EFFECT OF STORAGE TEMPERATURE, QUALITY OF CREAM AND HANDING PRACTICES ON SHELF LIFE OF BUTTER

By

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DECLARATION

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Affectionately Dedicated

to My Parents

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ABSTRACT

It has been complained by regular customers and retailers that large proportions of salted butter samples get deteriorated due to rancidity and microbial growth rather before its expected shelf life under domestic refrigeration conditions. which in turn greater extent of returns becoming inevitable and continuously faces to the problem profit loss. Therefore the objective of this research was to determine the root cause that leads to deprive the storage stability of "Highland" salted-butter in order to the shelf life under domestic refrigeration conditions.

First approach was to study the butter manufacturing process. Foremost, principle raw materials, equipments and machineries that are used in butter manufacturing were identified. Thereafter actual processing flow diagram along with workers traffic pattern was out lined to identify the most probable steps that may lead to cross contaminations. The quality of incoming cream is very important for shelf life of butter. Quality of incoming cream was evaluated using two quality parameters. The physico-chemical quality was evaluated by analyzing the titrable acidity, where as the microbiological quality was evaluated by performing total plate count. Another important factor is storage temperature of butter. It was evaluated using acidity level at two temperatures (10°C and -10°C). Free fatty acid level was identified using titration method, and continued for ten weeks. Finally, the results were statistically analyzed. Handling practices are very important in butter manufacturing. Handling practices was observed and identify bad manufacturing practices.

Acidity test (P value 0.009) indicates that there is a day to day variation in percentage acidity and thus, assuring the uniformity of end product is somewhat questionable. However, average acidity of the each cream sample is far less compared to the minimum allowable acidity i.e., 0.14%. The maximum CFU/ml shall be 50 for a cream if it is utilized for butter manufacturing. The two cream samples conform to the specifications where as one cream sample is somewhat inferior in microbiological quality. The average shelf-life of butter was determined using regression equation at these two temperatures. Sample of butter stored in butter cold room had a shelf-life of 46 weeks. On other hand, samples of butter stored in normal refrigerated condition had a shelf-life of 24 weeks. According to these results, storage temperature directly affects to shelf life of butter.

CONTENTS

CHAPTER 03: MATERIALS AND METHODOLOGY

CHAPTER 04: RESULTS AND DISCUSSION

CHAPTER 05: CONCLUSION AND RECOMMENDATION

LIST OF FIGURES

LIST OF TABLES

LIST OF ABREVIATIONS

Min	Minute
USDA	United State Department of Agrculture
AR	Analytical Reagent
LDL	Low Density Lipoprotein
EU	Europe Union
GMP	Good Manufacturing Practices
SFC	Solid Fat Content
Wt	Weight
TA	Titrable Acidity
hrs	Hours

CHAPTER 01

INTRODUCTION

1.1. Background:

Milk Industry of Lanka Company Ltd. (MILCO) is a leading dairy company in Sri Lanka, engaged in the process of manufacturing range of milk based products including butter, milk powder, condensed milk, sterilized milk, pasteurized milk, cheese, set yoghurt, drinking yoghurt, mangodelight, ghee etc. and marketed under the brand name "Highland".

Butter is essentially the fat of the milk. It is usually made from sweet cream and is salted. However, it can also be made from acidulated or bacteriologically soured cream and saltless (sweet) butters are also available. Butter is a water-in-oil emulsion, comprised of >80% milkfat, but also containing water in the form of tiny droplets, perhaps some milk solids-not-fat, with or without salt (sweet butter); texture is a result of working/kneading during processing at appropriate temperatures, to establish fat crystalline network that results in desired smoothness (compare butter with melted and recrystallized butter); used as a spread, a cooking fat, or a baking ingredient. The principal constituents of a normal salted butter are fat (80 - 82%), water (15.6 - 17.6%), salt (about 1.2%) as well as protein, calcium and phosphorous (about 1.2%). Butter also contains fat-soluble vitamins A, D and E. (Guelf University, 2007).

MILCO usually produces two types of butter, i.e., salted butter and non-salted butter and wrapped in an aluminium foil expecting a one year and nine month shelf life respectively within the cold chain managed up to ultimate consumers. It has been complained by regular customers and retailers that large proportions of salted butter samples get deteriorated due to rancidity and microbial growth rather before its expected shelf lives even under domestic refrigeration conditions, which in turn greater extent of returns becoming inevitable and continuously faces to the problem profit loss. Thus in present scenario MILCO is in the greatest challenge of improving the shelf life and struggling to finding out the detrimental causes that are leading to deprive the storage stability of butter.

It is believed that quality of incoming cream, handling practices and end product storage temperature are primary factors that directly affected to the shelf life of butter (Early,1998). In addition to accumulated practical experience, a good deal of science has now been incorporated in butter making, enhancing the shelf life and the quality of the product and the economy of manufacture. (Walstra *et al.*,1999). Therefore a detail investigation on these aspects is of utmost significance.

1.2. Overall Objective:

- To determine the root causes those deprive the storage stability of salted-butter with a view to extending the shelf life under domestic refrigeration conditions.

1.3. Specific Objectives:

- Studying the butter manufacturing process in order to identify the workers miss-handling practices that may lead to cross contaminations.
- Assessing the quality of incoming cream which is used for butter manufacture.
- Studying the effect of end product storage temperature for shelf life of butter.
- Establishing the specifications for quality of incoming cream and optimum temperature range for butter storage in market.

CHAPTER 02

LITERATURE REVIEW

2.1. Butter: An Overview

Butter is generally made from cream by means of churning and working. It contains somewhat over 80% fat, which is partly crystallized. The churning proceeds most easily with sour cream, at a temperature of around 15-20°C. Therefore, butter typically is a product originating from regions of temperature climate. In addition to accumulated practical experience, a good deal of science has now been incorporated in butter making, enhancing the shelf life and the quality of the product and the economy of manufacture. (Walstra *et al.*,1999).

2.1.1. Definition

Butter is a water-in-oil emulsion, comprised of >80% milkfat, but also containing water in the form of tiny droplets, perhaps some milk solids-not-fat, with or without salt (sweet butter); texture is a result of working/kneading during processing at appropriate temperatures, to establish fat crystalline network that results in desired smoothness (compare butter with melted and recrystallized butter); used as a spread, a cooking fat, or a baking ingredient.

The principal constituents of a normal salted butter are fat (80 - 82%), water (15.6 - 17.6%), salt (about 1.2%) as well as protein, calcium and phosphorous (about 1.2%). Butter also contains fat-soluble vitamins A, D and E.

Butter should have a uniform colour, be dense and taste clean. The water content should be dispersed in fine droplets so that the butter looks dry. The consistency should be smooth so that the butter is easy to spread and melts readily on the tongue (Goulf university, 2007).

2.1.2. History and Origin of Butter Making

Since even accidental agitation can turn cream into butter, it is likely that the invention of butter goes back to the earliest days of dairying, perhaps in the Mesopotamian area between 9000 and 8000 BCE. The earliest butter would have been from sheep or goat's milk; cattle are not thought to have been domesticated for another thousand years or so. An ancient method of butter making, still used today in some parts of Africa and the Near East, is taken in Palestine. A goat skin is half filled with milk, then inflated with air and sealed. It is then hung with ropes on a tripod of sticks and rocked to and fro until the butter is formed.

Butter was certainly known in the classical Mediterranean civilizations, but it does not seem to have been a common food, especially in Ancient Greece or Rome. In the warm Mediterranean climate, unclarified butter would spoil very quickly - unlike cheese, it was not a practical method of preserving the benefits of milk. The people of ancient Greece and Rome seemed to consider butter a food fit more for the northern barbarians (Andrew Dalby,2005).

2.1.3. Production and Consumption

Butter production in selected countries for 1997 is estimated at 5.3 million tons, up from both the January forecast and 1996. Prospects for butter trade have risen sharply since the January report with increases expected for both Oceania and the EU. Most of the increased exports appear to be destined for Russia and other former members of the Soviet Union. As mentioned, although imports appear to have been above expectations, the estimates for Russia were not revised at this time. The United State is large producer of butter from cow's milk, accounting for 12% of total world production. New Zealand and Germany follow close behind (USDA,Agricultural marketing service,1997).

2.1.4. Health and Nutrition

According to united state department of agriculture figures, one tablespoon of butter (14 grams) contains 100 calories, all from fat, 11 grams of fat, of which 7 grams are saturated fat, and 30 milligrams of cholesterol. In other words, butter consists mostly of saturated fat and is a significant source of dietary cholesterol. For these reasons, butter has been generally considered to be a contributor to health problems, especially heart disease. For many years, vegetable margarine was recommended as a substitute, since it is an unsaturated fat and contains little or no cholesterol. In recent decades, though, it has become accepted that the trans fats contained in partially hydrogenated oils used in typical margarines significantly raise "bad" LDL cholesterol levels as well. Trans-fat free margarines have since been developed.

Small amounts of butter contain only traces of lactose, so moderate consumption of butter is not generally a problem for those with lactose intolerance. People with milk allergies do need to avoid butter, which does contain enough of the allergy-causing proteins to cause reactions (Butter wikipidia,2007).

2.1.5. Classification of Butter Varieties

Butter is usually divided into two main categories: sweet cream butter and cultured or sour cream. Sour cream butter made from bacteriologically soured cream. Butter can also be classified according to salt content: unsalted, salted and extra salted (Tetra Pak Processing Systems AB,1995).

2.1.5.1. Salted butter

From the weight of the cream placed in the churn, it is possible to calculate how much salt must be added. It should be recommended that the salt is dissolve in the water portion of the butter and that a salt concentration of 2 per cent in the butter (Herrington,2000).

2.1.5.2. Unsalted Butter

All butter is not salted is usually called "sweet butter." (Herrington,2000).

2.1.5.3. Whey Butter

Whey which is produced by-product of cheese-making contains varying proportion of butter-fat, and the separation of this fat repays the trouble and expense incurred in obtaining it from the liquid (Harvey and Hill,1999).

2.1.5.4. Dehydrated Butter

The moisture content is being reduced to between 1 and 2 per cent. The fat then passes to a dehydrator where all further moisture is removed. It is called dehydrated butter (Harvey and Hill,1999).

2.1.5.5. Milk-blended Butter

It is manufactured from imported butter in to which an additional quantity of milk has been worked. Milk-blended butter must not contain more than 24 per cent of water (Harvey and Hill,1999).

2.1.5.6. Renovated Butter

The fat is melted, the cured removed by sedimentation, and air is blown through the liquid to disperse any offensive odours which may be present. The whole is then emulsified by churning with milk and is subsequently worked and washed, as in ordinary butter manufacture (Harvey and Hill,1999).

2.2 Milk Fat

Fat, one of the major constituents of milk, contributed to the physical properties of dairy products. The functional properties of milk fat are strongly related to its composition and to the amount and the type of crystals formed at the temperature of the application (Lopez *et al.*, 1995). Milk fat Gives desired composition, texture and meltability characteristics, hence butter, plastic (80% fat) cream. Cream is a colloidal dispersion that displays non-Newtonian behavior (inverse relationship between apparent viscosity and shear rate, or hysteresis) at high fat contents and/or storage temperatures below 40°C (Fox and McSweeney, 1998). An

increase in fat level has been found to increase apparent viscosity of cream while increasing temperatures result in a decrease in cream viscosity (Prentice *et al.*, 1999).

Milk and cream are examples of fat-in-water (or oil-in-water) emulsions. The milk fat exists as small globules or droplets dispersed in the milk serum. Their diameters range from 0.1 to 20 mm (1 mm = 0.001 mm). The average size is 3 - 4 mm and there are some 15 billion globules per ml. The emulsion is stabilized by a very thin membrane only 5 - 10 nm thick (1 nm = 10-9 m) which surrounds the globules and has a complicated composition.

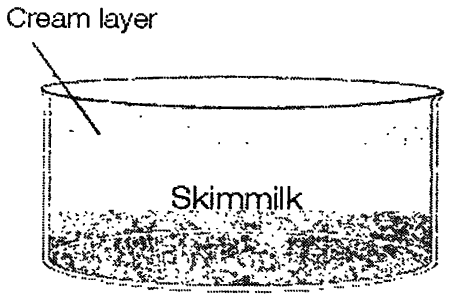


Fig. 2.1. Emulsion of fat and water

Milk fat consists of triglycerides (the dominating components), di- and monoglycerides, fatty acids, sterols, carotenoids (the yellow colour of the fat), vitamins (A, D, E, and K), and all the others, trace elements, are minor component. The spectrum of fatty acids present in milk is shown in table 2.1, 2.2.

If milk is left to stand for a while in a vessel, the fat will rise and form a layer of cream on the surface. The membrane consists of phospholipids, lipoproteins, cerebrosides, proteins, nucleic acids, enzymes, trace elements (metals) and bound water (Tetra Pak Processing Systems AB,1995).

Table 2.1. Principal saturated fatty acids in milk fat

Fatty acids	**%of total fatty acid content**
Butyric acid	3.0-4.5
Caproic acid	1.3-2.2
Caprylic acid	0.8-2.5
Capric acid	1.8-3.8
Lauric acid	2.0-5.0
Myristic acid	7.0-11.0
Palmitic acid	25.0-29.0
Stearic acid	7.0-3.0

Table 2.2. Principal unsaturated fatty acids in milk fat

Fatty acid (unsaturated)	%of total fatty acid content
Oleic acid	30.0-40.0
Linoleic acid	2.0-3.0
Linolenic acid	up to 1.0
Arachidonic acid	up to 1.0

As the fat globules are not only the largest particles in the milk but also the lightest (density at 15.5°C = 0.93 g/cm3), they tend to rise to the surface when milk is left to stand in a vessel for a while. The rate of rise follows Stokes' Law, but the small size of the fat globules makes creaming a slow process. Cream separation can however be accelerated by aggregation of fat globules under the influence of a protein called agglutinin. These aggregates rise much faster than individual fat globules. The aggregates are easily broken up by heating or mechanical treatment. Agglutinin is denaturated at time-temperature combinations such as 65°C/10 min or 75°C/2 min (Tetra Pak Processing Systems,1995).

Fat globule membrane protects the fat inside the globule from chemical and lipolytic action and extends protection against mechanical damage during handling. Fat globule membrane is hydrophilic. It is maintain fat and water emulsified. Fat globule consists of two basic layers. Inner core of fat surrounded by emulsified system with hydrophobic tail linked to the fat. Hydrophilic head attached to the surrounding aqueous layer (Early,1998).

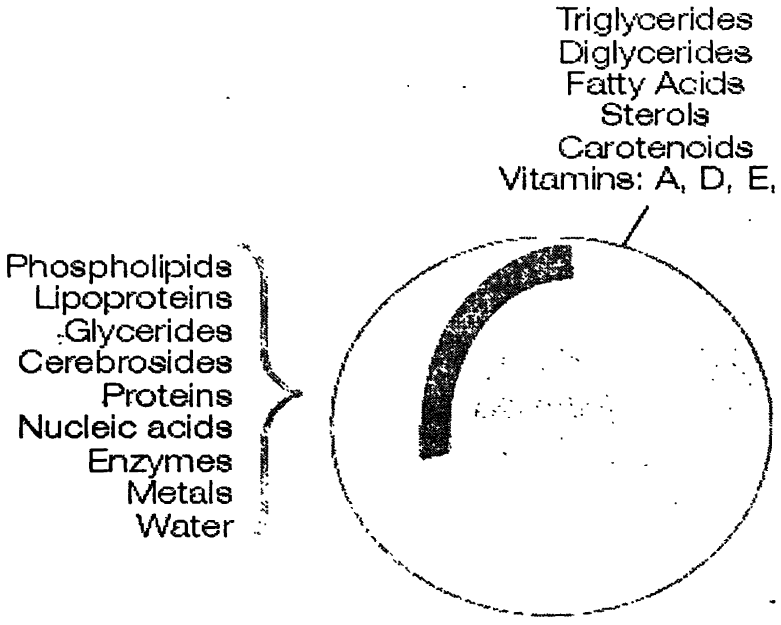


Fig. 2.2. Structure of fat globule

2.2.1. Free fatty acids

These already occur in fresh milk and lipolysis increases their amount. The shorter acids are somewhat soluble water. Fatty acids can, of course, dissociate into; their pK is about 4.8. In milk plasma, there are thus predominantly in the ionized form (i.e. as soaps), and these are much more soluble in pure water than the pure fatty acid are. Fatty acids dissolve well in oil, though only in the nonionized form; moreover, they tend to associate into dimmers. Obviously, the partition of the acids over the oil and water fractions is rather intricate. All in all, it means that the shorter acids (C4 and C6) are predominantly in the plasma, the longer ones (from C14) in the fat. The other acids are distributed between both fractions, though more go into the fat with decreasing pH (i.e., with ionization becoming weaker). This is all of much importance because it is the shorter acids that are responsible for the soapy-rancid flavor perceived after lipolysis. All this becomes even more complicated since the fatty acids, especially the long-chain ones, are surface-active and tend to accumulate in the oil-water interface. (Walstra *et al.*,1999).

The free fatty acid test measures the free fatty acid in butter and is essentially a titration of extracted fat with a weak alkali. Test result can be expressed in number of ways, including 'as % oleic acid' or as the volume or moles of titrant consumed. As discussed previously, free fatty acid level can rise during storage for several reasons, and high levels are not always associated with development of off-flavours (Deeth et al.,1979). However, the free fatty acid levels in good quality butter which has been packed and stored appropriately should remain close to the IDF specification of 0.3% as oleic acid (R.Early,1998).

2.2.2. The important of the milk fat

The fats are of great commercial importance. The chemistry of fats is of great interest to the food manufacturer. The fat deteriorates in storage, and a very slight degree of deterioration can cause serious changes in odor and flavor. The deterioration of is the chief cause of spoilage of those food which are sold in the relatively dry state. Such defect as rancidity in butter is al due to change in the fat present in those products. The study of these changes in fat and the discovery of methods for their control are of great importance to both the manufacturer and the consumer of many food products.

The fats are of particular importance in the dairy industry because the wholesale price of milk is based upon its fat content. This exaggerated idea of the importance of fat in determining the value of milk sold by farmers was used to make butter. (B.L.Herrington,2000).

2.3 Cream

Cream is the solid in many varieties. The fat content may range from 10% ("half-and half") to 48% ("double cream"). Although it may be used for several purposes, mostly it is something of a luxury and therefore an excellent flavour is paramount. Because of the high fat content, any off-flavour of the fat becomes concentrated. For instance, a milk with a fat acidity of 1 mmol per 100g fat will not be perceived to have a soapy-rancid flavor by most people, but a whipping cream made from it will definitely taste rancid. Hence, the milk should be impeccable with regard to lipolysis and fat oxidation.

Sometimes anhydrous milk fat is used in cream products and recombination is applied. This enhances the danger of an oxidation flavour and also, if impeccable in this respect, the taste of the product may be different (somewhat less rich) because of the absence of components from the milk fat globule membrane. One may improve on this by using a limited quantity of good-quality (dried) sweet cream butter milk. Here we will cover several products, chosen to illustrate most of the important technological and quality aspects of cream (Walstra *et al.*,1999).

2.3.1 Defects in the cream

Cream, in common with other foodstuffs, may be subject to certain defects or faults due to unsatisfactory processing or handling. The purchaser requires a clean-flavoured product which possesses god keeping qualities, has a natural colour and sufficient viscosity to appear reasonably rich. If the cream is to be whipped, it should whip easily and procedure a finished article of suitable stability. The principal defects are founded (W.M.C.Harvey and H.Hill,1999).

2.3.1.1 Poor keeping quality

This is extremely important, particularly if cream is to be stored, unrefrigarated, in the home for any length of time. Extended ageing of cream will injure its keeping quality, while other factors which affect this property are high bacterial content of the milk from which the cream is produced, and the use of improperly sterilized utensils, apparatus, and containers (W.M.C.Harvey and H.Hill,1999).

2.3.1.2 Poor flavour

Obviously, flavour depends upon the quality of the initial milk supply, and a clean, wholesome flavour is possible only when fresh milk of good quality is separated. Proper processing is essential so that the flavour is essential so that the flavour is not injured, and

cream should always be retailed in fresh condition. Ageing for lengthy periods in order to increase viscosity, be avoided. Bitter or rancid flavours may develop due to non-spore-forming bacteria of soil and water origin, causing spoilage at low temperatures. These obtain entrance to the milk supply from bedding, utensils, or water. Mould growth may also occur and cause deterioration of flavour. Tallowy flavours are due to the oxidation of the fat content by exposure to strong sunlight or on account of contamination by contact with unsuitable metal surfaces during processing. Rancid or bitter taints may arise due to the action of lipase, the fat-splitting enzyme. This enzyme may be secreted in the milk of a few cows only, but if mixed with the bulk supply, will taint the whole quantity (W.M.C.Harvey and H.Hill,1999).

2.3.1.3 Cream plug formation

This is caused by excessive agitation of the cream in partially-filled tanks or cans, or by high-speed pumps. Cream should be cooled quickly and excessive heating must always be avoided (Harvey and Hill,1999).

2.3.1.4 Poor Colour

This depends upon the solids-not-fat content and the size of the fat globules. Partially-churned cream does not possess the colour of cream with the natural emulsion. Homogenization will increase the colour as will building up the solids-not-fat content, but this later practice may affect the flavour (Harvey and Hill,1999).

2.3.1.5 "Thin" Body

Too much handling, pumping, or agitation, separation at a high temperature or separating warm milk, and ultra–rapid cooling are the causes of this defect. It is important that the natural clumping of the fat globules should be encouraged by the proper handling of the milk before separation, gentile handling of cream, and slow cooling below 55°F (Harvey and Hill,1999).

2.3.1.6 Serum separation

The factors responsible for this defect are excessive pumping causing partial churning of cream, high-temperature separation, and low fat content. This defect, which is evidenced by fat rising and serum separation, does not occur if the viscosity is sufficient to retain the fat globules in position throughout the serum. Serum separation may be prevented by a high pasteurization temperature, a low separation and gentle agitation (Harvey and Hill,1999).

2.3.1.7 "Oiling off"

Causative factors are the freezing of the cream during cooling, separating excessively hot or cold milk, pasteurization of very rich cream and the employment of very high temperatures, excessive agitation of warm cream prior to pasteurization, high-speed pumping by centrifugal pumps, slow cooling in pasteurizer and using an excessively hot heating medium. Homogenization at a pressure of 300 lb. per square inch will prevent this defect (Harvey and Hill,1999).

2.3.1.8 Feathery cream

This type of cream is highly acid, possesses calcium or magnesium salts in excess, an abnormal mineral content or has been homogenized at excessive pressures, and will feather when added to coffee (Harvey and Hill,1999).

2.3.1.9 Poor whipping qualities

This is a defect sometimes found in whipping cream and is due temperature and the type of whipping apparatus employed. Excessive ageing will cause this defect as will partial freezing and homogenization. Sugar, which is sometimes added, should be mixed with the cream towards the end of this process. If added too early, the whipping ability will be lowered (Harvey and Hill,1999).

2.4. Quality characteristics of butter

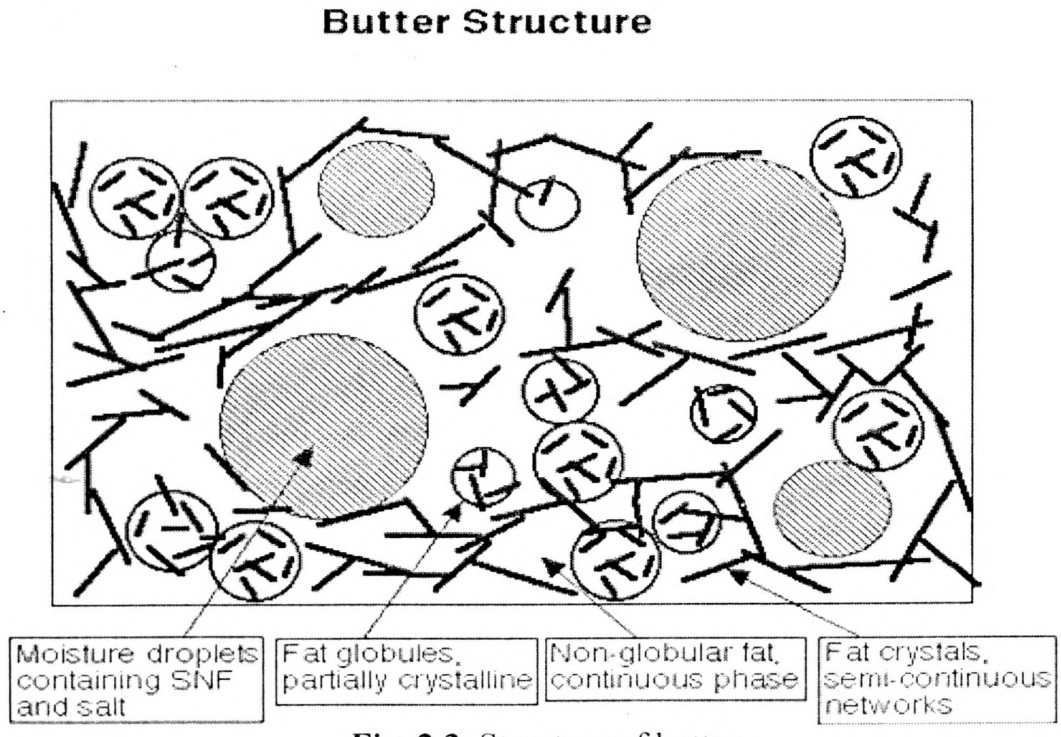


Fig. 2.3. Structure of butter

The size and extent of crystal networks both within the globules and within the non-globular phases is controlled to a large extent by milkfat's variable composition and by the aging process. The extent of globular versus non-globular fat is controlled to a large extent also by the amount of physical working applied to the butter post-churning (Goulf University, 1997).

2.4.1 Colour and Appearance

The natural golden or creamy yellow colour of butter arising from the presence of beta-carotene (which also has vitamin A activity) and its smooth, slightly matt surface appearance combine to give and image of richness so that, despite it is high fat content, butter does not seem to be overtly greasy and unpleasant. In contrast, margarines tend to be shinier and appear more greasy.

These features result from difference in the crystalline fat, variation in the size of the moisture droplets, and the way in which these droplets cause light to be absorbed and reflected at the product surface. In most butters, the fat crystals partially retain the spherical shape of the cream fat globules, while some globules survive intact, and most moisture droplets are of the size 10-15μm diameter. Smaller moisture droplets, at the same carotene level, lead to a paler product. In margarines, the desired effect is achieved by the addition of beta-caroten or other permitted yellow colours (Early,1998).

2.4.2 Flavour

The flavour of butter, either in the so-called sweet cream product or in the sour cream (lactic) variant, has been well accepted. The chemical compounds responsible are derived from:

- The milk fat itself, in the form of short chain fatty acids or lactones, ketones, and other compounds which develop during heating or mild oxidation;
- The phospholipids of the milk fat globule membrane;
- The action of bacterial cultures on the cream producing, e.g. lactic acid and diacetyl.

Butter flavour depends on the correct levels and balance of the many chemical compounds involved. For example, the process of lipolysis and oxidation, which give rice to some of the desirable flavour components will, if excessive, lead to rancidity and off-flavours.

Interestingly, butter flavour differs from its pure butterfat itself, again highlighting the importance of the emulsion structure and aqueous phase components. Moreover, during heating higher levels of lactones are formed from certain precursor fatty acids in milkfat and

readily imitated by the addition of lactones to yellow fats containing no milk fat, as the high addition rates required to give a buttery flavour for baking usually cause the products to be unpalatable when used as a bread spread (Early,1998).

2.4.3 Texture and mouthfeel

The physical properties of milkfat, derived from its unique fatty acid and triglyceride composition, combined with the properties arising from the microstructure of the fat and aqueous phases, serve to enhance the flavour by the way in which butter melts in the mouth.

Although butter and the other yellow fats appear solid, the fat is actually present as complex matrix of crystalline (solid) and liquid fat, and even a relatively hard product like butter only has 50-60% solid fat at 5°C. solid fat content (SFC) can easily be measured by pulsed nuclear magnetic resonance (NMR) techniques, after carefully tempering samples of extracted fat, and valuable information on the properties of yellow fats can be obtained from the melting profile (SFC vs. temperature).

Cows diet is largely fresh grass, the SFC values are lower, through the shapes of curve is similar. So at anytime milkfat contains relatively high levels of solid fat at temperatures bellow about 12°C. Above this temperature these levels fall sharply and by body temperature (37°C) they have reached zero. The heat required to cause this melting is drawn from the mouth and gives rise to a pleasant cooling sensation, while releasing flavour volatiles. This is the beneficial aspect of a steep melting profile, and this, combined with the way in which the w/o emulsion readily inverts in the mouth to the oil in water (w/o) from gives a balanced release of flavour and does not feel greasy or mouthcoating as some spreads can. The inversion to the o/w emulsion is aided by the presence of phospholipids (derived from the natural membranes around the fat globules).To obtain good plasticity there must be a suitable ratio of solid to liquid fat, with many small fat crystals which 'bind' in the liquid oil, and yet give a structure which will deform under shear. If this is not the case, faults such as brittleness or 'oiling-off' are likely. If fat crystals larger than 20μm are formed they cause a 'gritty' texture in the product. This can occur in some margarines when certain triglycerides predominate. In butter, the plasticity seems to be enhanced by the diversity of triglycerides and contribution of short chain fatty acids to the liquid fat phase (Early,1998).

The aqueous phase droplets in butter tent to be larger and the structure of their interface with the fat phase also seems to differ from margarines, where the added emulsifiers probably play a significant role. These factors nay also contribute to the oral properties and good flavour release of butter (Juriaanse and Heertye, 1988).

2.4.4 Spreadability

Spreadability depends largely on the SFC of the fat phase of a spread and begins to be acceptable at levels below 45% (and down to about 15%). That for butter this varies during the year but it is usually considered spreadable temperatures from 15-18 °C. While enough solid fat remains to give it body. This has meant that historically butter has generally rather been spreadable at the prevailing ambient temperatures or the occasional need to warm it before use was taken. The microstructure of butter also plays a part the way it spreads. The shapes and size of fat crystals and the bonds between them are important. A benefit of the churning process, by which most butter is still made, is the presence in butter of some globular fat' derived from the original cream (Early,1998).

2.4.5. Keeping quality

The physical entrapment of the disperse aqueous phase droplet un the continuous fat phase of liquid and solid fat, coupled with their microscopic size, restricts microbial growth due to limited nutrient availability. In the case of salted butters the concentration of salt in the aqueous phase is high enough to inhibit growth of many organisms. This means that butter can maintain an acceptable quality for several weeks at moderate ambient temperatures. And much longer if refrigerated or frozen.

These are probably the main features associated with the traditional use of butter although other factors such as the low level of spattering exhibited butter when used for frying are also important. This is due to the natural emulsifying action of the phospholipids derived from the cream fat globule membranes. The perceived value for money, the effects of advertising and recommendation on dietary factors cannot be ignored, and the response of the dairy industry will be described later (Early,1998).

2.4.6. Bacteria in Butter

Bacteria, particularly lactic acid bacteria, play and exceedingly important part in the manufacture of butter, and also its spoliation. In good quality butter which has been manufactured in a satisfactory manner, there is little food present for bacteria other than the fat. Very fortunately, organisms do not readily attack this fat, although it is not secure from attack by various yeasts and molds. Such other nourishment as may be present is seized upon by the lactic-acid bacilli which form acid by-products. These, in turn, deter the growth of unwanted putrefactive organisms. The organisms present in butter may be divided in to three groups, as non-pathogenic organisms, pathogenic organisms and abnormal bacteria (Harvey and Hill,1999).

2.5. Butter Yield Calculations

Technological limits to yield efficiency are defined by separation efficiency, churning efficiency, composition overrun, and package over fill.

2.5.1 Separation efficiency (Es):

Represents fat transferred from milk to cream

$$Es = 1 - fs/fm$$

Where fs = skim fat as percent w/w fm = milk fat as percent w/w

Separation efficiency depends on initial milk fat content and residual fat in the skim. Assuming optimum operation of the separator, the principal determining factor of fat loss to the skim is fat globule size. Modern separators should achieve a skim fat content of 0.04 - 0.07%.

2.5.2. Churning Efficiency (Ec):

Resents fat transferred from cream to butter

$$Ec = 1 - fbm/fc$$

Where fbm = buttermilk fat as percent w/w

fc = cream fat as percent w/w

Maximum acceptable fat loss in buttermilk is about 0.7% of churned fat corresponding to a churning efficiency of 99.3% of cream fat recovered in the butter. Churning efficiency is highest in the winter months and lowest in the summer months. Fat losses are higher in ripened butter due to a restructuring of the FGM (possibly involving crystallization of high melting triglycerides on the surface of the globules). If churning temperature is too high, churning occurs more quickly but fat loss in buttermilk increases. For continuous churns assuming 45% cream, churning efficiency should be 99.61 - 99.42%.

2.5.3. Composition Overrun

Churn Overrun = (Kg butter made - Kg fat churned)/Kg fat churned x 100 %

% Composition Overrun = (100 - % fat in butter)/% fat x 100 %

2.5.4 Package Fill control

Package fill control= (actual wt. - nominal wt.)/nominal wt. x 100%

An acceptable range for 25 kg butter blocks is 0.2 - 0.4% overfill. Overfill on 454 g a print is about 0.6%.

2.5.5 Other factors affecting yield

- Srinkage due to leaky butter (improperly worked).
- Srinkage due to moisture loss; avoided by aluminum wrap.
- Loss of butter remnants on processing equipment; % loss minimal in large scale continuous processing.

2.5.6 Plant Overrun

Plant efficiency or plant overrun is the sum of separation, churning, composition overrun and package fill efficiencies. In summary the theoretical maximum efficiency values are:

- Separation Efficiency 98.85
- Churning Efficiency 99.60
- Composition overrun (% fat) 23.30
- Package overfill 0.20

These values can be used to predict the expected yield of butter per kg of milk or kg of milk fat received (Goulf University, 2007).

2.6. Overview of the Buttermaking Process

The butter making process involves quite a number of stages. The continuous buttermaker has become the most common type of equipment used. The cream can be either supplied by a fluid milk dairy or separated from whole milk by the butter manufacturer. The cream should be sweet (pH >6.6, TA = 0.10 - 0.12%), not rancid and not oxidized. If the cream is separated by the butter manufacturer, the whole milk is preheated to the required temperature in a milk pasteurizer before being passed through a separator. The cream is cooled and led to a storage tank where the fat content is analyzed and adjusted to the desired value, if necessary. The skim milk from the separator is pasteurized and cooled before being pumped to storage. It is usually destined for concentration and drying.
The cream goes to pasteurization at a temperature of 95oC or more. The high temperature is needed to destroy enzymes and micro-organisms that would impair the keeping quality of the butter.

If ripening is desired for the production of cultured butter, mixed cultures of S. cremoris, S. lactis diacetyl lactis, Leuconostocs, are used and the cream is ripened to pH 5.5 at 21oC and then pH 4.6 at 13oC. Most flavour development occurs between pH 5.5 - 4.6. The colder the temperature during ripening the more the flavour development relative to acid production. Ripened butter is usually not washed or salted.

In the aging tank, the cream is subjected to a program of controlled cooling designed to give the fat the required crystalline structure. The program is chosen to accord with factors such as the composition of the butterfat, expressed, for example, in terms of the iodine value which is a measure of the unsaturated fat content. The treatment can even be modified to obtain butter with good consistency despite a low iodine value, i.e. when the unsaturated proportion of the fat is low.

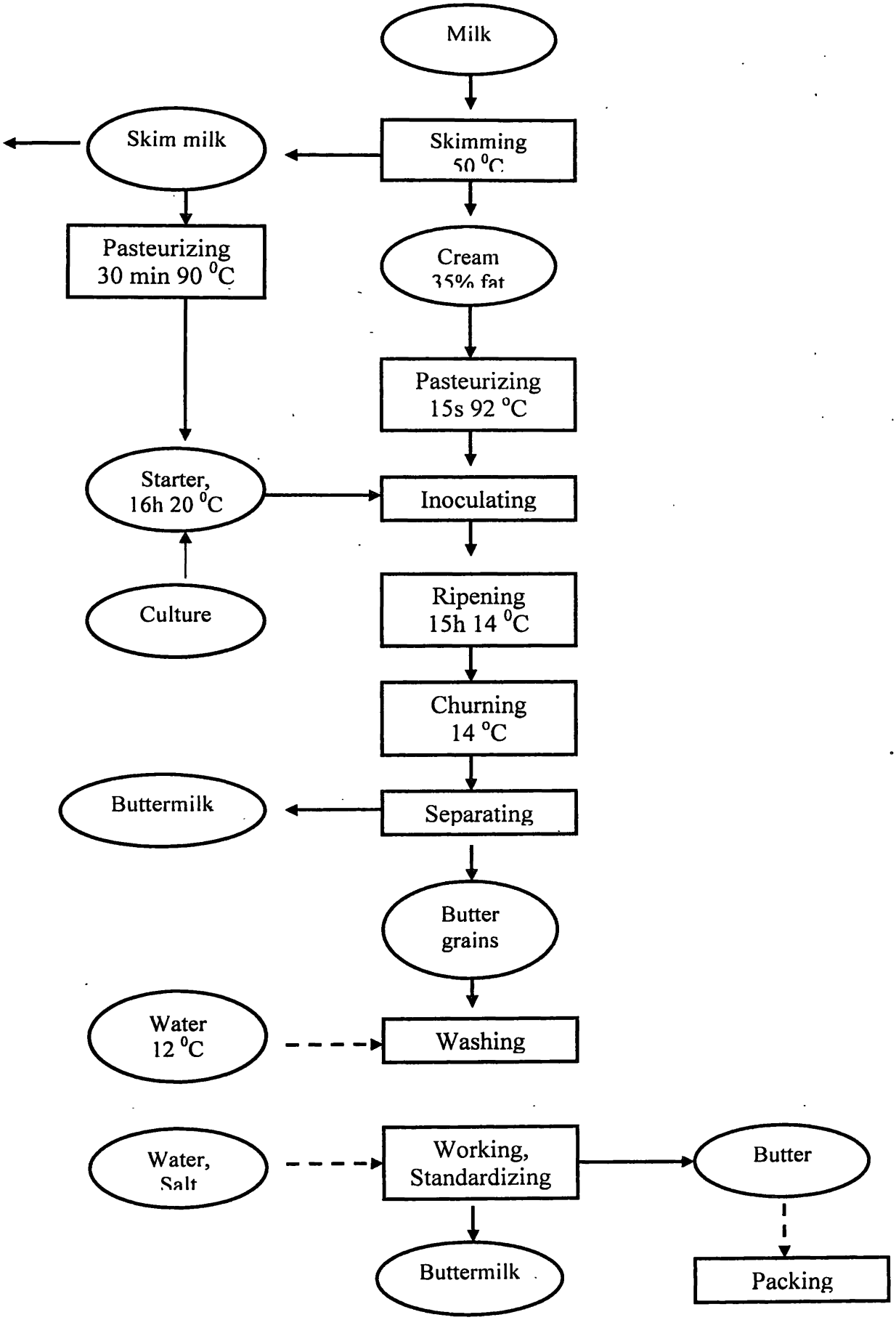
As a rule, aging takes 12 - 15 hours. From the aging tank, the cream is pumped to the churn or continuous buttermaker via a plate heat exchanger which brings it to the requisite temperature. In the churning process the cream is violently agitated to break down the fat globules, causing the fat to coagulate into butter grains, while the fat content of the remaining liquid, the buttermilk, decreases.

The machine stops when the grains have reached a certain size, whereupon the buttermilk is drained off. With the continuous buttermaker the draining of the buttermilk is also continuous. After draining, the butter is worked to a continuous fat phase containing a finely dispersed water phase. It used to be common practice to wash the butter after churning to remove any residual buttermilk and milk solids but this is rarely done today.

Salt is used to improve the flavour and the shelf-life, as it acts as a preservative. If the butter is to be salted, salt (1-3%) is spread over its surface, in the case of batch production. In the continuous buttermaker, a salt slurry is added to the butter. The salt is all dissolved in the aqueous phase, so the effective salt concentration is approximately 10% in the water.

After salting, the butter must be worked vigorously to ensure even distribution of the salt. The working of the butter also influences the characteristics by which the product is judged - aroma, taste, keeping quality, appearance and colour. During working, fat moves from globular to free fat. Water droplets decrease in size during working and should not be visible in properly worked butter. Overworked butter will be too brittle or greasy depending on whether the fat is hard or soft. Some water may be added to standardize the moisture content. The finished butter is discharged into the packaging unit, and from there to cold storage (Goulf University, 2007).

2.6.1 Butter production Process operations



2.6.2 The raw material for Butter

The cream must be of good bacteriological quality, without taste or aroma defects. The iodine value is the deciding factor in the selection of manufacturing parameters. Unless corrected, fat with a high iodine value (high unsaturated fat content) will produce greasy butter. Butter of acceptable consistency can be obtained from both hard fat (iodine value down to 28) and soft fat (iodine value up to 42) by varying the ripening treatment to suit the iodine value.

Cream containing antibiotics or a disinfectant is unsuitable for the manufacture of acidified butter. If harmful micro-organisms have been given the chance to develop, the cream cannot be used, even if they can be rendered inactive by heat treatment. Strict hygiene is therefore essential in all stages of the production process (Tetra Pak Processing Systems,1995)

2.6.3 Cream Separation

In a centrifugal separator the disc stack is equipped with vertically aligned distribution holes. Figure 2.4. shows schematically how fat globules are separated from the milk in the disc stack of a centrifugal separator.

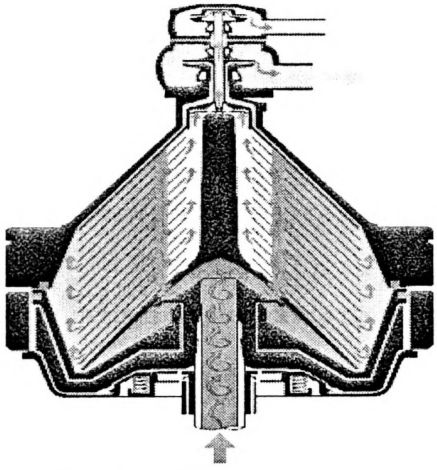


Fig. 2.4. Cream separator

In a centrifugal separator bowl the milk enters the disc stack through the distribution holes. The milk is introduced through vertically aligned distribution holes in the discs at a certain distance from the edge of the disc stack. Under the influence of centrifugal force the sediment and fat globules in the milk begin to settle radially outwards or inwards in the separation channels, according to their density relative to that of the continuous medium (skimmilk).

As in the clarifier, the high-density solid impurities in the milk will quickly settle outwards towards the periphery of the separator and collect in the sediment space. Sedimentation of solids is assisted by the fact that the skimmilk in the channels in this case moves outwards towards the periphery of the disc stack. The cream, i.e. the fat globules, has a lower density

than the skimmilk and therefore moves inwards in the channels, towards the axis of rotation. The cream continues to an axial outlet. The skimmilk moves outwards to the space outside the disc stack and from there through a channel between the top of the disc stack and the conical hood of the separator bowl to a concentric skimmilk outlet (Tetra Pak Processing Systems,1995).

2.6.4 Cream Pasteurisation

Cream is pasteurised at a high temperature, usually 95°C or higher, normally without any holding time. The heat treatment should be sufficient to result in a negative peroxidase test. This vigorous treatment kills not only pathogenic bacteria but also other bacteria and enzymes that could affect keeping quality. The heat treatment should not be so intense that there will be defects, such as a cooked flavour. Pasteurization serves to kill microorganisms, to enactive enzymes to make the cream a better substrate for the starter bacteria and to render the butter more resistant to oxidative deterioration. Sometimes the cream is pasteurized in a vacreator, which implies that the cream is put under vacuum to cool, due to which some off-flavors are (partly) removed (R.Early,1998).

2.6.5 Vacuum deaeration

If necessary, any undesirable flavouring substances of a volatile nature can be removed by vacuum treatment. The cream is first heated to 78°C and then pumped to a vacuum chamber where the pressure corresponds to a boiling temperature of 62°C. The reduced pressure causes volatile flavouring and aromatic matter to escape in the form of gas when the cream is flash-cooled. After this treatment the cream is returned to the heat exchanger for pasteurisation and cooling, and then continues to the ripening tank. Onion off-flavour is a very common defect during the summer, when various onion plants grow in the fields. Sorting of the cream is sometimes necessary to avoid strong flavours (Tetra Pak Processing Systems,1995).

2.6.6 Churning

The cream is churned after temperature treatment and after souring where applicable. Butter is traditionally made in cylindrical, conical, cubical or tetrahedral churns with adjustable speed. Axial strips and dashers are fitted inside the churn. The shape, setting and size of the dashers in relation to the speed of the churn are factors that have an important effect on the end product. Modern churns have a speed range that permits selection of the most suitable working speed for any set of butter parameters. Before transfer to the churn the cream is stirred and the temperature adjusted. The churn is usually filled to 40 – 50% to allow space for foaming (Tetra Pak Processing Systems,1995).

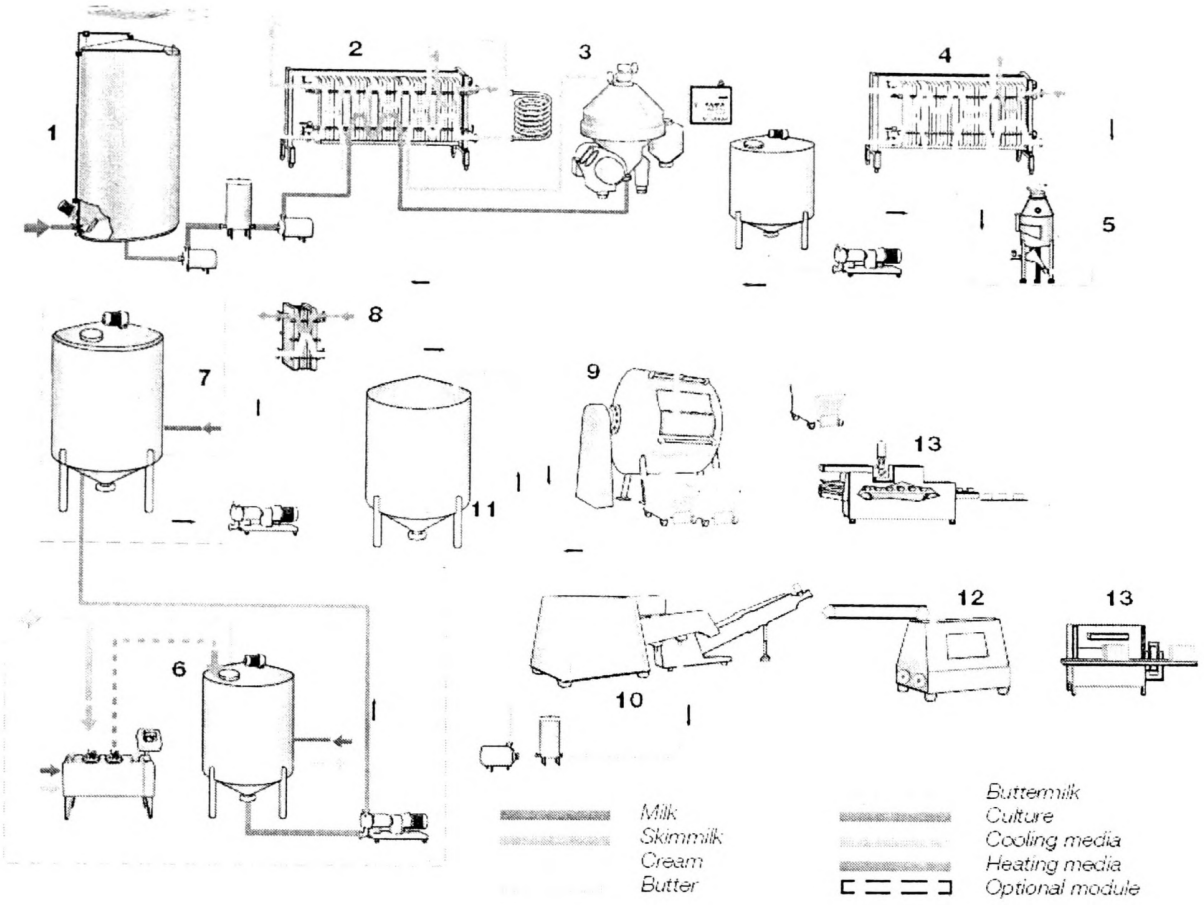


Fig. 2.5. Industrial butter making plant

2.6.7. The background Science of Butter Churning

Milk fat is comprised mostly of triglycerides, with small amounts of mono- and diglycerides, phospholipids, glycolipids, and lipo-proteins. The trigylcerides (98% of milkfat) are of diverse composition with respect to their component fatty acids, approximately 40% of which are unsaturated fat firmness varies with chain length, degree of unsaturation, and position of the fatty acids on the glycerol. Fat globules vary from 0.1 - 10 micron in diameter. The fat globule membrane is comprised of surface active materials: phospholipids and lipoproteins. Fat globules typically aggregate in three ways: flocculation, coalescence, Partial coalescence (Goulf University, 2007).

Many milk products foam easily. Skim milk foams copiously with the amount of foam being very dependent on the amount of residual fat - fat depresses foaming. The foaming agents are proteins, the amount of proteins in the foam are proportional to their contents in milk. Foaming is decreased in heat treated milk, possibly because denaturated whey proteins produce a more brittle protein layer at the interface. Fats

tend to spread over the air-water interface and destabilize the foam; very small amounts of fats (including phospholipids) can destabilize foam.

During the interaction of fat globules with air bubbles the globule may also be disrupted (this is the only way that fat globules can be disrupted without considerable energy input). Disruption of the fat globule by interaction between the fat globule and air bubbles is rare except in the case of newly formed air bubbles where the air-water interfacial layer is still thin. If part of the fat globule is solid, churning will result, hence the term "flotation churning" -from repeated rupturing of air bubbles and resulting coalescence of the adsorbed fat.
In spite of the above comments on the destabilization of foams by fat, milk fat is essential for the formation of stable whipped products which depend on the interaction between fat globules, air bubbles and plasma components (esp. proteins).

When cream is beaten air cells form more slowly partly because of higher viscosity and partly because the presence of fat causes immediate collapse of most of the larger bubbles. If most of the fat is liquid (high temperature) the fat globule membrane is not readily punctured and churning does not occur -at cold temperature where solid fat is present, churning (clumping) of the fat globule takes place. Clumps of globules begin to associate with air bubbles so that a network of air bubbles and fat clumps and globules form entrapping all the liquid and producing a stable foam. If beating continues the fat clumps increase in size until they become too large and too few to enclose the air cells, hence air bubbles coalesce, the foam begins to "leak" and ultimately butter and butter milk remain(Goulf university, 2007).

2.7. Hygiene in butter manufacture

A key issue for product safety is the risk of cross-contamination occurring during the process from the internal factory environment. Cross-contamination could arise from a wide range of sources and the inherent risks in a particular processing area must be understood. Most of these issues are managed through adherence to Good Manufacturing practice (GMP) (Mortimore and Wallace,1998).

2.7.1 Layout

The facility layout should be considered carefully to minimize the cross-contamination risks. The should include adequate segregation of raw materials and finished product. Depending on the type of operation, fully segregation between raw and cooked product may be required, and

in most facilities the outer packaging stages, both for raw materials and finished products, will need to be kept separate form the main risk area.

Availability of the requires services and facilities for manufacture of the product should also be considered. This will include the availability of potable water, and adequate cleaning facilities for plant, equipment and environment, along with the connection of all required service in the correct area, e.g. steam heating and cooling facilities.

The number of holding stages and associated times should also be considered at this stage as it is important that there is adequate space for holding the required amount of product at each stage without causing a cross-contamination, and that the appropriate temperature-controlled facilities are available.

The patterns of movement of staff and equipment should also be assessed here, with the provision of adequate hygiene facilities, such as changing and rest rooms and hand wish stations, along with canteen and recreational facilities (Mortimore and Wallace,1998).

2.7.2 Buildings

The fabric of the building itself could pose a hazard or safety risk to the product, through harborage of pests and others contamination, or through physical contamination or through physical contamination due to poor design and maintenance. Surface should be non-porous and easy to keep clean, with all cracks filled and sealed and overhead services should be well maintained to prevent physical hazards falling into the product, and drains should be designed and serviced so that the flow is always away from the production areas, with no chance for back flow or seepage. Adequate pest proofing and cleaning schedules should be drawn up for all facility buildings. All food manufacturing areas should be constructed such that these issues are managed (Mortimore and Wallace,1998).

2.7.3 Equipment

Equipment should be designed to minimize any cross- contamination risk. This could arise through parts of the equipment breaking off and gaining entry to the product as physical hazards, alternatively, if equipment has any dead-leg areas, is difficult to clean or is poorly cleaned, microbiological build-up could contaminate the product. Chemical contaminate could arise through lubricants or cleaning residues remaining on the equipment food contract surfaces. Remember also to ensure that you can clean around and under equipment. If it is too close to the floor to clean underneath, the equipment should be sealed around the base.You will also need to consider what the equipment is made of. For example, it is stainless steel or it is mild steal which may corrode leaving a surface prone to microbiological contamination.

It painted and could your product be at risk from paint flakes. It have any wooden parts or brush attachments that cannot be effectively cleaned (Mortimore and Wallace,1998).

2.7.4. People

Food handlers and other personnel with access to the food processing area could cross-contaminate the product with microbiological, chemical physical hazards. The process layout and movement patterns should be considered in order to minimize this risk, along with the appropriate

Here also you will need to take at the types of protective clothing required, along with frequencies of changing and laundering procedures. You should already have considered changing facilities; amenities and hand wash station as part of the building layout, but cross-check whether you have made sufficient provision. All personnel in a food plant should be trained in Good Hygiene practice. Steps should also be taken to ensure that this knowledge is fully utilized (Mortimore and Wallace,1998).

2.7.5. Cleaning

There must be sufficient facilities for the cleaning of equipment, people, plant and buildings, and these should be situated to enable their convenient use. Cleaning areas should not cause a cross- contamination risk to the process. Cleaning schedules should be prepared for all areas and staff must be adequately trained to carry out cleaning activities effectively (Mortimore and Wallace,1998).

2.7.6. Chemicals

Strong facilities must be provided for any chemicals that are required for use in the manufacturing areas. These must prevent the risk of product contamination. All chemicals must be properly labeled and must not be decanted into food containers. All personnel handling chemicals must be trained in their safe use (Mortimore and Wallace,1998).

2.7.7. Raw Materials

Raw materials can act as cross-contaminants if they gain access to the wrong product, or if they added in excess quantities. This can have serious consequences in the case of allergenic raw materials entering a product where they are not labeled. Handling areas for raw materials must be carefully planned, and areas used for more than one type of ingredient may require through cleaning between uses.

Make sure that you know how all your raw materials need to be handled, and put appropriate measures in place. You may be buying in something in a safe condition but it is easy to make

it unsafe through improper handling, e.g. leaving perishable goods sitting no a loading bay for several hours (Mortimore and Wallace,1998).

2.7.8. Storage

Storage areas must be properly planted to minimize damage and cross-contamination issues. /consider whether you have adequate segregation, temperature and humidity control, and ensure that all storage areas are properly pest proofed. All materials should be stored off the floor and in sealed bags or containers. Part-used containers must be resealed after each use, and strict stock rotation should be employed (Mortimore and Wallace,1998).

2.7.9. Products

Residues of other products can be also cause a serious hazard if allergenic material is present or if they affect the intrinsic nature of the product that is contaminated. Production lines should be spatially separated to prevent cross-contamination and handling and cleaning producers should be planned appropriately. Consider any extra control required if personnel are switched between lines or departments; will there be an addition risk from their protective clothing (Mortimore and Wallace,1998).

2.7.10. Packaging

Packaging areas and handling practices should be managed and controlled to prevent any cross-contamination risk. The packaging itself could be a major hazard, e.g. glass fragments, or could introduce micro-organisms to the product. Make sure that your packaging is suitable for the job and won't be damaged during product storage and distribution, and consider whether you have the correct coding and usage instructions printed legibly (Mortimore and Wallace,1998).

CHAPTER 03

MATERIALS AND METHODOLOGY

3.1. Preliminary study on butter manufacturing process

Salted-butter manufacturing process was studied over one month period at MICO company in Narahenpita. Foremost, principle raw materials, equipments and machineries that are used in actual butter manufacturing were identified. Thereafter actual processing flow diagram along with workers traffic pattern was out lined and in order to identify the most probable steps that may lead to cross contaminations.

3.1.1. Identification of bad handling practices in the butter processing line

The following components in the process line were evaluated to identify...

- Design and facilities of the building and equipment: location and location of equipment.
- Control operation: incoming milk and cream requirement and basic butter processing operation.
- Maintenance and sanitation: cleaning system, maintenance systems were observed.
- Personal hygiene: bad handling practices in the process line and health statues (illness and injuries) of personal.
- Product information: information on label.
- Transportation: safe of product and safe of contaminations

3.2. Assessing the quality of incoming cream that is used for butter manufacturing

Quality of incoming cream was evaluated using two quality parameters. The physico-chemical quality was evaluated by analyzing the titrable acidity where as, the microbiological quality was evaluated by performing total plate count.

3.2.1. Analysis of the titrable acidity:

Materials:

Standard sodium hydroxide solution (0.1 N)
Phenolphthalein indicator (0.05%)
Distilled water

Equipments:

Pipettes (10 ml)
Titration flask (100 ml)
Burettes (25 ml)
Beaker (50 ml)
Stand

Methodology:

- A representative sample of cream was obtained from butter processing line into beaker.
- 9 ml of well mixed cream was pipetted into a 100ml titration flask and 20 ml of distilled water was added.
- 1ml of 0.05% phenolphthalein indicator was added.
- The burette was filled with (0.1 N) NaOH solution, drained out a portion to insure that there are no air bubbles in the colomn.
- While agitating the sample continuously sodium hydroxide solution was slowly drained from the burette until the 1st permanent pink colour is obtained.
- The volume of standard sodium hydroxide solution required for titration was measured.
- This was replicated 10 times and titrable acidity was calculated as percentage of lactic acid and compared against the prescribed level of acidity.
- Three cream samples that were obtained in three different days were analyzed for titrable acidity according to the same procedure.

Calculations:

Percentage of acidity = [Volume of NaOH used x 0.009 x 100] / 9

3.2.2. Total plate count:

Materials:

Distilled water
Peptone power
Milk agar

Equipments:

Sterile test tubes
Cotton wool
Aluminum foil
Culture tubes
Water bath
Autoclave
Pipettes
Incubator
Electronic balance (±0.00g)
Quebec colony counter

Methodology:

Sterilization of glassware, tools and working table:

- Pipettes, Erlenmeyer flasks and Petri dishes were wrapped with aluminium foils and placed in a hot air oven maintained at 240°C for 2 ½ hrs.
- Inoculation loop was sterilized using Bunsen flame until it turns red hot.
- 70% Ethanol was used to sterilize the working table, inoculation chamber etc.

Preparation of peptone water:

- A solution of peptone water was prepared by dissolving 1g of peptone in a 1litre of distilled water.
- 9ml thereof were measured into series of milk dilution bottles and capped well.
- They were sterilized at 121 ^{0}C for 30 min in a laboratory autoclave.

Preparation of milk agar culture media:

- 24g of milk agar powder was accurately weighed and transferred into a beaker containing 500 ml distilled water.
- Contents were gently heated using a water bath with slight agitation.
- More distilled water was added to make up the volume to 1 litre.
- pH of the broth was measured using a pH meter and adjusted it to 7.0 by adding drops of NaOH solution.
- 10 ml of culture media was dispensed to 27 culture tubes.

- Mouth of each tube was covered using cotton plugs and further tightened by applying aluminum foils and rubber bands.
- Finally they were placed inside an iron basket, transferred into an autoclave and sterilized at 121°C for 30 minutes.

Preparation of dilution series:

- 1 ml of cream from sample A, B and C was pipetted out and transferred into sterilized dilution bottles containing 9 ml of peptone water separately.
- Bottle was shacked well and labeled as 10^{-1}.
- 1ml from each of first dilutions (10^{-1}) were measured into a three dilution bottles containing 9ml of sterilized peptone water, shacked thoroughly and labeled as 10^{-2}.
- Similarly, 1ml from each of second dilutions (10^{-2}) were measured into a dilution bottles containing 9ml of sterilized peptone water, shacked thoroughly and labeled as 10^{-3}.

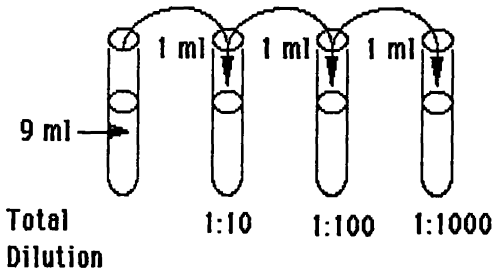


Fig 3.1. A graphical representation of preparing the dilution series

Culturing method

- The media contained in each culture tube was emptied into sterilized culture plates.
- 1ml from each dilution (9 dilutions) was transferred into labeled culture plates containing liquefied media and mixed thoroughly. (Each dilution was triplicated)
- After solidification of the media all the plates (27) were kept in inverted position inside the incubator maintained at 35°C for 24-48 hrs.

Counting method

- The petri plates which afford the counting were kept on the slanting platform of the colony counter.
- The number of colonies was counted with the help of digital reading meter.

3.3. Determination of free fatty acid percentage of butter

Materials:

Standard sodium hydroxide solution (0.1 N)
Phenolphthalein indicator (0.05%)
Ethyl alcohol
Ether

Equipments:

Titration flask (100 ml)
Burettes (25 ml)
Beaker (50 ml)
Stand

Methodology:

- A sample of butter (10g) was weighed into a titration flask and melted by keeping them inside the oven.
- Equal amount of ethyl alcohol and ether was mixed and prepared a solution.
- 50ml thereof was mixed with melted butter.
- Adding two drops of phenolphthalein, it was titrated with 0.1N sodium hydroxide until the faint pink color appeared.
- This process was continued for 10 weeks.
- Acidity development of butter was assessed against time.

Calculations:

Amount of Fatty acid = (end point result x 5.61) /weight of the butter

Amount of Free fatty acid = fatty acid/2

CHAPTER 04

RESULTS AND DISCUSSION

4.1.1. Principle raw materials used in butter making

- Fresh milk cream (Base raw material)
- Salt (Flavouring agent)
- Potassium sorbate (preservative)
- Ascorbic acid (Anti-oxidant)
- Aluminum foil (Packaging material)

The fat content of incoming cream may range from 35% to 40%. Cream that was used for butter manufacturing was tested for fat, acidity and also microbiologically to ensure their quality. Salt, potassium sorbate, ascorbic acids were used in powder form at final stage of churning (after washing of butter grains). Aluminum foil was used as packaging material at packaging stage.

4.1.2. Equipments and machineries used in butter Manufacturing

- Butter churner
- Packaging machine
- Tank (for storage butter milk)
- Trolley

There are two butter churners in MILCO Narahenpita branch that can churn 900L of milk cream at once. Churning speed of that churners can be controlled by using speed regulating system according to the operation requirement. At final stage, the packaging machine is used for molding and packaging of end product. A large tank is used to store buttermilk that is generated as a by-product of butter making process, which is intended for further processing. Also there is an extra storage tank near the main tank to store excess buttermilk that can't be stored in main tank. A conveying trolley is used to convey butter between processing room and butter cold room.

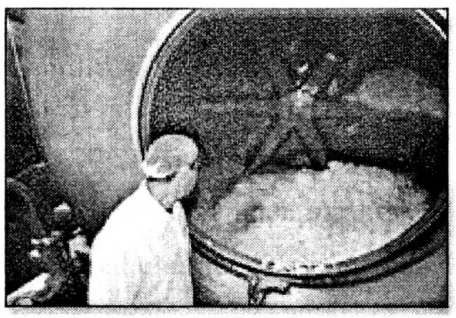
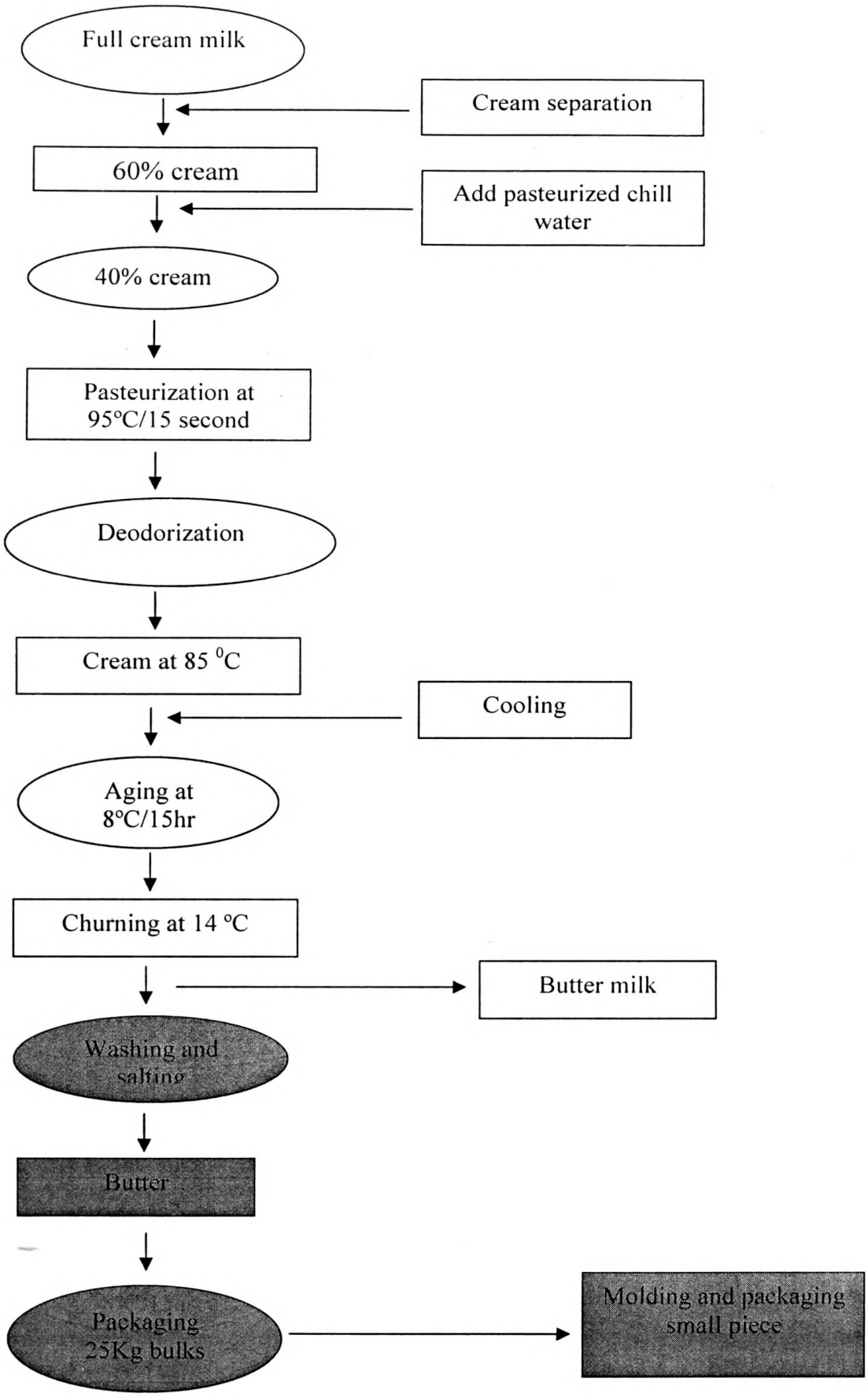


Fig. 4.1 Structure of butter

4.1.3. Actual processing flow diagram of Highland butter manufacturing



*** Critical steps that cross contamination can occur**

4.1.4. Design and available function of building and equipment:

It was observed that some facilities are not at appropriate level. Equipments are very old and their functions were not at appropriate level and some equipments have corroded. Some amount of butter milk is removed with waste water. Floor is not level. Therefore cleaning water doesn't remove properly. Roof is damaged and therefore rain water leaks down to the processing area an rainy days. There are no proper places for storing of chemicals and salt.

Buildings and equipments are playing a major role in butter manufacturing. Because there are risks of cross-contaminations during the processing and any holding stages. Microbiological, chemical and physical safety issues should be considered. Damaged roof can introduce many physical and biological hazards. Unless equipment is not well maintained, there a can be loss of butter quality. All chemicals and ingredients should be placed at suitable condition. Waste should be deposited properly otherwise it may be lead to an environmental effect (e.g. Butter milk).

4.1.5. Control operations:

Quality requirements of incoming cream are in normal range. However day to day variation is observed. Basic butter processing operations and handling practices are take place in normal range. Workers handle the butter without glows.

In the control operation, raw materials should be inspected critically because some raw materials are highly susceptible to hazardous microbes and also contaminate the final product. It may cause big damage to product quality. Day to day variations do not directly affect the basic quality requirement of the product. But it may affect the uniformity of butter. Also, workers involvement affect to the quality of butter. Because microbiological cross-contaminations may be happen. The risk of unsafe food should be reduced by taking preventive measure to assure the safety and quality of butter at an appropriate stage in the operation.

4.1.6. Maintenance and sanitation:

Maintenance and sanitation systems are not at an appropriate level. Some equipments are not well maintained.

Maintenance and sanitation facilitate to continuing effective control of food hazards, pests, waste and other agent likely to contaminate food. It's essential to improve the proper hygienic and maintenance facilities to enhance the quality of the butter.

4.1.7. Personal Hygiene

Some workers didn't wear glows, caps, boots and masks in working. It may be lead to contaminations.

Butter makers and other personnel who access to the butter processing area could cause to cross-contaminate the product with microbiological, chemical physical hazards. Therefore they need to wear special protective clothing such as boots, overcoats, caps, etc, along with frequencies of changing and laundering procedures. Hand washes should be place in suitable manner. Personnel behaviors should be improved.

4.1.8. Transportation:

Transportation is another important factor. In transportation butter quality may be reduced. Preventive measures during transportation should be applied to protect food from potential source of contaminations, from damage and to provide an environment unconductive to the growth of microorganisms.

When something goes wrong with a food product there may be localized or widespread illness and suffering, and the cost of the company concerned can be huge. Even when no illness has been caused, the discovery of safety hazards in a product intended for consumption can lead to prosecution for the company. Routine prosecutions often result from foreign material being discovered in butter, but microbiological hazards have the potential to case much greater impact.

4.2. Assessing the quality of incoming cream that is used for butter manufacturing

Titrable acidity:

Table 4.1. Titrable acidity of cream samples obtained from the process line on three consecutive days.

Sample code	% Acidity	P>0.05
A	0.10900 ± 0.00699	0.009
B	0.11700 ± 0.00483	
C	0.11250 ± 0.00354	

Sample A, B and C are three cream samples obtained from butter manufacturing line at three consecutive days and their corresponding average acidities are 0.109%, 0.117% and 0.112% respectively as shown in the table 4.1. P value (0.009) indicates that there is a day to day variation in % acidity and thus assuring the uniformity of end product is somewhat questionable. However, average acidity of the each cream sample is far less compared to the minimum affordable acidity i.e., 0.14% (reference). This implies that all the cream samples

used for butter manufacturing were in acceptable chemical quality. Based on two of the above parameters it can be realized that effect of day to day variation in acidity can be ignored and thus cream is of favorable quality as far as chemical quality is concern.

Micro biological quality:

Table 4.2. Total plate count for cream samples

Cream code	CFU /ml ('000)	Average CFU/ml ('000)
A	45	42
	39	
	43	
B	58	59
	59	
	61	
C	49	46
	41	
	48	

According to the prescribed standards, the maximum CFU/ml shall be 50 for a cream if it is utilized for butter manufacturing. Table 4.2. shows that cream sample "A" and "C" conforms to the specifications where as cream sample "B" is somewhat inferior in microbiological quality. According to Food and Environmental Hygiene department (2001) cream sample with 50CFU/ml or less is considered as Class A, Therefore cream samples "A" and "C" can be categorized as class A and cream sample "B" is to class B.

4.3. Free fatty acid level

Samples of A1and A2 are butter samples obtained from butter manufacturing line at one batch. Cream sample A is representative raw material quality.

Table 4.3. Free fatty acid levels of butter stored in cold room (Sample code A1, at -10°C)

Storage time (week)	%Free fatty acids
0	0.3366
1	0.3366
2	0.3489
3	0.3506
4	0.3635
5	0.3642
6	0.3646
7	0.3768
8	0.3775
9	0.3786
10	0.3786

Table 4.4. Free fatty acid levels of butter stored in normal refrigerated condition (Sample code A2, at 10°C)

Storage time (week)	%Free fatty acids
0	0.3366
1	0.3489
2	0.3643
3	0.3923
4	0.3927
5	0.4047
6	0.4063
7	0.4195
8	0.4207
9	0.4343
10	0.4461

The Anderson-Darling test's p-value indicates that, at α levels (0.05) less than 0.133 (A1) and 0.608 (A2), there is evidence that the % of acidity follow a normal distribution. These two data was checked for variances and p-value is 0.024. Therefore variances are not equal. In t-test p-value is 0.008. P-value is less than α level. Therefore, % of free fatty acids in -10°C (A1) and % of free fatty acids in 10°C (A2) are statically significant different. Development of free fatty acids of these two samples is showed using time series test in Fig.4.2.

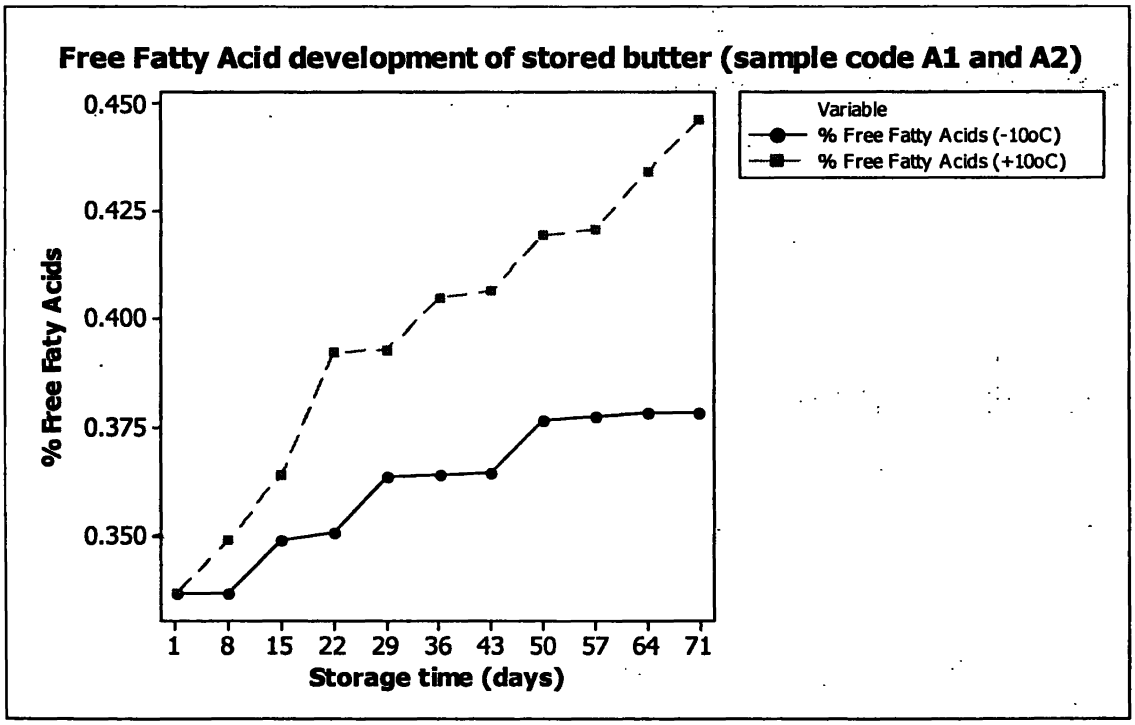


Fig.4.2. Development of free fatty acid against the time

Acidity development in above two temperatures statically can be measured. Using simple liner regression, these equations are obtained. Regression equation of butter sample stored in cold room (Sample code A1) is;

% of Free Fatty Acid (butter stored in cold room) = 0.338 + 0.00470 Week

Regression equation of butter sample stored in normal refrigerated condition (sample codeA2) is;

% of Free Fatty Acid (butter stored in normal refrigerated condition) = 0.346 + 0.0102 Week

Using optimum % level of free fatty acid, maximum shelf life of butter stored in cold room and maximum shelf life of butter stored in normal refrigerated condition can be calculated. USDA, Agricultural marketing service recommended free fatty acid content of not more than 0.6 present (as oleic acid) prior to first use(USDA, Agricultural marketing service,2005).Using above recommendation, calculated shelf-life is bellow.

Table 4.5. Calculated shelf-life of stored butter samples (sample code A1 and A2)

Sample	Shelf-life (weeks)
Butter stored in cold room	56
Butter stored in normal refrigerated condition	25

Samples of B1and B2 are butter samples obtained from butter manufacturing line at one batch. Cream sample B is representative raw material quality.

Table 4.6. Free fatty acid levels of butter stored in cold room (Sample code B1, at -10°C)

Storage time (week)	**Free fatty acid level**
0	0.3082
1	0.3085
2	0.3359
3	0.3362
4	0.3489
5	0.3499
6	0.3635
7	0.3646
8	0.3646
9	0.3768
10	0.3790

Table 4.7. Free fatty acid levels of butter stored in normal refrigerated condition (Sample code B2, at 10°C)

Storage time (week)	Free fatty acid level
0	0.3082
1	0.3210
2	0.3359
3	0.3366
4	0.3496
5	0.3768
6	0.3787
7	0.3900
8	0.4063
9	0.4182
10	0.4207

The Anderson-Darling test's p-value indicates that, at α levels (0.05) less than 0.294 (B1) and 0.567 (B2), there is evidence that the % of acidity follow a normal distribution. These two data was checked for variances and p-value is 0.149. It is less than 0.05 significant level. Therefore variances are equal. In t-test p-value is 0.194. P-value is less than α level. Therefore, % of free fatty acids in -10°C (A1) and % of free fatty acids in 10°C (A2) are not statically significant different. Development of free fatty acids of these two samples is a showed using time series test Fig.4.3.

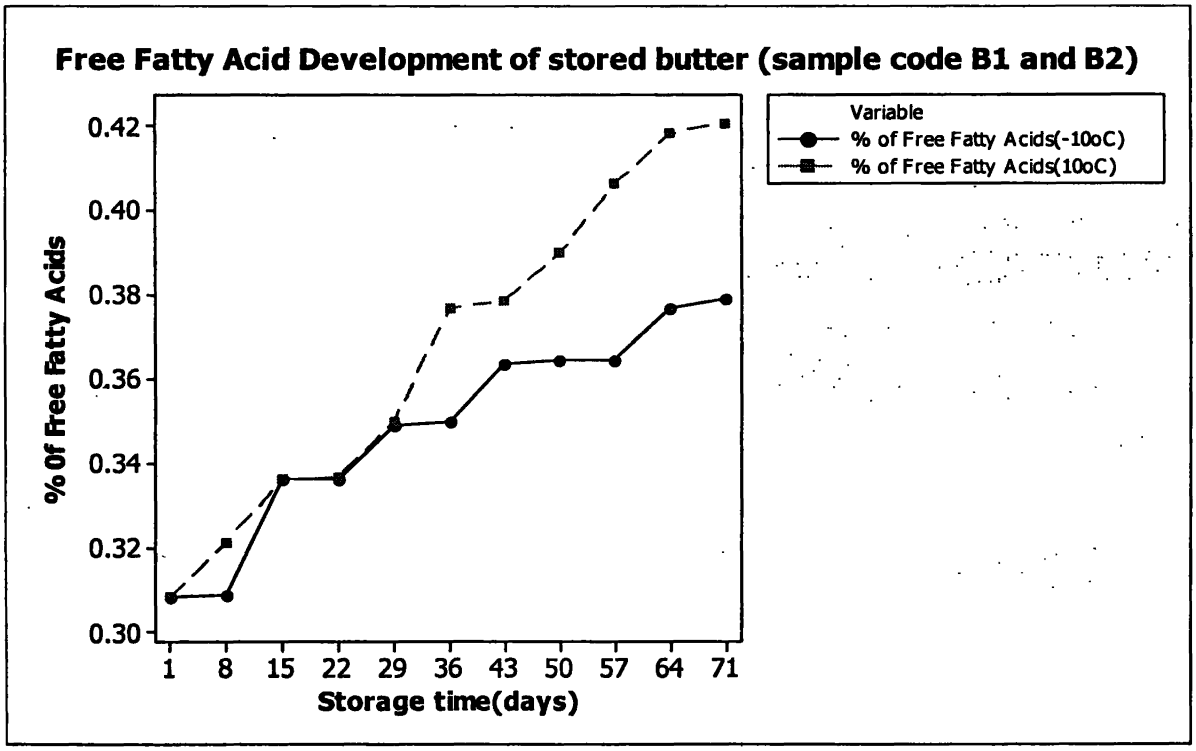


Fig.4.3. Development of free fatty acid against the time

Acidity development in above two temperatures statically can be measured. Using simple liner regression, these equations are obtained. Regression equation of butter sample stored in cold room (sample code B1) is;

% of Free Fatty Acid (butter stored in butter cold room) = 0.313 + 0.00713 Week

Regression equation of butter sample stored in normal refrigerated condition (sample code B2) is;

% of Free Fatty Acid (butter stored in normal refrigerated condition) = 0.306 + 0.0118 Week

Using optimum % level of free fatty acid, maximum shelf life of butter stored in cold room and maximum shelf life of butter stored in normal refrigerated condition can be calculated

Table 4.8. Calculated shelf-life of stored butter samples (Sample code B1 and B2)

Sample	Shelf-life (weeks)
Butter stored in cold room	40
Butter stored in normal refrigerated condition	25

Samples of C1and C2 are butter samples obtained from butter manufacturing line at one batch. Cream sample C is representative raw material quality.

Table 4.9. Free fatty acid levels of butter stored in cold room (Sample code C1, at -10°C)

Storage time (week)	**Free fatty acid level**
0	0.2936
1	0.3076
2	0.3091
3	0.3226
4	0.3339
5	0.3356
6	0.3363
7	0.3492
8	0.3499
9	0.3646
10	0.3646

Table.4.10. Free fatty acid levels of butter stored in normal refrigerated condition (Sample code C2, at 10°C)

Storage time (week)	Free fatty acid level
0	0.2936
1	0.3079
2	0.3356
3	0.3492
4	0.3628
5	0.3760
6	0.3790
7	0.3923
8	0.4067
9	0.4317
10	0.4335

The Anderson-Darling test's p-value indicates that, at α levels (0.05) less than 0.693 (C1) and 0.929 (C2), there is evidence that the % of acidity follow a normal distribution. These two data was checked for variances and p-value is 0.043. Therefore variances are equal. In t-test p-value is 0.034. P-value is less than α level. Therefore, % of free fatty acids in -10°C (C1) and % of free fatty acids in 10°C (C2) are statically significant different. Development of free fatty acids of these two samples is a showed using time series test in graph 4.3.

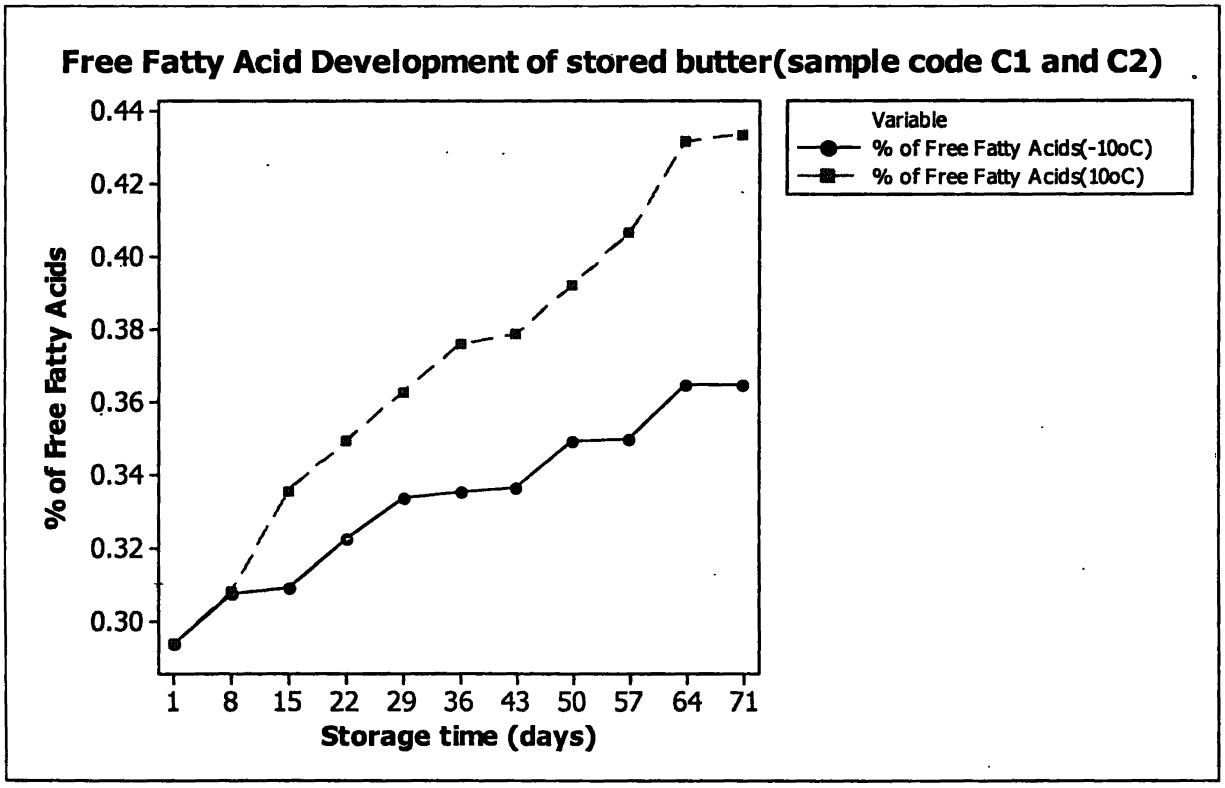


Fig.4.4. Free fatty acid development against the time

Acidity development in above two temperatures statically can be measured. Using simple liner regression, these equations are obtained. Regression equation of butter sample stored in cold room (Sample code C1) is;

% of Free Fatty Acid (butter stored in butter cold room) = 0.299 + 0.00692 Week

Regression equation of butter sample stored in normal refrigerated condition (sample code C2) is;

% of Free Fatty Acid (butter stored in normal refrigerated condition) = 0.301 + 0.0137 Week

Using optimum % level of free fatty acid, maximum shelf life of utter stored in cold room and butter stored in normal refrigerated condition can be calculated.

Table 4.11. Calculated shelf-life of stored butter samples (Sample code C1 and C2)

Sample	Shelf-life (weeks)
Butter stored in cold room	43
Butter stored in normal refrigerated condition	22

According to above samples, average shelf-life of butter was determined using regression equation at these two temperatures. Sample of butter kept at -10°C had 46 weeks average shelf-life. But sample of butter kept at 10°C had 24 weeks average shelf-life. According to these calculations, sample of butter at -10°C has long shelf life than sample of butter at -10°C. Therefore temperature is directly affected to shelf life of butter

Small proportions of free fatty acids are found are always present in fresh milkfat; larger percentages are found in the fat isolated from milk or cream that has been subjected to bacterial action or in fat that has been stored for some time. Lipolysis of milkfat, resulting in the production of free fatty acids.

The composition of free fatty acids in fresh milk to be similar to that of acid bound in the milk glycerides. Those during storage of butter relatively more of the even-numbered fatty acid are liberated. The liberated are considered a consequence of unanalyzed hydrolysis of the milk lipids. The rate of fatty acid liberation varied directly with temperature, no detectable lipid hydrolysis thanking place during storage at 4°C. The composition of free fatty acids, after storage, was similar to that in the parent lipids (Alford and Johnson1987).

The primary objective of this test was to evaluate free fatty acid development of butter made with milk cream. The influence of the fatty acid profile effected rancidity and product properties. Fatty acid development in two temperatures was compared in this research.

Butter primarily tends to become rancid due to the action of atmospheric oxygen, light, heat, water, metals, enzymes (lipases) and microorganisms. When butter becomes rancid it breaks down into glycerol and fatty acids. Due to its high water content, the size of the water droplets in the water-in-oil emulsion being < 6 μm, butter is subject to rapid microbiological spoilage at normal temperatures. Since the droplets are isolated one from the other, microbial growth occurs within individual droplets and if these are very small, multiplication cannot even occur. If the droplets are > 6 μm, the contact area with the fat rises sharply as the size of the droplets increases. These factors still operate at temperatures of -12°C and the microbes are inactivated only once the temperature falls below this value. Rancid butter becomes yellow to brown and the flavor becomes harsh.

Rancidity ,which is a frequent cause of consignments of butter being condemned, may be regarded as the final stage of staleness , and is the result of decomposition of the butter-fat into glecerine and fatty acids, and of oxidation. This taints is often observed in varying degrees during hot weather, and presents considerable difficulties in the way of eliminations. It may generally be assumed to indicate advanced deterioration of the product. Rancidity and moulds are closely allied (Harvey and Hill,1999).

Bacteria, mold, water, air, light, enzymes and some metals can accelerate souring. Souring results in the formation of free fatty acids, such as butyric acid and caproic acid, and the butter becomes soapy in flavor.

Table.4.9. The maximum duration of storage for chilled and frozen butter:

Temperature	Relative humidity	Max. duration of storage
-1 - +4°C	75 - 80%	1 - 2 months
-10°C	80 - 85%	3 months
-15°C	80 - 85%	4 - 6 months
-23 - -18°C	80 - 85%	8 - 12 months

Butter is transported in either chilled or frozen form. The advantages of transport in frozen form are, firstly, a longer storage life and, secondly, greater stackability of the product. These advantages are counterbalanced by the greater refrigeration capacity required throughout the transport chain.

Butter must be flash-frozen to protect it from losses in quality. The rapid cooling result in the formation of only small ice crystals, which have no negative effects. If, however, the temperature is reduced very slowly, relatively large ice crystals are formed which can result in loss of quality (Butter wikipidia, 2005).

CHAPTER 05

CONCLUSION AND RECOMENDATIONS

5.1. Conclusion

In processed butter manufacturing the design and facilities of the building and equipment, control operation, maintenance and sanitation, personal hygiene, product information and transportation and also bad manufacturing practices influence the shelf life of butter. The factors result in microbiological and physical quality loss of butter.

The day today variation in percentage acidity of the cram has a significant effect on the quality of the butter. However, this quality variation was within the level of acceptable of 0.14% (minimum).

According to the prescribed standards, the maximum CFU/ml shall be 50 for a cream if it is utilized for butter manufacturing. The study show that two cream samples conform to the microbiological quality where as one cream sample is somewhat inferior in microbiological quality.

Butter stored in butter cold room at had 46 weeks average shelf-life. However butter stored in normal refrigerated condition had 24 weeks average shelf-life. According to these results, sample of butter stored in butter cold room has long shelf life than sample of butter stored in normal refrigerated condition. Therefore temperature is directly affected to shelf life of butter.

Therefore, shelf-life of salted butter is influenced good manufacturing practices, quality of incoming cream and storage temperature of butter. Butter can get maximum shelf life to shelf life of butter be prolonged considerably to improving good manufacturing practices, microbial load, chemical quality of cream and using frozen type storage conditions.

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APPENDIX I

Compare the titrable acidity in cream samples (samples code A, B and C)

Descriptive Statistics: Sample A, Sample B, Sample C

```
Variable      Mean     StDev
Sample A   0.10900   0.00699
Sample B   0.11700   0.00483
Sample C   0.11250   0.00354
```

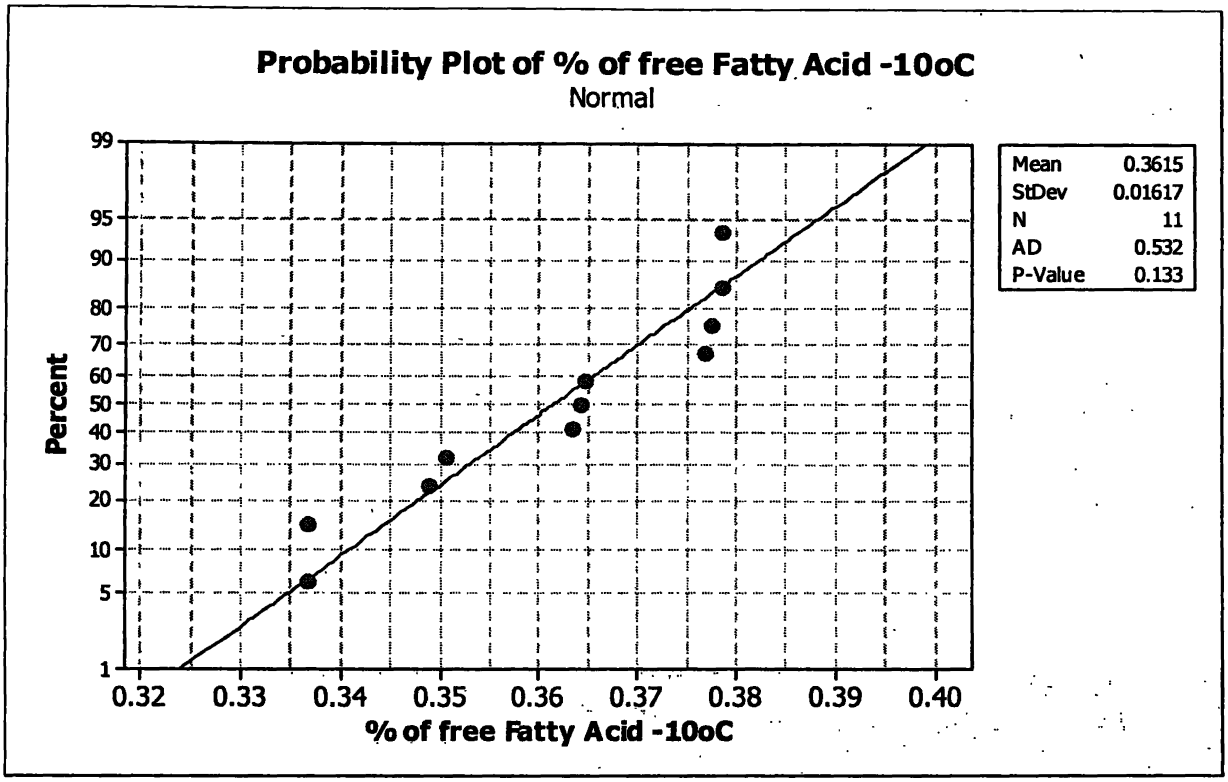
One-way ANOVA: responses versus Treatment

```
Source      DF         SS          MS      F      P
Treatment    2  0.0003217   0.0001608   5.70  0.009
Error       27  0.0007625   0.0000282
Total       29  0.0010842

S = 0.005314   R-Sq = 29.67%   R-Sq(adj) = 24.46%


                                Individual 95% CIs For Mean Based on
                                Pooled StDev
Level    N     Mean     StDev  ------+---------+---------+---------+---
1       10  0.10900  0.00699  (-------*--------)
2       10  0.11700  0.00483                    (-------*--------)
3       10  0.11250  0.00354           (-------*--------)
                                ------+---------+---------+---------+---
                                    0.1080    0.1120    0.1160    0.1200

Pooled StDev = 0.00531


Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 2.00

Intervals for level mean minus smallest of other level means

Level      Lower     Center     Upper  ----+---------+---------+---------+-----
1      -0.008247  -0.003500  0.001247  (-------*-------)
2       0.000000   0.008000  0.012747               (-----------*-------)
3      -0.001247   0.003500  0.008247            (-------*-------)
                                       ----+---------+---------+---------+-----
                                        -0.0060    0.0000    0.0060    0.0120
```

Sample A, B and C are three cream samples obtained from butter manufacturing line at three consecutive days and their corresponding average acidities are 0.109%, 0.117% and 0.112% respectively as shown in the table 4.1. P value (0.009) indicates that there is a day to day variation in % acidity and thus assuring the uniformity of end product is somewhat questionable.

APPENDIX II

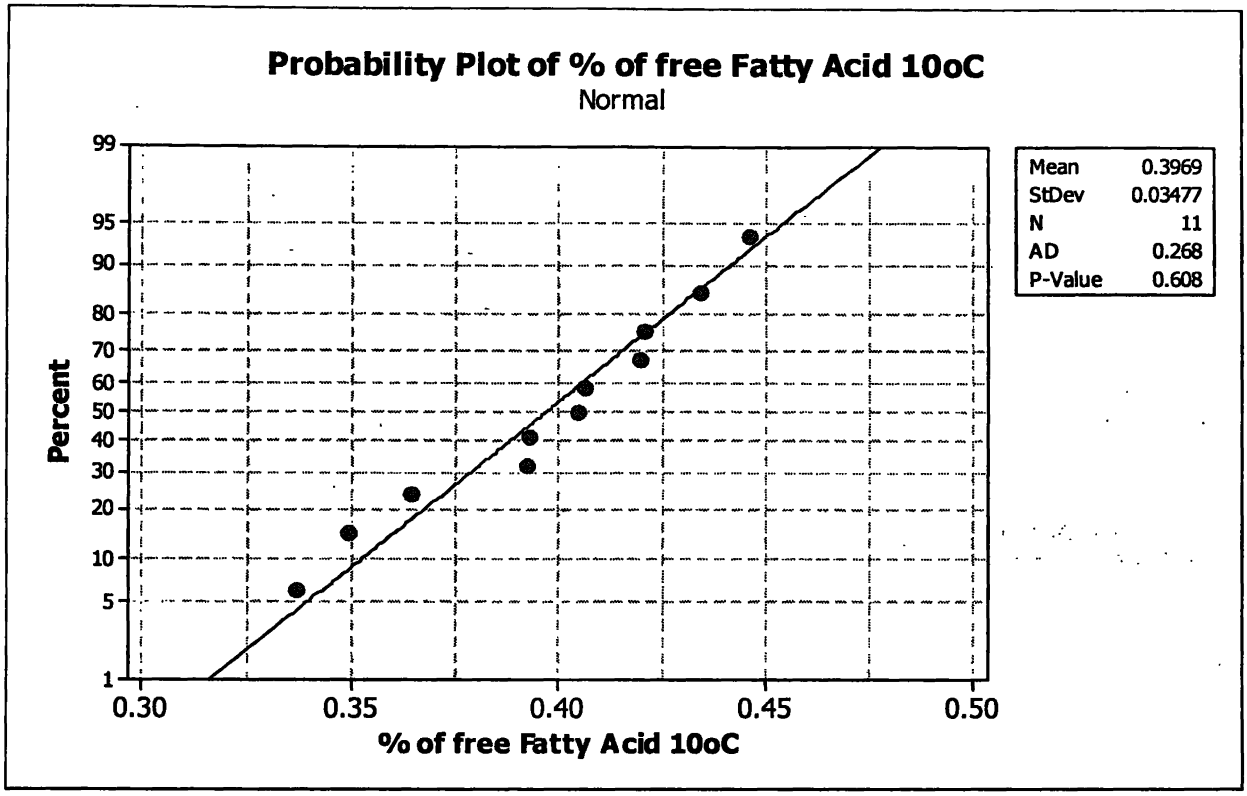
Normality test for butter sample stored in butter cold room (sample code A1)



P-value of normality test is 0.133. Significance level 0.05 < 0.133, therefore data is normally distributed.

APPENDIX III

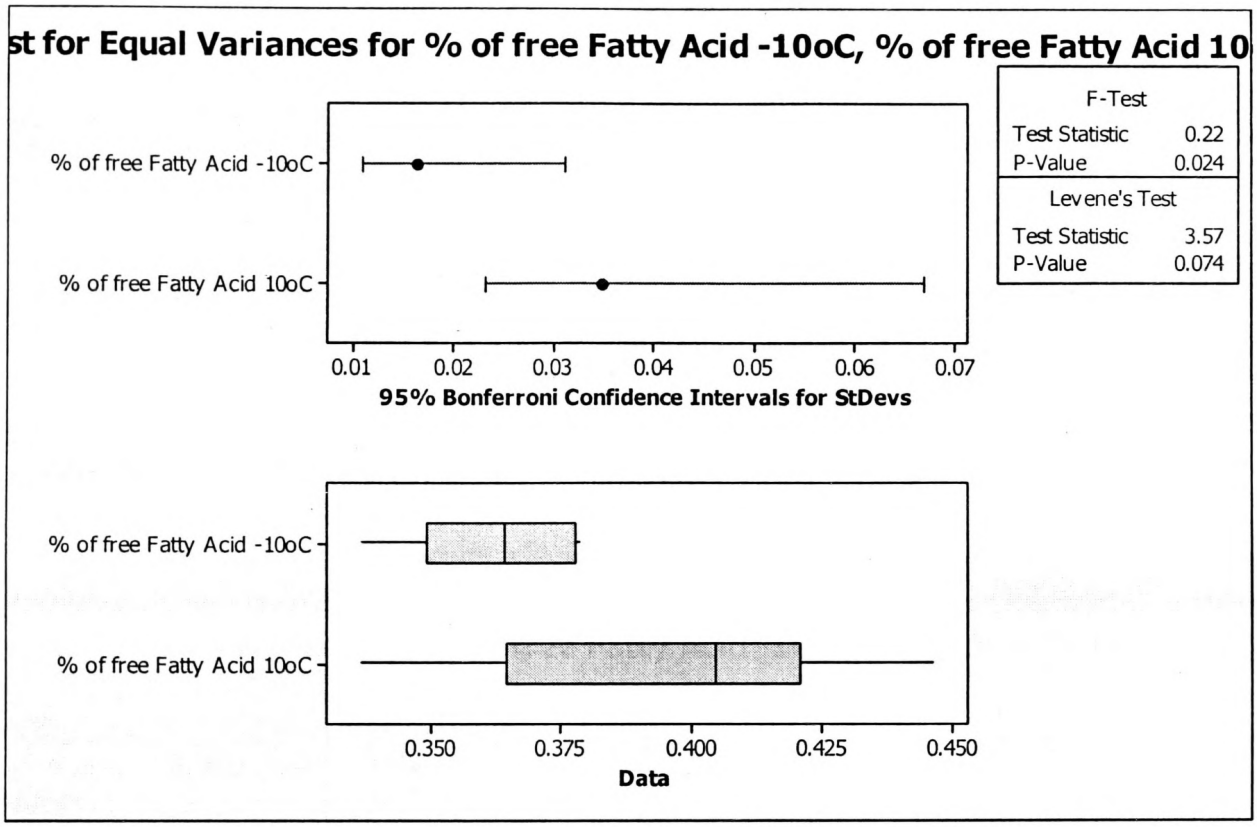
Normality test for butter sample stored in normal refrigerated condition (sample code A2)



P-value of normality test is 0.608. Significance level 0.05 < 0.608, therefore data is normally distributed.

APPENDIX IV

Variance test for butter samples stored in butter cold room and normal refrigerated condition (sample code A1 and A2)



Test for Equal Variances: % of free Fatty Acid -10oC, % of free Fatty Acid 10oC

```
95% Bonferroni confidence intervals for standard deviations

                              N      Lower      StDev      Upper
% of free Fatty Acid -10oC   11  0.0107634  0.0161660  0.0310700
 % of free Fatty Acid 10oC   11  0.0231480  0.0347670  0.0668199


F-Test (normal distribution)
Test statistic = 0.22, p-value = 0.024


Levene's Test (any continuous distribution)
Test statistic = 3.57, p-value = 0.074
```

Under variance test, p-value is 0.024. It is lower than significance level (0.024<0.05). Therefore variances are not equal.

APPENDIX V

Sample code A1 and A2 analyze using static's

Section 1

Two sample t-test for butter sample code A1 and A2

Two-Sample T-Test and CI: % of free Fatty Acid -10oC, % of free Fatty Acid 10oC

```
Two-sample T for % of free Fatty Acid -10oC vs % of free Fatty Acid 10oC

                    N    Mean   StDev  SE Mean
% of free Fatty    11  0.3615  0.0162   0.0049
% of free Fatty    11  0.3969  0.0348    0.010


Difference = mu (% of free Fatty Acid -10oC) - mu (% of free Fatty Acid
10oC)
Estimate for difference:  -0.035445
95% CI for difference:  (-0.060240, -0.010651)
T-Test of difference = 0 (vs not =): T-Value = -3.07  P-Value = 0.008  DF =
14
```

In t-test p-value is 0.008. P-value is less than α level. Therefore, % of free fatty acids in -10°C (A1) and % of free fatty acids in 10°C (A2) are statically significant different.

Section 2

Regression analysis of butter sample A1 and A2

Regression Analysis: % of free Fatty Acid -10oC versus Week

```
The regression equation is
% of free Fatty Acid -10oC = 0.338 + 0.00470 Week


Predictor       Coef    SE Coef       T      P
Constant    0.337986   0.002527  133.73  0.000
Week       0.0047027  0.0004272   11.01  0.000


S = 0.00448057   R-Sq = 93.1%   R-Sq(adj) = 92.3%


Analysis of Variance

Source           DF         SS         MS       F      P
Regression        1  0.0024327  0.0024327  121.18  0.000
Residual Error    9  0.0001807  0.0000201
Total            10  0.0026134
```

Regression Analysis: % of free Fatty Acid 10oC versus Week

```
The regression equation is
% of free Fatty Acid 10oC = 0.346 + 0.0102 Week
```

Predictor	Coef	SE Coef	T	P
Constant	0.345750	0.004430	78.04	0.000
Week	0.0102391	0.0007488	13.67	0.000

S = 0.00785380 R-Sq = 95.4% R-Sq(adj) = 94.9%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	0.011532	0.011532	186.96	0.000
Residual Error	9	0.000555	0.000062		
Total	10	0.012087			

Unusual Observations

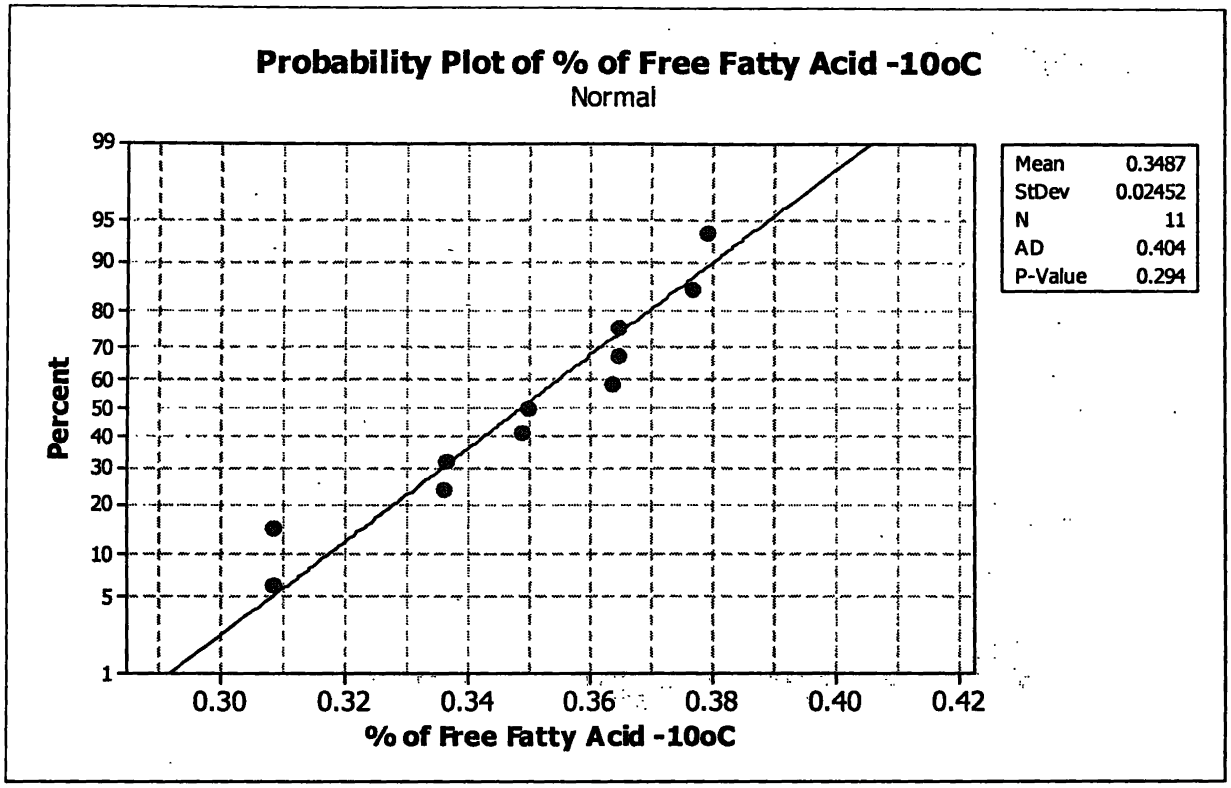
Obs	Week	% of free Fatty Acid 10oC	Fit	SE Fit	Residual	St Resid
4	3.0	0.39230	0.37647	0.00280	0.01583	2.16R

R denotes an observation with a large standardized residual.

Using above liner regression equation, shelf life of salted butter samples was calculated. It is 56 and 25 respectively.

APPENDIX VI

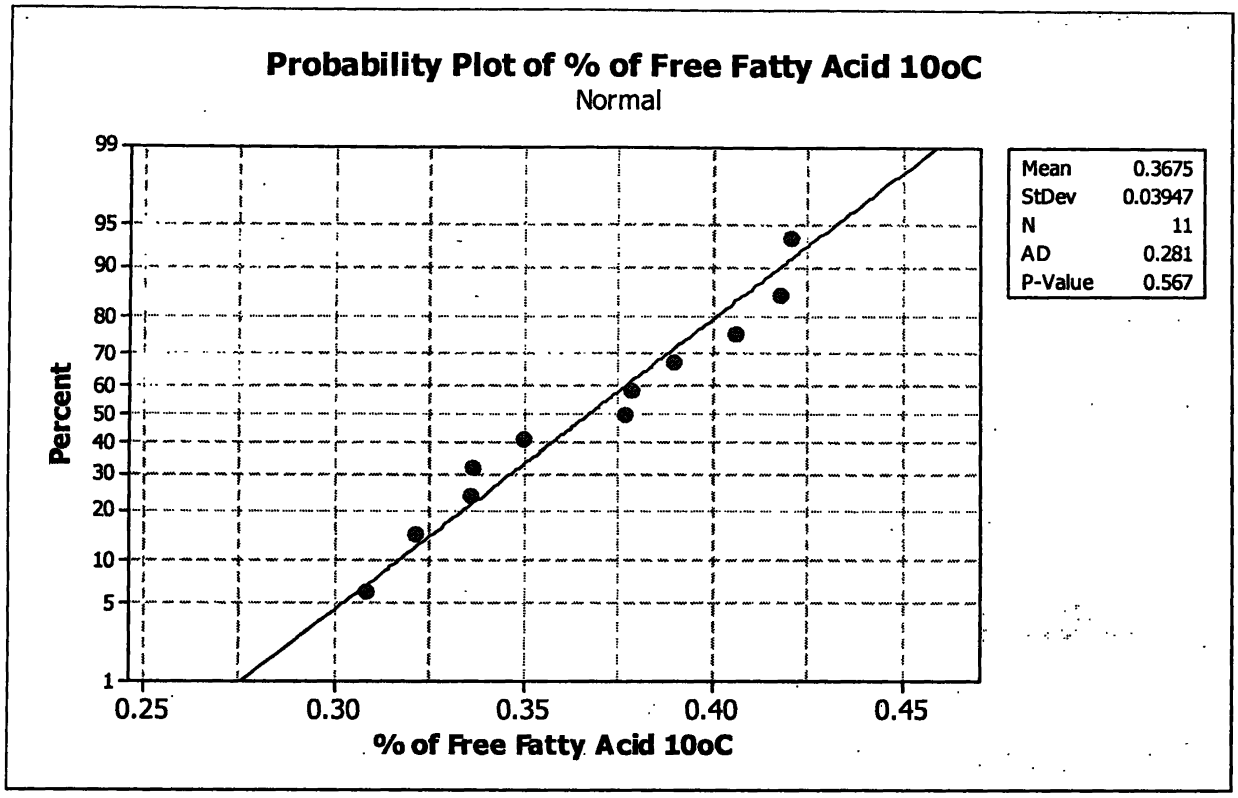
Normality test for butter sample stored in butter cold room (sample code B1)



P-value of normality test is 0.294. Significance level 0.05 < 0.294, therefore data is normally distributed.

APPENDIX VII

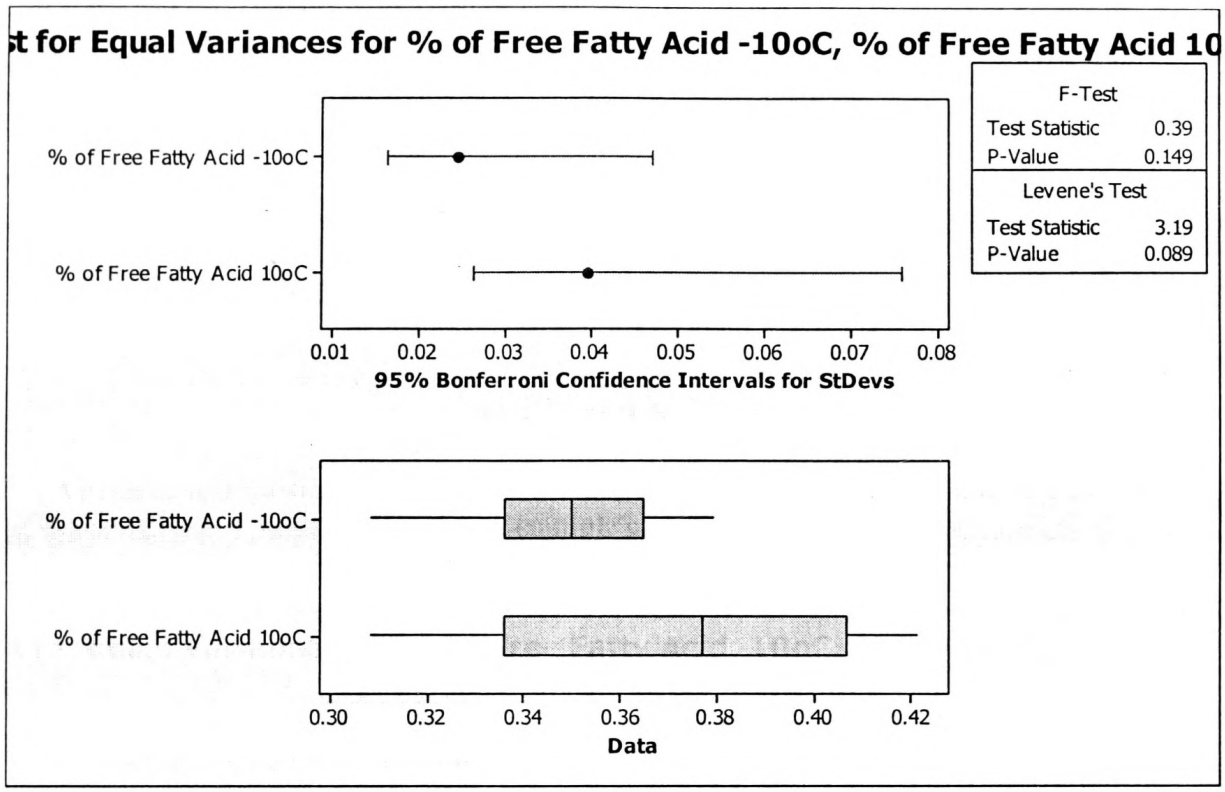
Normality test for butter sample stored in normal refrigerated condition (sample cod B2)



P-value of normality test is 0.567. Significance level 0.05 < 0.567, therefore data is normally distributed.

APPENDIX VIII

Variance test for butter samples stored in butter cold room and normal refrigerated condition (sample code A1 and A2)



Test for Equal Variances: % of Free Fatty Acid -10oC, % of Free Fatty Acid 10oC

```
95% Bonferroni confidence intervals for standard deviations

                              N      Lower      StDev      Upper
% of Free Fatty Acid -10oC   11  0.0163227  0.0245157  0.0471176
 % of Free Fatty Acid 10oC   11  0.0262777  0.0394675  0.0758540


F-Test (normal distribution)
Test statistic = 0.39, p-value = 0.149


Levene's Test (any continuous distribution)
Test statistic = 3.19, p-value = 0.089
```

Under variance test, p-value is 0.149. It is grater than significance level (0.149>0.05). Therefore variances are equal.

APPENDIX IX

Sample code B1 and B2 analyze using static's

Section 1

Two sample t-test for butter sample code B1 and B2

Two-Sample T-Test and CI: % of Free Fatty Acid -10oC, % of Free Fatty Acid 10oC

```
Two-sample T for % of Free Fatty Acid -10oC vs % of Free Fatty Acid 10oC

                 N    Mean   StDev  SE Mean
% of Free Fatty   11  0.3487  0.0245   0.0074
% of Free Fatty   11  0.3675  0.0395    0.012


Difference = mu (% of Free Fatty Acid -10oC) - mu (% of Free Fatty Acid
10oC)
Estimate for difference:  -0.018718
95% CI for difference:  (-0.047940, 0.010504)
T-Test of difference = 0 (vs not =): T-Value = -1.34  P-Value = 0.196  DF =
20
Both use Pooled StDev = 0.0329

MTB > Regress '% of Free Fatty Acid -10oC' 1 'Week';
SUBC>   Constant;
SUBC>   Brief 2.
```

In t-test p-value is 0.196. P-value is less than α level. Therefore, % of free fatty acids in -10°C (B1) and % of free fatty acids in 10°C (B2) are not statically significant different.

Section 2

Regression analysis of butter sample B1 and B2

Regression Analysis: % of Free Fatty Acid -10oC versus Week

```
The regression equation is
% of Free Fatty Acid -10oC = 0.313 + 0.00713 Week


Predictor       Coef    SE Coef      T      P
Constant    0.313068   0.003819  81.99  0.000
Week       0.0071336  0.0006455  11.05  0.000


S = 0.00676955   R-Sq = 93.1%   R-Sq(adj) = 92.4%


Analysis of Variance

Source          DF         SS         MS       F      P
Regression       1  0.0055978  0.0055978  122.15  0.000
Residual Error   9  0.0004124  0.0000458
Total           10  0.0060102

MTB > Regress '% of Free Fatty Acid 10oC' 1 'Week';
SUBC>   Constant;
SUBC>   Brief 2.
```

Regression Analysis: % of Free Fatty Acid 10oC versus Week

The regression equation is
% of Free Fatty Acid 10oC = 0.308 + 0.0118 Week

Predictor	Coef	SE Coef	T	P
Constant	0.308436	0.002979	103.54	0.000
Week	0.0118036	0.0005035	23.44	0.000

S = 0.00528105 R-Sq = 98.4% R-Sq(adj) = 98.2%

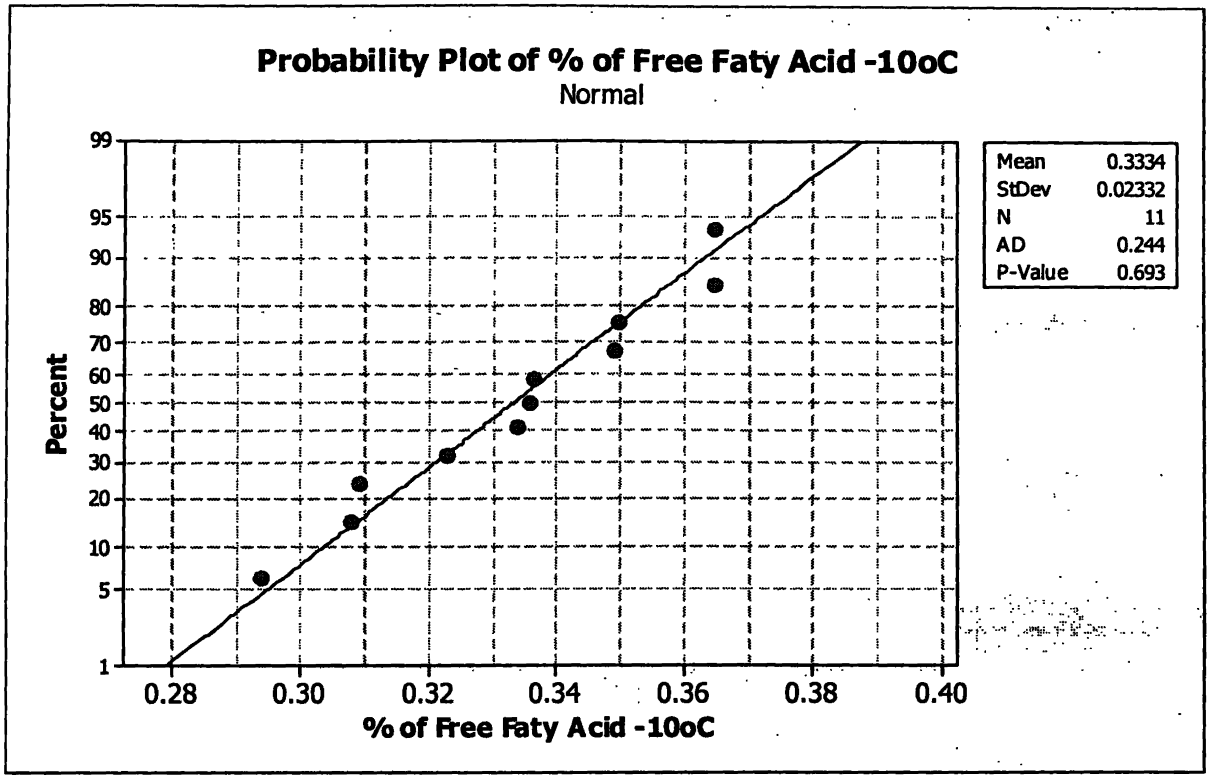
Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	0.015326	0.015326	549.52	0.000
Residual Error	9	0.000251	0.000028		
Total	10	0.015577			

Using above liner regression equation, shelf life of salted butter samples was calculated. It is 40 and 25 respectively.

APPENDIX X

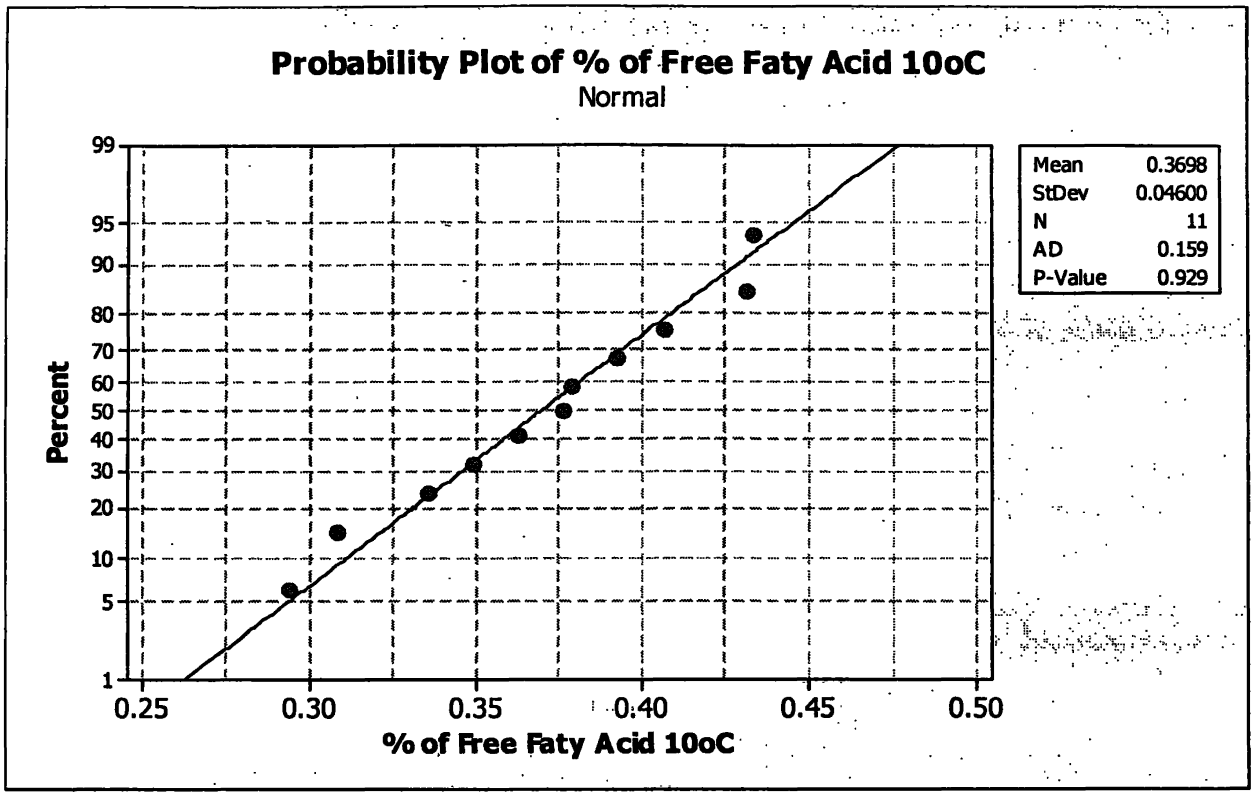
Normality test for butter sample stored in butter cold room (sample code C1)



P-value of normality test is 0.693. Significance level 0.05 < 0.693, therefore data is normally distributed.

APPENDIX XI

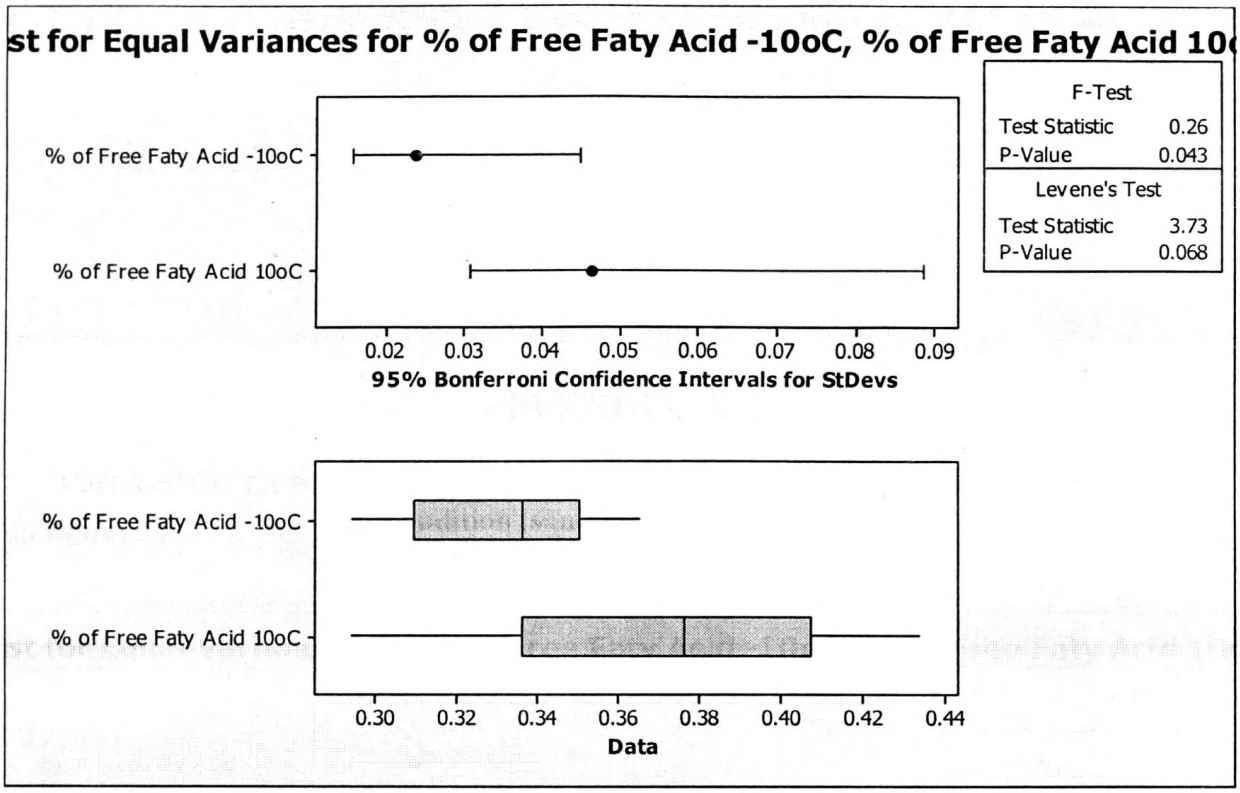
Normality test for butter sample stored in normal refrigerated condition (sample code C2)



P-value of normality test is 0.929. Significance level 0.05 < 0.929, therefore data is normally distributed.

APPENDIX XII

Variance test for butter samples stored in butter cold room and normal refrigerated condition (sample code C1 and C2)



Test for Equal Variances: % of Free Faty Acid -10oC, % of Free Faty Acid 10oC

```
95% Bonferroni confidence intervals for standard deviations

                              N      Lower      StDev      Upper
% of Free Faty Acid -10oC    11  0.0155278  0.0233218  0.0448230
 % of Free Faty Acid 10oC    11  0.0306280  0.0460014  0.0884117


F-Test (normal distribution)
Test statistic = 0.26, p-value = 0.043


Levene's Test (any continuous distribution)
```

Under variance test, p-value is 0.024. It is lower than significance level (0.024<0.05). Therefore variances are not equal.

APPENDIX XIII

Sample code C1 and C2 analyze using static's

Section 1

Two-Sample T-Test and CI: % of Free Faty Acid -10oC, % of Free Faty Acid 10oC

```
Two-sample T for % of Free Faty Acid -10oC vs % of Free Faty Acid 10oC

                    N    Mean   StDev  SE Mean
% of Free Faty A   11  0.3334  0.0233   0.0070
% of Free Faty A   11  0.3698  0.0460    0.014


Difference = mu (% of Free Faty Acid -10oC) - mu (% of Free Faty Acid 10oC)
Estimate for difference:  -0.036482
95% CI for difference:  (-0.069835, -0.003129)
T-Test of difference = 0 (vs not =): T-Value = -2.35  P-Value = 0.034  DF = 14

MTB > Regress '% of Free Faty Acid -10oC' 1 'Week';
SUBC>   Constant;
SUBC>   Brief 2.
```

In t-test p-value is 0.034. P-value is less than α level. Therefore, % of free fatty acids in -10°C (C1) and % of free fatty acids in 10°C (C2) are statically significant different.

Section 2

Regression analysis of butter sample C1 and C2

Regression Analysis: % of Free Faty Acid -10oC versus Week

```
The regression equation is
% of Free Faty Acid -10oC = 0.299 + 0.00692 Week


Predictor       Coef    SE Coef       T      P
Constant    0.298773   0.002483  120.35  0.000
Week       0.0069182  0.0004196   16.49  0.000


S = 0.00440112   R-Sq = 96.8%   R-Sq(adj) = 96.4%


Analysis of Variance

Source          DF         SS         MS       F      P
Regression       1  0.0052647  0.0052647  271.80  0.000
Residual Error   9  0.0001743  0.0000194
Total           10  0.0054391

MTB > Regress '% of Free Faty Acid 10oC' 1 'Week';
SUBC>   Constant;
SUBC>   Brief 2.
```

Regression Analysis: % of Free Faty Acid 10oC versus Week

The regression equation is
% of Free Faty Acid 10oC = 0.301 + 0.0137 Week

Predictor	Coef	SE Coef	T	P
Constant	0.301191	0.003863	77.97	0.000
Week	0.0137309	0.0006530	21.03	0.000

S = 0.00684838 R-Sq = 98.0% R-Sq(adj) = 97.8%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	0.020739	0.020739	442.20	0.000
Residual Error	9	0.000422	0.000047		
Total	10	0.021161			

Using above liner regression equation, shelf life of salted butter samples was calculated. It is 43 and 22 respectively.

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