.82**
Other Costs		
Total Direct and Indirect Cost	420.00	4,300,577.82
Contractor's fee	21.00	215,028.89
Contingency	42.00	430,057.78
Fixed Capital Investment (FCI)	**483.00**	**4,945,664.49**
Working Capital Investment (WCI)	**85.24**	**872,764.32**
Total Capital Investment (TCI)	**568.24**	**5,818,428.82**

5.3.1.2. Total Product Cost

$$TotalProductCost = ManufacturingCost + GeneralExpenses$$

$$Manufacturing\ Cost\ = Direct\ Production\ Cost + Fixed\ Charges + Plant\ Overhead\ Costs$$

$$General\ Expense = \text{Administrative Costs} + \text{Distribution and Selling Costs} + \text{Interest}$$

Direct production costs

- ✓ Raw Materials = 20% of total product cost
- ✓ Operating labor = 10% of total product cost
- ✓ Direct supervisor and clerical labor = 10% of operating labor
- ✓ Utilities = 10% of total product cost
- ✓ Maintenance and repairs = 2% of fixed capital investment

- ✓ Operating supplies = 10% of Maintenance and repairs cost
- ✓ Laboratory charges = 10% of operating labor
- ✓ Patents and royalties = 10% of total product cost

$$Direct\ production\ cost = 0.52 * Total\ Product\ Cost + 108{,}804.62\ birr$$

Fixed Charges

- ✓ Depreciation = 10% of fixed capital investment for machinery and equipment + 2% of building value
- ✓ Local taxes = 1% of fixed capital investment
- ✓ Insurance = 1% of fixed capital investment

$$Fixed\ charges = 204{,}994.21\ \text{birr}$$

Plant Overhead costs

- ✓ 5% of total product cost

$$Plant\ overhead\ cost = 0.05 * Total\ Product\ Cost$$

Administrative cost

- ✓ 2% of total product cost

$$Administrative\ cost = 0.02 * Total\ Product\ Cost$$

Distribution and selling cost

- ✓ 10% of total product cost

$$Distribution\ and\ selling\ cost = 0.1 * Total\ Product\ Cost$$

Interest

- ✓ 8% of total capital investment

$$Interest = 465{,}474.31\ \text{birr}$$

$$Manufacturing\ Cost\ = 0.57 * Total\ Product\ Cost + 313{,}798.83\ birr$$

$$General\ Expense = 0.12 * Total\ Product\ Cost + 465{,}474.31\ \text{birr}$$

$$TotalProductCost = 0.69 * Total\ Product\ Cost + 779{,}273.14\ birr$$

$$0.31 * TotalProductCost = 779{,}273.14\ birr$$

$$\boldsymbol{TotalProductCost = 2{,}513{,}784.32\ \text{birr}}$$

5.3.2. Financial Evaluation

- Daily production = 100 kg
- 13 public holidays and 52 Sundays = 65 non working days
- Annual production = 30,000 kg
- One pack size = 0.500 kg
- Plant Capacity = 60,000 packs per year
- Supply of raw material = 2548 liter raw milk, 100 liter milk collected daily from 26 different small scale farms
- Supply of packages = imported

$$Unit\ production\ cost = \frac{2{,}513{,}784.32\,\frac{\text{birr}}{\text{year}}}{60{,}000\,\frac{packs}{year}} = 41.89\,\frac{birr}{pack}$$

$$\text{Profit margin} = 50\% * \text{Unit Production Cost} = 20.945\,\frac{birr}{pack}$$

$$\text{Unit product selling price} = \text{Unit production cost} + \text{Profit margin} = 62.84\,\frac{birr}{pack}$$

$$Sales = 60{,}000\,\frac{packs}{year} * 62.84\,\frac{birr}{pack} = 3{,}770{,}400.00\,\frac{birr}{year}$$

$$Gross\ earnings = Total\ product\ sales - Total\ product\ cost$$

$$Gross\ earnings = 3{,}770{,}400.00\,\frac{birr}{year} - 2{,}513{,}784.32\,\frac{\text{birr}}{\text{year}}$$

$$Gross\ earnings = 1{,}256{,}615.68\,\frac{birr}{year}$$

$$Income\ Tax = 30\% * Gross\ earnings = 376{,}984.70\ Birr/year$$

$$Net\ Profit = 879{,}630.98\ birr/year$$

Return on Investment (ROI)

$$Return\ on\ Investment = \frac{Annual\ Profit}{Total\ Capital\ Ivestment} * 100\%$$

$$ROI = \frac{879{,}630.98\ birr}{5{,}818{,}428.82\ \text{birr}} * 100\%$$

$$ROI = 15.1\%$$

Payback Period

$$Pay\ back\ period = \frac{Total\ capital\ investment}{net\ profit\ per\ year + Average\ Depriciation\ Per\ year} * 100\%$$

$$Pay\ back\ period = \frac{5{,}818{,}428.82\ \text{birr}}{879{,}630.98\ birr + 106{,}080.92\ birr}$$

$$Pay\ back\ period = 5.9\ years$$

Break Even Point

Annual Direct production costs

$$Direct\ production\ cost = 0.52 * Total\ Product\ Cost + 108{,}804.62\ birr$$

$$Direct\ production\ cost = 0.52 * 2{,}513{,}784.32\ \text{birr} + 108{,}804.62\ birr$$

$$Direct\ production\ cost = 1{,}415{,}972.47\ birr$$

Annual Plant Capacity = 60,000 packs per year

$$Unit\ Direct\ production\ cost = \frac{1{,}415{,}972.47\ birr}{60{,}000\ pack} = 23.60\ birr/pack$$

Fixed Charges

$$Fixed\ charges = 204{,}994.21\ \text{birr}$$

Plant overhead cost

$$Plant\ overhead\ cost = 0.05 * Total\ Product\ Cost$$

$$Plant\ overhead\ cost = 0.05 * 2{,}513{,}784.32\ \text{birr}$$

$$Plant\ overhead\ cost = 125{,}689.22\ \text{birr}$$

General Expense

$$General\ Expense = 0.12 * Total\ Product\ Cost + 465{,}474.31\ \text{birr}$$

$$General\ Expense = 0.12 * 2{,}513{,}784.32\ \text{birr} + 465{,}474.31\ \text{birr}$$

$$General\ Expense = 767{,}128.43\ \text{birr}$$

At break even

$$TotalProductCost = Total\ sales\ income$$

$$Direct\ Production\ Cost + Fixed\ Charges + Plant\ Overhead\ Costs + General\ Expense = Total\ sales\ income$$

$$n * unitDirect\ Production\ Cost + Fixed\ Charges + Plant\ Overhead\ Costs + General\ Expense = n * unit\ product\ selling\ price$$

Where; n = production capacity

$$n * 23.60\frac{birr}{pack} + 204{,}994.21\ \text{birr} + 125{,}689.22\ \text{birr} + 767{,}128.43\ \text{birr} = \text{n} * 62.84\frac{birr}{pack}$$

$$n * 39.24\frac{birr}{pack} = 1{,}097{,}811.86\ \text{birr}$$

$$n = 27{,}976.85\ packs$$

Break even production capacity = $27{,}976.85\ packs$ = 46.6% of the plant capacity

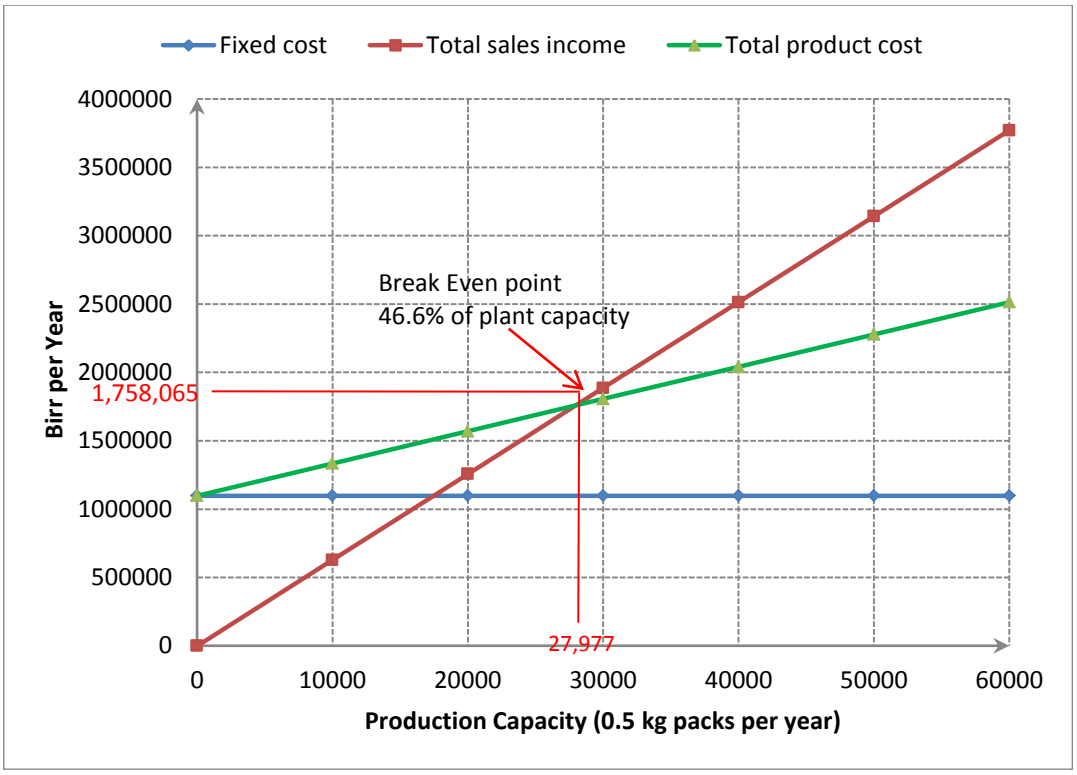


Figure 5: Breakeven point summary

CHAPTER SIX

6. Conclusions and Recommendations

To minimize post-harvest losses it is wise to process milk into shelf stable products. Ghee is one of the most shelf stable dairy products available in today's market. The major objective of this paper work is to assess the effect of different Ghee processing methods on shelf stability and yield. Therefore, different Ghee processing methods, namely Direct Cream Method (DCM), Creamery Butter Method (CBM), and a modified Ethiopian Butter Method (EBM) were evaluated. Ghee products prepared by these methods were subjected to a harsh environmental condition, to compare the performance of each product. Through exposure of products to high environmental temperature it was possible to accelerate the rate of deterioration.

Overall yield was highest for CBM with a yield of 91.3% on the basis of fat in butter. 2.26% and 2.68% fat losses were registered during separation and churning, respectively. Thus a total of approximately 13.64% fat loss was registered. DCM Ghee yield was 73.0% on the basis of fat in cream. Fat loss during separation was 10.04%. In total fat loss associated with this processing method was 37%. But it should be noted that fat loss during separation could be minimized to as low as 1%. The high fat loss in this case, i.e. during cream separation, was due to operational error. Both EBMwa (Ethiopian Butter Method without additives) and EBMa (Ethiopian Butter Method with additives) methods resulted in better yield of Ghee on the basis of fat in butter than DCM; i.e. 82.9% and 79.8%, respectively. However, a possible fat loss of 7.52% to 36.69% could occur during churning. Churning the milk at lower pH (4.5) and higher temperature (15 – 17^{o}C) will significantly reduce fat loss. To reduce churning time reducing the acidity to a pH level of 5.0 should be used. Yield of Ghee residue was highest, 11%, for EBMa, followed by 7.9% for DCM, 3.2% for EBMwa and 2.5% for CBM. A significant amount of fat can be lost with Ghee residue during processing. Overall Ghee yield will be higher for a processing method with low amount of Ghee residue.

Even though sensory analysis scores at the beginning of shelf stability test favored CBM Ghee, after 26 days storage at elevated temperature DCM Ghee, EBM_a Ghee and CBM Ghee scored highest, second highest and least, respectively. EBMa Ghee had the lowest sensory characteristics at the beginning of the tests but did not deteriorate as expected.

At elevated temperature storage, EBMa Ghee performed better, scoring lowest peroxide value, followed by EBMwa, DCM and CBM Ghee. The fact that Ghee from EBMa performs better when compared to EBMwa under stressed conditions indicates that the additives had positive effect in retarding the oxidation process, which resulted less formation of peroxides.

Sserunjogi, *et al.* (1998) reported that generally, fresh cream/butter Ghee has a longer shelf life than ripened cream/ butter Ghee. Likewise, in this study, DCM Ghee scored the longest shelf life (7.5 month) followed by EBMa Ghee (3.45 months) and CBM Ghee (2.7 months). On the other hand, percentage outturn was highest for CBM Ghee (91%) followed by EBMwa Ghee (83%), EBMa Ghee (80%), and DCM Ghee (73%).

To conclude, though percentage outturn of DCM Ghee was low, its better shelf stability performance at elevated temperatures outweighs the low yield. Moreover, fat loss during processing can be minimized via the application of centrifugal Ghee residue clarifiers at industrial level. Therefore, for the techno-feasibility study shown in chapter 5, DCM Ghee processing line was chosen. In addition, EBM Ghee processing line has been included to show Ghee product more acceptable by the local community can also be processed side to side. But further research should be carried out on selection and specification of additives amount.

Finally, as Ashenafi (2006) and other researchers elaborated food preparation is predominantly a household phenomenon in Ethiopia. Every household appears to process food starting from raw ingredients to the final products. In case where fermentation is important to obtain a certain product, the microorganisms naturally present on the raw ingredients or in the containers spontaneously take care of the process. I believe this reality must change soon; and food products, such as Ghee, should be processed at industrial level in order to insure the safety and continuous availability of quality fat product to the society.

Suggestion for Future Work

Various physical, chemical and biological methods have been proposed for reducing cholesterol in foods especially dairy products. These include blending of milk fat with vegetable oils, extraction with organic solvent, adsorption with activated charcoal and saponin, vacuum distillation, molecular distillation, degradation of cholesterol by cholesterol oxidase and removal of cholesterol by supercritical carbon dioxide. (Kumar, *et al.*, 2010)Many of the above listed physical and chemical methods have one or more limitations for their application in food processing. Studying which method is appropriate for effective reduction of cholesterol without affecting other nutrients is important.

References

Abdalla, M. (1994) Milk in the rural culture of contemporary Assyrians in the Middle-East. *Milk and Milk Products from Medieval to Modern Times*, (ed. P. Lysaght), pp. 27–39, Canongare Press, Edinburgh.

Abou-Donia, S. A. & EI-Agamy, S. I. (1993) Ghee. *Encyclopedia of Food Science, Food Technology and Nutrition*, (eds. R. Macrae, R. K. Robinson & M. J. Sadler), Vol. 6, pp. 3992–3994, Academic Press, London.

Ahmed, M.A.M., Ehui, S., Yemesrach, A., 2003. Dairy Development in Ethiopia. ILRI working paper no. 58. Addis Ababa, Ethiopia.

Anonymous (2003) *Dairy Processing Handbook*, 2nd edn., pp. 293–299, Tetra Pak Processing Systems A/B, Lund, Sweden.

AOAC (2000). AOAC. Official Method 936.15 Standard Solution of Hydrochloric Acid. *Standard Sodium Hydroxide Method* . AOAC International.

AOAC (2000). AOAC. Official Method 936.16 Standard Solution of Sodium Hydroxide. *Standard Potassium Hydrogen Phthalate Method* . AOAC International.

AOAC (2000). AOAC. Official Method 940.28 Fatty Acids (Free) in Crude and Refined Oils. *Titration Method, National Cottonseed Products Association–AOAC Method* . AOAC International.

AOAC (2000). AOAC. Official Method 942.27 Standard Solutions of Sodium Thiosulfate. AOAC International.

AOAC (2000). AOAC. Official Method 965.33 Peroxide Value of Oils and Fats. *Titration Method AOCS–AOAC Method* . AOAC International.

Ashenafi, M. (2006). A Review on the Microbiology of Indigenous Fermented Foods and Beverages of Ethiopia. *Ethiop. J. Biol. Sci* , 189 - 245.

Banks, W. (1991). Chemical composition of milk lipids. *Bulletin of the IDF 260* , 3 - 5.

Berg, J. C. (1988). Dairy Technology in the Tropics. Wageningen, Netherlands: Pudoc.

Bradley, J. R. (2010). Moisture and Total Solids Analysis. In S. S. Nielsen, *Food Analysis* (pp. 85-91). New York: Springer Science+Business Media, LLC.

Codex, A. (2007). Milk and Milk Products. *Codex Standard for Milk fat Products, Codex Stan A-2-1973* . Rome, Italy: FAO and WHO.

CSA (2000). *Report on Livestock, Poultry and Beehives Population (Private Peasant Holidings),volume 2.* Addis Ababa: The Federal Democratic Republic of Ethiopia Centeral Statistical Agency.

CSA (2010). *Report on Crop and Livestock Product Utilization (Private Peasant Holdings, Meher Season), Volume VII.* Addis Ababa: The Federal Democratic Republic of Ethiopia Centeral Statistical Agency.

CSA (2011). *Report on Livestock and Livestock Characteristics (Private Peasant Holdings) Volume II.* Addis Ababa: The Federal Democratic Republic of Ethiopia Centeral Statistical Agency.

Sukumar, D. (1990). Indian Dairy Products. In D. Sukumar, *Outlines of Dairy Technology* (pp. 433-463). N. Delhi: Kalyani Publishing House.

Ethiopian Standards (2009). Butter-Determination of moisture, non-fat solids and fat content, part 3: calculation of fat content. *ES ISO 8851-3:2009* . Addis Ababa, Ethiopia: Quality and Standards Authority of Ethiopia (QSAE).

Ethiopian Standards (2009). Butter-Determination of moisture, non-fat solids and fat contents, part 2:Determination of non-fat solids content. *ES ISO 8851-2:2009* . Addis Ababa, Ethiopia: Quality and Standards Authority of Ethiopia (QSAE).

Ethiopian Standards (2009). Milk fat products - Specification. *ES 3465:2009* . Addis Ababa, Ethiopia: Quality and Standards Authority of Ethiopia (QSAE).

FAO/WHO (1997) *Draft Revised Standard for Milk Fat Products,* (A–2) 37–39, Food and Agriculture Organization of the United Nations, Rome.

FAO/WHO (2006) Codex Alimentarius standard for butter, A-1-1971, revised 1–1999, amended 2003 and 2006, Food and Agriculture Organization of the United Nations, Rome. http://www.codexalimentarius.net/web/standard list.jsp.

Fellows, P. (2008). *Butter and Ghee: Technical brief.* Rugby, Warwickshire: Practical Action, The Schumacher Center for Technology and Development.

Francesconi, G. N., Heerink, N., & D'Haese, M. (2010). Evolution and challenges of dairy supply chains: Evidence from supermarkets, industries and consumers in Ethiopia. *Food Policy* , 60–68.

Ganguli, N. C., & Jain, M. K. (1972). Ghee: Its Chemistry, Processing and Technology. *Journal of Dairy Science* , 19-25.

Gonfa, A., Foster, H. A., & Holzapfel, W. H. (2001). Field survey and literature review on traditional fermented milk products of Ethiopia. *International Journal of Food Microbiology* , 173 – 186.

Hamid, A. D. (1993) *The Indigenous Fermented Foods of the Sudan: A Study in African Food and Nutrition,* CAB International, Wallingford.

Illingworth, D., Patil, G. R., & Tamime, A. Y. (2009). Anhydrous Milk Fat Manufacture and Fractionation. In A. Y. Tamime, *Dairy Fats and Related Products* (pp. 108 - 157). Chichester: John Wiley & Sons Ltd.

ILRI. (2004). *Milk and Dairy Products, Post-Harvest Losses and Food Safety in sub-Saharan Africa and the Near East - regional approaches to national challenges.* FAO Action progamme for the pevention of food losses; International Livestock Research Institute.

Irz, D. X., Shankar, D. B., & C.S.Srinivasan, D. (2003). *Dietary Recommendations in the Report of a Joint WHO/FAO Expert Consultation on Diet, Nutrition and the Prevention of Chronic Diseases (WHO Technical Report Series 916, 2003): Potential Impact on Consumption, Production and Trade of Selected Food Products.* International Federation of Agricultural Producers and the Institute of European Food Studies.

Indian Standards (1975). Method for Sensory Evaluation of Ghee (Clarified Butterfat). *Indian Standard IS:7770-1975* . N.Delhi, India: Indian Standard Institution.

Jha, Y. (1988). Dairying Instructional - cum - practical manual. In D. A. Sacheti, *Milk and Milk Products* (pp. 99 - 105). New Delhi: National Council of Educational Research and Training.

Joyner, N. T., & Mclntyre, J. E. (1938). The Oven Test as an Index of Keeping Quality. *oil and soap* , 184 - 186.

Kilcast, D., & Subramaniam, P. (2000). *The stability and shelf-life of food.* Cambridge: Woodhead Publishing Limited.

Kumar, M., Sharma, V., Lal, D., Kumar, A., & Seth, R. (2010). A comparison of the physico-chemical properties of low-cholesterol ghee with standard ghee from cow and buffalo creams. *International Journal of Dairy Technology* , 252 - 255.

Labuza, T. P. (1984). Application of Chemical Kinetics to Deterioration of Foods. *Journal of Chemical Education* , 348-358.

Lazic´, Z. R. (2004). *Design of Experiments in Chemical Engineering: a practical guide.* Weinheim: WILEY-VCH Verlag GmbH & Co. KGaA.

Montgomery, D. C. (2001). *Design and analysis of experiments.* New York: John Wiley & Sons, Inc.

Mortensen, B. K. (2011). Anhydrous Milk Fat/Butter Oil and Ghee. In *Butter and Other Milk Fat Products* (pp. 515-521). Denmark: Elsevier Ltd.

O'Connor, C. B. (1995). Rural Dairy Technology. *ILRI Training Manual 1* . Addis Ababa, Ethiopia: International Livestock Research Institute.

O'Keefe, S. F., & Pike, O. A. (2010). Fat Characterization. In S. S. Nielsen, *Food Analysis* (pp. 241 - 260). New York: Springer Science+Business Media, LLC.

O'Mahony, F. (1988). ILCA Manual No. 4. *Rural dairy technology Experiences in Ethiopia* . Addis Ababa, Ethiopia: International Livestock Centre for Africa.

O'Mahony, F., & Bekele, E. (1985). *Traditional buttermaking in Ethiopia and possible improvements* . Addis Ababa, Ethiopia: International Livestock Center for Africa.Bulletin 22.

Pagote, C. N., & Bhandari, V. (1988). Antioxidant properties and nutritive value of Ghee. *Indian Dairyman* , 73 - 77.

Peters, M. S., & Timmerhaus, K. D. (1991). *Plant Desing and Economics for Chemical Engineers.* New York: McGraw-Hill, Inc.

Walstra, P., Wouters, J. T., Geurts, T. J. (2006). *Dairy Science and Technology, Second Edition.* New York: Taylor & Francis Group, LLC.

Ragnarsson, J. O., & Labuza, T. P. (1977). Accelerated Shelf-Life Testing for Oxidative Rancidity in Foods. *Food Chemistry* , 291 - 308.

Ranganna, S. (1986). Sensory Evaluation. In S. Ranganna, *Handbook of analysis and quality control for fruit and vegetable products* (pp. 594 - 645). New Delhi: Tata Mc Graw-Hill publishing.

Richardson, T., & Korycka-Dahl, M. (1988). Lipid Oxidation. In P. F. Fox, *Developments in Dairy Chemistry* (pp. 241 - 263). London: Applied Science Publishers Ltd.

Robinson, R. K. (2002). *Dairy Microbiology Handbook; The microbiology of milk and milk products.* New York: John Wiley & Sons.Inc.

Ranganna, S. (1986). Edible Oils and Fats. In S.Ranganna, *Handbook of analysis and quality control for fruit and vegetable products* (pp. 237 - 238). New Delhi: Tata Mc Graw-Hill Publishing.

Sadler, G. D., & Murphy, P. A. (2010). pH and Titratable Acidity. In S. S. Nielsen, *Food Analysis* (pp. 219 - 234). New York: Springer Science+Business Media, LLC.

Shahidi, F., & Zhong, Y. (2005). Lipid Oxidation: Measurement Methods. In F. Shahidi, *Bailey's Industrial Oil and Fat Products* (pp. 357-380). Newfoundland: John Wiley & Sons, Inc.

Singh, R. P., & Heldman, D. R. (2009). *Introduction to Food Engineering.* NEW YORK: Elsevier Inc.

Singh, S., Ram, P. B., & Mittal, k. S. (1979). Effect of phospholipids and method of manufacture on flavor and keeping quality of Ghee. *Indian Journal of Dairy Science* , 161-167.

Smit, G. (2003). *Dairy Processing: Improving Quality.* New York: CRC press.

Smith, J. S. (2010). Evaluation of Analytical Data. In S. S. Nielsen, *Food Analysis* (pp. 53 - 65). New York: Springer Science+Business Media, LLC.

Sripad, S., Kempanna, C., & Bhat, G. S. (1996). Effect of Alcohol extract of defatted Ghee residue on the shelf life of Ghee. *Indian Journal of Dairy and Biological Sciences* , 82 - 84.

Sserunjogi, M. L., Abrahamsen, R. K., & Narvhus, J. (1998). A Review Paper: Current Knowledge of Ghee and Related Products. *Int. Dairy Journal* , 677 - 688.

Stone, H., & Sidel, J. L. (2004). *Sensory Evaluation Practices.* California: Elsevier Academic Press.

Taoukis, P. S., Labuza, T. P., & Saguy, I. S. (1997). Kinetics of Food Deterioration and Shelf-Life Prediction. In E. Rotstein, R. P. Singh, & K. J. Valentas, *HandBook of Food Engineering Practice.* New York: CRC Press, LLC.

Taub, I. A., & Singh, R. P. (1998). *Food Storage Stability.* New York: CRC press.

Tsegaye, M., & Ashenafi, M. (2005). Fate of Escherichia coli O157:H7 during the processing and storage of Ergo and Ayib, traditional Ethiopian dairy products. *International Journal of Food Microbiology* , 11 – 21.

Unnikrishnan, V., & Rao, M. B. (1977). Copper and iron in Ghee and their influence on oxidative deterioration. *Journal of Food Science and Technology* , 167 - 169.

Urbach, G. & Gordon, M. H. (1994) Flavours derived from fats. *Fats in Food Products*, (eds. D. P. J. Moran & K. K. Rajah), pp. 347–405, Blackie Academic and Professional, London.

Wilbey, R. A. (1997). Estimating shelf-life. *International Journal of Dairy Technology* , 64-67.

Y.H.Hui, Goddik, L. M., Hansen, A. S., Josephsen, J., Nip, W.-K., Stanfield, P. S., et al. (2004). *Hand book of food and Beverage Fermentation Technology*. New York: Marcel Dekker, INC.

Zkanli, O. O., & Kaya, A. (2007). Storage stability of butter oils produced from sheep's non-pasteurized and pasteurized milk. *Food Chemistry* , 1026–1031.

Appendices

1. **Reagents Preparation/Standardization Procedures and Tables for Titratable Acidity Determination**

Phenolphthalein indicator solution

0.5036-gram phenolphthalein was dissolved in 50 ml 95% ethanol.

Standard sodium hydroxide solution

0.1 M standardized sodium hydroxide solutions were prepared according to the procedures outlined in AOAC 936.16, standard KHP method.(AOAC, 2000)

To standardize, first carbon dioxide free water was prepared by boiling distilled water for 15 – 20 minutes Erlenmeyer flask. After boiling the flask was stoppered with a rubber stopper and cooled to room temperature.

Dry 3 gram Potassium Acid Phthalate ($KHC_8H_4O_4$) was dried in an oven at 120 ^oC for 2 hours and cooled in a closed bottle inside a desiccator.

Sodium hydroxide solution was prepared by adding 50 ml H_2O to a flask containing 50 g NaOH (reagent quality containing <5% Na_2CO_3). The flask was tightly closed after completely dissolving the NaOH pellets, and set aside for 10 days. Taking 5.4 ml of the NaOH solution prepared 10 days earlier and mixing it with one liter CO_2 free distilled water prepared 0.1 M stock sodium hydroxide solution.

Finally, 0.8 gram of the dried $KHC_8H_4O_4$ was weighed accurately into 250 ml flask. 50 ml cool CO_2-free water was added and swirling gently dissolved the acid. Then the dissolved acid was titrated using the stock NaOH base solution as a titrant, and using glass-electrode pH meter as indicator. Titration was stopped when the pH reading nearly reached 8.6. The volume of NaOH required was recorded and used to calculate molarity using the following formula.

Equation 44

$$Molarity\left(\frac{mol}{L}\right) = \frac{grams\ of \mathrm{KHC_8H_4O_4}}{ml\ NaOH * 204.229} * 1000$$

Standardization was done in triplicate; results are summarized in Table 15 and Table 16

Table 15: Summary of Data on NaOH Standardization for Titratable Acidity Determination (NaOH standard solution Batch 1)

Standardization of sodium hydroxide solution (Batch 1)				
	Replicate 1	Replicate 2	Replicate 3	Blank
$KHC_8H_4O_4$ (g)	0.5434	0.6300	0.5380	0
Temperature (^oC)	25.9	26	27	
pH	9.07	8.54	9.23	9.2
NaOH (ml)	23.5	27.2	24	0.05
Molarity (mol/liter)	0.113464336	0.113619613	0.109991556	
Molarity	0.1135 ±	0.0035		

Table 16: Summary of Data on NaOH Standardization for Titratable Acidity Determination (NaOH standard solution Batch 2)

Standardization of sodium hydroxide solution (Batch 2)				
	Replicate 1	Replicate 2	Replicate 3	Blank
$KHC_8H_4O_4$ (g)	0.8001	0.8055	0.8031	0
Temperature (^oC)	26	26	26	26
pH	8.4	9.5	9.0	9.2
NaOH (ml)	33.25	34.0	33.9	0.05
Molarity (mol/liter)	0.1180	0.1162	0.1162	
Molarity	0.1171 ±	0.0018		

2. Reagents Preparation/Standardization Procedures and Tables for Peroxide Value Determination

Acetic acid–chloroform solution

One-liter acetic acid-chloroform solution was prepared by mixing 600 ml glacial acetic acid (CH_3COOH) with 400 ml chloroform ($CHCl_3$).

1% starch solution

0.55 gram soluble starch was added in 50 ml cold H_2O and boiled for 1 min while stirring.

Saturated Potassium iodide solution

100.01 gram Potassium iodide (KI) was dissolved in freshly boiled 60 ml H_2O. Solution was stored in dark.

Sodium thiosulfate standard solution

24.98-gram sodium thiosulfate ($Na_2S_2O_3$.$5H_2O$) was dissolved in one liter distilled H_2O and gently boiled for 5 minute. The solution was transferred, while hot, into clean heat-resistant storage bottle and stored in cool dark place.

The prepared solution was standardized according to procedures outlined in AOAC official method 942.27. (AOAC, 2000)

To standardize, first three gram potassium dichromate ($K_2Cr_2O_7$) was dried for two hours in an oven at 100°C. 2.5% potassium iodide solution (wt/vol), enough for three replicates, was prepared by dissolving 6.024 gram KI in 240 ml boiled and cooled distilled water.

One molar Hydrochloric acid solution was prepared according to procedure in AOAC 936.15.(AOAC, 2000)8.6 ml concentrated HCl (36.5% – 38%) pipetted in to 100 ml volumetric flask containing 20 ml distilled water, and then diluted to 100 ml using distilled water. The flask was closed tightly and labeled appropriately.

Then 0.20–0.23 gram of the dried $K_2Cr_2O_7$was weighed accurately into three 250 ml flasks each. 80 ml KI solution was added in to each flask followed by 20 ml 1M HCl. Then the flasks were placed in dark for 10 minutes and titrated using the $Na_2S_2O_3$ solution prepared earlier.0.5 ml 1% starch solution was added after most of I_2 has been consumed. Titration was stopped when the color changed from dark blue to faint blue color. The amount of sodium thiosulfate solution needed was recorded and its molarity was calculated using the formula below.

Equation 45

$$Molarity\left({}^{mol}/_{L}\right) = \frac{grams\ K_2Cr_2O_7 * 1000}{ml\ Na_2S_2O_3 * 49.032}$$

Standardization results are summarized in Table 17 and Table 18

Since addition of only 0.1 ml 0.1 M $Na_2S_2O_3$caused color change, it was necessary to dilute the standardized 0.1 M $Na_2S_2O_3$to 0.001 M. Thus, 10 ml 0.1 M $Na_2S_2O_3$solution was diluted using freshly boiled and cooled water to one liter.

Table 17: Summary of Data on Na2S2O3 Standardization for Peroxide Value Determination (Shelf stability analysis week 1)

Standardization of sodium thiosulfate solution (week 1)			
	Replicate 1	Replicate 2	Replicate 3
$K_2Cr_2O_7$ (g)	0.2032	0.2176	0.2171
$Na_2S_2O_3$ (ml)	41.4000	43.5000	43.2000
Molarity (mol/liter)	0.1001	0.1020	0.1025
Molarity	0.1015	±	0.0021

Table 18: Summary of Data on Na2S2O3 Standardization for Peroxide Value Determination (Shelf stability analysis week 2)

Standardization of sodium thiosulfate solution (week 2)		
	Replicate 1	Replicate 2
$K_2Cr_2O_7$ (g)	0.2030	0.2278
$Na_2S_2O_3$ (ml)	47.5000	46.9000
Molarity (mol/liter)	0.0872	0.0991
Molarity	0.0931	±0.0142

3. Reagents Preparation/Standardization Procedures and Tables for Free Fatty Acids Determination

Standard sodium hydroxide solution

Standard sodium hydroxide solution was prepared according to AOAC 936.16, standard potassium hydrogen phthalate method as described above in titratable acidity determination. Standardization results are summarized in

Table 19 and Table 20.

1% Phenolphthalein indicator solution

0.57-gram phenolphthalein was dissolved in 50 ml 95% ethanol and stored in tightly closed volumetric flask.

Neutralized ethanol

100 ml 95% ethanol was neutralized by adding 2 ml phenolphthalein solution and enough 0.1M NaOH to produce faint permanent pink color.

Table 19: Summary of Data on NaOH Standardization for Free Fatty Acid Value Determination (Shelf stability analysis week 1)

Standardization of sodium hydroxide solution (week 1)						
	Replicate 1	Blank 1	Replicate 2	Blank 2	Replicate 3	Blank 3
$KHC_8H_4O_4$ (g)	0.7987	0.0000	0.8376	0.0000	0.8185	0.0000
Temperature (^oC)	26.0000	26.0000	26.0000	26.0000	26.0000	26.0000
pH	8.5000	9.1000	8.4500	8.9000	8.7700	8.7000
NaOH (ml)	37.9000	0.0500	40.0000	0.0500	38.9000	0.0500
Molarity (mol/L)	0.1033		0.1027		0.1032	
Molarity	0.1030	±	0.0006			

Table 20: Summary of Data on NaOH Standardization for Free Fatty Acid Value Determination (Shelf stability analysis week 2)

Standardization of sodium hydroxide solution (week 2)						
	Replicate 1	Blank 1	Replicate 2	Blank 2	Replicate 3	Blank 3
$KHC_8H_4O_4$ (g)	0.4331	0.0000	0.4342	0.0000	0.4227	0.0000
Temperature (^oC)	25.0000	25.0000	25.0000	25.0000	25.0000	25.0000
pH	8.9800	8.8800	8.6900	9.0100	9.1200	8.7700
NaOH (ml)	20.4000	0.0500	20.3000	0.0500	19.7000	0.0500
Molarity (mol/L)	0.1042		0.1050		0.1053	
Molarity	0.1046	±	0.0010			

4. **Ghee Production Chart**[Source: (Jha, 1988, pp. 103-104)]

Particular of cream/butter

- Type _____
- Quantity (Kg) _____
- Fat % _____
- Acidity % (as lactic acid) _____
- Amount of fat (Kg) _____
- Particulars _____

Heating

- Temperature of cream/butter (^{o}C) _____
- Source of Heating _____
- Container _____
- Time heating started (Hrs, min) _____
- Temperature and time foaming subsided _____
- Time heating finished (Hrs, min) _____
- Highest temperature reached (^{o}C) _____

Straining

- Type of strainer _____
- Temperature of Ghee at the time of straining (^{o}C) _____
- Quantity of Ghee residue obtained (Kg) _____
- Color of residue _____
- Fat in Ghee residue (%) _____

Ghee

- Quantity of Ghee (Kg) _____
- Color of Ghee _____
- Acidity (%) _____
- Moisture content of Ghee (%) _____

Package used

- Type _____
- Size _____

Storage temperature (^{o}C)

- _____

5. Sensory Evaluation Card for Ghee[Source:(IS, 1975, p. 7)]

Name …… Date……

Code No…… Time ……

Characteristics	Maximum Score	Sample score
1. Color	10	……..
2. Odor	30	……..
3. Flavor	60	……..

I prefer this one ☐

Name ……. Date ……

Code No…… Time ……

Characteristics	Maximum Score	Sample score
1. Color	10	……..
2. Odor	30	……..
3. Flavor	60	……..

I prefer this one ☐

6. Ghee Products Peroxide Value and Free Fatty Acid Value Change During Storage

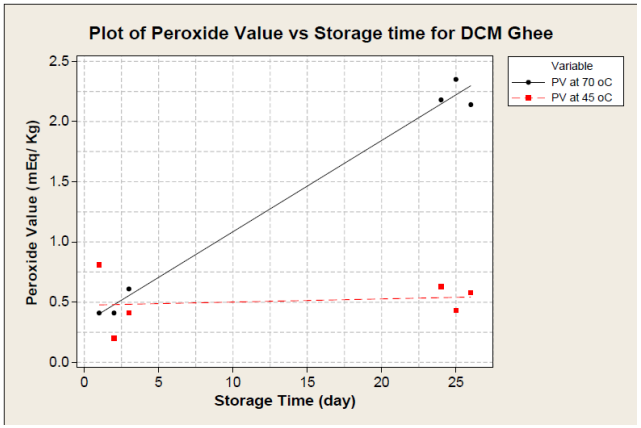


Figure 6: PV changes of Ghee produced by Direct Cream Method at 45 and 70 ^{o}C

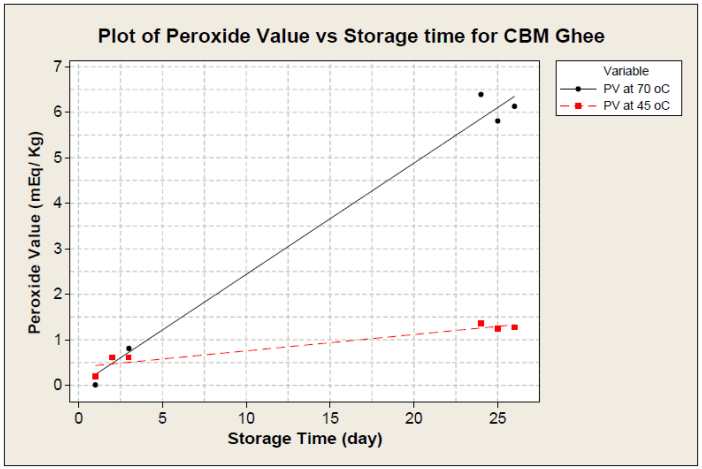


Figure 7: PV changes of Ghee produced by Creamery Butter Method at 45 and 70 ^{o}C

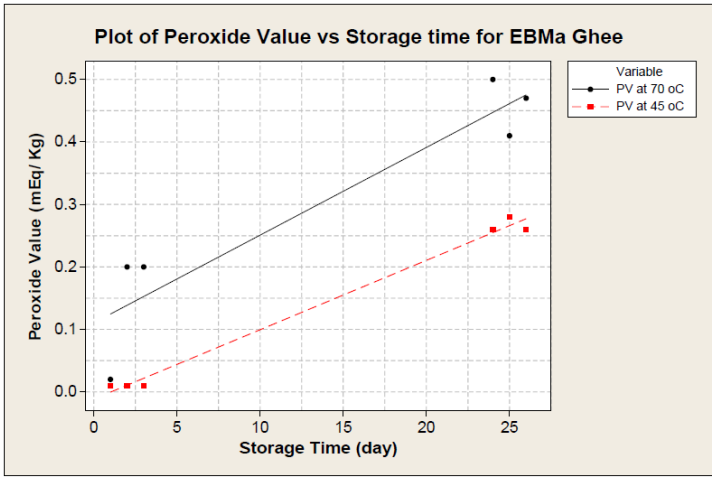


Figure 8: PV changes of Ghee produced by Ethiopian Butter Method with additives at 45 and 70 ^{o}C

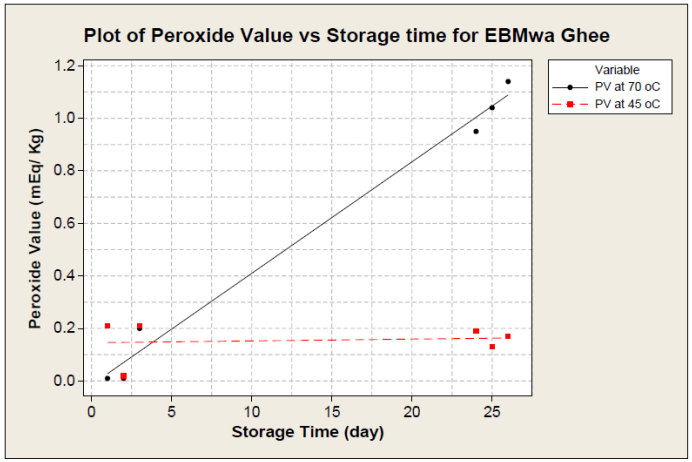


Figure 9: PV changes of Ghee produced by Ethiopian Butter Method without additives at 45 and 70 ^{o}C

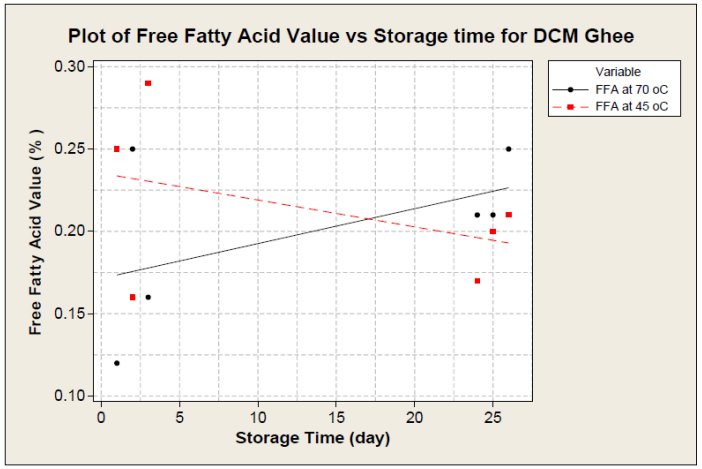


Figure 10: FFA_v changes of Ghee produced by Direct Cream Method at 45 and 70 ^oC

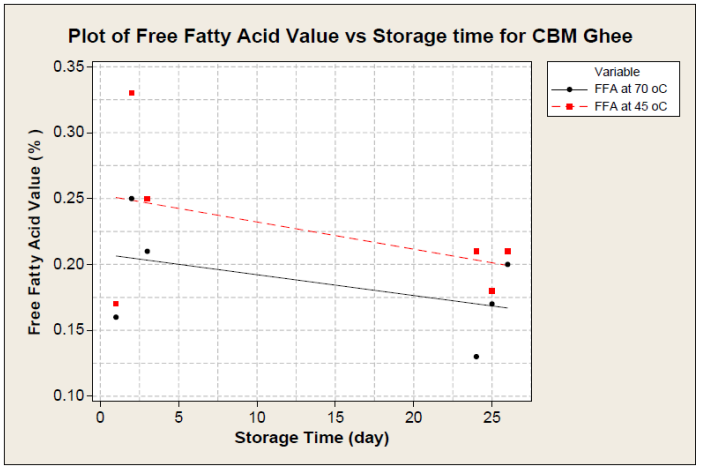


Figure 11: FFA_v changes of Ghee produced by Creamery Butter Method at 45 and 70 ^oC

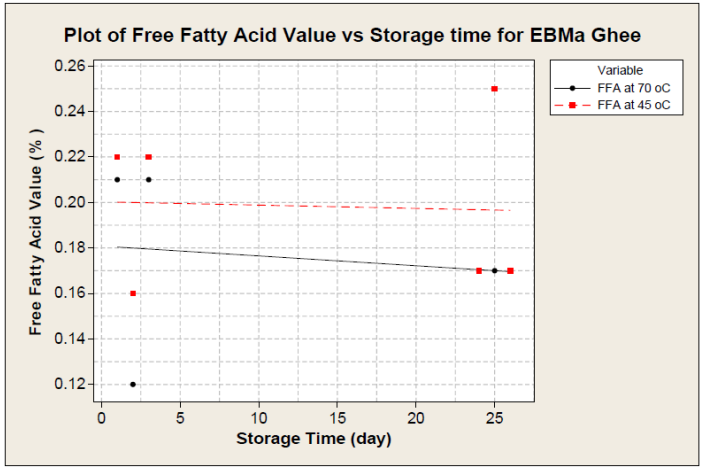


Figure 12: FFA_v changes of Ghee produced by Ethiopian Butter Method with additives at 45 and 70 ^oC

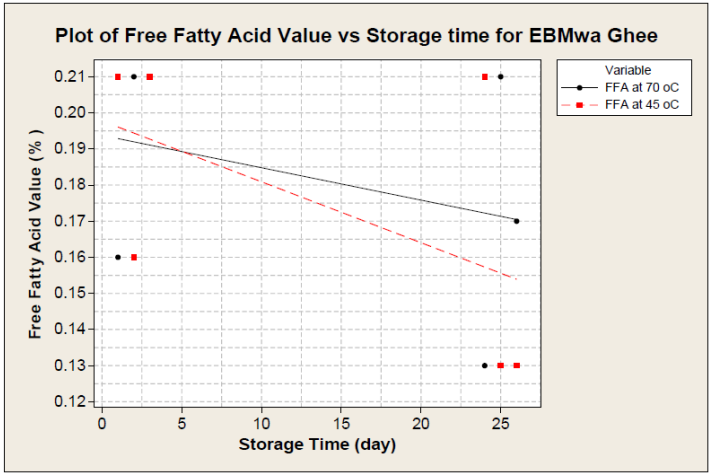


Figure 13: FFA_v changes of Ghee produced by Ethiopian Butter Method without additives at 45 and 70 ^oC

Declaration

I, the undersigned, declare that this thesis is my original work, has not been presented for a degree in any other University, and that all sources of materials used for the thesis have been duly acknowledged.

Name: Simon Hailu Habtegebriel.

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Place: Addis Ababa

Date of submission: _____

Advisor

Professor Yogesh Kumar Jha

Signature: _____

Date: _____