To my mother and father

STABILITY OF BUTTER OILS

A MASTER'S THESIS

in

Food Engineering

University of Gaziantep

by

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ABSTRACT

STABILITY OF BUTTER OILS

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The physical and chemical characteristics and thermal stability of butter oils produced from sheep's and cow's milk by using different production methods were studied. Thermal stability of samples was estimated by using Accelerated Shelf-life Testing Method.

Results were compared for sheep's butter oils produced from (i) pasteurized and nonpasteurized milk, (ii) pasteurized milk and sweet yogurt and (iii) sweet and sour yogurt, and for cow's butter oils produced from (i) pasteurized milk and sweet yogurt, (ii) sweet and sour yogurt, and(iii) sweet yogurt using 3 and 6 h of melting.

Samples were stored at 60, 70 and 80°C in the dark and the reaction was monitored by measuring peroxide, thiobarbituric acid and free fatty acid values. The peroxide and thiobarbituric acid values increased as the temperature increased. The increase of the acid value of all samples was not significant. A slight increase in free fatty acid value showed that hydrolytic reaction was not responsible for the deterioration of butter oil samples in thermal stability studies. Oxidative changes compared to hydrolytic changes was of greater significance in thermal stability of butter oil samples.

Thermal stability was highest in butter oil produced from sweet yogurt (27.0 days at 60°C) using sheep's milk. Butter oil produced from sweet yogurt using 6 h of melting time had higher thermal stability (37.1 days at 60°C) when it was compared to the other butter oils prepared from cow's milk. Butter oil produced from cow's milk had higher

thermal stability than that sheep's milk. Thermal stabilities were highest in butter oils produced in November and December.

Activation energies were estimated from PV data using Arrhenius equation.

Keywords: butter oil, thermal stability, accelerated shelf-life testing, peroxide value, thiobarbituric acid value, free fatty acid value.

ÖZET

SADEYAĞLARIN DAYANIRLILIĞI

ÖZBAYRAM, Oya
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Farklı metotlar kullanılarak koyun ve inek sütlerinden üretilen sadeyağlarının fiziksel ve kimyasal özellikleri ve ısıl dayanırlılığı çalışıldı. Sadeyağı numunelerinin ısıl dayanırlığı Hızlandırılmış Raf Ömrü Test Metodu kullanılarak tahmin edildi.

Sonuçlar (i) pastörize ve pastörize edilmeyen süt, (ii) pastörize ve tatlı yoğurt ve (iii) tatlı ve ekşi yoğurttan üretilen koyun sadeyağları ve (i) pastörize süt ve tatlı yoğurt, (ii) tatlı ve ekşi yoğurt ve (iii) tatlı yoğurt 3 ve 6 saat eritme zamanı kullanılarak üretilen inek sadeyağları için karşılaştırıldı.

Numuneler 60, 70 and 80°C lerde karanlıkta saklandı ve reaksiyon peroksit, tiyobarbitürik asit and serbest yağ asit değerleri ölçümleriyle takip edildi. Peroksit ve tiyobarbitürik asit değerleri sıcaklık artışı ile yükseldi. Tüm numunelerde asit değerindeki artma önemli değildi. Serbest yağ asit değerinde önemli olmayan artma ısıl dayanırlılık çalışmalarında, hidrolitik reaksiyonun sadeyağlarının bozulması için sorun olmadığını gösterdi. Oksidasyon değişimi hidrolitik değişimle kıyaslandığında sadeyağı numunelerinin ısıl dayanırlılığında daha önemliydi

Koyun sütü kullanılarak tatlı yoğıuttan üretilen sadeyağında ısıl dayanırlılık daha yüksekti (60°C de 27 gün). İnek sütü kulanılarak elde edilen diğer sadeyağları ile kıyaslandığı zaman tatlı yogurtan 6 saat eritme zamanı kullanılarak üretilen sadeyağı daha yüksek ısıl dayanırlılığa sahipti (60°C de 37.1 gün). İnek sütü kullanılarak üretilen sadeyağı koyun sütünden üretilen sadeyağından daha yüksek ısıl dayanırlılığa sahipti.

Kasım ve Aralık'ta üretilen sadeyağlarında ısıl dayanırlılıklar daha yüksekti.

Aktivasyon enerjileri peroksit değerlerinden Arrhenius eşitliği kullanılarak tahmin edildi.

Anahtar kelimeler: sade yağ, ısıl dayanırlılık, hızlandırılmış raf ömrü testi, peroksit değeri, tiyobarbitürik asit değeri, serbest yağ asit değeri.

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ABBREVIATIONS

AOM	: Active Oxygen Method
ASLT	: Accelerated Shelf Life Test
CB	: cream butter
CLA	: conjugated linoleic acid
CLY	: butter oil produced from cow's sour yogurt
CPB	: butter oil produced from cow's pasteurized milk in November
CSY1	: butter oil produced from cow's sweet yogurt in November
CSY2	: butter oil produced from cow's sweet yogurt in May
CSYM1	: butter oil produced from cow's sweet yogurt using 3 h melting
CSYM2	: butter oil produced from cow's sweet yogurt using 6 h melting
DC	: direct cream
FAME	: fatty acid methyl ester
FFA	: free fatty acid
HDL	: good cholestrol
HTST	: high temperature short time
LDL	: bad cholesterol
MA	: malonaldehyde
MB	: milk butter
OAM	: Oxygen Absorption Method
SLY	: butter oil produced from sheep's sour yogurt in September
SNB	: butter oil produced from nonpasteurized milk
SPB1	: buttter oil produced from sheep's pasteurized milk in March
SPB2	: butter oil produced from sheep's pasteurized milk in May
SSY1	: butter oil produced from sheep's sweet yogurt in May
SSY2	: butter oil produced from sheep's sweet yogurt in September
PS	: prestratification

PV : peroxide value

TBA : thiobarbituric acid value

CHAPTER I
INTRODUCTION

1.1. Butter oil

1.1.1. Definition of Butter Oil (Anhydrous Milk Fat)

The clarified butter, more properly called 'butter oil' in many parts of India and Egypt, is usually prepared from cow's milk, buffalo milk or mixed milk. Butter oil is the anhydrous form of milk fat. In the Middle East, butter oil is commonly made from goat, sheep or camel milk and it is known as 'maslee' or by some variant of the Arabic term 'samn'. In India, Africa, and other tropical regions butter oil is commonly marketed and used in an anhydrous form, after separation of the fat from the milk serum by heating and settling [1-6].International Dairy Federation (IDF), defined butter oil as a product exclusively obtained from milk, cream or butter from various animal species. It is obtained by means of processes which result in the almost total removal of moisture and solids-not-fat and which gives the product a particular physical structure.

Butter oil is the anhydrous form of milk fat produced in Europe and the U.S.A. Butter oil is prepared by melting butter between a temperature range of 50-80°C, whereas the similar product 'ghee' which is produced in Egypt, India and other Middle East Asian is prepared by melting butter at 110-140°C [2,4,7]. The making butter oil is mostly a home industry. It is produced at home and cottage levels from cow's, sheep's and goat's milk butter, after the separation of the fat from the milk serum by heating and settling. On a small scale, butter oil is made by a rendering process. The butter is melted slowly and the fat is allowed to separate by gravity from the serum. The serum is drained off and oil is drawn off into containers [7,8].

1.1.2. Nutritional Aspects of Butter Oil

The body can make use of the carbohydrate, protein, and fat in the diet interchangeably and so use one to compensate for the lack of another. Protein in the diet is not used to supply caloric energy that should be furnished by the carbohydrate and fat in the diet. A diet in which the fat supplies 20 % of the total calories appears to furnish an adequate supply for human requirements. They are most concentrated as dietary energy source providing 9 kcal/g more than twice that supplied by proteins and carbohydrates.

Besides their many functional roles in foods, fats also have positive nutritional aspects. Fats carry and aid the absorption of the fat-soluble vitamins: A, D, E and K. Fat is vital to the growth and development of the human body [9]. Milk fat is unusual as a source of fat. It is made by mammary tissue to nourish a young, growing calf. This is in contrast to all other sources of edible fats and oils, which are synthesized largely to nourish plants or to store fat in animals. It is important, therefore, to focus on the nutritional properties that are unique to milk fat. Milk fat is a source of lipid nutrients, fat-soluble vitamins and essential fatty acids. About 70 % of the fatty acids in milk are saturated, of which about 60 % are long-chain fatty acids. The monoenes, mainly 18:1, constitute most of the remainder, with the dienes and trienes together only accounting for about 3 % [1]. Milk fat is more easily digested and larger amounts of it can be absorbed without producing a digestive disturbance than any other common, edible fat.

Milk fat enhances the digestion and distribution of its own fatty acids and fat-soluble vitamins. These are host of fatty acids in varying amounts in milk fat that have potential to influence digestion, nutrient absorption and metabolism. The structure of milk fat enhances the digestion and distribution of these fatty acids. Milk fat may do the same for fat-soluble vitamins. Since several antioxidant vitamins (which consumers recognize as important in disease prevention) are fat soluble, this is an important attribute. While milk fat tends to raise the ''bad cholesterol'' (LDL) in humans, milk fat tends to raise the ''good cholesterol'' (HDL) as well [1].

Conjugated linoleic acids (CLA), and butyric acids are other potentially beneficial components of milk fat. Although CLA is present in small amounts in milk, it appears to be in a biologically active form. Giving CLA to laboratory animals has revealed potent anti-cancer effects [1]. Milk fat is a significant source of dietary butyric acid. It was previously thought that the only value of butyric acid is a source of energy, it also exhibits a very interesting additional effect. Butyric acid promotes the differentiation of the cells that line the intestinal wall. This has two benefits. Normal cells are healthier and more active. Even more intriguing is that by promoting differentiation of transformed cells, butyric acid appears to cause these pre-cancerous cells to die [1,2].

1.1.3. Utilization of Butter Oil

A major portion of butter oil is utilized for culinary cooking and frying of different foods. Uses of various products related to ghee or butter oil have been documented from different parts of the world [1]. In the USA, chocolate containing partially lipolysed butter oil is actually preferred. Also, butter oil is added to shortbread to increase its buttery flavor. When butter oil is fractionated, flavors are preferentially fractionated into the lower melting fraction. This makes the low melting fraction particularly useful for applications where high flavor intensity is desirable.

Butter oil is used in France and elsewhere in Western Europe for shallow frying. In deep-frying, butter oil has the advantage over vegetable oils in that it has superior flavor and oxidative stability. It contains protective agents, which contribute to its own stability and, in blends, protect other less stable oils during deep-frying. As an industrial ingredient, anhydrous milk fat can be used for recombining skim milk powder into fluid milk and is also used widely in the ice cream and chocolate industries and in the production of baby foods and ready-cooked meals. Ice cream manufactured with butter oil has an enhanced creamy flavor and that rich smooth moist texture associated with the highest quality ice cream. Bakery applications such as cakes, puff pastries and cookies can benefit from milk fat fractions. European bakers have been using milk fat fractions to give their croissants and puff pastries lighter and flakier texture. High melting fraction of milk fat can increase storage stability of preventing fat bloom, a whitish discoloration and texture change caused by unstable cocoa crystals [1,2].

1.1.4. The Structure and Nature of the Fat of Milk

Milk fat exists in milk in the form of minute globules in a true emulsion of the oil-in-water type, the fat globules, being in the dispersed phase. Milk fat is excreted in the form of droplets ranging in size from 1-12 mm in diameter with a mean size of about 4 mm. These globules comprise almost exclusively triglyceride but are stabilized by complex surface membrane rich in phospholipids [10,11].

Each globule of fat is surrounded by a very thin film of protein, or the serum of milk, concentrated on the surface and held in place by surface attraction or adsorption. This concentration of the milk proteins around the fat globule is one factor assisting in maintaining the stability of the fat emulsion in milk. The concentrated layer surrounding the fat globules is composed of certain protein and fat-like substances, especially lecithin, a substance having many of the properties of fat, and found in milk in small quantities.

Milk fat is not a single chemical compound but a variable mixture of several different glycerides. Each glyceride is the result of the union of glycerol and one or more organic acids. The organic acids contained in milk fat and fats from other sources other than milk fat are commonly termed 'fatty acids'.

Glycerol is written $C_3H_5(OH)_3$ and butyric acid, for example, is written, C_3H_7COOH. Milk fat is a triglyceride. This is true for most of animal fats. It means that 1 molecule of glycerol is combined with 3 molecules of the fatty acid. When it is realized that glycerol combines with one or more of the fatty acids to form the glyceride of the fatty acid, it may be exceedingly complex [11].

1.1.4.1. Fatty Acids of Milk Fat

It has been the common practice to divide fatty acids of milk into two general groups, the volatile and the non-volatile. While these terms are not strictly accurate as applied to the fatty acids of milk fat, this grouping serves a useful purpose in indicating general properties.

The volatile acids include: butyric, caproic, caprylic, capric, lauric and small quantities of others. Butyric, caprylic, and capric of this group are classed as soluble fatty acids. Non-volatile are: myristic, palmitic, oleic, stearic and small quantities of a few others. The glycerides of the volatile group make up about 17 % of the total in milk fat. This group of fats gives the characteristic flavors to butter and cream. Butyric acid is the most important acid found in this group. Butyric acid contributes to butter its characteristic taste and, on being liberated, is largely responsible for the rancid flavor which sometimes occurs in dairy products.

Fatty acid distribution of milk fat is given in Table 1[2].

Table 1. Fatty acid distribution of milk fat.

Soluble acids	Formula	Average percentage	Range
Butyric	C_3H_7COOH	2.932	2.410 to 4.230
Caproic	$C_5H_{11}COOH$	1.898	1.290 to 2.400
Caprylic	$C_7H_{15}COOH$	0.786	0.527 to 1.041
Capric	$C_9H_{19}COOH$	1.570	1.187 to 2.008
Insoluble acids			
Lauric	$C_{11}H_{23}COOH$	5.849	4.533 to 7.687
Myristic	$C_{13}H_{27}COOH$	19.784	15.554 to 22.618
Palmitic	$C_{15}H_{31}COOH$	15.167	5.782 to 22.863
Stearic	$C_{17}H_{35}COOH$	14.907	7.803 to 20.370
Oleic	$C_{17}H_{33}COOH$	31.895	25.273 to 40.313

The glycerides of the fatty acids found in the non-volatile group constitute 82.7 % of milk fat. This group of fatty acids is particularly important because of their relation to the hardness or softness of milk fat and their ultimate relation to the texture of butter. Oleic acid is the most variable of this group, and likewise possesses the lowest melting point. It is, therefore, the chief acid influencing the hardness or softness of milk fat.

Milk fat probably contains a larger number of the fatty acids than any other fat found in nature. It is, therefore, one of the most complex natural fats known in nature.

1.2. Other Constitutes of Milk

1.2.1. Gross Composition of Milk

Milk is the liquid food secreted by the mammary gland for the nourishment of the newly born, containing water, fat, proteins, lactose, and minerals (ash).The principal constituent of milk including fat, protein, milk sugar or lactose, and the minerals of milk which collectively are referred to as ash. Table 2 presents a general idea for the composition of cow's milk [12].

1.2.1.1. Water in Milk

Milk is a natural liquid food containing a high percentage of water (87 %). Milk is actually concentrated food designed to produce rapid growth in young mammals and contains more solid material than many of our other common foods. Water is the medium in which all the other components of milk (total solids) are dissolved or suspended. A small percentage of the water in milk is hydrated to the lactose and salts; and some is bound in the proteins [12].

Table 2. Composition of cow's milk.

Component	Composition (%)	Micro components
Fat	3.75	Some diglycerides but mainly triglycerides(C_4-C_{18}, $C_{18\text{-}2}$, $C_{20\text{-}3}$)
Phospholipids	0.05	Lecithin, Cephalin, Sphingomyelin
Proteins	3.35	Caseins:2.78 % α-casein:1.67 %; β-casein:0.62 %; α-casein:0.12 %; β-casein:0.37 % Whey proteins:0.60 % α-lactalbumin:0.13 %; α-lactoglobulin:0.35 %; immunoglobulin:0.08 %; serum albumin:0.04 %
Lactose	4.95	Milk sugar
Salts (minerals)	0.90	Calcium, magnesium, sodium, potassium, iron, Copper, phosphates, chlorides, sulphates
Water	87	

1.2.1.2. Proteins in Milk

Although a substantial number of individual proteins are found in milk, they are usually classified into two distinct groups: caseins and whey proteins. Cow's milk contains approximately 3.5 % protein (2.9 % casein and 0.6 % whey proteins). Casein may be defined most simply as the protein precipitated by acidifying skim milk to a pH value near 4.6 at 20°C. The proteins remaining after casein has been removed from skim milk are known as 'whey proteins' or 'milk-serum' proteins. The principal protein of milk, casein, makes up 80 % of the total, while serum proteins, lactalbumin and lactoglobulin, make up the remaining 20 %. The α– and β-caseins are phosphoproteins with 5-13 serine phosphate residues per molecule, while the d-caseins are fragments of β-casein. κ-casein is a glycoprotein [10,12].

1.2.1.3. Lactose in Milk

Lactose is the major component of the solids in milk, typically comprising 4.5-4.9 %. It is a disaccharide consisting of β-D-galactose linked to α-D-glucose. Lactose is a reducing sugar and, under appropriate conditions of pH, temperature and water activity, will react with free amino groups in milk-protein. This is called the Maillard reaction and is in fact a complex series of reactions. The first stage involves glycosylamine formation and rearrangement. This is followed by dehydration to yield furfurals, fission and further degradation. The final stage consists of conversion of the degradation products into high molecular weight compounds. These reactions limit the shelf life of some dried dairy products [12].

1.2.1.4. Minerals in Milk

The mineral content of milk is an analytical value indicating the amount of noncombustible matter in milk. In normal milk the value remains rather constant at about 0.7 % [12]. The mineral system found in milk is almost as complex as that of the protein.

Main minerals in milk are: calcium, magnesium, sodium, potassium, chloride, inorganic phosphate, and citrate. Sodium, potassium and chloride are the main monovalent ions and, together with lactose, these ions regulate the total ionic strength of milk- such regulation is a biological necessity to maintain milk in isotonic equilibrium with blood and intracellular fluid in the mammary gland. Therefore, chloride concentrations in milk are at their lowest at the peak of lactation and increase towards the end of lactational cycle. Variations in the total calcium and phosphate concentration are less marked and are related to the total protein content of milk. The citrate concentration of milk is important because the ion is a very effective buffer of both calcium and hydrogen ions [10,12].

1.2.1.5. Acidity of Milk

Fresh milk is slightly acid in reaction, having a pH of 6.5 to 6.7. Because of many variables, it is difficult to give a definite value for titratable acidity, but normal milk usually varies between 0.15 and 0.18 % expressed as lactic acid.

1.2.1.6. Enzymes of Milk

Milk contains a number of enzymes. An enzyme is a biologic catalyst elaborated by a living cell, milk enzymes being elaborated by the cells of the mammary tissue. The enzymes produced by bacteria in the milk cannot be considered as normal components of milk. Enzymes are proteins. They are denatured and inactivated by high temperatures, they possess a pH of optimum activity, and they exhibit specificity for certain substrates. The principal, naturally occurring enzymes in milk are catalase, peroxidase, xanthine oxidase, alkaline and acid phosphatases amylases, proteases, lipases, and aldolase [1,3].

1.3. Manufacture of Butter Oil

The first step of butter oil manufacture involves the preparation of the raw material, i.e. whole milk, cream or butter. If milk is the starting material, it is normally allowed to ferment before it is churned to butter. Methods of butter oil manufacture vary with respect to the material used (milk, cream, butter), the intermediate treatment of raw materials, and the handling of the semi-finished or fully formed product.

Four methods of butter oil making are known: the indigenous milk butter (MB) process (desi method), the direct cream (DC) method, the cream butter (CB) method, and the prestratification (PS) method; as illustrated in Figure 1 [1,4].

In the milk butter method, also known as the desi method, sour whole raw milk is churned to butter. The butter is then converted to butter oil. The desi method involves the souring of raw milk in earthenware vessels which have been used previously as a milk container, and which contain an inoculum of bacteria within pores of the wall. The fermented milk is churned to butter. The butter is then boiled in an open pan to allow evaporation of the water without charring the proteins. The butter oil is transferred while hot and stored in earthen vessels [13].

In the direct cream method, which is a technologically improved method, fresh cream, cultured cream or washed cream may be used. This process omits the butter production step as the cream is directly converted to butter oil [1].

The cream butter method is a three-step process of butter oil manufacture whereby milk is separated into cream, which is then churned into butter or butter granules which are then processed into butter oil.

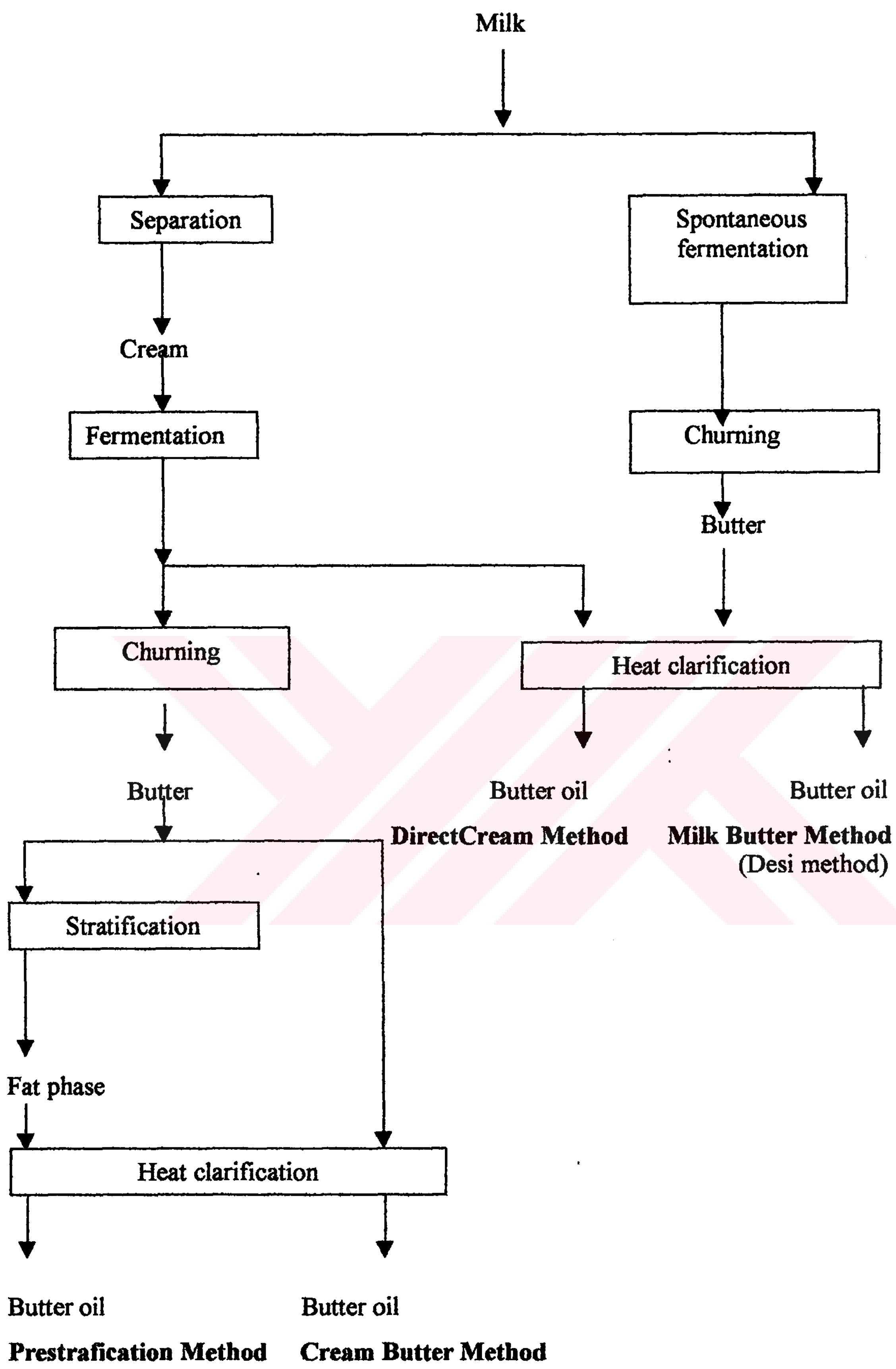


Figure 1. Flow diagram illustrating four methods of butter oil manufacture.

The churning operation is judged to be at an end when the granules of butterfat are of about pea size. At this point churning is stopped, the buttermilk is drained off [1].

The prestratification method which is also known as the 'clarified butter method' is particularly suitable if large quantities of butter are available. The method involves melting butter at 80-85°C for 30 min to separate it into the top layer composed of floating denatured protein particles and impurities probably suspended by entrapped air bubbles, the middle layer of almost clear fat, and the bottom layer of buttermilk serum. The buttermilk is drained off and the fat layer is heated together with the top layer to a temperature of 110°C to remove more moisture and developed flavor [1,4].

1.3.1. Pasteurization of Milk

Pasteurization is the process of heating a foodstuff, usually a liquid, for a definite time at a definite temperature and thereafter cooling it immediately. Pasteurization means the exposure of all products to a heat treatment, which will destroy all pathogenic microorganisms and nearly all other bacteria, and yet not alter the flavor or composition of the product. All yeasts and molds generally are killed by pasteurization. Pasteurization is not same as sterilization because it usually destroys only 95 to 99 % of the bacteria present, whereas in sterilization the heat treatment is sufficient to destroy completely all-living organisms present. Pasteurization is defined to mean that every particle of a dairy product shall have been heated in properly operated equipment to specified temperature and held continuously at or above that temperature for the specified time [2]. Pasteurization also destroys lipase and other natural milk enzymes.

The two accepted methods for milk pasteurization are (1) the batch (holding) method of heating every particle of milk to not less than 63°C and holding at this temperature for not less than 30 min and (2) the high temperature-short time (HTST) method of heating every particle of milk to not less than 72°C (161°F) and holding for not less than 15 sec [11]. Pasteurized milk is not sterile and so it must be quickly cooled to prevent multiplication of surviving bacteria. Pasteurization at these temperatures does not produce an objectionable cooked flavor in milk and has no important effect upon the nutritional value of milk. Batch pasteurization is carried out in heated vats provided with an agitator to ensure uniform heating, a cover to prevent contamination during the holding period, and a recording a thermometer to trace a permanent record of the time-temperature treatment. But batch pasteurization of milk has been replaced with HTST pasteurization. In HTST pasteurization, raw milk held in a cool storage tank is pumped through a plate-type heat exchanger and brought to temperature. The key to the process rests in ensuring that every particle of the milk remains at not lower than 71.7°C for no less than 15 sec. This is accomplished by pumping the heated milk through a holding tube of such length and diameter that it takes every milk particle at least 15 sec to pass through the tube [14].

1.3.2. Fermentation (Yogurt)

1.3.2.1. Yogurt Technology

Yogurt is pasteurized milk or low-fat milk coagulated to a custard-like consistency with a mixed lactic acid culture containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus.* These organisms grow together symbiotically, which are responsible for the lactic acid fermentation.

The growth association between the two organisms (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) of the yogurt starter culture is termed a symbiosis. High heat treatment of the milk used in the manufacturing yogurt is essential to denature whey proteins, thus increasing the capacity of the proteins to bind water and liberate amino acids. These promote the growth of *L. bulgaricus. L. bulgaricus* enzymatically hydrolyzes proteins providing amino acids, especially valine that stimulate the growth of *S.thermophilus. S.thermophilus* uses oxygen, thus making the oxygen tension more favorable for *L.bulgaricus.*[5,15].

High incubation temperatures (41 to 45°C) are required by these bacteria, and their balance in culture is determined by the temperature selected. The release of stimulatory factors by yogurt starter cultures takes place during the incubation period, and while *L.bulgaricus* provides the essential nutrients, i.e. amino acids for *S.thermophilus*, the latter produces a formic acid-like compounds which promote the growth of *L.bulgaricus*. It is also noticeable that the rate of acid development of *S.thermophilus* and *L.bulgaricus* increases with increase in incubation temperature, up to maxima relation to acid production. Although the activity of mixed strains is optimum at 45°C, it is recommended that, in order to maintain and/or achieve a ratio of 1:1 between *S.thermophilus* and *L.bulgaricus*, the organisms should be propagated together at 42°C using a 2 % inoculation rate. The catabolism of lactose by *S.thermophilus* and *L.bulgaricus* results mainly in the production of lactic acid and although the conversion process consists of many different biochemical reactions, it can be simplified by the following reaction:

$$\underset{\text{Lactose}}{C_{12}H_{22}O_{11}} + \underset{\text{Water}}{H_2O} \longrightarrow \underset{\text{Lactic acid}}{4C_3H_6O_3}$$

1.3.2.2. Fermentation Process

During the manufacture of yogurt, the heat-treated milk is cooled to the incubation temperature of the starter culture (*S.thermophilus* and *L.bulgaricus*), and in general the milk is fermented at 40-45°C, i.e. the optimum growth condition for the mixed culture (the short incubation method). In some cases the incubation period can be as short as 2.5 hours, assuming that the starter culture (3 %) is an active one method and the ratio between rods and the cocci is well balanced. However, the longer incubation method, i.e. overnight, can be used and the incubation conditions are 30°C for around 18 hours or until desired acidity is reached [16]. While the cooled milk is pumped to the fermentation tanks, the starter culture is normally metered directly into the milk, or alternatively, if a multi-purpose tank is being used, the starter culture is added either manually or, if the volume of the tank is large, the desired quantity of the starter is pumped to the tank. The biochemical reactions responsible for the formation of the gel/coagulum takes place. The formation of the yogurt gel is the result of the following biological and physical actions on milk:

The yogurt starter culture utilizes the lactose in milk for its energy requirements, and as a result the production of lactic acid and other relevant compounds becomes inevitable.

1. The gradual development of lactic acid starts to destabilize the casein micelle/denatured whey protein complex by solubilisation of the calcium phosphate/citrates.

2. Aggregates of casein micelles and/or the individual micelles group together and partially coalesce as the pH approaches the isoelectric point, i.e. pH 4.6-4.7.

1.3.2.3. Cooling

Yogurt production is a biological process, and cooling is one of the popular methods used to control the metabolic activity of the starter culture and its enzymes. Cooling of the coagulum commences directly after the product reaches the desired acidity, e.g. around pH 4.6 or 0.9 % lactic acid depending on the type of yogurt produced, the method of cooling used and/or the efficiency of the heat transfer.

Since the yogurt organisms show limited growth activity around 10°C, the primary objective of cooling is to drop the temperature of the coagulum from 30-40C to <10°C (best around 5°C) as quickly as possible so as to control the final acidity of the product [5,16].

1.3.3. Churning (Butter making)

When milk or cream is held at a favorable temperature and subjected to continuous agitation, the fat globules coalesce progressively, forming small granules of butter. A small quantity of other milk solids and of water is caught and held with the fat during the process, but the bulk of these constituents is separated from the fat and constituents the product known as buttermilk.

When cream is churned, it is observed that more or less foaming and whipping take place due to the incorporation of the air, the cream increases in viscosity, and small grains of fat become visible to the eye. As churning proceeds, the cream undergoes a decided change in physical make up: the cream appears granular; small granules of butter become more clearly defined; the cream adheres to the walls of the churn, and drops more like a semi solid mass; suddenly the breaking point is reached, butter comes; the granules of butter are distinct; there is definite separation of the fat and serum of the cream. With continued agitation the granules become larger, and make the distinction between the buttermilk and the butter granules even more definite.

The churning operation results in breaking up of the fat-in-water emulsion existing in the cream, with resulting separation of the butter oil in relatively large masses.

Cream has its fat globules suspended in water such that the water is the continuos phase and the fat globules are dispersed or discontinuous phase. The fat globules each are surrounded by a kind of phospholipid membrane, which contains lecithin, that helps to keep the globules emulsified or suspended in the water phase. The mechanical agitation of churning breaks this membrane like surface of the fat globules and causes globules to collide with one another [3,12,17].

The churning properties appear to be related to the plasticity of the butterfat; hence the cream must not only be brought to the proper temperature, but also be held that temperature long enough for a sufficient degree of fat crystallization to occur. During churning the fat globules must be sufficiently soft and plastic to coalesce readily, yet not soft enough for large aggregates of fat to appear while uncoalesced globules remain.

The temperature should be such that churning will not require an inordinate time; at high temperatures churning is quickly completed, but an excessive amount of fat will remain in the buttermilk, and the butter tends to be grassy in texture.

The conventional butter churn is a cylinder, rotated on a horizontal axis. As the churn rotates it raises the cream by means of shelves attached to the sides of the churn. After butter is formed, the continued raising and dropping of the butter from the shelves imparts the necessary working. The churns were usually made up of wood. The wooden churn, being porous, is difficult to sterilize and gradually wears out with use. The churns used consist of large horizontal cylinders, usually made up of wood, but occasionally of nonrusting metal, equipped with large doors for charging and withdrawal of the product. They are designed to turn on the longitudinal axis at about 25-30 rpm. The churning operation is judged to be at an end when the granules of butterfat are of about pea size. At this point churn is stopped, the buttermilk is drained off.

It is desirable that the churning operation consumes the least amount of time compatible with a minimum fat loss in the buttermilk. Further, it is desirable butter have a firm, waxy body and not appear as a slushy, salvy mass. As a general rule, any condition, which tends to make churning easier or which results in the formation of butter granules in a comparatively short period causes a relatively high fat loss in the buttermilk and slushy, salvy butter at churn.

1.4. Rancidity

The term rancidity is often used in a general way to designate the development of any unpleasant odour and flavour in fats and oils. The term may be applied differently in various industries to spoilage or deterioration, which occurs in particular manner. Rancidity is the development of an off-flavour by the chemical reactions, which make the food unacceptable on a consumer market level. In other definition, rancidity is the characteristics, unpalatable odor and flavour of edible fats and oils following oxidative or hydrolytic degradation. In addition to unpalatability, rancidity may give rise to toxic levels of certain products, e.g. aldehydes, hydroperoxides, and epoxides.

Many factors affect the onset and development of rancidity including degree of unsaturation of the oil, the type and the concentration of antioxidants, pro-oxidants and trace metals present, availability of oxygen, surface area, moisture content, temperature and degree of exposure to light [18].

The important chemical reactions leading to rancidity are summarized in Figure 2. Two well-known pathways are recognized: oxidation (process I) and hydrolysis (process II). Oxidation leads to oxidative rancidity and involves oxygen attack of glycerides. Hydrolysis leads to hydrolytic rancidity and involves hydrothermal or enzymic (lipase) hydrolysis to free fatty acids and other products.

1.4.1. Hydrolytic Rancidity

Hydrolytic rancidity is catalyzed by lipase and results in the formation of glycerol and free fatty acids. Some of these fatty acids have low flavor thresholds and their accumulation is responsible for unpleasant flavor in many dairy products. These flavors are variously described as rancid, butyric, bitter, unclean, soapy and astringent. The essence of the reaction of hydrolytic rancidity may be represented as:

$$\begin{array}{l} CH_2 - OOC\text{-}R_1 \\ | \\ CH - OOC\text{-}R_2 \\ | \\ CH_2 - OOC\text{-}R_3 \end{array} + 3H_2O \xrightarrow{\text{Lipase}} \begin{array}{l} CH_2O \\ | \\ CHOH \\ | \\ CH_2O \end{array} + COOR_1 + COOR_2 + COOR_3$$

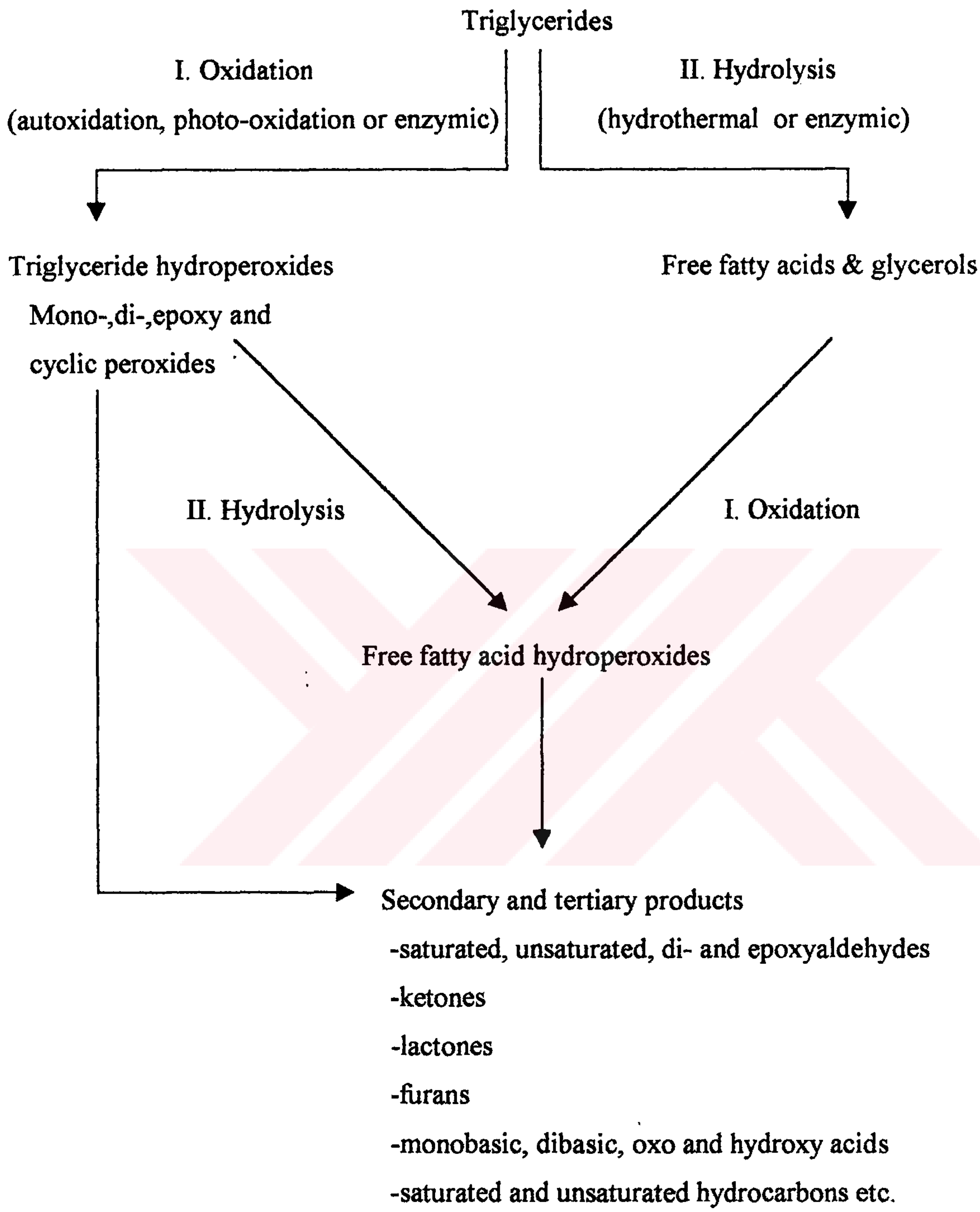


Figure 2. Overall reaction scheme for (I) oxidative and (II) hydrolytic rancidity

This reaction states that; as the water is present, this causes the hydrolysis of fat to tri-, di-, mono-glycerides and glycerol with the formation of free fatty acids.

The flavors developed by lipase action depend on the composition of the fat. Hydrolytic rancidity is especially important in butter and products containing milk fat because hydrolysis releases short-chain fatty acids such as butyric, caproic, and capric, resulting in particularly disagreeable flavors and odors [12,19]. The short chain fatty acids are volatile at room temperature. Some of these acids detectable by smell and taste even when present in very small quantities. Although hydrolysis may produce long-chain free fatty acids, these do not usually contribute much to off-flavors unless oxidation also occurs.

1.4.2. Oxidative Rancidity (Autoxidation)

Lipids in biological systems can undergo oxidation, leading to deterioration. In food, these reactions can lead to rancidity and the possible formation of toxic compounds. The oxidative deterioration of lipids involves primarily autoxidation reactions which are accompanied by various secondary reactions having oxidative and nonoxidative characteristics [20-22].

1.4.2.1. Mechanism of Lipid Autoxidation

The autoxidation of unsaturated fatty acids has been proposed to occur by a free radical chain mechanism involving three stages: (1) initiation, the formation of free radicals; (2) propagation, the free radical chain reactions; and (3) termination, the formation of non-radical products [20,23,24].

Initiation:	$RH + O_2$	$\longrightarrow R\cdot + H\cdot$
Propagation:	$R\cdot + O_2$	$\longrightarrow ROO\cdot$
	$ROO\cdot + RH$	$\longrightarrow ROOH + R\cdot$
Termination:	$R\cdot + R\cdot$	$\longrightarrow RR$
	$R\cdot + ROO\cdot$	$\longrightarrow ROOR$

where RH is any unsaturated fatty acid; R· is a free radical formed by removing a labile hydrogen from a carbon atom adjacent to a double bond; and ROOH is a hydroperoxide, one of the major initial oxidation products that decompose to form compounds responsible for off-flavors.

The classical route of autoxidation depends on the production of free lipid radicals from triglycerides by their interaction with oxygen to form a peroxy radical, the latter, in turn, abstracting hydrogen from another glyceride or fatty acid molecule to yield a hydroperoxide and a further radical. In this way, propagation of the reaction is ensured. Termination occurs when radicals R· react to produce stable products.

Regardless of the mechanism of formation, hydroperoxides will decompose further into the final flavor components. The hydroperoxides formed in the autoxidation of unsaturated fatty acids are unstable and readily decomposes. The latter conversion is known to proceed via the formation of alkoxy group. Depending on the mode of this reaction, the secondary oxidation products so formed may be aldehydes, ketones, alcohols or hydrocarbons. The main products of hydroperoxide decomposition are saturated and unsaturated aldehydes.

Other products, such as unsaturated ketones, saturated and unsaturated alcohols, saturated and unsaturated hydrocarbons, and semi-aldehydes are observed in the decomposition of hydroperoxides of oxidized lipid systems [12,22].

The overwhelming consideration in regard to lipid deterioration is the resulting off-flavors. Aldehydes, both saturated and unsaturated, impart characteristic off-flavors at minute concentrations. Terms such as painty, nutty, melon-like, grassy, tallowy, oily, carboard, fishy, cucumber, etc. are used to characterize the flavors imparted by individual saturated and unsaturated aldehydes, as well as by mixtures of these compounds. In addition to aldehydes, other secondary products of lipid oxidation, such as unsaturated ketones and alcohols, impart characteristic flavors.

1.4.3. Measurement of Rancidity

Factors that affect the quality of fats and oils determine the physicochemical characteristics of these products. The principal measurements are; peroxide value (PV), free fatty acid (FFA), thiobarbituric acid reaction (TBA), and anisidine value. The oxidation stability of lipids reflects their potential to resist the action of atmospheric oxygen. This is an essential parameter for the quality of foodstuffs and determinant of their shelf life. The fatty acid composition and amount of anti- and pro-oxidative substances are known to be key factors in oxidation stability [25].

Since an extremely minor order of oxidative deterioration of milk fat is sufficient to influence consumer acceptance and also its nutritional quality, numerous methods have been developed for assessing the extent of oxidation.

Levels of PV and FFA are the commonest criteria used to evaluate the shelf life of butter oil. However, interpretation of shelf life based on increase in PV and/or FFA should be applied with caution. The PV test measures only the reactive lipid hydroperoxide intermediates in the oxidation reaction. It is relevant early in the life of the product, or when oxygen is readily available in the sample and oxidation is proceeding exponentially. Under conditions of limited oxygen, such as in a sealed container where oxygen consumed by lipid oxidation cannot be replaced, the PV may fall to levels found in fresh milk fat. Yet in this case, the flavor will be objectionable due to the conversion of lipid hydroperoxides to the final flavorful reaction compounds.

1.4.3.1. Peroxide Value (PV)

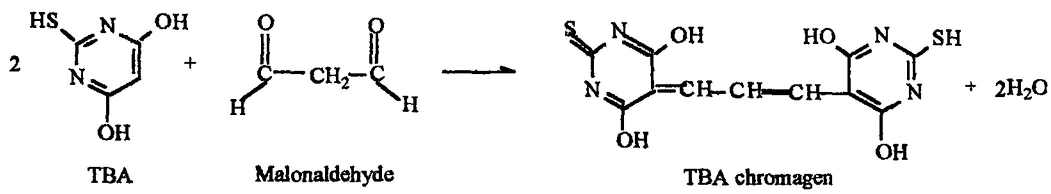
The peroxide value of a fat has long been associated with its state of rancidity [26]. Peroxides are the primary products of oxidation, and hence, measurement of their concentration gives an idea about the extent of oxidation. The most common method to determine PV relies on the oxidation of iodide ion to iodine in an acidic medium by lipid peroxides and measurement of the released iodine titrimetrically.

PV is reported as meq O_2/kg fat. It was noted that the iodometric method is not satisfactory for milk fat, since considerable deterioration occurs especially at PV below the limits that can be accurately determined [19]. To overcome this limit, liberated iodine is converted into a blue starch-iodine complex before titration. It is essential, during the determination to deaerate all solutions, since presence of oxygen can lead to further formation of peroxides.

Although, PV is a measure of extent of oxidation, theory is limited due to the transitory nature of the peroxides. The peroxides are intermediate products in the formation of carbonyl and hydroxy compounds. There is a sharp relationship between oxygen-containing functional groups and oxidation time. This indicates that the peroxide value passes through a maximum and is very sensitive to temperature changes [19].

1.4.3.2. Thiobarbituric Acid (TBA) Test

The thiobarbituric test is one of the more commonly used methods for the detection of lipid oxidation. This test is based on the reaction of malonic di-aldehyde (OHC-CH-CHO), a product of, particularly, polyunsaturated fatty acids containing fat, with 2 molecules of thiobarbituric acid (TBA) and the resultant red pigments are measured colorimetrically [27,28].



A mechanism exists for the formation of malonaldehyde, a secondary product in the oxidation of polyunsaturated fatty acids. The mechanism is based on that no color developed for linoleate even at peroxide values of 2000 or greater, but that for fatty acids with three or more double bonds, the molar yield of the TBA color increased with the degree of unsaturation. These results indicate that, only peroxides which possessed unsaturation, b, d to the peroxide group are capable of undergoing cyclization with the ultimate formation of malonaldehyde. Such peroxides could only be produced from fatty acids containing three or more double bonds. It therefore becomes imperative to know the fatty acid profile of the sample to be tested. Structure of TBA is altered by acid and heat treatment as well as by the presence of peroxides and recommended that blank determinations be carried out in conjunction with the test. Color development during the TBA test is usually assessed by measuring the absorbance of the red pigment at 532-540 nm [20].

1.4.3.3. Free Fatty Acid (FFA)

FFA are undesirable in milk fat products because shorter-chain homologues are primarily responsible for the rancid flavor typified by butyric acid. The short-chain FFAs have been reported also to catalyze the fat oxidation reaction. However, FFA contribute to the normal flavor of butter oil and their level has been closely related to the flavor quality [19].

Despite numerous variations and modifications, the methods for measuring FFA are classified broadly as (a) titrimetric, (b) colorimetric and (c) chromatographic. Most general titrimetric method is the using petroleum ether or diethyl ether, along with absolute alcohol was used to dissolve the fat. In this method, long persistence of end point and the easy color change are obtained. Application of gas chromatography (GC), gas-liquid chromatography (GLC), and high performance liquid chromatography (HPLC) in determining individual FFA provides useful and accurate information and is helpful in trouble-shooting and in research [19].

1.4.3.4. Flavor

Milk fat surves both as a precursor of flavors, and as a solvent for flavors from the aqueous phase of milk. Triglycerides can be broken down into glycerol and fatty acids by chemical or biochemical means in process called lipolysis. Heat, lipolysis and oxidation release flavors from their triglyceride precursors. During the manufacture of butter oil, clarification affects the flavor quality. Degree of heating for clarification depends on the local taste. Commercially, butter oil is made by heating either cream or butter to remove the water. More flavor is produced if the cream or butter have been cultured. From its method of manufacture it is to be expected that butter oil contains all the compounds produced by the action of heat on butter fat. The flavor of butter oil is due to three factors: a) the flavor produced by culturing the milk, b) the effect of heat on the components of the butter serum in an essentially all-fat environment, c) the effect of heat on the butter fat itself [29,30]. The highly prized characteristic flavor of butter oil is due to a complex mixture of compounds produced during the various stages of processing. The heating process generates flavor compounds through the interaction of protein degradation products, lactose and minerals, and possibly through the degradation of FFA and lactose. Carbonyls (aldehydes and ketones) and lactones are believed to play significant role in the typical flavor of butter oil.

1.5. Storage Stability and Accelerated Shelf Life Testing (ASLT)

The expected shelf life of a product depends upon both the potential environmental conditions that the product will be exposed to and how much of the initial quality can be lost before the product should no longer be sold to the consumer for any number of reasons, including an unacceptable loss in nutrient value, an undesirable change in flavor or color, or the development of an undesirable texture.

In the production of any food, one important aspect is the knowledge of the shelf life. This life must at least exceed the minimum distribution time required from processor to the consumer. A major aim of the shelf life concept is the prediction of the change in quality of a particular food as a function of both time and environmental conditions including temperature, relative humidity, oxygen transparency and oxygen permeability of packaging material. In order to predict the approximate storage stability of a food product, knowledge is needed about the reactions which would take place in the food under specified conditions [31].

The following steps should be followed in designing a shelf life test for quality loss in food:

1. The microbiological safety and quality parameters for the proposed formulation and process are determined.
2. From an analysis of the ingredients and the process which chemical reactions are likely to be the major causes of quality loss are determined.
3. The package to be used for the shelf life test is selected.
4. The storage temperatures (at least two) are selected.
5. Using the shelf life plot and knowing the desired shelf life at the average distribution temperature. If no information is available on the probable Q_{10} value, then more than two temperatures are needed.
6. Which tests will be used and how often they will be conducted at each temperature are decided.
7. To determine the order and to decide whether test frequency should be increased or decreased, the data is plotted as it is collected.
8. From each test storage condition, k or q_s are estimated, the appropriate shelf life plot is made and estimate the potential shelf life at the desired (final) storage condition is

estimated [31-37].

In general, the end of the acceptable shelf life is defined as that time when stored samples are perceived as being different by a certain amount. This perception could be based on testing by an in-house, established sensory panel (preferably) or a small but select group of product development scientists using statistically designed tests, or by measurement of a key quality factor. Thus, to establish the rate of deterioration, one must transform the published data into a kinetic plot. Unless, transformed, these data can lead to erroneous results in prediction of true shelf life,
since the data are for specific conditions which may not be applicable to the particular product in question, especially if one chooses the wrong mode of deterioration.

The Arrhenius model, or Q_{10} model can be used to describe how much faster a reaction will go if the product is held at some other temperature, including high abuse temperatures. If the temperature-accelerating factor is given, then extrapolation to lower temperatures, such as those found during distribution, could be used to predict expected product shelf life.

In many cases, the Q_{10} is not given, so one must is not given, so one must is not given, so one must establish the mathematical relationship. Most reactions fit a zero-or first-order mathematical expression:

$$\frac{-dA}{dt} = k\,(A)^n \qquad \text{(Equation 1)}$$

where A= the quality factor measured in some units of amount, n= the reaction order, and k= the rate constant; the slope of the plot of the appropriate reaction extent A vs. time t.

Other orders besides zero and first are possible. Thus, by using the literature to evaluate the rate constant k at two or three different temperatures, one could then, on the basis of the Arrhenius relationship, extrapolate with a straight line on a plot of log k vs. reciprocal absolute temperature (K^{-1}) to predict the reaction at other reaction at any other temperature.If it is impossible to establish an Arrhenius plot, a simple plot of the log of the time to end of shelf life q_s vs. temperature in °C can be used, as long as the extrapolation does not go beyond a 30°C range; this is called the shelf life plot. It should be noted that this plot also helps to establish the Q_{10}, which is simply the ratio of shelf life at two temperatures 10°C apart. The value of either plot (log k vs. K^{-1} or log Q_s vs. °C) is that data can be collected at high temperature and used to extrapolate to shelf life at some lower temperatures. This is the principle behind accelerated shelf life testing [35-37].

1.5.1. Measurement of Oxidation Stability

The susceptibility of an oil or fat to autoxidative degradation can generally be assessed in terms of oxidation stability. The quality assessment of fats with regard to freshness, keeping properties, and storage ability can be carried out by either static or dynamic methods. In the former, analytical determinations are made of various characteristics (such as the peroxide number) relating to the degree of auto-oxidation which has already taken place. Dynamic methods, on the other hand, enable a better forecast to be made of the future response of a fat to oxidative influences, i.e., its oxidation stability. In these methods, the fat is subjected to air at certain temperatures and other defined reaction conditions. The periodic determination of peroxide numbers or other measurable values enables oxidation curves to be plotted.

When the autoxidation of a fat is followed experimentally, either by measuring the amount of oxygen absorbed or by determining the peroxide value of the fat, it is found that the course of oxidation exhibits two distinct phases. During the initial phase, oxidation proceeds at a relatively slow and more or less uniform rate. Then, after a certain critical amount of oxidation has occurred, the reaction enters a second phase characterized by a rapidly accelerating rate of oxidation, and an eventual rate many times greater than that observed in the initial phase. The point at which the sample begins to smell and taste rancid coincides more or less with the beginning or the early part of the second phase. The initial period of relatively slow oxidation of a fat is termed 'induction period' or 'induction time'.

The induction time obtained is in many cases a good indication not only of the keeping properties, but also of the momentary state of quality of a fat or a fatty product.

1.5.1.1. ASLT Methods

Methods for detecting rancidity are essential in determining the stability of fats. Stability may be defined as the resistance to autoxidation under predescribed conditions of aging and is measured in units of time required for the product (a) to acquire a state of oxidation which correlates with organoleptic detection of rancid odor and flavor, or (b) to reach the end of the induction period, if oxygen absorption measurements or peroxide analyses are used. Under usual conditions of storage, this time is too long for practical evaluation, therefore, it is necessary to employ accelerated oxidation tests.

Basically, four parameters are manipulated in ASLT procedures to speed up the oxidation and development of rancidity in fats and oils: temperature, oxygen, added metals, and reactant contact.

The most commonly used methods are explained as follows:

The Schaal Oven Test: The simplest of the commonly used accelerated methods for determining stability of fats or fat products is the oven or Schaal test. Since it is run at temperatures only moderately greater than those found in ordinary storage conditions, it provides an index of keeping quality which more nearly rates a product as a user will find it. The sample is simply incubated in an oven at 63°C or 145°F until it develops a specific peroxide number or rancid odor and flavor.

Oxygen Absorption Methods (OAM): Many versions of the OAM are available. The sample is kept at atmospheric pressure in oxygen at 100°C. The end point is taken as the time when a marked drop in pressure occurred. The temperature used in these methods is considerably higher than that used in the Schaal Oven Test.

Active Oxygen Method (AOM): This method is based on the principle that the aging and rancidification of a fat is greatly accelerated by aeration at a constant elevated temperature. While an organoleptic evaluation may be used to indicate the rancid point, the results are based on the peroxide content of the fat after correlation with organoleptic rancidity. The apparatus consists of a constant temperature bath and aerating train contrived to deliver a continuous flow of air to the sample at a constant rate. It involves continuously aerating the sample at 208°F (97.8°C), and by periodic titration, determining the time required for a specific peroxide number to develop. The peroxide number chosen as the end point should represent an average value for the beginning of definite rancidity in the kind of fat under test. The original method was designed for lard and a peroxide number of 20 meq/kg was suggested.

Rancimat Method: Rancimat method is based on the conductometric determination of volatile degradation products and features automatic plotting of the conductivity against time. The evaluation is performed graphically after completion of the experiment. In this method, volatile low molecular weight products developed in oil oxidized by air passing air through it at increased temperature are trapped in water and detected. The Rancimat instrument measures an increase in electrical conductivity. From the curves representing these changes, the induction period of oxidation is determined. The labor required for this method is considerably less as it is not necessary to perform titrations at regular intervals [3,37].

1.6. Rancidity and Storage Stability of Butter Oil (Anhydrous Milk Fat)

Milk fats are recognized as major contributors in determining consumer acceptability of most dairy products. Deterioration (lipolysis and oxidation) of milk fat due to several factors causes flavor impairment, lowers nutritional quality, creates serious problems in storage stability. Oxidative deterioration of butter oil is one of the major concerns of the dairy industry. The on-set of rancidity in butter oil may be usually due to oxidation of unsaturated glycerides leading to development of peroxides and/or due to hydrolysis of glycerides resulting into increased level of free fatty acids [6,10,19,38].

Butter oil may deteriorate as a result of development of oxidized and/or rancid flavors. The heat treatment involved in the manufacture of butter oil should destroy most bacteria and the moisture content is too low to allow normal growth of most organisms.

The keeping quality of butter oil is governed by such factors as the ripening of cream, method of manufacture, clarification temperature and permeability of the packaging material to air and moisture, type of animal feed [4,39].

The storage stability of butter oil is attributed to the low moisture content (0.2%) and the high content of phospholipids (400 mg/kg) and perhaps the free amino acids, which are liberated from the phospholipid-protein complex into the fat phase. The low acidity of butter oil and presence of natural antioxidants are also believed to contribute to the extension of its shelf-life. Generally fresh cream butter oil has a longer shelf life than ripened cream butter oil.

Degradation of milk fat occurs by two distinct routes- lipoysis and oxidative rancidity. The oxidative rancidity is more complex, unpredictable and more common in milk products than the lipolytic rancidity. Oxidative degradation is by oxidation of the polyunsaturated fatty acids in milk. Because of their unsaturated nature, phospholipids are more prone to oxidation than neutral fat. In the autoxidation of milk fat, the free radical process is assumed to originate in the phospholipid. The fat portion of milk exists as small discrete globules, neatly enveloped in a biological membrane of complex composition and structure. The major function of this membrane is to facilitate the dispersion of the fat in the aqueous phase of milk. Milk fat globule membrane material constitutes only 2-6 % of the total mass of the fat globules. The phospholipids of the milk fat globule membrane play a significant role in oxidation and the development of oxidized flavor defects [40,41].

In milk fat, a hydrogen atom is removed from the methylene group adjacent to the double bond in mono and unconjugated polyunsaturated fatty acids. Oxygen then adds to the stabilized free radical to form a peroxide. A chain of reactions then ensues which terminates in the formation of aldehydes and ketones, which give rise to oxidized off-flavors. Oxidation of milk fat is enhanced by high storage temperatures, by exposure to light, by heavy metal contamination and by exposure to oxygen. It is retarded by the presence of natural antioxidants [42,43].

Effect of ripening of cream, manufacturing temperature and packaging materials of butter oil were studied by Singh et al. [44]. He concluded that; the clarification temperature did show a definite effect on flavor of fresh butter oil. It was found that; the flavor of butter oil was deteriorated with storage. The deterioration was fastest in ripened cream sample. The keeping quality of butter oil packaged in plastic bottles was not different from that in brown glass bottles. They concluded that; the FFA content increased both by ripening and by heating to a higher temperature.

The role of polar carbonyls, produced during heating in the preparation of butter oil, on its oxidative stability was investigated by Sserunjogi et al. [1]. The contents of polar carbonyls were higher in ripened cream butter oil compared to that prepared from fresh cream and butter. This is to be expected since the fermentation process itself produces polar carbonyls. The rate of formation of peroxides was lowest in ripened cream butter oil followed by fresh cream and cream butter oil. This may indicate that the presence of higher quantities of polar carbonyls in butter oil decreases its rate of oxidation.

Textural changes may also occur in butter oil during storage [1]. The fat in butter oil crystallizes with the formation of solid, semi solid and liquid layers. Butter oil stored at 20°C or below has been reported to solidify uniformly with fine crystals. However, sample stored above 20°C and below 30°C solidifies with a loose structure. It has been suggested that butter oil should be stored at temperatures below 20°C to avoid layer formation.

Storage stability of commercial anhydrous milk fat and hydrogenated oils were studied by Iskander et al. [7]. During storage, the FFA and PV values increased. The PV showed a faster increase at 35°C than at 20°C. The increase at 20°C was mostly below 20 meq O_2/kg, which had no practical effect on flavor changes. This suggested that oxidative rancidity could be a major problem if the fat is stored at temperatures above 20°C. The rate of increase in peroxide in peroxide values was much higher during storage at 35°C than 20°C. The PV increased after 6 months of storage at 20°C from about 1.1-2.0 to 4.5-5.5 in most fat samples. Since the maximum PV attained were mostly less than 20, this has no practical significance for the oxidative rancidity development. However, at 35°C all samples showed a considerable increase in PV as the storage time proceeded.

Amr found that, both storage time and type of treatment had highly significant effects on the peroxide value and free fatty acid content of the anhydrous butter oil [6]. It was concluded that, the PV of the product was higher at the onset of the storage period. This was due mainly to the high PV in the butter which is not surprising as the butter was collected over a period of 4 days and kept without refrigeration. Another factor that contributed to high PV in the anhydrous butter oil was the heating process itself. By regarding a PV of 10 meq O_2/kg as the end of the induction period, it can be seen that butylated hydroxy anisole (BHA)-butylated hydroxy toluene (BHT) helped prolong this period to ~ 10 months. Also, results indicated that hydrolytic rancidity is not a major problem in the storage of anhydrous butter oil. The low FFA contentent is due mainly to the low moisture content of the product and the heat treatment it receives which inactivates most lipases present in the butter.

Wade et al.[42] studied the autoxidative stability of anhydrous milk fat with and without antioxidants. He found that; ascorbyl palmitate at a concentration of 100 ppm exhibited antioxidant activity by extending the time to reach a peroxide value of 0.5 meq O_2/kg from 4 to 14 h at 98°C. Also, they found that; BHT was more effective than BHA at the same concentration of 200 ppm. The time to reach a peroxide value of 0.5 meq O_2/kg was about 9h in case of BHA but nearer 11h for BHT. The BHT at 200 ppm was a less effective antioxidant than ascorbyl palmitate at the same concentration.

The keeping quality of synthetically flavored butter oil in comparison to butter oil and ghee packaged in tin and polyethylene packages was analyzed by Wadhwa et al. [45]. Samples were analyzed by 37°C storage temperature. They concluded that; as the oxidative changes compared to hydrolytic changes are of greater significance in the deterioration of flavored butter oil (FBO) and butter oil (BO), tin packages should be preferred to polyethylene packages for the storage. In tin containers, the shelf-life of FBO was 75 days in comparison to BO (30 days). In polyethylene containers, the shelf-life of FBO was 60 days in comparison to BO (30 days). FBO and BO developed more peroxides as compared to ghee during storage under both packaging conditions. The development of greater oxidative rancidity in BO and FBO is possibly due to their lower levels of natural antioxidants like phospholipids.

Effect of temperature on rate of autoxidation of milk fat was studied by Hamm et al. [46]. In this study, milk fat was autoxidized at 50, 35, 21, 4 and −10°C, and the reaction monitored by peroxide and thiobarbituric acid values, and organoleptic examination. It was concluded that; PV and TBA values showed significant correlation with flavors at higher temperatures, but at lower temperatures no increases in PV and TBA values could be observed.

Fearon [39] studied to determine whether increased content of monounsaturated fatty acids adversely affected the oxidative stability of milk fat by using the naked oat as the animal feed. The oxidative stability of milk fats from cows offered naked oats and barley-based diets were compared in shelf-life tests. Milk fat from cows offered the diet containing naked oats, although containing a higher portion of monounsaturated fatty acids, had a significantly longer induction period (13 days) than milk fat from cows offered the control (barley- based) diet (9 days).

1.7. The Aim of Present Study

In the production and the storage of any food, one important aspect is the knowledge of the shelf-life. Major aim of the shelf-life concept is the prediction of the change in quality of a particular food as a function of both time and environmental conditions. Butter oil is a special fat product from milk, and therefore it is considerable that its stability and storage is important. Therefore, aim of the present study is to estimate the storage stability of butter oil by considering the effects of processing methods and storage conditions compare the experimental results obtained from ASLT.

CHAPTER II
MATERIALS AND METHODS

2.1. Materials

2.1.1. Sample

Cow and sheep's milk containing 2.9-3.2 % fat were obtained from a local farm.

2.1.2. Chemicals

All chemicals used in this work were supplied from Merck, Sigma, Riedel-deHaen, and Carlo Erba Reagent. Deionized water was used for the preparation of solutions.

2.2. Methods

2.2.1. Preparation of Butter Oil

Butter oil samples were prepared from two sources: sheep's and cow's milk as described on the following diagram:

MILK

SHEEP

- Pasteurized-Nonpasteurized milk
  (in March)
- Pasteurized milk-Sweet yogurt
  (in May)
- Sweet yogurt-Sour Yogurt
  (in September)

COW

- Pasteurized milk-Sweet yogurt
  (in November)
- Sweet yogurt-Sour yogurt
  (in May)
- Sweet yogurt using different melting times
  (in December)

Butter oils produced each matched run were analyzed and their stabilities were compared to find the most stable one. Processing method, giving more stable butter oil, was used to compare with a new processing method in next run.

2.2.1.1. Butter Oil Produced from Nonpasteurized Milk

Milk was directly cooled to 10°C for 30 min by waiting in iced water bath without applying any heating procedure and it was churned with a butter churner (Eskay Yayık, Gaziantep) at 10°C for 45 min. Butter produced was melted at 40°C and extracted using hexane. The organic phase (hexane and butter oil) above serum phase was filtered and hexane was then evaporated from butter oil-hexane mixture in a vacuum oven under

2.2.1.2. Butter Oil Produced from Pasteurized Milk

Milk was pasteurized by heating at 72°C for 15 sec. Pasteurized milk was cooled to 10°C for approximately 30 min by waiting in iced water bath and it was churned. After churning, butter produced was melted in oven at 70°C.The melted butter was continuously stirred and the oil phase above the serum phase was allowed to separate for 3 h. The oil phase was clarified by filtering through several layers of cheese cloth into a glass beaker.

2.2.1.3. Butter Oil Produced from Sweet Yogurt

Pasteurized milk was cooled to 42°C. 3 % (w/v) previous day yogurt (Pınar Yogurt) was added into pasteurized milk to produce yogurt. It was incubated for 3 h in an incubator at 42°C to reach a pH of 4.60. Then, it was cooled to 10°C for 30 min and churned for 45 min. After churning, the butters produced were melted in an oven at 70°C for 3 h and clarified as explained in section 2.2.3

2.2.1.4. Butter Oil Produced from Sour Yogurt

Pasteurized milk was cooled to 42°C. 3% (w/v) previous day yogurt was added to produce sour yogurt. It was incubated for 6 h in an incubator at 42°C to reach a pH of 3.80. Then, it was cooled to 10°C in iced water bath and churned for 45 min.After churning, butter oils produced were melted in an oven at 70°C for 3 h and was clarified.

2.2.1.5. Butter Oil Produced from Sweet Yogurt using Different Melting Times

Milk was pasteurized and was cooled to 42°C. 3% (w/v) previous day yogurt added to milk to produce yogurt. It was incubated for 3h in an incubator at 42°C to reach a pH of 4.60. Then, it was cooled to 10°C and was churned. After churning, butter produced was divided into two parts. One of them was melted in an oven at 70°C for 3h at and the other were melted at 70°C for 6h. They were clarified.

2.2.2. Analysis of Butter Oil

2.2.2.1. Storage Conditions

Clarified butter oil samples produced from methods described above were stored in glass jars open to atmosphere in temperature controlled ovens at 60°C, 70°C and 80°C in the dark to perform stability tests. PV, TBA and FFA levels were followed periodically. The samples were taken from different jars at different time intervals.

2.2.2.2. Peroxide Value (PV)

Peroxide values were determined by iodometric titration method, as described in BS 684,14 [47].5 g of butter oil, in duplicate was weighed in a glass stoppered 250 ml erlenmeyer flask. 10 ml of the chloroform was added to the sample flask and stirred vigorously to dissolve the sample. Then, 15 ml of the glacial acetic acid and 1ml of saturated aqueous solution of freshly prepared potassium iodide were added. The flask was closed, stirred for 1 minute and left for 5 minutes away from the light. After that, 75 ml of water and 1 ml of 1% starch solution (as an indicator) were added. The liberated iodine was titrated with the standard volumetric 0.01N $Na_2S_2O_3$ sodium thiosulphate solution. A blank test was carried out in parallel with the determination. The peroxide value was calculated as follows:

$$\text{PV (meq of } O_2\text{/kg of fat)} = \frac{(S-B)*N*1000}{m}$$

where;

S=ml of 0.01 N $Na_2S_2O_3$ used for sample; B=ml of 0.01 N $Na_2S_2O_3$ used for blank

N=normality of $Na_2S_2O_3$; m=mass of sample in gram

2.2.2.3. Thiobarbituric Acid Value (TBA)

TBA determination was carried out as follows[48]:

TBA Reagent: 0.02 M 2-thiobarbituric acid in 90% glacial acetic acid was prepared. 0.2883 g of thiobarbituric acid was brought into solution in 100 ml of 90% glacial acetic acid by warming slightly in a boiling water bath.

1 g of butter oil sample was taken into a glass-stoppered test tube and 5 ml of TBA reagent was added. Then, tube was stoppered and the contents were mixed. Then, the tube was immersed in a boiling water bath for 35 min. A distilled water-TBA reagent blank was also prepared and treated like the sample. After heating, it was cooled in tap water for 10 min. A portion was transferred to a cuvette and the optical density of the sample was read against the blank at a wavelength of 538 nm in spectrophotometer (Novaspec II model spectrophotometer). The optical density value was converted to the moles of malonaldehyde per gram of oil sample by using the standard curve.

2.2.2.3.1. Preparation of Standard Curve for TBA

A standard curve was prepared by making appropriate dilutions of the 1×10^{-3}M 1,1,3,3-tetraethoxypropane standard solution, to give amounts ranging from 1×10^{-8} to 7×10^{-8} moles of malonaldehyde in 1 ml. These dilutions were reacted with TBA reagent and the optical density of them were measured at the wavelength of 538 nm in Novaspec II model spectrophotometer. Samples with an optical density higher than 0.5 were diluted to a known degree to get proper values.

2.2.2.4. Free Fatty Acids (FFA)

FFA was determined by titration method, as defined in AOCS Official method Ca 5a-40 as oleic acid [49].

5 g of butter oil samples, in duplicate, were weighed into 250 ml erlenmeyer flasks. Firstly, 20 ml of ethyl alcohol (95%) and 20 ml of diethyl ether were mixed and 1 ml of 1% phenolphthalein indicator was also added. This mixture was neutralized with 0.01 N sodium hydroxide (NaOH) solution. That freshly neutralized solvent was added to sample flask, swirled and titrated until the pink color persisted for 30 sec.

The FFA content was calculated as % oleic acid.

$$\text{FFA (\% oleic acid} = \frac{V*N*28.2}{m}$$

where;

N=the normality of NaOH; V=volume of NaOH consumed; m=mass of sample in g

2.2.2.5. Fatty Acid Composition

Fatty acid methyl esters (FAME) of the butter oil samples were prepared using 14% (w/v) BF_3 in methanol and analyzed in gas chromatography (GC) according to the AOAC Official method Ce 2-66 [50]. 350 mg of lipid sample was weighed into a 50 ml flask. 6 ml of approximately 0.5 M methanolic sodium hydroxide and a boiling chips were added. The flask was attached to the condenser, and it was boiled under reflux until droplets of fat disappear (>10 min). Through the condenser by means of automatic pipette, 7 ml of commercially prepared 14% BF_3 in methanol solution was added, and boiling was continiued to boil for one min. The flask was removed from heat and condenser, and enough saturated salt solution was added to float the heptane solution of the methyl esters into the neck of the flask. 1 ml of heptane solution was transferred into a test tube and a small amount of sodium sulfate was added to dry the heptane solution. One ml of FAME mixture was injected (by a Hamilton syringe # 701) into GC equipped with a flame ionization detector (column; 6 m long stainless steel column packed with 4% DEGS ON 80/100 mesh GDMS, carrier gas; N_2-20 psi, column temperature; 180°C isothermal, injection port temperature; 210°C, flame ionization temperature; 230°C, H_2 pressure; 20 psi, O_2 pressure; 20 psi. Identification of FAME was based on the flame ionization detection of commercial reference standard mixtures and by comparing their relative retention times under the same experimental conditions. Peak areas were measured with electronic integrator. Since peak area is proportional to component mass for fatty acid esters, the percentage composition of the mixture can be determined from peak areas with good accuracy. So, the relative fatty acid composition was determined by calculating peak areas and reported as weight percentage of total fatty acids [51].

2.2.2.6. Color Values

Color values of butter oil samples were measured according to Hunter color system by HunterLab ColorFlex. Sample was placed into the cell of the apparatus and the L, a, b, and YI values were determined.

L measures the lightness; a measures redness when positive, gray when zero, and greenness when negative; b measures yellowness when positive, gray when zero, and blueness when negative.

2.2.2.7. Refractive Index

Refractive index of butter oil sample was measured by Abbe Refractometer [52].

2.2.2.8. Iodine Value

The iodine value was determined by Hanus Method as described by AOAC [53]. 0.5 gram butter oil was weighed into a 500 ml glass stoppered flask and dissolved in 10 ml $CHCl_3$. 25 ml of Hanus reagent was added and let to stand for 30 min exactly in the dark by shaking occasionally. Then, 10 ml of 15 % KI solution was added, shaken thoroughly, and 100 ml of freshly boiled and cooled water was also added in order to wash down any free I on the stopper. The solution was titrated with 0.1 N standard $Na_2S_2O_3$ until yellow solution turns almost colorless. A few drops of 1 % starch solution was added as indicator and titrated again until blue color entirely disappeared. A blank test was carried out in parallel with the determination. IV was calculated as follows;

$$\text{Iodine value} = \frac{12.69 * N * (B-S)}{m}$$

where;

S=ml of 0.1 N $Na_2S_2O_3$ solution used for sample; B=ml of 0.1 N $Na_2S_2O_3$ solution used for blank; N=normality of $Na_2S_2O_3$ solution; m=mass of samples in gram

2.2.2.9 Saponification Value

The SV determination was determined as described in AOAC [54]. 2 g of the butter oil sample was weighed into a conical flask and exactly 25 ml of the 0.5 M alcoholic (in 95 % ethanol) potassium hydroxide solution was added. The flask was attached to a reflux condenser and heated in boiling water for 1 hour by shaking frequently. Then, 1 ml of phenolphthalein (1 % in ethanol) solution was added as indicator and the excess alkali was titrated while hot with 0.5 M hydrochloric acid solution. A blank test was carried out in parallel with the determination. SV was calculated as mg KOH required to saponify 1 g oil;

$$\text{Saponification value} = \frac{(B-S)*28.05}{m}$$

where;

B=ml of 0.5 M HCl required by blank; S=ml of HCl required for sample;

m=mass of sample in g

2.2.2.10. Arrhenius Kinetic Model

In order to estimate the Arrhenius parameters, two-step ordinary linear least squares fit was applied to each set of experimental data.

The increase in peroxide concentration with time was applied to the zero, half, first and second order models. The error mean squares were significantly larger in zero, half and second order models compared to that of first order. Therefore, present study suggests a first reaction, and the measured data fitted to the model proposed in eq 1:

$$dC/dt = k*C \qquad (1)$$

where;

C is the peroxide value (meq O_2/kg fat) at time t and k is the rate constant (day^{-1}).

With respect to the application of the Arrhenius equation to a first order reaction, this means first regressing ln(C) versus time at each temperature to obtain the rate constants, and then regressing ln(k) versus 1/T to obtain the estimates for E_a and k_o.

The Arrhenius equation was used as the basis for the development of a mathematical description of the temperature sensitivity of the product as in the eq 2:

$$k = k_o * \exp(-E_a/R*T) \qquad (2)$$

where;

k_o=pre-exponential factor (day^{-1}); E_a=activation energy (kJ/mol);

T=absolute temperature (K); R=gas constant (8.314 J/mole*K)

2.2.2.11. Induction Period (IP)

Induction period based on PV was determined using permissible limit of 10 meq O_2/kg of fat [54].

CHAPTER III
RESULTS AND DISCUSSION

The aim of this research is to estimate the storage stability of butter oils produced by different methods using sheep's and cow's milk and to compare the stability results obtained from ASLT studies. The result of experimental studies and the treatment of the resultant data are given in graphical and tabular forms and discussed separately for butter oil samples.

3.1. Butter Oils Produced from Sheep's Milk

Table 3 presents the physical and chemical characteristics of butter oil samples produced from nonpasteurized (SNB)-pasteurized (SPB1), pasteurized (SPB2)-sweet yogurt (SSY1) and sweet yogurt (SSY2)-sour yogurt (SLY).

Table 3. Initial physical and chemical characteristics of sheep's butter oils.

Analysis	SNB	SPB1	SPB2	SSY1	SSY2	SLY
-Peroxide value (meq O_2/kg fat)	0.98	1.21	0.66	1.02	1.45	1.28
-FFA (% oleic acid)	0.15	0.28	0.17	0.21	0.17	0.24
-TBA value (mg MA/kg fat)	0.13	0.22	0.15	0.27	0.28	0.51
-Color values						
L	5.45	6.27	6.31	4.64	6.17	6.47
a	0.35	0.34	-0.31	-0.12	0.24	0.17
b	-0.25	0.03	0.73	0.93	0.13	0.18
YI	5.36	1.49	6.27	14.81	16.95	17.07
-Iodine value (ml 0.1N $Na_2S_2O_3$/g fat)	26.7	26.4	26.1	26.3	25.6	25.8
-Saponification value (ml 0.5N HCl/g fat)	232	230	232	234	235	234
-Refractive index (35°C)	1.4595	1.4598	1.4595	1.4598	1.4595	1.4598

Values indicate that there are small differences in iodine and saponification values of butter oil samples. Processing methods were effective on the initial peroxide and TBA values. Color values were also slightly different due to formation of browning compounds in heating process of milk and yogurt during pasteurization and melting process.

The course of oxidation was followed in butter oil samples by testing of oils at regular intervals using PV and TBA determinations. Figures 3-8 show the change in PV values of SNB, SPB1, SPB2, SSY1, SSY2 and SLY at 60, 70 and 80°C, respectively. PV increased as the temperature increased. It can be seen that the traditional method for determining PV serves as an indicator of butter oil quality. This method does not distinguish between various unsaturated fatty acids that undergo oxidation; it also does not supply information about the secondary oxidation products formed by hydroperoxide decomposition. However, it generally be stated that the PV is an indicator of the primary level of oxidation. The initial stage of slow oxidation (the induction period) can be measured as the time required to reach an end point of oxidation corresponding either to a level of detectable rancidity (a defined peroxide value) as in this study or to as sudden change in oxidation rate [39].

In early stages of oxidation (induction period) any antioxidants present in a lipid containing product will play a role in inhibiting or delaying the onset and progress of oxidation. By the end of the induction period, however, the antioxidant molecules will all have been involved in reaction and PV will increase rapidly.

PV of butter oil samples were followed up to permissible limit of 10 meq O_2/kg [54]. IP values obtained from PV graphs using permissible limit were presented in Table 4. When IP values of each matched run were compared, the thermal stability results were highest in SPB1, SSY1 and SSY2 and those were lowest in SNB, SPB2 and SLY. It has found that the thermal stability was highest in butter oil produced from sweet yogurt (SSY2) in September.

TBA values also increases as the storage time and temperature increased (Table 5). PV and TBA values indicate that the rate of increase in PV and TBA values of SNB, SPB2 and SLY were higher than those of SP1, SSY1 and SSY2 during the storage at all temperatures studied.

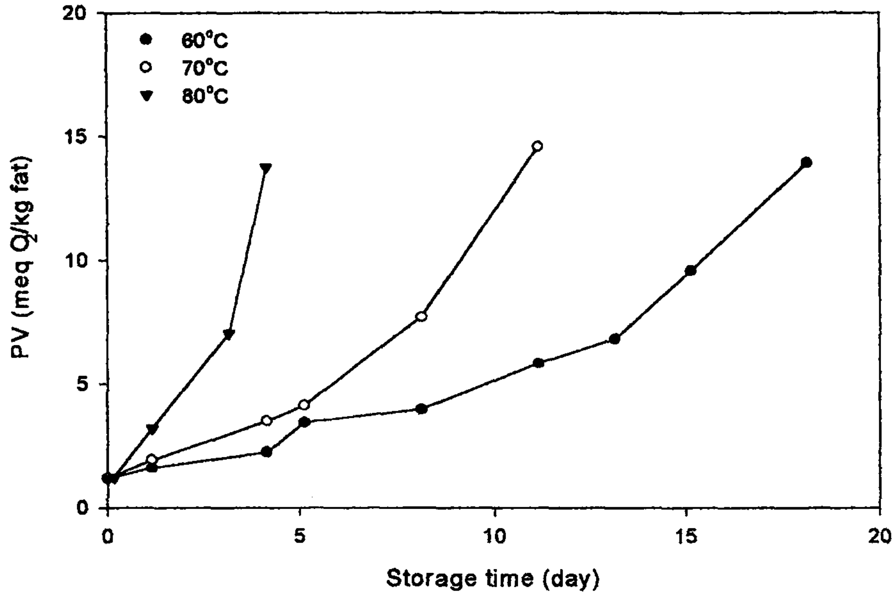


Figure 3. PV changes of butter oil produced from SNB at 60, 70, and 80°C.

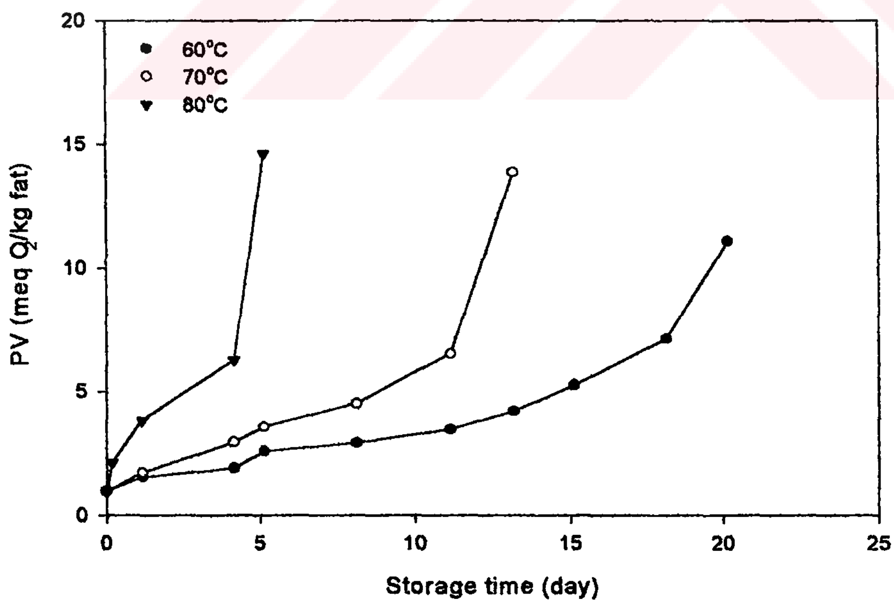


Figure 4. PV changes of butter oil produced from SPB1 at 60, 70, and 80°C.

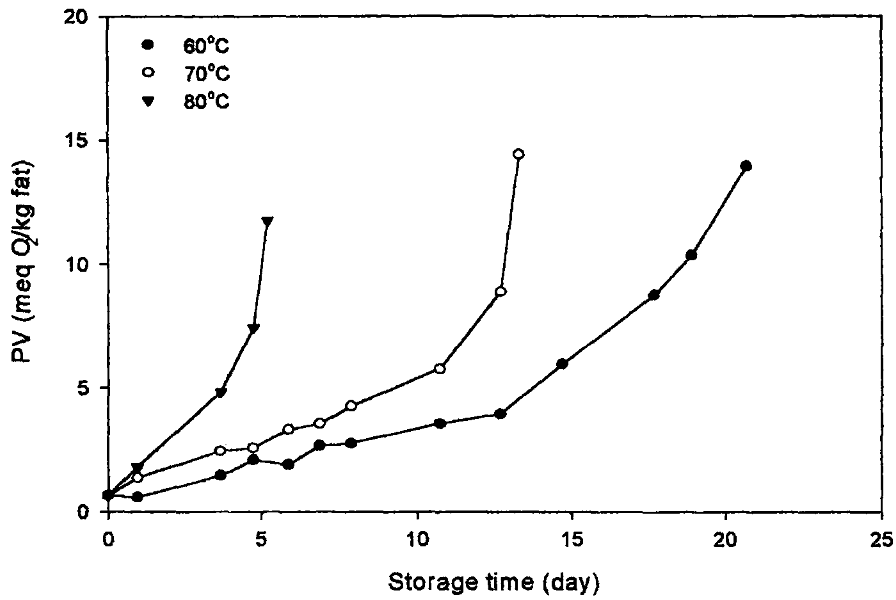


Figure 5. PV changes of butter oil produced from SPB2 at 60, 70, and 80°C.

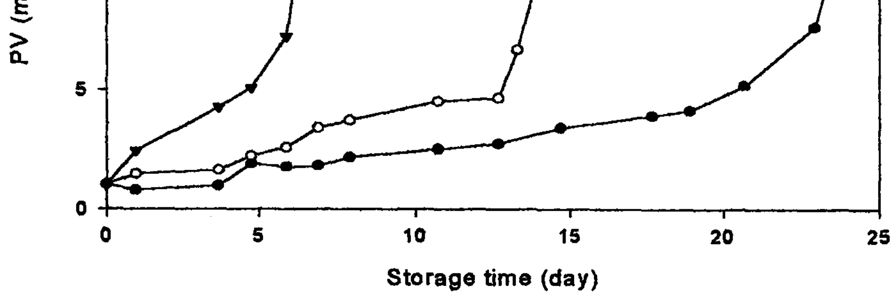


Figure 6. PV changes of butter oil produced from SSY1 at 60, 70, and 80°C.

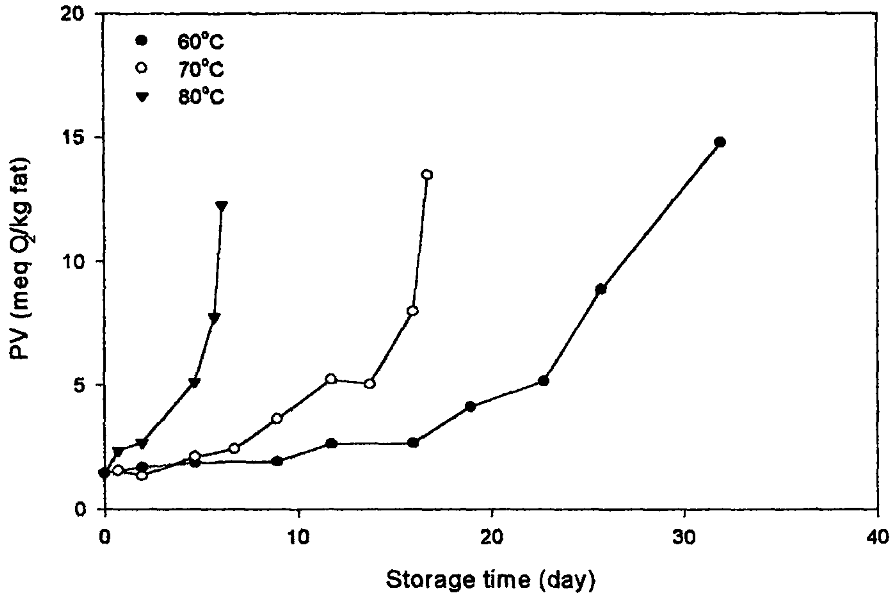


Figure 7. PV changes of butter oil produced from SSY2 at 60, 70, and 80°C.

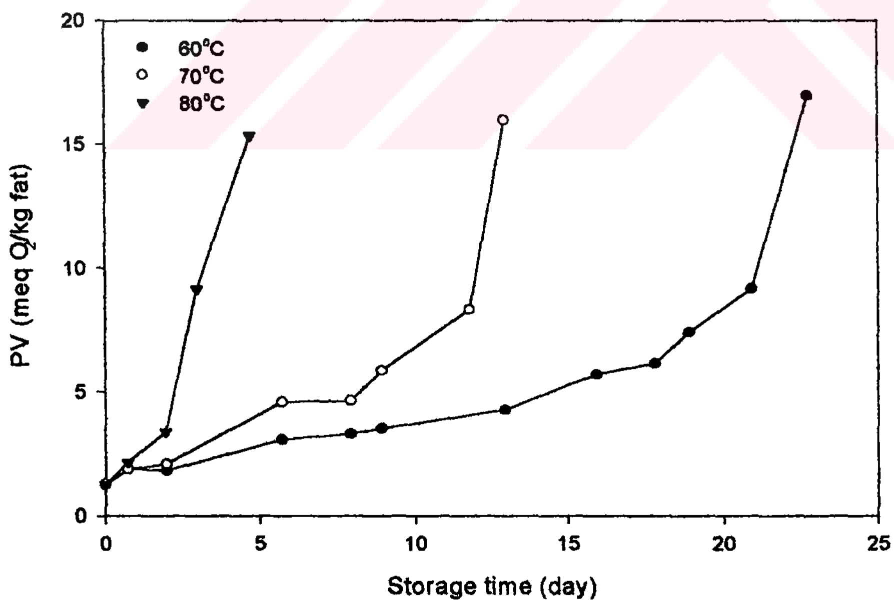


Figure 8. PV changes of butter oil produced from SLY at 60, 70, and 80°C.

Table 4. IP values of sheep's butter oils.

Temperature	SNB	SPB1	SPB2	SSY1	SSY2	SLY
60°C	15.6	19.5	18.7	23.5	27.0	21.2
70°C	9.2	12.1	12.8	14.0	16.2	12.1
80°C	4.7	4.8	5.0	6.2	5.9	3.2

The stability of SPB1 can be attributed to pasteurization and heat treatment of milk. As a result of heat treatment, microorganisms and enzyme activities which could initiate lipid oxidation are killed. Heat treatment was not applied to butter oil produced from SNB in melting process. Therefore, the butter oil is more susceptible to enzymic oxidation. PV and TBA values also support this result.

Stabilities of SSY1 and SSY2 were higher than those of SPB2 and SLY during the storage at all temperatures studied. This can be attributed to stability of fermented milk products due to possible antioxidant production during fermentation and antioxidant characteristic of browning reaction products formed in melting process [58].

Iskander and Shalabi reported that oxidative rancidity development of in the fat was inhibited by the treatment of ghee with fermented milk or whey [59]. Taylor and Richardson have also suggested that formation of sulphydryl groups could be responsible for the antioxidant activity of solid non fat [60].

The color change due to browning reaction products observed in melting and clarification process shows the formation of browning products. It can be seen from Table 3 that Yellowness Index values of butter oils produced from yogurt are significantly higher than that of milk.

Butter oil produced from soured butter (SLY) has certain advantages over the sweet butter variety. The aroma is richer, the fat yield higher and there is less risk of reinfection after heat treatment because the bacterial culture suppresses undesirable microorganisms. The disadvantage of butter oil produced from soured butter is that it is more easily prey to oxidation defects, giving it metallic taste. This tendency is emphasized if the slightest trace of copper or other heavy metal is present, thus considerably worsening the butter oil's keeping properties [61].

Table 5. TBA values of sheep's butter oils.

Butter Oil	60°C		70°C		80°C	
	Time (day)	TBA (mg MA/kg fat)	Time (day)	TBA (mg MA/kg fat)	Time (day)	TBA (mg MA/kg fat)
SNB	0.0	0.22	0.0	0.22	0.0	0.22
	1.1	0.37	1.1	0.62	1.1	1.06
	4.1	0.55	4.1	0.97	4.1	2.18
	8.1	0.84	8.1	1.42		
	13.1	1.39	11.1	2.05		
	15.1	1.96				
SPB1	0.0	0.13	0.0	0.13	0.0	0.13
	1.1	0.26	1.1	0.25	1.1	0.29
	4.1	0.38	4.1	0.65	4.1	1.61
	8.1	0.73	8.1	0.71		
	13.1	1.21	11.1	1.59		
	15.1	1.45				
	18.1	1.98				
SPB2	0.0	0.15	0.0	0.15	0.0	0.15
	3.7	0.67	3.7	0.62	1.0	1.18
	5.9	1.29	5.9	0.91	3.7	2.69
	7.9	1.75	7.9	2.73		
	12.7	1.33	10.7	1.27		
	17.7	2.31	12.7	2.81		
SSY1	0.0	0.27	0.0	0.27	0.0	0.27
	3.7	0.55	3.7	0.78	1.0	0.47
	5.9	1.17	5.9	1.31	3.7	1.97
	12.7	1.30	7.9	1.53		
	17.7	1.65	10.7	1.75		
	20.7	2.25	12.7	1.76		
SSY2	0.0	0.28	0.0	0.28	0.0	0.28
	4.7	0.66	0.7	0.59	0.7	0.74
	8.9	0.85	4.7	0.69	4.7	1.07
	13.7	0.87	6.7	0.64		
	15.9	1.05	8.9	1.07		
	18.9	1.16	11.8	1.42		
	22.7	1.56	13.7	1.6		
	26.7	1.91	15.9	2.12		
	28.9	2.15				
SLY	0.0	0.51	0.0	0.51	0.0	0.51
	6.7	0.85	0.7	0.59	0.7	0.87
	8.9	0.93	4.7	0.96	4.7	2.51
	11.8	1.19	6.7	1.32		
	18.9	1.22	8.9	1.46		
	18.9	1.49	11.8	1.81		
	20.9	1.74				

Tables 6-8 present initial and final fatty acid profiles of butter oils. The major fatty acids of butter oil samples were myristic, palmitic, stearic and oleic acids. These results are in agreement with the previous studies [7,8,55]. The unsaturated (U)/saturated (S) fatty acid ratio was obtained by dividing the total unsaturated fatty acids by the total saturated fatty acids.

Table 9 lists the FFA values of butter oils. Although the initial FFA is highest in SNB values obtained during storage indicate that the rate of increase in FFA is very low. There was only a slight increase in the FFA content of the samples at all temperatures. These results suggest that butter oil samples were stable to hydrolytic rancidity at elevated temperatures. This can be explained by high temperature storage of samples which inhibit the lipolytic activity [56]. At the end of storage the FFA values were still within the permissible range of 0.5 % oleic acid [54].

Table 6. % Fatty acid composition of butter oils produced from SNB and SPB1.

Fatty Acids	SNB				SPB1			
	Initial	Final			Initial	Final		
		60°C	70°C	80°C		60°C	70°C	80°C
C6	0.78	0.16	0.31	0.34	0.56	0.35	0.41	0.36
C8	0.35	0.42	0.48	0.32	0.98	0.51	0.62	0.46
C10	1.81	1.53	1.79	1.44	1.52	1.44	1.16	1.41
C12	2.24	2.10	2.28	2.42	2.39	1.89	1.80	1.60
C14	12.05	12.67	11.85	11.98	11.45	12.09	12.47	12.77
C14:1	0.59	0.35	0.42	0.51	0.81	0.63	0.54	0.26
C16	29.83	30.25	30.81	30.63	30.06	31.14	31.30	31.08
C16:1	2.96	2.50	2.69	2.61	3.31	3.16	2.89	3.13
C18	12.07	12.96	12.51	12.48	10.57	11.24	11.45	11.77
C18:1	31.08	32.67	32.45	32.21	31.63	32.48	32.06	32.29
C18:2	2.81	1.98	1.86	2.04	3.05	2.18	2.09	1.97
C18:3	0.74	0.37	0.24	0.31	0.95	0.35	0.42	0.44
Total	97.31	97.96	97.69	97.29	97.28	97.46	97.21	97.54
Saturated (S)	59.13	60.09	60.03	59.61	57.53	58.66	59.21	59.45
Unsaturated (U)	38.18	37.87	37.66	37.68	39.75	38.80	38.00	38.09
Others*	2.69	2.04	2.31	2.71	2.72	2.54	2.79	2.46
U/S	0.65	0.63	0.63	0.63	0.69	0.66	0.64	0.64

Table 7. % Fatty acid composition of butter oil produced from SPB2 and SSY1.

Fatty Acids	SPB2				SSY1			
	Initial	Final			Initial	Final		
		60°C	70°C	80°C		60°C	70°C	80°C
C6	0.60	0.37	0.33	0.15	0.74	0.42	0.50	0.28
C8	0.58	0.53	0.41	0.34	0.43	0.51	0.38	0.65
C10	0.72	1.28	1.05	0.86	1.79	2.01	1.93	1.61
C12	1.90	1.91	1.73	2.12	2.60	2.43	2.61	2.28
C14	13.05	13.65	13.39	13.48	14.29	14.52	14.02	13.86
C14:1	0.22	0.19	0.18	0.21	0.44	0.34	0.41	0.22
C16	29.54	29.74	30.06	29.87	28.55	29.86	29.61	30.37
C16:1	3.80	3.62	3.77	3.35	3.06	2.43	2.28	2.59
C18	11.87	11.98	13.05	13.22	11.94	12.22	12.09	12.22
C18:1	31.21	31.87	32.05	31.44	30.09	31.11	31.25	30.82
C18:2	3.19	2.08	1.67	2.31	3.23	1.63	1.94	2.16
C18:3	0.88	0.37	0.19	0.25	0.76	0.21	0.32	0.47
Total	97.56	97.59	97.88	97.60	97.92	97.69	97.34	97.53
Saturated (S)	58.26	59.46	60.02	60.04	60.34	61.97	61.14	61.27
Unsaturated (U)	39.30	38.13	37.86	37.56	37.58	35.72	36.20	36.26
Others*	2.44	2.41	2.12	2.40	2.08	2.31	2.66	2.47
U/S	0.67	0.64	0.63	0.63	0.62	0.58	0.59	0.59

Table 8. % Fatty acid composition of butter oil produced from SSY2 and SLY.

Fatty Acids	SSY2				SLY			
	Initial	Final			Initial	Final		
		60°C	70°C	80°C		60°C	70°C	80°C
C6	0.24	0.38	0.18	0.35	0.40	0.37	0.22	0.36
C8	0.53	0.46	0.43	0.48	0.38	0.14	0.24	0.45
C10	1.24	1.32	1.18	1.25	1.72	1.28	1.34	1.74
C12	2.08	1.83	1.61	1.99	2.13	1.86	2.08	2.04
C14	10.14	10.61	11.29	9.75	12.42	13.05	12.95	11.52
C14:1	0.52	0.45	0.27	0.18	0.41	0.38	0.25	0.36
C16	31.49	31.73	32.51	31.50	31.06	31.59	31.73	32.05
C16:1	1.36	0.79	0.93	1.06	1.21	1.06	0.91	0.84
C18	11.42	11.78	12.01	12.23	10.05	10.95	11.07	11.25
C18:1	36.07	36.51	36.40	36.77	35.01	35.81	35.56	35.36
C18:2	1.92	0.81	0.85	1.02	2.01	1.04	0.96	1.19
C18:3	0.80	0.48	0.23	0.69	0.87	0.33	0.28	0.41
Total	97.81	97.15	97.71	97.27	97.67	97.86	97.59	97.57
Saturated (S)	57.14	58.11	59.21	57.55	58.16	59.24	59.63	59.41
Unsaturated (U)	40.67	39.04	38.50	39.72	39.51	38.62	37.96	38.16
Others*	2.19	2.85	2.29	2.73	2.33	2.14	2.41	2.43
U/S	0.71	0.67	0.65	0.69	0.68	0.65	0.63	0.64

Table 9. FFA values of sheep's butter oils.

Butter Oil	60°C		70°C		80°C	
	Time (day)	FFA (% oleic acid)	Time (day)	FFA (% oleic acid)	Time (day)	FFA (% oleic acid)
SNB	0.0	0.28	0.0	0.28	0.0	0.28
	4.1	0.29	4.1	0.30	4.1	0.30
	8.1	0.29	8.1	0.31	8.1	0.31
	13.1	0.31	13.1	0.33		
	15.1	0.34				
	18.1	0.36				
SPB1	0.0	0.15	0.0	0.15	0.0	0.15
	4.1	0.17	4.1	0.16	4.1	0.17
	8.1	0.20	8.1	0.18	8.1	0.23
	13.1	0.24	13.1	0.24		
	15.1	0.26				
	18.1	0.30				
SSB2	0.0	0.17	0.0	0.17	0.0	0.17
	5.9	0.19	5.9	0.22	5.9	0.26
	10.7	0.23	10.7	0.30	6.9	0.32
	17.7	0.34	17.7	0.33	7.9	0.33
SSY1	0.0	0.21	0.0	0.21	0.0	0.21
	10.7	0.24	5.9	0.23	1.0	0.22
	17.7	0.25	10.7	0.26	3.7	0.25
	20.7	0.28	20.7	0.28		
	24.7	0.31				
SSY2	0.0	0.17	0.0	0.17	0.0	0.17
	4.7	0.21	4.7	0.23	4.7	0.22
	7.9	0.26	7.9	0.27	5.7	0.26
	15.9	0.29	15.9	0.31	7.9	0.29
	20.9	0.32	17.8	0.34		
	39.9	0.33				
SLY	0.0	0.24	0.0	0.24	0.0	0.24
	4.7	0.25	4.7	0.28	4.7	0.30
	7.9	0.32	7.9	0.31	5.7	0.33
	15.9	0.35	12.9	0.39	6.7	0.35
	20.9	0.38				
	22.7	0.42				

The Arrhenius parameters and initial concentrations estimated using two-step linear least squares were summarized in Table 10 for a first order kinetic. Table 10 lists the estimated initial peroxide (C_o) and rate constant (k) values at three temperatures. Also it is seen that k values increase as the temperature is increased. E_a values of butter oils were also presented in Table 10. Those E_a values are in reasonable agreement with the data (50-80 kJ/mol) presented by Labuza for lipid oxidation [57].

Table 10. Statistical analysis of first order kinetic parameters for sheep's butter oils from PV data.

Samples	Parameters	60°C	70°C	80°C
SNB	C_0 (meq O_2/kg)	1.43 ± 0.11	1.42 ± 0.01	1.27 ± 0.24
	k (day^{-1})	0.12 ± 0.01	0.21 ± 0.01	0.57 ± 0.01
	R^2	0.9833	0.9925	0.9883
	E_a		75.9	
	R^2		0.9687	
SPB1	C_0 (meq O_2/kg)	1.03 ± 0.17	0.98 ± 0.36	1.22 ± 0.78
	k (day^{-1})	0.11 ± 0.02	0.19 ± 0.03	0.47 ± 0.13
	R^2	0.9661	0.9412	0.9255
	E_a		70.8	
	R^2		0.9861	
SPB2	C_0 (meq O_2/kg)	0.82 ± 0.08	0.84 ± 0.28	0.62 ± 0.33
	k (day^{-1})	0.14 ± 0.01	0.20 ± 0.03	0.55 ± 0.11
	R^2	0.9921	0.9254	0.9715
	E_a		68.5	
	R^2		0.9350	
SSY1	C_0 (meq O_2/kg)	0.54 ± 0.18	0.66 ± 0.32	0.57 ± 0.32
	k (day^{-1})	0.12 ± 0.02	0.19 ± 0.04	0.47 ± 0.09
	R^2	0.9040	0.9259	0.9475
	E_a		66.4	
	R^2		0.9568	
SSY2	C_0 (meq O_2/kg)	0.76 ± 0.14	0.76 ± 0.32	1.01 ± 0.54
	k (day^{-1})	0.09 ± 0.01	0.16 ± 0.03	0.39 ± 0.09
	R^2	0.9783	0.9054	0.9275
	E_a		69.8	
	R^2		0.9790	
SLY	C_0 (meq O_2/kg)	0.83 ± 0.31	1.02 ± 0.43	1.87 ± 0.57
	k (day^{-1})	0.13 ± 0.02	0.20 ± 0.04	0.45 ± 0.07
	R^2	0.9087	0.9194	0.9554
	E_a		63.0	
	R^2		0.9747	

3.2. Butter Oils Produced from Cow's Milk

Table 11 presents the physical and chemical characteristics of butter oil samples produced from pasteurized milk (CPB)-sweet yogurt (CSY1), sweet yogurt (CSY2)-sour yogurt (CLY) and sweet yogurt using 3 h melting (CSYM1)-sweet yogurt using 6 h melting (CSYM2).

Table 11. Initial physical and chemical characteristics of cow's butter oils.

Analysis	CPB	CSY1	CSY2	CLY	CSYM1	CSYM2
-Peroxide value (meq O_2/kg fat)	0.25	0.21	0.47	0.38	0.49	0.56
-FFA (% oleic acid)	0.18	0.16	0.16	0.21	0.14	0.17
-TBA value (mg MA/kg fat)	0.21	0.24	0.31	0.37	0.47	0.44
-Color values						
L	7.32	5.48	6.21	6.38	6.17	5.88
a	0.21	0.13	0.23	0.15	-0.10	0.08
b	0.17	0.36	0.11	0.18	0.37	0.41
YI	15.86	18.45	21.89	20.24	18.12	24.10
-Iodine value (ml 0.1N $Na_2S_2O_3$/g fat)	35.10	35.50	35.4	35.6	35.7	35.4
-Saponification value (ml 0.5N HCl/g fat)	224.0	227.0	226.3	225.1	225.7	226.2
-Refractive index (35°C)	1.4632	1.4327	1.4605	1.4598	1.4605	1.4600

Values indicate that there are small differences in physical and chemical characteristics of the butter oil samples. These results are in agreement with those reported for butter oil by Iskander et al [7]. Yellownes Index was slightly higher than that of sheep's butter oil due to both heating effect during pasteurization and melting process and feeding of cow.

The course of oxidation was followed in the butter oil samples by testing of oils at regular intervals using PV and TBA determinations. Figures 9-14 show the change in PV values of CPB, CSY1, CSY2, CLY, CSYM1 and CSYM2 at 60, 70 and 80°C, respectively.

IP values obtained from PV graphs using permissible limit were presented in Table 12. When IP values of each matched run were compared, the thermal stability results were highest in CSY1, CSY2 and CSYM2 and those were lowest in CPB, CLY and CSYM1.

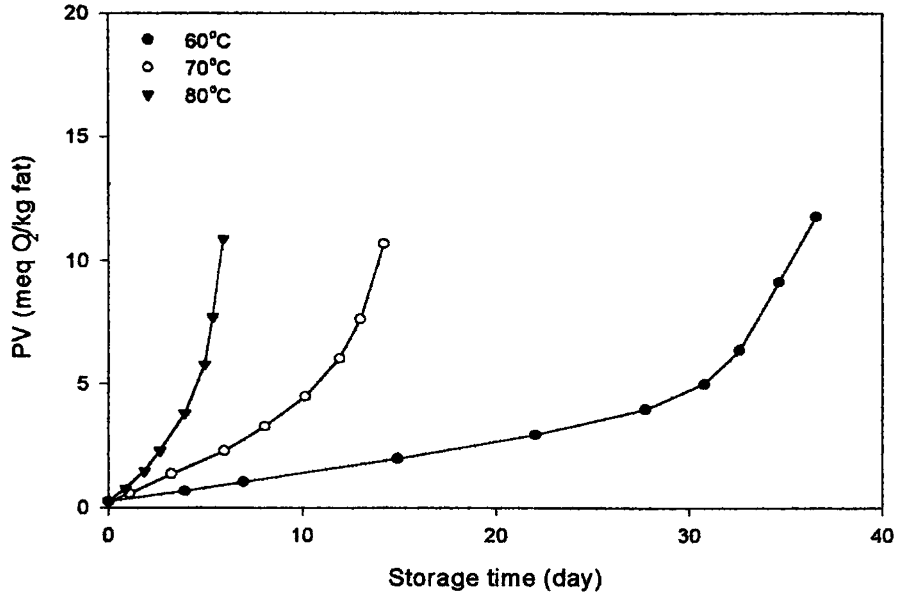


Figure 9. PV changes of butter oil produced from CPB at 60, 70, and 80°C.

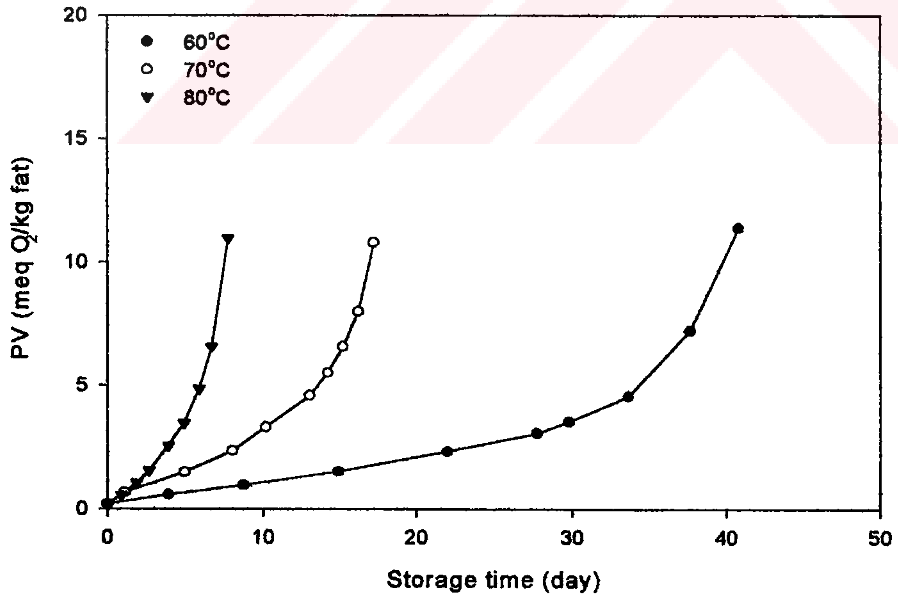


Figure 10. PV changes of butter oil produced from CSY1 at 60, 70, and 80°C.

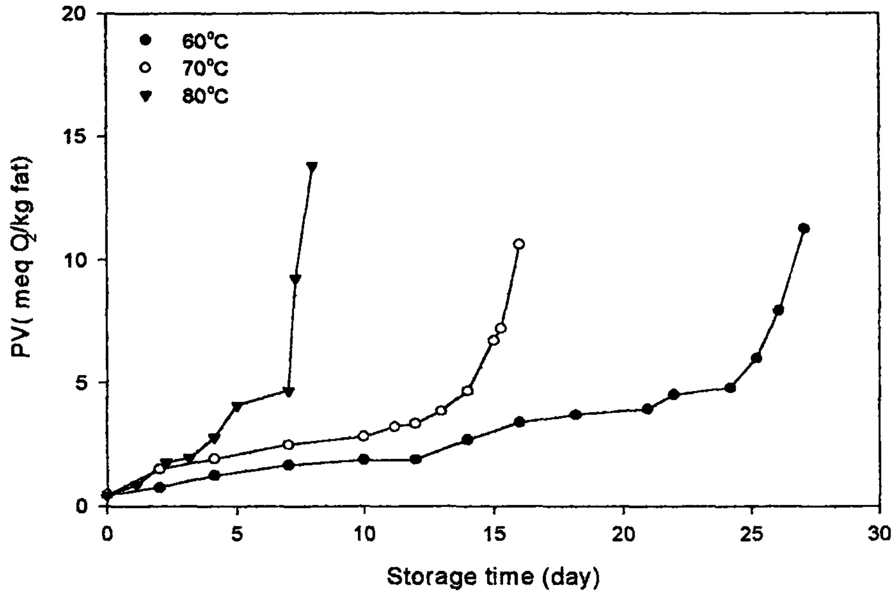


Figure 11. PV changes of butter oil produced from CSY2 at 60, 70, and 80°C.

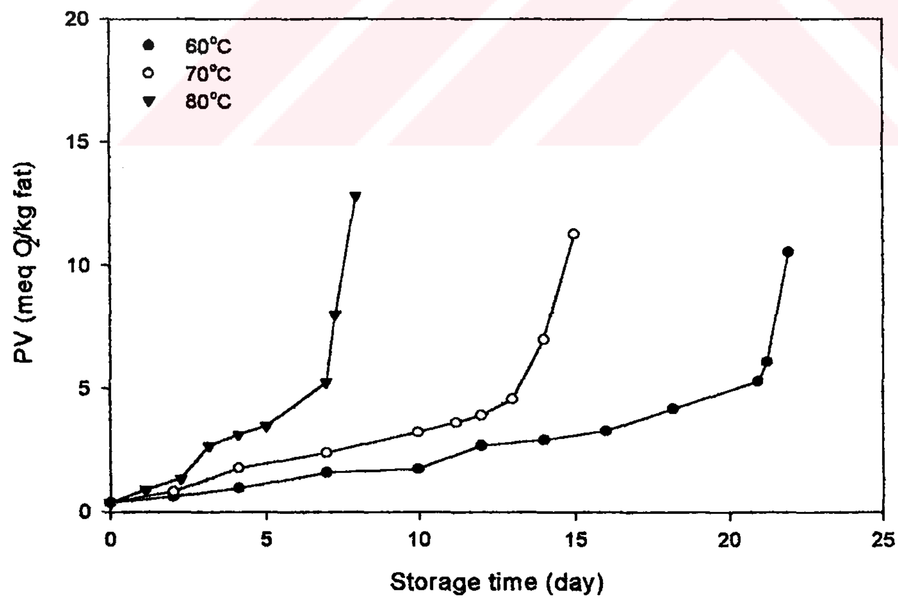


Figure 12. PV changes of butter oil produced from CLY at 60, 70, and 80°C.

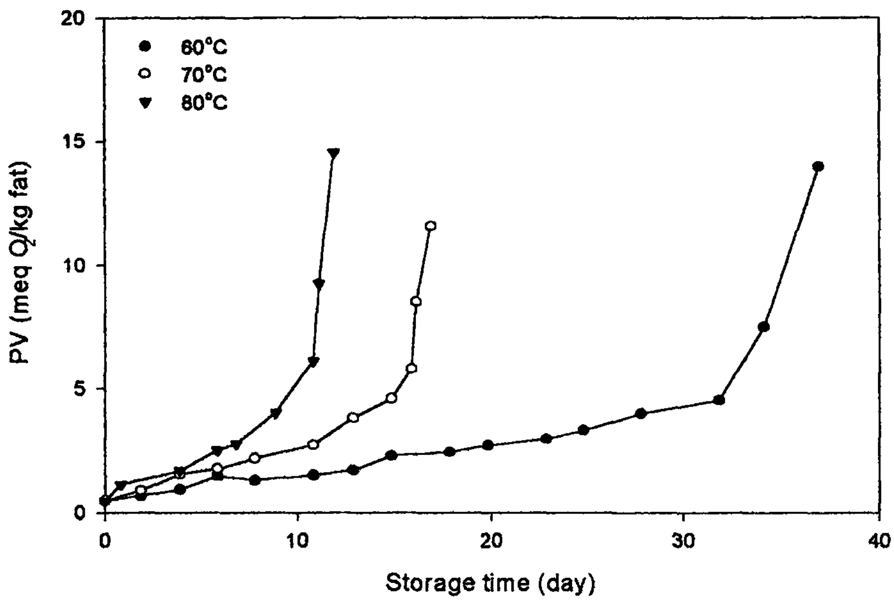


Figure 13. PV changes of butter oil produced from CSYM1 at 60, 70, and 80°C.

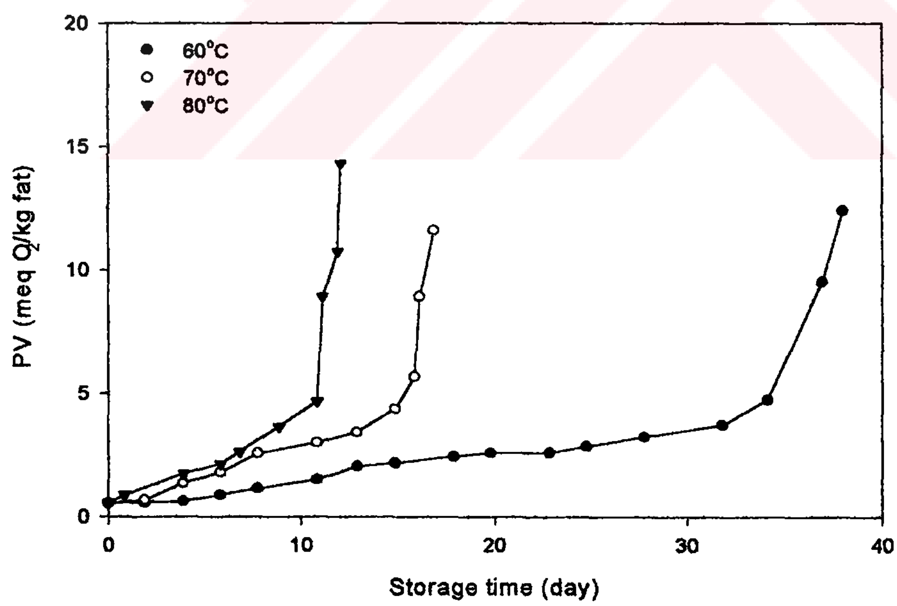


Figure 14. PV changes of butter oil produced from CSYM2 at 60, 70, and 80°C.

It has found that the thermal stability was highest in butter oil produced from sweet yogurt (CSY1).

TBA values also increases as the storage time and temperature increased (Table 13). PV and TBA values indicate that the rate of increase in PV and TBA values of Among each matched run, CSY1, CSY2 and CSYM2 were higher than those of CPB, CLY and CSYM1 during the storage at all temperatures studied.

Stability was highest in butter oil produced from sweet yogurt using 3 h melting time in November (CSY1) and was lowest in butter oil produced from sour yogurt (CLY) in May. When CSYM2 produced using same process of CSY1 was compared with butter oil produced from sweet yogurt using 6 h melting time(CSYM2) in further study in December, stability of CSYM2 was higher than that of CSYM1. If CSYM2 was produced in November stability would be highest in CSYM2. This can be attributed to stability of fermented milk products as explained for sheep's butter oil produced from sweet yogurt. Also, it was assumed that using 6 h holding time during melting increased antioxidant production due to browning. However, it can be seen from Table 12 that IP values of CSYM1 and CSYM2 are nearly equal and 6 h holding time during melting is not feasible when energy consumption is considered. Excess heating may also lead to the formation of undesirable mutagenic products [62]. As it was explained in section 3.1, long fermentation, causing sourness, decreased the stability of CLY.

Table 12. IP values of cow's butter oils.

Temperature	CPB	CSY1	CSY2	CLY	CSYM1	CSYM2
60°C	36.0	40.5	26.7	22.0	35.2	37.1
70°C	14.2	17.0	15.9	14.7	16.5	16.4
80°C	5.7	7.5	7.4	7.6	11.2	11.6

Tables 14-16 present the initial and final fatty acid profiles of butter oils at 60, 70 and 80°C. It was found that major fatty acids of butter oil samples were myristic, palmitic, stearic and oleic acids [7,8,55]. The unsaturated (U)/saturated (S) fatty acid ratio was obtained by dividing the total unsaturated fatty acids by the total saturated fatty acids.

Table 13. TBA values of cow's butter oils.

Butter Oil	60°C		70°C		80°C	
	Time (day)	TBA (mg MA/kg fat)	Time (day)	TBA (mg MA/kg fat)	Time (day)	TBA (mg MA/kg fat)
CPB	0.0	0.21	0.0	0.21	0.0	0.21
	3.9	0.34	3.9	0.42	1.9	0.46
	6.9	0.45	5.9	0.63	2.7	0.65
	15.0	0.61	8.0	0.74	3.9	0.89
	22.0	0.88	10.2	0.9	4.9	1.16
	27.7	0.97	13.0	1.23	5.9	1.44
	32.6	1.14	14.3	1.38		
	36.6	1.35				
CSY1	0.0	0.24	0.0	0.24	0.0	0.24
	3.9	0.35	4.9	0.46	2.7	0.44
	8.7	0.43	8.0	0.58	4.9	0.71
	15.0	0.52	13.0	0.92	6.7	1.07
	22.0	0.69	14.3	1.07	7.7	1.32
	27.7	0.78	16.3	1.28		
	33.6	1.06				
	37.7	1.24				
CSY2	0.0	0.31	0.0	0.31	0.0	0.31
	6.9	0.73	6.9	0.82	4.1	1.39
	16.0	1.09	11.2	0.96	7.3	1.50
	21.9	1.73	16.0	1.95		
	24.4	2.23		:		
CLY	0.0	0.37	0.0	0.37	0.0	0.37
	6.9	0.64	6.9	0.83	4.1	1.50
	16.0	1.01	11.2	1.44	8.3	1.95
	21.9	1.28	15.0	1.76		
	27.1	2.13				
CSYM1	0.0	0.47	0.0	0.47	0.0	0.47
	6.9	0.96	6.9	0.74	6.9	1.08
	11.9	1.15	11.9	1.45	12.0	2.01
	21.9	1.79	17.8	2.80		
	38.9	2.36				
CSYM2	0.0	0.47	0.0	0.47	0.0	0.47
	6.9	0.96	6.9	0.74	6.9	1.08
	11.9	1.15	11.9	1.45	12.0	2.01
	21.9	1.79	17.8	2.80		
	38.9	2.36				

Table 14-16 present the fatty acid profiles of cow's butter oils. It was found that the major fatty acids were myristic, palmitic, stearic and oleic acids.

Table 14. % Fatty acid composition of butter oil produced from CPB and CSY1.

Fatty Acids	CPB				CSY1			
	Initial	Final			Initial	Final		
		60°C	70°C	80°C		60°C	70°C	80°C
C6	0.59	0.47	0.38	0.25	0.63	0.38	0.45	0.54
C8	0.73	0.52	0.41	0.34	0.67	0.50	0.60	0.43
C10	1.96	1.81	1.05	1.12	1.79	1.82	1.29	1.47
C12	2.52	2.35	2.19	2.27	2.35	1.76	1.31	1.58
C14	10.27	11.65	11.41	11.43	9.50	10.20	10.46	10.43
C14:1	1.82	1.49	0.94	1.09	1.81	1.46	1.35	0.98
C16	31.38	31.45	31.82	31.81	29.19	30.57	30.51	30.91
C16:1	3.06	2.42	3.77	3.35	2.30	1.75	2.05	2.29
C18	10.05	11.07	11.41	11.67	11.60	12.20	12.18	12.06
C18:1	31.45	31.57	31.76	31.75	33.75	34.09	34.15	34.30
C18:2	2.94	2.13	2.25	2.27	3.14	2.34	2.38	2.35
C18:3	0.81	0.18	0.23	0.25	0.73	0.18	0.20	0.19
Total	97.58	97.11	97.62	97.60	97.46	97.25	96.93	97.53
Saturated (S)	57.50	59.32	58.67	58.89	55.73	57.43	56.80	57.42
Unsaturated (U)	40.80	37.79	38.95	38.71	41.76	39.82	40.13	40.11
Others*	2.42	2.89	2.38	2.40	2.51	2.75	3.07	2.47
U/S	0.69	0.64	0.66	0.66	0.75	0.69	0.70	0.7

Table 15. % Fatty acid composition of butter oil produced from CSY2 and CLY.

Fatty Acids	CSY2				CLY			
	Initial	Final			Initial	Final		
		60°C	70°C	80°C		60°C	70°C	80°C
C6	0.68	0.37	0.51	0.31	0.34	0.36	0.45	0.21
C8	0.92	0.86	0.75	0.79	0.68	0.41	0.72	0.84
C10	1.06	1.34	1.78	1.65	1.47	1.19	1.33	1.14
C12	1.92	1.83	2.72	2.04	2.63	2.51	2.27	2.45
C14	10.14	9.68	10.41	10.52	10.63	11.08	10.81	10.84
C14:1	0.46	0.31	0.52	0.28	0.36	0.31	0.28	0.49
C16	32.59	33.01	32.97	32.33	32.11	33.08	32.61	32.73
C16:1	3.08	3.79	3.04	3.11	3.25	2.71	2.60	2.78
C18	5.26	5.81	5.35	5.62	5.89	6.48	6.54	6.43
C18:1	37.43	38.14	36.30	38.51	36.84	37.06	36.98	37.21
C18:2	3.03	2.21	2.43	2.28	2.85	2.05	2.23	2.37
C18:3	1.44	0.68	0.72	0.65	1.28	0.57	0.52	0.36
Total	98.01	98.03	97.50	98.09	98.33	97.81	97.34	97.85
Saturated (S)	52.57	52.90	54.49	53.26	53.75	55.11	54.73	54.64
Unsaturated (U)	45.64	45.13	43.01	44.83	44.58	42.70	42.61	43.21
Others*	1.79	1.97	2.50	1.91	1.67	2.19	2.66	2.15
U/S	0.87	0.85	0.79	0.84	0.83	0.77	0.78	0.79

Table 16. % Fatty acid composition of butter oil produced from CSYM1 and CSYM2.

Fatty Acids	CSYM1				CSYM2			
	Initial	Final			Initial	Final		
		60°C	70°C	80°C		60°C	70°C	80°C
C6	0.23	0.44	0.54	0.19	0.49	0.36	0.50	0.18
C8	0.71	0.66	0.81	0.79	0.59	0.57	0.48	0.81
C10	1.17	0.86	1.32	1.06	1.92	1.14	1.39	1.43
C12	1.51	3.08	2.46	2.32	1.48	1.21	1.96	1.55
C14	6.72	7.01	7.23	7.74	8.73	9.12	10.43	9.46
C14:1	0.54	0.41	0.33	0.16	0.15	0.19	0.18	0.28
C16	33.79	33.56	33.29	34.17	32.61	33.82	33.02	32.94
C16:1	4.51	3.30	3.38	3.53	3.36	2.47	2.29	2.62
C18	8.47	8.82	8.11	8.71	10.07	10.25	9.85	11.28
C18:1	36.46	37.21	37.52	37.08	35.26	35.81	36.04	35.08
C18:2	2.83	2.03	2.18	1.97	2.30	1.79	1.52	1.64
C18:3	0.85	0.37	0.41	0.31	0.81	0.42	0.24	0.36
Total	97.79	97.75	97.58	98.03	97.74	97.15	97.90	97.63
Saturated (S)	52.60	54.43	53.76	54.98	55.86	56.47	57.63	57.65
Unsaturated (U)	45.19	43.32	43.82	43.05	41.88	40.68	40.27	39.98
Others*	2.21	2.25	2.42	1.97	2.26	2.85	2.10	2.37
U/S	0.86	0.79	0.81	0.78	0.75	0.72	0.70	0.69

Table 17 lists the FFA values of cow's butter oils. There was only a slight increase in the FFA content of the samples at all temperatures. These results suggest that butter oil samples were stable to hydrolytic rancidity at elevated temperatures. This can be explained by high temperature storage of samples which inhibit the lipolytic activity. At the end of storage the FFA values were still within the permissible range.

The Arrhenius parameters and initial concentrations estimated using two-step linear least squares were summarized in Table 18 for a first order kinetic. Table 18 lists the estimated initial peroxide (C_o) and rate constant (k) values at three temperatures. It is seen that k values increase as the temperature is increased. Those E_a values are in reasonable agreement with the data presented by Labuza for lipid oxidation [57].

Table 17. FFA values of cow's butter oils.

Butter Oil	60°C		70°C		80°C	
	Time (day)	FFA (% oleic acid)	Time (day)	FFA (% oleic acid)	Time (day)	FFA (% oleic acid)
CPB	0.0	0.18	0.0	0.18	0.0	0.18
	6.9	0.20	3.2	0.24	2.7	0.21
	15.0	0.24	8.0	0.27	3.9	0.23
	22.0	0.25	10.2	0.31	4.9	0.28
	27.7	0.26	13.0	0.33	5.9	0.32
	32.6	0.28	14.3	0.35		
	36.7	0.36				
CSY1	0	0.16	0.0	0.16	0.0	0.16
	8.7	0.19	4.9	0.17	2.7	0.19
	15.0	0.24	8.0	0.21	4.9	0.23
	22.0	0.26	10.2	0.24	6.7	0.24
	27.7	0.27	14.3	0.25	7.7	0.26
	33.6	0.28	16.3	0.27		
	37.7	0.30				
CSY2	0.0	0.31	0.0	0.31	0.0	0.31
	6.9	0.73	6.9	0.82	4.1	1.39
	16.0	1.09	11.2	0.96	7.3	1.50
	21.9	1.73	16.0	1.95		
	24.4	2.23				
CLY	0.0	0.37	0.0	0.37	0.0	0.37
	6.9	0.64	6.9	0.83	4.1	1.50
	16.0	1.01	11.2	1.44	8.3	1.95
	21.9	1.28	15.0	1.76		
	27.1	2.13				
CSYM1	0.0	0.14	0.0	0.14	0.0	0.14
	10.8	0.20	10.8	0.21	6.8	0.26
	34.1	0.26	14.8	0.28	12.0	0.28
	38.9	0.30	17.8	0.31	13.0	0.31
CSYM2	0.0	0.17	0.0	0.17	0.0	0.17
	18.0	0.23	11.9	0.26	6.8	0.27
	34.1	0.28	16.1	0.28	11.1	0.30
	38.9	0.34	17.8	0.31	13.0	0.34

Table 18. Statistical analysis of first order kinetic parameters for cow's butter oils from PV data.

Samples	Parameters	60°C	70°C	80°C
CPB	C_0 (meq O_2/kg)	0.20 ± 0.08	0.59 ± 0.09	0.63 ± 0.09
	k (day^{-1})	0.11 ± 0.01	0.20 ± 0.01	0.47 ± 0.03
	R^2	0.9743	0.9866	0.9913
	E_a			
	R^2			
CSY1	C_0 (meq O_2/kg)	0.22 ± 0.06	0.47 ± 0.10	0.46 ± 0.05
	k (day^{-1})	0.10 ± 0.01	0.18 ± 0.01	0.41 ± 0.02
	R^2	0.9805	0.9844	0.9953
	E_a			
	R^2			
CSY2	C_0 (meq O_2/kg)	0.48 ± 0.18	0.29 ± 0.15	0.33 ± 0.23
	k (day^{-1})	0.11 ± 0.01	0.21 ± 0.03	0.46 ± 0.09
	R^2	0.9033	0.9106	0.9127
	E_a			
	R^2			
CLY	C_0 (meq O_2/kg)	0.40 ± 0.21	0.18 ± 0.11	0.42 ± 0.23
	k (day^{-1})	0.14 ± 0.03	0.27 ± 0.04	0.41 ± 0.07
	R^2	0.8781	0.9313	0.9268
	E_a			
	R^2			
CSYM1	C_0 (meq O_2/kg)	0.28 ± 0.12	0.22 ± 0.16	0.13 ± 0.12
	k (day^{-1})	0.10 ± 0.01	0.23 ± 0.05	0.39 ± 0.08
	R^2	0.9078	0.9067	0.9320
	E_a			
	R^2			
CSYM2	C_0 (meq O_2/kg)	0.27 ± 0.12	0.20 ± 0.10	0.09 ± 0.01
	k (day^{-1})	0.10 ± 0.01	0.23 ± 0.05	0.41 ± 0.09
	R^2	0.8967	0.8915	0.9240
	E_a			
	R^2			

CHAPTER IV
CONCLUSIONS

Butter oils produced from different methods using sheep's and cow's milk were studied to determine physical and chemical characteristics and thermal stability. In the light of the results given in Chapter III, followings were concluded;

1. Major fatty acids of butter oil samples were myristic, palmitic, stearic, and oleic acids.
2. PV and TBA values increased significantly with increasing temperature and oxidative change was of greater significance in thermal stability of butter oils
3. The rate of increase in FFA values was very low. These results suggest that butter oil samples were stable to hydrolytic rancidity at elevated temperatures because of the low moisture content (0.2%).
4. Rate constants of PV data increased significantly as the temperature increased.
5. Activation energies (E_a) were estimated based on PV data. All E_a values were in agreement with the lipid oxidation range.
6. Thermal stability was highest in butter oil produced from sweet yogurt and using sheep's milk. For cow's milk, butter oil produced from sweet yogurt using 6 h of melting time had slightly high thermal stability.
7. Among all the samples studied, butter oil samples produced from sweet yogurt using cow's milk in November and December were the most stable as the induction periods were considered.
8. When sheep's and cow's butter oil samples were compared in terms of thermal stability, butter oil sample produced from sweet yogurt using cow's milk was more stable than butter oil sample produced from sweet yogurt using sheep's milk.

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