STORAGE STABILITY OF MARGARINE

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By

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ABSTRACT

STORAGE STABILITY OF MARGARINE

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In this study, Rancimat Method, which is one of the Accelerated Shelf-Life Test (ASLT) methods, was used to predict the shelf-life of margarines, and two margarine samples (packaged with parchment (A) and plastic (B)) were studied to predict their storage stabilities. The samples were stored at two different temperatures (4°C with a 35 % relative humidity and 14°C with 70, 85 and 98 % relative humidities).

The parameters, peroxide value (PV), free fatty acid (FFA) and total mold and yeast count, that affect margarine deterioration were studied at the mentioned conditions above.

The shelf-lives that obtained from ASLT results by extrapolation to low temperatures were 134 and 77 days for Margarine (A), and 125 and 63 days for Margarine (B) at 4 and 14°C respectively. Q_{10} values calculated from ASLT results, based on formic acid formation, were 2.05 and 2.10, and from normal storage results, based on peroxide formation, were 1.33 and 1.34 for Margarine (A) and (B) respectively.

No hydrolytic deterioration was observed on both Margarine (A) and (B). Also, there were no mold and yeast growth on Margarine (B) neither at high nor low relative humidities. But, mold and yeast growth was observed after 73rd day at 98 % R.H. and 90th day at 85 % R.H. for Margarine (A).

It was observed that parchment packaged Margarine (A) deteriorated in a shorter time than plastic packaged Margarine (B) at R.H. above 70 %. The shelf-life of Margarine (A) was ended due to microbial growth.

On the other hand, there was no microbial growth in Margarine (B), therefore, its shelf-life at high R.H. ended due to oxidative rancidity.

It was concluded that Tangent Line Method is a healthy method to decide the end of shelf-life of a fat containing food product.

Key words: Margarine, Accelerated Shelf-Life Test, Storage Stability, Rancidity, Peroxide Value, Free Fatty Acid, Microbial Count, Rancimat.

ÖZET

MARGARİNİN SAKLAMA DAYANIKLILIĞI

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Bu çalışmada, Hızlandırılmış Raf—Ömrü Test (HRÖT) metodlarından Rancimat Metodu, margarin nümunelerinin raf ömrünü tahmin etmek için kullanıldı ve parşimentle (A) diğeri plastikle (B) paketlenmiş iki margarin nümunesinin saklama dayanıklılığının ölçülmesine çalışıldı. Bunun için, nümuneler iki farklı sıcaklıkta (4°C % 35 bağıl nemde, 14°C, % 70, 85 ve 98 bağıl nemlerde) saklandı.

Bahsedilen bu şartlarda, margarinin bozulmasına sebep olan parametrelerden peroksit değeri (PD), serbest yağ asidi (SYA), toplam küf ve maya sayımı çalışıldı.

HRÖT sonuçlarından elde edilen ve düşük sıcaklıklarda ekstrapolasyon sonucu bulunan dayanma ömürleri sırasıyla 4 ve 14 derecelerde Margarin (A) için 134 ve 77 gün ve Margarin (B) için 125 ve 63 gün olarak belirlendi. Formik asit oluşumu kriter olarak alındığında, HRÖT sonuçlarından hesaplanan Q_{10} değerleri Margarin (A) için 2.05 ve (B) için 2.1 olarak bulunmuştur. Peroksit oluşumunun kriter olarak alındığı normal saklama sonuçlarından Q_{10} değerleri bu margarinler

için sırasıyla 1.33 ve 1.34 olarak hesaplanmıştır.

Her iki nümunede de hidrolitik bozunmaya rastlanmamıştır. Margarin (B)'de yüksek veya düşük bağıl nemlerde herhangi bir küf ve maya büyümesi de görülmemiştir. Fakat Margarin (A)'da % 98 bağıl nemde 73'üncü ve % 85'de ise 90'ıncı günden sonra küf ve maya büyümesi gözlenmiştir.

Parşiment ambalajlı Margarin (A) % 70'in üzerindeki bağıl nem ortamlarında plastik ambalajlı Margarin (B)'den daha kısa bir sürede bozulduğu gözlendi. Margarin (A)'nın raf ömrü mikrobiyal üremeden dolayı sona ermiştir. Margarin (B)'de ise mikrobiyal üreme gözlenmediğinden raf ömrü bu yüksek bağıl nemlerde (A)'dan daha fazla olmuş ve oksidasyon nedeniyle sona ermiştir.

Bu çalışmada Teğet Eğrisi Metodunun yağ içeren bir gıda maddesinin raf ömrünü tayin etmede kullanılması gereken sağlıklı bir metod olduğu sonucuna varıldı.

Anahtar kelimeler: Margarin, Hızlandırılmış Raf-Ömrü Testi, Saklama Dayanıklılığı, Ransidite, Peroksit Değeri, Serbest Yağ Asidi, Mikroorganizma Sayımı, Ransimat.

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LIST OF ABBREVIATIONS

ASLT : Accelerated Shelf-Life Test

PV : Peroxide Value

FFA : Free Fatty Acid

IT : Induction Time

a_w : Water Activity

PEC : Proximity Equilibration Cell

T : Temperature

R.H. : Relative Humidity

CHAPTER I

INTRODUCTION

1.1. Lipid Structure

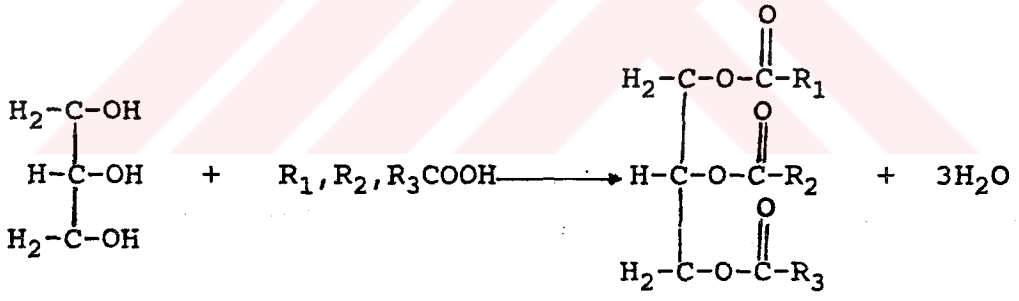
Lipids have been defined as a heterogeneous group of naturally occurring substances which are insoluble in polar solvents but soluble in organic solvents. Lipids contain carbon, oxygen, while some lipids also contain phosphorus and nitrogen. Most lipids are soft solids or liquids at room temperature [1]. The word "fat" is ordinarily used to refer to triglycerides that are solids, or more correctly, semisolid, whereas the word "oil" is used for triglycerides that are liquid at room temperature [2].

Edible fats are complex mixtures of triglycerides and small amounts of other substances occurring naturally or are derived through processing and storage of the fats. The natural fats are made up mostly of mixed triglycerides with only trace amounts of the mono- and di-glycerides and little or no free fatty acids. In contrast, processed fats may contain up to 20 % of the mono- and di-glycerides. The fat-associated substances are important. As examples: The fat-soluble vitamins, sterols, and phospholipids are of

nutritional importance; the free fatty acids are an index of the degree of hydrolysis of a triglyceride; the presence of peroxides, aldehydes, and ketones are indicative of the amount of oxidative deterioration that has taken place in the fat. Furthermore, certain of the sterols, phospholipids, carotenoid pigments, metallic impurities may contribute to the oxidative deterioration of the fat [1].

1.1.1. Mono-, Di- and Triglyceride

A triglyceride is the condensation product of one molecule of glycerol, a trihydroxy alcohol, with three molecules of monocarboxylic acid, fatty acids, to yield three molecules of water and one molecule of a triglyceride [3].



Naturally occurring fats are always mixtures of different triglycerides and the individual triglyceride may have three different fatty acids. Such molecules are known as mixed glycerides.

Triglycerides, on standing, may undergo partial hydrolysis to form di-glycerides and mono-glycerides. The

extent of hydrolysis depends upon the presence of water, heat, and hydrogen or hydroxide ions. The reaction mixture consists of mono-, di-, and triglycerides together with unreacted glycerol [1].

1.1.2. Fatty Acids

Most fatty acids are unbranched monocarboxylic acids which vary in length and degree of saturation or unsaturation [1]. Chemically speaking, a fatty acid is a long-chain aliphatic carboxylic acid. Although there are a considerable number and variety of naturally occurring fatty acids, we can simplify matters by providing generalizations concerning those acids that occur most frequently in nature:

1. Most are monocarboxylic acids containing linear hydrocarbon chains with an even number of carbon atoms,generally in the range of C_{12}-C_{20}. Shorter and longer chain acids, branched and cyclic chain acids, and acids of odd-number carbon content do occur but at a much lower frequency.

2. Unsaturation is common but largely confined to the C_{18} and C_{20} acids. When two or more double bonds exist they are almost always separated by a single methylene group, that is,

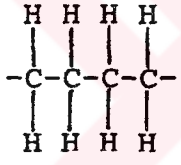
$$-CH=CH-CH_2-CH=CH- \quad \text{and} \quad -CH=CH-CH_2-CH=CH-CH_2-CH=CH-$$

3. In the unsaturated acids, the double bonds are

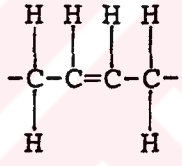
nearly always in the *cis* configuration.

The many C-C and C-H nonpolar bonds in the hydrocarbonchain confer considerable nonpolar character to the entire molecule even though there is one polar COOH group. Obviously any substance composed in part of one or more fatty acids will also be largely nonpolar [4].

Those fatty acids in which all carbon atoms in the chain contain 2 hydrogen atoms and thus contain no double bonds are termed saturated. The fatty acids which contain double bonds are termed unsaturated. The degree of unsaturation of an oil depends upon the average number of double bonds in its fatty acids.



Carbon chain of a saturated acid



Carbon chain of an unsaturated acid

The saturated fatty acids range from C_4 to C_{30}, but fatty acids of greater than C_{20} comparatively rare. The most common saturated fatty acids found in animal fats are palmitic ($C_{15}H_{31}COOH$) and stearic acid ($C_{17}H_{35}COOH$). Saturated fatty acids of less than 16 carbon chain length are frequently found in plant fats. In animal fats, fatty acids containing less than 16 carbons occur only in small quantities. Higher fatty acids such as arachidic acid ($C_{19}H_{39}COOH$) may also occur

in animal fats but branched-chain acids are uncommon.

The saturated acids, C-4 through C-8, are liquid at room temperature (20°C); above C-10 the fatty acids are solids. Butyric acid is miscible with water in all proportions. As the molecular weight increases solubility decreases rapidly; caproic, caprylic and capric acids are slightly soluble in water whereas lauric acid and higher homologues are water insoluble [1]. The most common saturated fatty acid is palmitic and only very small amounts of stearic acid are present in oils. Oils containing linolenic acid, such as soybean oil, are unstable. Such oils are usually selectively hydrogenated to reduce the linolenic acid content before using in foods [5].

The melting points of the saturated acids exhibit a progressive increase as the carbon chain length gets longer. Differences in the melting points of the acids are reflected in the melting points of the simple glycerides and also in those of mixed triglycerides. Thus milk fats and vegetable fats of the coconut oil type, which contain large proportions of C_6 to C_{12} fatty acids, have lower melting points than fats with an equivalent degree of unsaturation that are composed substantially of glycerides of C_{16} and C_{18} fatty acids.

Unsaturated fatty acids are found in the fats of animals, plant, and marine animals. Those occurring in animal fats are principally monoethenoic acids (oleic and palmitic) because animal cells are unable to synthesize unsaturated

fatty acids containing more than one double bond. In contrast di-, and triethenoic acids (linoleic and linolenic) are found primarily in plant fats and oil while polyunsaturated acid (arachidonic) are found in fish oils [1].

A large number of unsaturated fatty acids occur naturally and are more difficult to isolate, purify, and characterize than the saturated acids. Their study is further complicated by the fact that they are less stable and are readily converted to position (difference in position of the double bond) and geometric (*cis*, *trans*) isomers. The naturally occurring acids usually contain an even number of carbon atoms (most frequently 18). In most cases the double bonds have the *cis*-configuration, and a preferred position for a double bond is between the ninth and tenth carbon atoms in the fatty acid chain.

Unsaturated fatty acids are more chemically reactive than the saturated fatty acids. The presence of a double bond permits the addition of hydrogen atoms to the double bond, in the presence of a suitable catalyst (palladium, platinum, nickel, and copper) to give the corresponding saturated fatty acid. Thus, oleic, linoleic and linolenic acids give stearic acid upon hydrogenation. Since the melting point of a fat is increased by this procedure, vegetable oils can be hydrogenated to yield semisolid creamy products that are used extensively as shortening agents for pies, cakes, and other bakery products. The hydrogenation process must be controlled

in order to obtain the desired type of product.

In addition to hydrogenation, unsaturated fatty acids are susceptible to oxidation because of the double bond. Exposure to oxygen of the air causes a fat containing polyunsaturated fatty acids to undergo gradual formation of peroxides together with a mixture of volatile aldehydes, ketones, and acids. The reaction is catalyzed by trace metals or the enzyme lipoxidase.

Some of the more important saturated and unsaturated fatty acids are listed in Table 1.

Table 1. The fatty acid composition of vegetable oils used in margarines [6,7].

Fatty acids g/100 g fat	Soybean	Cotton-seed	Corn	Palm	Sunflower-seed	Olive
Capric(10:0)*	-	0.5	-	-	-	-
Lauric(12:0)	0.1	0.4	-	0.2	-	-
Myristic(14:0)	0.2	0.8	-	1.1	0.1	-
Palmitic(16:0)	10.7	22.0	10.7	42.0	5.8	12.1
Stearic(18:0)	3.9	2.2	1.7	4.3	4.1	2.7
Arachidic(20:0)	0.2	0.2	0.3	0.3	0.3	0.5
Total saturated	15.1	26.1	12.7	47.9	10.3	15.3
Palmitoleic(16:1)	0.3	0.6	0.1	0.5	0.1	1.0
Oleic(18:1)	22.8	18.1	24.6	37.9	21.7	71.8
Linoleic(18:2)	50.8	50.3	57.3	9.0	66.4	10.2
Linolenic(18:3)	6.8	0.4	0.8	0.3	0.3	0.8
Total unsaturated	80.7	69.6	82.8	47.7	88.5	83.8

* (x:y): x = number of Carbon atom, and y = number of double bond.

1.2. Rancidity

Rancidity is the characteristic, unpalatable odour and flavour of edible oils, fats and fat-containing foods following oxidative or hydrolytic degradation [8]. Another way to define rancidity is the development of an off flavour which makes the food unacceptable on a consumer market level. This process can occur in raw food stuffs, refined or used edible oils and processed foods containing edible oils. In addition 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 the degree of unsaturation of the oil, the type and concentrations of antioxidants, pro-oxidants and trace metals present, availability of oxygen, degree of exposure to light, temperature, relative humidity of storage environment and moisture content or water activity of food products. In Fig. 1, the effect of water activity on the stability of foods is graphically illustrated.

Rancidity development in fatty food products is suppressed by careful choice and maintenance of the cooking oil, careful processing, storage and selection of packaging material and the control of moisture content and transition metal contamination (particularly copper). Careful monitoring of rancidity in such foods and also the cooking oils is therefore important in establishing proper processing,

packaging and storage conditions to maintain a suitable shelf life for the product.

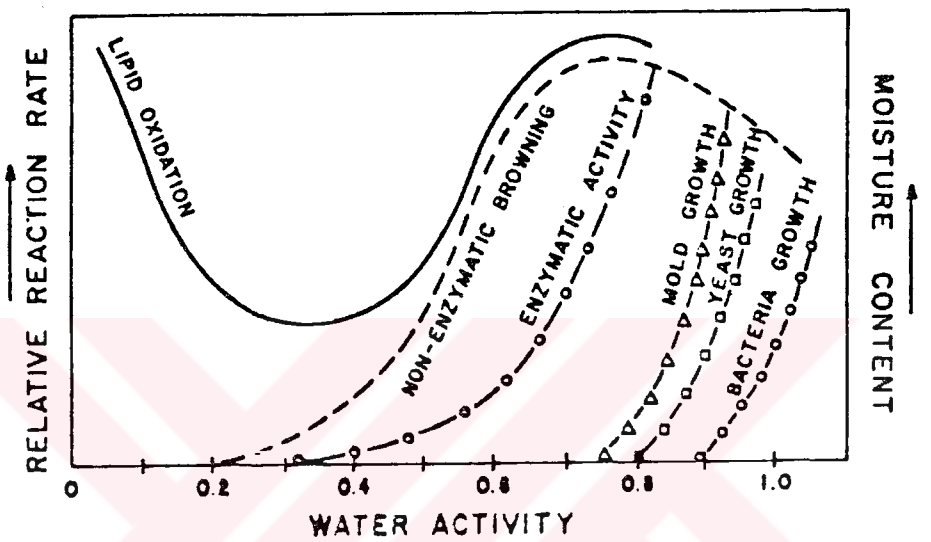


Figure 1. Stability of foods as a function of water activity [9].

1.2.1. Hydrolytic Rancidity

In hydrolytic rancidity the off-flavour is due to the hydrolysis of a fat with the liberation of free fatty acids. Hydrolytic rancidity is noticeable in fats such as butter because the volatile fatty acids have a disagreeable odor and taste. In contrast, hydrolytic changes in fats which contain few volatile fatty acids, are not evident on the basis of odor and taste [1]. Hydrolytic rancidity is not as important

in oils containing predominantly fatty acids of longer chain length except when they are used as frying media, as in snack food preparation. During frying, where heat and water are present, free fatty acids may develop rapidly if poor processing techniques are adopted. Moreover, there is no additive which will effectively prevent free fatty acid formation. Indeed, antioxidants capable of delaying the onset of oxidative rancidity will not prevent free fatty acid formation owing to chemical hydrolysis [8]. In the field of dairy science, rancidity refers usually to hydrolytic changes resulting from enzyme activity [5].

1.2.2. Oxidative Rancidity

The unsaturated bonds present in all fats and oils represent active centers which, among other things, may react with oxygen. This reaction leads to the formation of primary, secondary and tertiary oxidation products which may make the fat or fat-containing food unsuitable for consumption [5]. The reaction between the unsaturated fat and oxygen is a complex, free radical mechanism and results in the production of low-molecular-weight volatiles such as aldehydes, ketones, acids, and alcohols. Some of these, such as hexanal, impart an off odor to the food making it unacceptable even at a very low extent of reaction. For example, with cereals, only 2-3 ppm of hexanal leads to an unacceptable flavour even though only 0.1 % of the lipid may have reacted [10]. The

degeneration of vegetable and animal fats, detected in the incipient stage by alterations in organoleptic properties (i.e., rancid odor and flavour), is due to enzymatic and/or chemical processes.

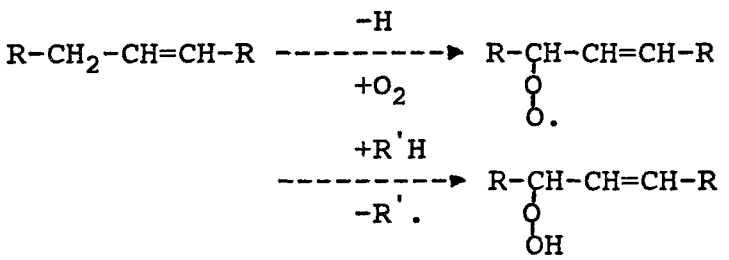
Biochemical processes are primarily conditioned by the water content and the activity of tissue enzymes and microorganisms, while chemical alteration are generally due to the effect of atmospheric oxygen, even when present only in small quantities. These oxidation processes, which only occur slowly at normal ambient temperature, are known as "auto-oxidation."

Auto-oxidation of unsaturated fatty acids in vegetable oils and the products formed decreases the biological availability of these essential dietary components and produces not only flavour deterioration but also potentially unsafe or toxic materials when these oils are heated during processing and cooking [11].

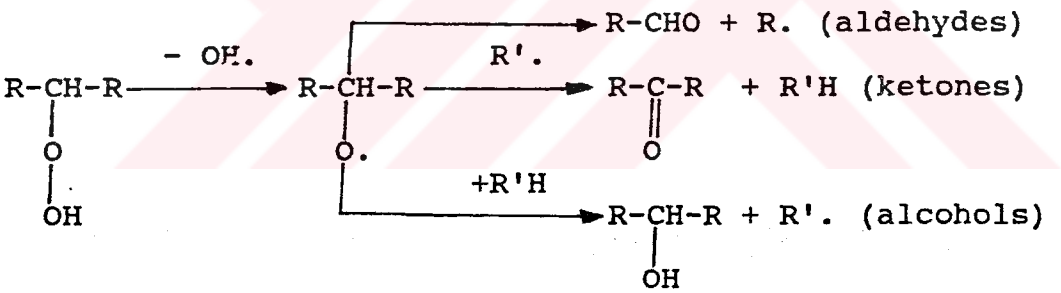
1.2.3. Chemistry of Rancidity

Various reaction mechanisms are possible, yet the oxidation process is primarily initiated by the formation of radicals as a result of the homolytic splitting-off of hydrogen atoms in the alpha position with respect to the double bond [12]. For this reason, oils and fats containing unsaturated fatty acids (such as oleic, linoleic, and linolenic acids) are particularly susceptible to oxidation.

The carbon radicals thus formed react with oxygen to form peroxide radicals, which then enter into chain reactions to form organic peroxides as the primary products:



Decomposition of the organic peroxides then leads to secondary products, such as alcohols and carbonyl compounds, which can be further oxidized to carboxylic acids.

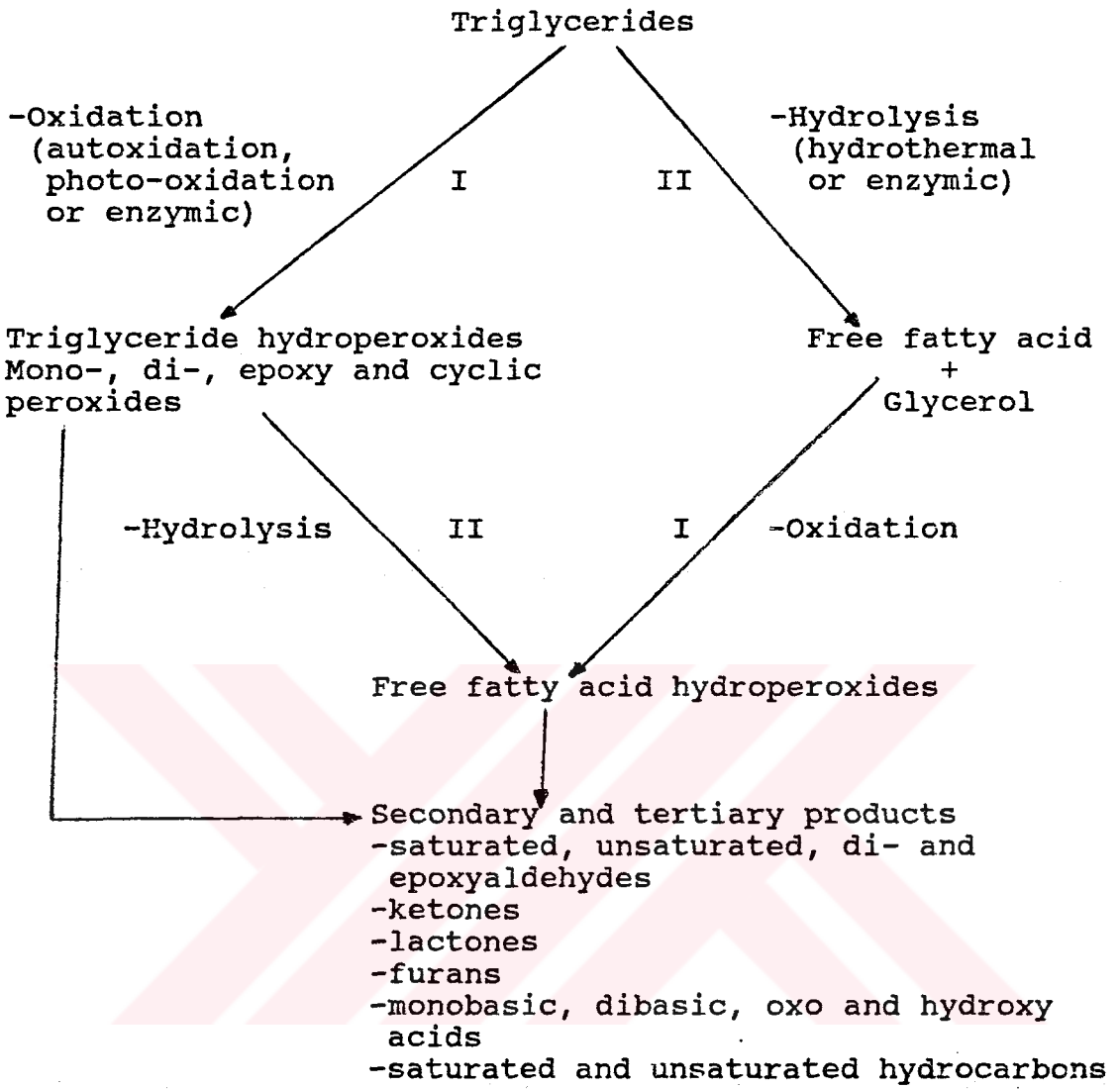


The primary radical formation is particularly encouraged by exposure to light and heat, but, like the secondary reactions, is also catalyzed by porphyrins (hemoglobin derivatives, chlorophyll) and heavy metals (copper, iron, manganese, nickel, etc.). The susceptibility of unsaturated fatty acids to auto-oxidation is dependent on the availability of allylic hydrogens for reaction with peroxy

radicals. The abstraction of the hydrogen on the carbon adjacent to the double bond is favoured because of the formation of a very stable "allyl radical" in which the electrons are delocalized over the three carbon atoms. This leads to a mixture of isomeric hydroperoxides due to the resonance structure of the allylic system [11].

The important chemical reactions leading to rancidity are summarised in Scheme 1. [8]. Two well known pathways are recognised, viz, oxidation (process I) and hydrolysis (process II). Oxidation leads to oxidative rancidity and involves oxygen attack of glycerides. The process is initiated by heat, pro-oxidants, certain enzymes (lipoxygenases) or light. Hydrolysis leads to hydrolytic rancidity and involves hydrothermal or enzymic (lipase) hydrolysis to free fatty acids (FFA) and other products. Oxidative rancidity can be suppressed by minimising exposure to oxygen, high temperatures, transition metals and light, and by the addition of antioxidants. Hydrolytic rancidity, on the other hand, is suppressed by low temperature and moisture content, and deactivation of lipases.

The classical route of auto-oxidation depends on the production of free lipid radicals from triglycerides (or fatty acids formed by hydrolytic rancidity) by their interaction with oxygen [11]. The resulting radical then combines with oxygen to form a peroxy radical, the latter, in turn, abstracting hydrogen from another glyceride or fatty

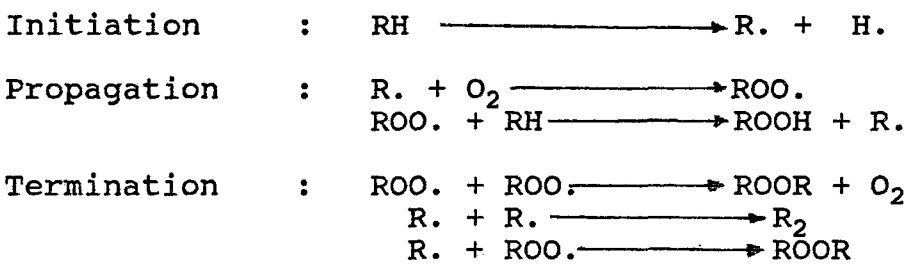


Scheme 1. Overall reaction scheme for (I) oxidative and (II) hydrolytic rancidity [8].

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, e.g., RH or RR as shown in Scheme 2. In general, the rate of oxidation increases rapidly with greater unsaturation in the fatty acid moiety of the glycerides. For

example, the relative rates of oxidation of stearic, oleic, linoleic, and linolenic acids which have no, one, two and



Scheme 2. Generalized mechanism for the autoxidation reaction [11].

three double bonds, respectively, are in the ratio 1:100:1200:2500. Regardless of the mechanism of formation, hydroperoxides will decompose further into the final flavour components. The latter conversion is known to produce via the formation of alkoxy free radicals, which decompose by cleavage on either side of the alkoxy group.

Depending on the mode of this reaction the secondary oxidation products so formed may be aldehydes, ketones, alcohols or hydrocarbons. The aldehydes, which are powerful flavour compounds with very low flavour thresholds, are in great part responsible for the rancid flavour of fats and oils. As the aldehydes themselves are oxidised, tertiary oxidation products including free fatty acids of low relative molecular mass are formed. Although aldehydes, alcohols and hydrocarbons may arise from the hydroperoxides produced by photo-oxidation or classical auto-oxidation, hydrolytic

reactions (Scheme 1) are believed to be the main source of fatty acids such as oleic, linoleic and linolenic acids, which can undergo more rapid auto-oxidation than intact triglycerides [8].

1.3. Margarine Manufacture

Before edible oils are processed, the seed must be cleaned using sorters, sieves, aspirators and electromagnets to remove dirt, dust, stone and iron fragments, foreign seeds, glass fragments of textiles etc [13]. After cleaning, the oilseeds are pressed by hot or cold pressing and the oil is extracted from crushed seeds with a suitable solvent by continuous or batch extraction principle.

Crude or raw oils must be subjected to refining before they are used.

1.3.1. Refining of Edible Oils And Fats

Modern refining practice is adapted to the composition of the crude oils and their intended uses and takes care to preserve the oils.

The following are the steps:

a. degumming
b. neutralisation
c. bleaching
d. deodorisation

Degumming is usually carried out with a dilute brine or phosphoric acid solution or acid alkaline phosphate, to

remove the resin and gum particles, proteins and phosphatides from crude fats. Degumming improves the storage stability of the oils because the gum materials are a good nutrient base for microorganisms and increase the danger of microbial breakdown of the fat. The removal of phosphatides and gummy materials is also important for the commercial processing of the fats, because during hydrogenation, gums and phosphatides can poison the catalyst (nickel) or render them inactive.

Neutralisation is frequently combined with degumming and is usually carried out by spraying or stirring in dilute caustic solution, followed by washing (bubbling water through) the oil. this also, to a large extent, bleaches the crude oil and removes traces of heavy metals (Cu, Fe) which promote autoxidative decomposition of the fat. The soaps formed during neutralisation, separate off as compact, dark coloured 'soapstock' which can be drawn off and used in the soap industry.

Bleaching is carried out with absorbants (bleaching earths, such as bentonite, florisil or active carbon), which absorbs the soap and gum residues, traces of heavy metals and colouring agents both natural and those which have been formed during storage. Bleaching also to a large extent removes oxidation products still remaining in the oil (hydroperoxides, peroxides) which might affect keepability of the oil and result in flavour changes due to decomposition

and further oxidative reaction.

Deodorisation is the most difficult stage of the whole process of refining. The aim is to remove those quality reducing flavour and odour materials which are naturally present in the fat or those which have been formed secondarily by oxidative or hydrolytic reactions (chemical, enzymatic or microbial).

Deodorisation is usually carried out as a steam distillation which removes the relatively volatile compounds which give an undesirable smell or taste to the oil. Steam is injected under vacuum to protect the hot oil from oxidation by oxygen in the atmosphere and also prevent hydrolysis of triglycerides by steam.

1.3.2. Hardening of Fats

The term hardening includes all processes which tend to raise the melting point of a glyceride mixture by the addition of hydrogen to the double bonds of the unsaturated fatty acids using nickel catalyst. The unsaturated fatty acids of the oleic and linoleic acid series present in glycerides are changed into saturated fatty acids with a higher melting point.

$$-CH=CH- \longrightarrow -CH_2-CH_2- \quad \text{(exothermic reaction)}$$

The degummed and neutralised vegetable oils or animal

fats are warmed and mixed with very finely divided nickel. This is prepared from nickel formate or nickel carbonate by reduction with hydrogen and added in amounts of 0.01-0.2 %. Hydrogen is then injected at a temperature of 160-220°C to give close contact with the oil under pressure.

Under these conditions some of the unsaturated fatty acids are transformed into saturated fatty acids. Some rearrangements (elaidinisation) of the fatty acids and rearrangement of the position of double bonds (positional isomerism) can also take place, so that so-called 'iso-oleic acids' are formed.

By varying the conditions of the reaction (catalyst, temperature, pressure etc.) it is possible to carry out the hardening in steps, so that only certain groups, for instance the fatty acids with several double bonds, are partially hydrogenated, whereas the oleic and linoleic acid are to a large extent preserved. In this way hydrogenation can be controlled and softer or harder fats of any desired melting point or consistency obtained.

Hardening of edible fats is always restricted to a partial saturation of double bonds, because complete hydrogenation, with few exceptions, would yield very high melting point triglycerides, (saturated fats difficult to digest). Generally edible fats are hydrogenated to a melting point of 32-37°C, which produces malleable fats of an even consistency with a melting point in the physiological range.

The constitution of the hydrogenated fat or oil depends on the type and composition of the starting material as well as the (controllable) hydrogenation conditions.

Hydrogenated fats are usually further refined to remove traces of nickel catalyst, free fatty acids, discolouration etc. This final refining consists of further gentle neutralisation, bleaching and deodorisation.

During vigorous hardening, fat soluble vitamins, (with the exception of vitamin E, tocopherol), and essential fatty acids, all of which are nutritionally valuable, may to a large extent be destroyed. The loss of essential nutrients such as linoleic acid and vitamin A during the hardening of edible fats is improved by the addition of high grade vegetable oils and vitamin A supplements.

1.3.3. Crystallization

The margarine emulsion is submitted to shock cooling and rapid solidification, partly to fix the emulsion by the mechanical restraint to separation in the solid state, and thus aid the effect of the emulsion stabilizers, partly to ensure the correct type of crystallization of the solid glycerides to obtain the desired consistency and smooth texture [14].

Cooling and solidification of the margarine emulsion require, however, not only extraction of heat to ensure solidification of the preferred proportion of solid

glycerides, but cooling of the whole emulsion to temperatures as low as 2-3°C with ice-water cooling, and far below freezing point with some dry cooling systems to reach the degree of supercooling which in many cases is necessary to obtain the right crystal size and formation.

Rapid and slow cooling can be applied for margarine crystallization. If a molten blend containing a large number of different glycerides is allowed to cool very slowly, crystallization is gradual according to the setting points of the individual glycerides and macroscopic crystals are formed by the solid glycerides, leading to a granular texture. Rapid cooling leads to formation of microscopic crystals and smooth texture and this is further assisted by stirring during cooling. Instantaneous or shock cooling gives a smoooth texture without stirring.

1.4. Shelf-Life Concept for Food Products

Shelf-life is the time range in which the food products keep on their quality and it also gives an information about the freshness of the food products.

A major aim for food scientist is the prediction of the change in quality of a particular food as a function of both time and environmental conditions because the information obtained is needed in the food industry so that they can

1. evaluate the effect of the addition of new ingredients or additives on shelf-life,

2. set an open-date for the food package (e.g., a "use by" or "best if used by" date) so that consumers are better informed in handling the product,

3. insure that the food meets the compliance standards if nutritional labelling is used.

In order to make a useful predictions about shelf-life, the researcher needs information regarding

i. the potential major modes for loss of quality of the products,

ii. the environmental conditions the food will be exposed to including temperature, relative humidity and light,

iii. whether it is packaged in a semi-permeable container, and, if so, the permeability of that film to oxygen, water vapor, and light,

iv. the kinetics of the reactions leading to loss of quality or nutritional value as a function of the reaction phase conditions in the food and the external environment.

The major reactions which lead the quality loss of food products are the followings [10];

1. Microbiological decay of foods
2. Senescence
3. Enzymatic chemical deterioration
4. Non-enzymatic-browning
5. Lipid oxidation
6. Vitamin loss
7. Protein loss
8. Color changes
9. Sensory changes
10. Physical deterioration

In designing a shelf-life test for quality loss in food the following steps should be taken into consideration.

-Determine the microbiological safety and quality parameters for the proposed formulation and process.

-Determine from an analysis of the ingredients and the process which chemical reactions are likely to be

the major causes of quality loss.

-Select the package to be used for the shelf-life test.

-Select the storage temperatures (at least two).

-Using the shelf-life plot and knowing the desired shelf-life at the average distribution temperature, determine how long the product must be held at each test temperature.

-Decide which tests will be used and how often they will be conducted at each temperature.

-Plot the data as it is collected to determine the order of the reaction.

-From each test storage condition estimate shelf-life, make the appropriate shelf-life plot and estimate the potential shelf-life at the desired storage condition.

A useful model in estimating the shelf-life is to accelerate the test conditions. With the aid of this method, the shelf-life of a food product can be predicted in a short period of time.

1.4.1 Accelerated Shelf Life Testing (ASLT) Methods

The first step in a typical ASLT study is to select a suitable method for testing the food product under consideration. Next, a sample is placed under the conditions of the test and the end of shelf-life (induction time) is measured, i.e. the time, usually in hours, required to reach a specified end-point. The last and the most difficult step is to translate the value for the induction period obtained into actual product shelf-life in months of storage.

Basically, four parameters are manipulated in ASLT procedures to speed up the oxidation and development of rancidity in foods or oils. These are temperature, oxygen, added metals and reactants contact. Increased temperature is the most common and effective means of accelerating the oxidation. Some commonly used ASLT methods for oxidative stability of fats or oils are explained below [15].

1.4.1.1. The Schaal Oven Test

It is frequently used for the analysis of vegetable oils. In experimental procedure of this test, samples are prepared under standard conditions and stored in ovens at temperature of approximately 60-63°C. The samples are evaluated daily until they become rancid. Then induction periods are recorded and peroxide values are measured [16].

1.4.1.2. 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 marked drop in pressure occurred. In order to get a sharp end-point with vegetable oil, it is necessary to replace the air with oxygen. The temperature used in these methods is considerably higher than that used in the Schaal Oven Test [3].

1.4.1.3. Active Oxygen Methods (AOM)

Active Oxygen Method or The Swift Stability Test (SST) is a widely used test for determining the oxidation stability of edible oils and fats. The method measures the time (in hours) required for a sample of fat or oil to attain a pre-determined peroxide value under specific conditions. The length of this period of time is assumed to be an index of resistance to rancidity [17]. The stability of fats is usually measured by the AOM. This method suffers from the disadvantage of being time consuming, labor intensive and wasteful. The most successful of these is the version based on the observation that in an oxidizing oil, volatile acids are formed at the end of the induction period. The air emerging from the oil in the AOM test can be led into water and the acids are titrated potentiometrically or conductometrically [18].

1.4.1.4. 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 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 then determined. The labour required for this method is considerably less as it is not necessary to perform titrations at regular intervals [19,20].

1.4.2. Evaluation of Accelerated Shelf-Life of Foods

In order to accelerate a shelf-life study of food deterioration as well as to get data which can be applied over a broader range of conditions, certain chemical laws can be used. There are two general ways to predict product shelf-life. The most common method is to select some single abuse condition, expose the food to it, test it two or three times during some specific period, generally sensory methods, and then extrapolate the results to normal conditions. Another approach is to assume that certain principles of chemical kinetics apply with respect to temperature dependency such as the Arrhenius and the simple shelf-life plot approach.

1.4.2.1. Arrhenius Approach

One of the most accepted models is that of Arrhenius in which the temperature effect is incorporated into an exponential model of the rate constant in the form [10].

$$\ln(k) = \ln(A) - \frac{E_a}{R*T} \qquad (1)$$

where k is the rate constant, A is the preexponential constant, E_a is the activation energy (kj/mol), R is the universal gas (j/mol.K) constant and T is the absolute temperature (K). A semilog plot of k (on the log scale) versus 1/T as shown in Fig. 2 gives a straight line. Data collected at high temperatures can be extrapolated to obtain shelf-life at some lower temperatures.

Most data for modes of deterioration in the literature do not give rates or rate constants but rather are in the form of overall shelf-life (end-point analysis) as a function of temperature. Using the above mathematical model the end point data (induction time or end of shelf-life) can be transformed into a shelf-life plot which is usually a straight line.

1.4.2.2. Simple Shelf-Life Plot Approach

This approach has been derived from Arrhenius model in which the rate constant of a food reaction is inversely proportional to the time to reach some degree of quality loss [11]. If only a small temperature range is considered, most food data yield a linear plot when log (shelf-life) versus T(°C) as shown in Fig. 3. The relationship is derived directly from the Arrhenius equation (1) where

$$\ln Q_{10} = \frac{10 * E_a}{R * (T) * (T+10)} \qquad (2)$$

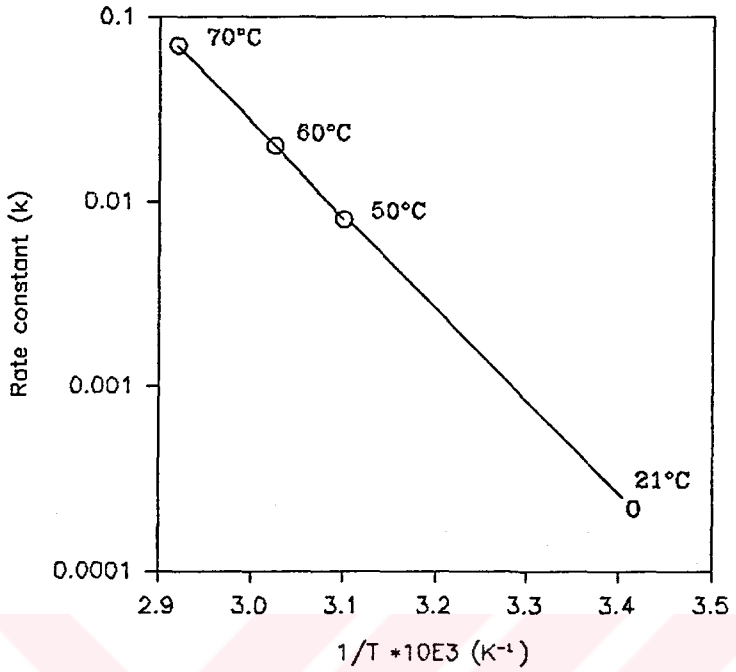


Figure 2. Typical Plot of log (k) versus invers absolute temperature showing projection to lower temperature [10].

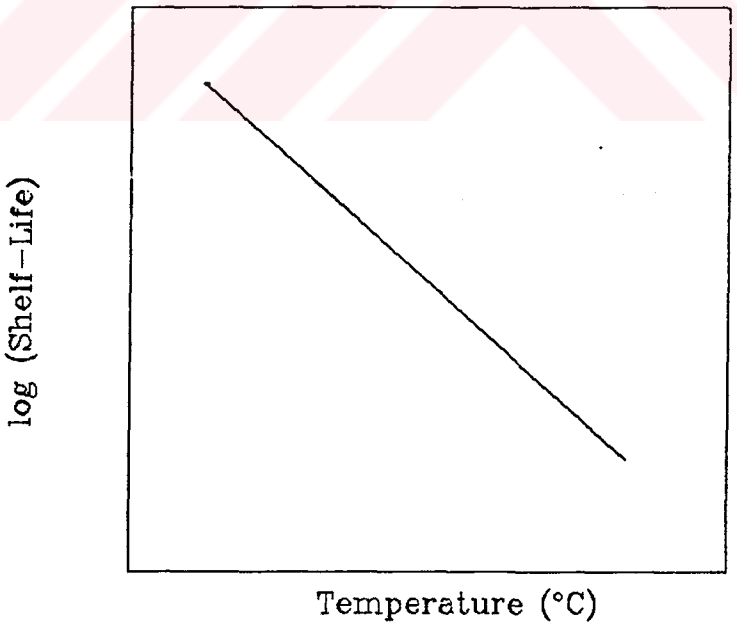


Figure 3. A pseudo-transformation of log of shelf-life versus temperature [10].

Instead of activation energy concept, Q_{10} approach have been used for temperature acceleration. Q_{10} is the decrease in shelf-life for a 10°C temperature increase. By using 0°C as an arbitrary reference temperature the equation for the shelf-life in Fig. 3 may be written

$$\theta_s=\theta_{ref}*e^{-b*(T-T_{ref})}=\theta_0*e^{-b*T} \qquad (3)$$

where; θ_s : is the shelf-life at T

θ_{ref} : is the shelf-life at reference temperature T_{ref}

θ_0 : is the shelf-life at T=0

b : is the slope in Δ°C

Then, substituting into equation (3) for shelf-life at T and T+10°C, followings can be obtained

$$Q_{10}=e^{10*b} \qquad (4)$$

and

$$(5) \quad b=\frac{E_a}{R*(T)*(T+10)}$$

By taking the ratio of shelf-life between any two temperatures 10°C apart, the Q_{10} value of the reaction can be

found. Q_{10} value shows the temperature sensitivity of a food deterioration. In determining Q_{10} value it is assumed that following equation is valid.

$$Q_{10} = \frac{Reaction\ Rate\ at\ (T+10)}{Reaction\ Rate\ at\ (T)} \qquad (6)$$

or

$$Q_{10} = \frac{Shelf\text{-}Life\ at\ (T)}{Shelf\text{-}Life\ at\ (T+10)} \qquad (7)$$

Whereas, this is true for small temperatures ranges. In general Q_{10} value must be constant.

1.5. Previous Works Done Related to The Present Study

Current interest in the study of ASLT and storage stability of food products in the presence of certain external factors is apparent from the relatively large number of papers appearing in the last few years.

There are many studies on lipid deterioration, stability and shelf-life determination. In order to measure the lipid oxidation in foods, no single method has yet been established to represent all oxidative qualities of lipids because of the complexity of the reactions between lipids and other substances.

The measurement of hydrolytic and oxidative rancidity,

by chemical and physical methods as free fatty acid, peroxide value, Kreis test, Anisidine value and infrared, refractive index, spectroscopy, gas chromatographic method, has been reviewed [8]. The oxidative stability of a set of several samples of various vegetable oils has been determined in duplicate using the Schaal test at 60°C [16]. The repeatability was the same whether the peroxide value or the gravimetric method were used to determine the course of oxidation. The repeatability was not affected by the degree of refining, and by the presence of phospholipids. Aged or stored oils showed rather poor repeatability of induction periods. The stability of fats has been measured by active oxygen method (AOM) [18]. In this study, procedure has included the direct recording of oxygen absorption of fats. The most successful of this is the version based on the observation that in an oxidizing oil, volatile acids are formed at the end of the induction period.

In recent years, there has been an effort to study the factors affecting the lipid deterioration and prediction of food shelf-life. The effects of temperature on the rates of chemical reactions [21], catalytic effect of heavy metals, light, and effect of water activity on lipid oxidation have been studied [9]. In these studies, it has been observed that high temperature and presence of metal ions accelerate but high water activity decreases lipid oxidation. On the other hand ASLT methods have been applied for processed foods [15].

Temperature was the dominant acceleration factor and its effect on the rate of lipid oxidation was best analysed in terms of the overall activation energy for lipid oxidation.

The extensive literature survey made by the author using library and computer facilities of Higher Education Council, Turkish Scientific and Technical Research Center and METU-Ankara, showed that no work has been reported for the study of both ASLT and storage stability at normal conditions for margarines.

1.6. The Aim of Present Study

Rancidity of edible oils and fatty foods due to lipid oxidation is a serious problem in some sectors of food industry. Factors which contribute to this problem are the increased emphasis of polyunsaturated dietary lipids, the fortification of certain foods with some metals, packaging type and some external factors such as temperature, light, oxygen and environmental humidity. Because of the unfortunate consequence of lipid oxidation in foods it is critical that information about the oxidative stability of susceptible food items be obtained before they are marketed.

The food manufacturer would like to employ methods which can give a reasonably accurate indication of the product shelf-life in a relatively short period of time.

Therefore, in the present study the aim was to determine the shelf-life of margarine packaged with parchment and

plastic by using Accelerated Shelf-Life Testing (ASLT) method in a short period of time. The shelf-life determinations were carried out not only by ASLT method but also at normal storage conditions (changing the temperature and relative humidity) so that a comparison between shelf-life obtained by ASLT and by normal storage conditions could have been made.

1.7. Methods and Systems Used in This Study

The susceptibility of an oil or fat to auto-oxidative degeneration can generally be assessed in terms of oxidation stability. In the food industry, the oxidation stability of fats plays an important part in ensuring the quality of food products. The condition and keeping qualities of an oil or fat are primarily assessed in terms of induction time (end of shelf-life). The induction time can be determined rapidly and reliably with a compact, commercially available instrument by conductometric measurement of volatile reaction products formed at elevated temperatures [12].

In this study, ASLT method was chosen to predict the shelf-life in a short time and for this aim an ASLT apparatus was set up.

The evaluation of ASLT parameters is also essential for deciding to which parameter gives a better result in a typical ASLT studies. Basically, four parameters are manipulated in ASLT procedures to speed up the oxidation and development of rancidity in foods or oils. These are listed

in Table 2.

As an accelerated parameter, temperature was chosen because increased temperature is the most common and effective means of accelerating the oxidation.

Table 2. Common Acceleration Parameters [3].

PARAMETER	NORMAL RANGE
Temperature (°C)	60-140
Oxygen Pressure (psia)	3-165
Added Metals (ppm)	25-100
Reactants Contact	Variable

Normal storage conditions were chosen to determine the actual shelf-life of margarine and to make a comparison with the shelf-life obtained by ASLT. The temperatures 4 and 14°C were chosen as refrigerated and market temperature respectively. The effects of relative humidity of the storage environment on the stability of margarine also were studied.

Peroxide (for oxidative rancidity) and Free Fatty Acid (for hydrolytic rancidity) values were chosen to make a criteria about the end of shelf-life of margarine samples because these parameters are very good indicator for shelf-life determination of fatty foods such as margarine.

Microbial count is also an important parameter at high relative humidities for shelf-life determination.

CHAPTER II

EXPERIMENTAL

2.1. Materials

Margarine samples used in the present study which had two different packaging type, parchment (A) and plastic (B) packages, were obtained from an Oil-Company two days after production. The compositional analysis have been achieved, then, the samples were divided into three lots.

First lot was used for ASLT method. Second lot was stored in refrigerated incubator at two selected temperatures 4°C (as refrigerated with a relative humidity of 35 %) and 14°C (as market condition with a relative humidity of 70 %) to obtain shelf-life at normal storage temperatures. Third lot was stored in desiccators which had different relative humidities (85 and 98 %). The Peroxide Value (PV), Free Fatty Acid (FFA) value and microbial analysis were done periodically for second and third lots as quality indicating parameters in order to see the quality change during normal storage conditions and decide to the end of shelf-life of margarine samples.

Freshly prepared triple distilled water was used in all

experiments and all chemicals used in chemical and microbiological analysis which had an analytical grade were obtained from Merck, Sigma and Oxoid Chemical Company.

2.2. Compositional Analysis

Water, salt, fat, benzoic acid content were determined as described in AOAC [22].

Metal content was determined using an Atomic Absorption Spectrophotometer Alpha Model-4 [23].

2.2.1. Calibration Curves

A series of solutions were prepared in various concentration to plot a calibration curve. A set of absorbance data was obtained and plotted against concentration for the determination of metal content (as copper and iron) in mg/kg of margarine.

These calibration curves are shown in Fig. 4 for copper and Fig. 5 for iron.

2.3. Water Activity

In order to determine the effect of high relative humidity on the deteriorative parameters (PV, FFA, and microbial growth) at 14°C, it was necessary to prepare the conditions having higher relative humidities than that of margarine samples. Firstly, the water activity values for margarine samples were determined from a calibration curve

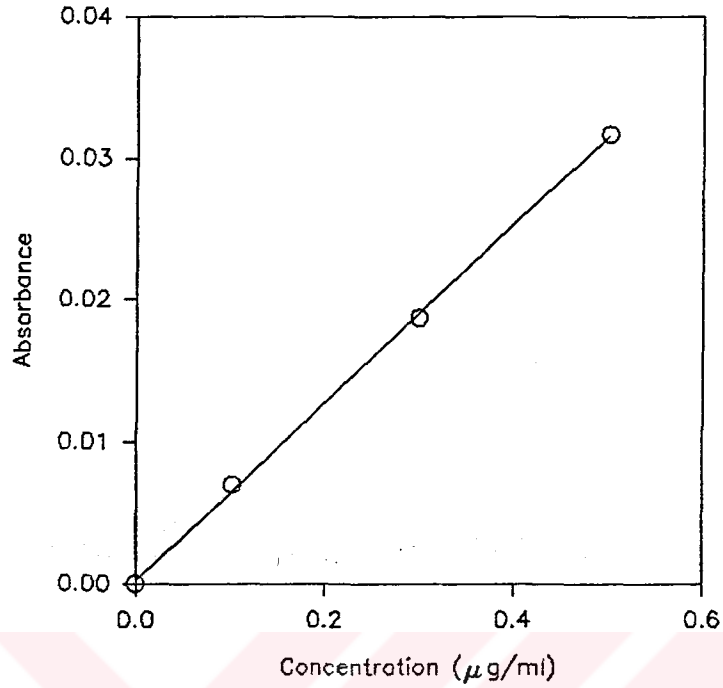


Figure 4. Plot of calibration line for Copper.

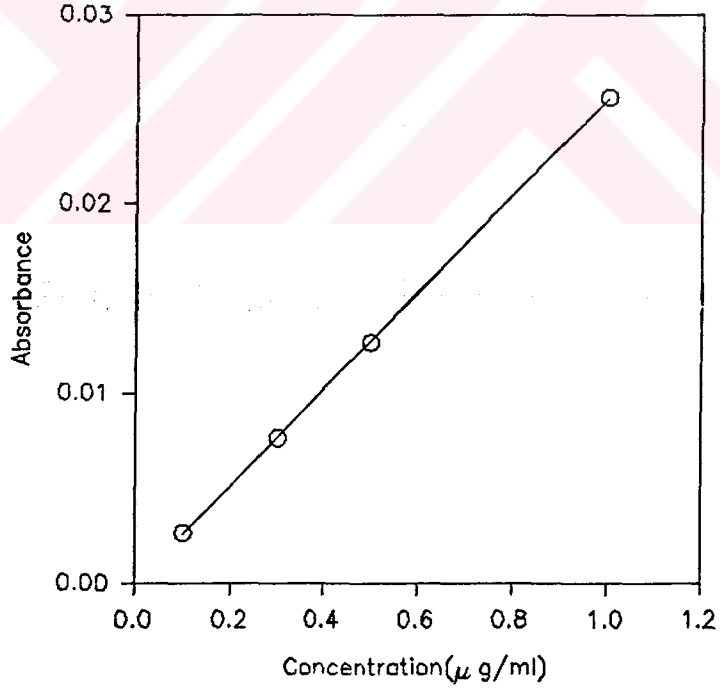


Figure 5. Plot of calibration line for Iron.

and then two storage conditions which had different relative humidities were selected.

2.3.1. Calibration Curve

Water activity of margarine samples were determined by the Proximity Equilibration Cell (PEC) method [24]. Three different saturated salt solutions (KI, NH_4Cl and K_2SO_4) with different water activities (0.7098, 0.7989 and 0.9739 respectively) were prepared to plot a calibration curve. Such calibration plot is to be shown in Fig. 6.

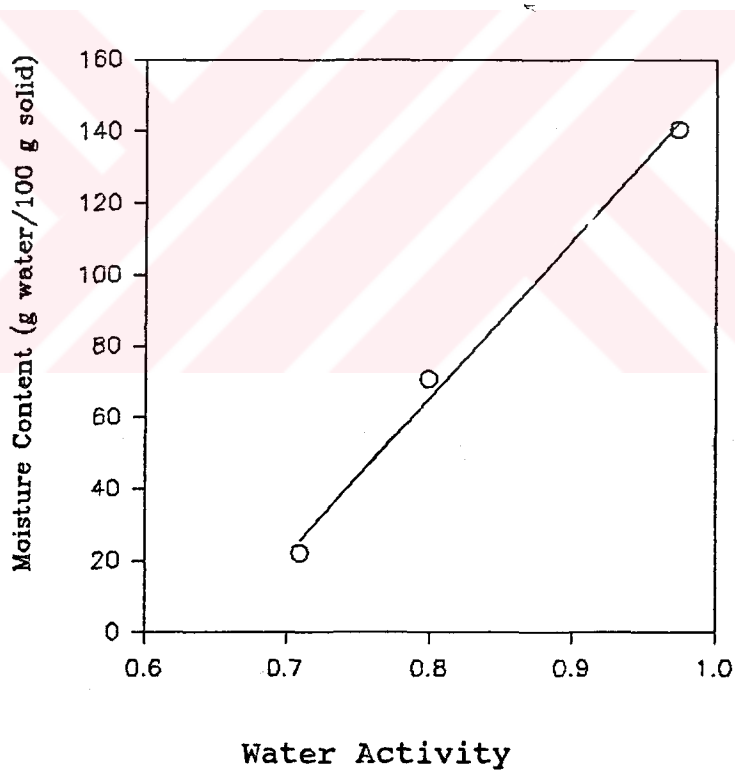


Figure 6. Plot of calibration line for determination of water activity of margarine samples.

2.4. Quality Analysis

Peroxide Value (PV) was determined as described in AOCS Official Method Cd 8-53 [25]. This method determines all substances, in terms of milli-equivalents of peroxides per 1000 grams of sample, which oxidize potassium iodide under the conditions of the test. These are generally assumed to be peroxides or other similar products of fat oxidation.

Free Fatty Acid (FFA) content was determined as described in AOCS Official Method Ca 5a-40 [26]. The percentage of FFA in most types of fats and oils is calculated as % oleic acid.

2.4.2. Total Microbial Count

Total microbial count was performed for mold and yeast grown on a Davis's Yeast Salt Agar.

2.5. ASLT Measurements

2.5.1. Apparatus

The ASLT method used in the present study was based on the principles of both the AOM and Rancimat method. It can be named as ASLT apparatus or rancimat apparatus. This apparatus setup was designed and established for this type of studies in Food Engineering Department as shown in Fig. 7.

In designed ASLT apparatus, the air was supplied by an air pump (manufactured by F.G. Bode & CO) and passed through a series of washing columns. The columns consist of triple

distilled water and 2% $K_2Cr_2O_7$ in 1% H_2SO_4 respectively. Also a condenser was mounted on the second column. Then the air was dried and filtered in a column containing glass wool and Na_2SO_4. Washed, dried and filtered air was compressed into reaction vessel contains margarine sample. The reaction vessel was placed in a heating block. The volatile compounds which were formed in reaction vessel passed through a glass pipe into the absorption vessel and trapped by triple distilled water in the absorption vessel. A second condenser was mounted on the absorption vessel to prevent loss of volatile compounds. The greater part of these compounds is formic acid, probably produced by the oxidative decomposition of aldehydes and ketones or organic peroxides, which can easily change the conductivity of the triple distilled water. Measurements were controlled conductometrically with a platinium electrode by using a Conductivity-Meter LBR model.

2.5.2. Method of Measurement

The ASLT measurements were carried out for each margarine sample by the following procedure.

Approximately 10 gr of margarine sample were weighed accurately into the reaction vessel and the vessel was placed in the thermostat. The absorption vessel containing a platinium electrode was filled with 300 cm^3 of water. When all the connections between the washing columns, reaction vessel and absorption vessel were performed, the reaction

vessel was allowed to reach thermal equilibrium with the thermostat fluid at selected temperature for a few minutes. The air flow-rate was adjusted to 18lt/hr. Conductivities of triple distilled water in which formic acid had dissolved were measured at every fifteen minutes time intervals. A steeply rise in conductivity showed that the reaction had completed.

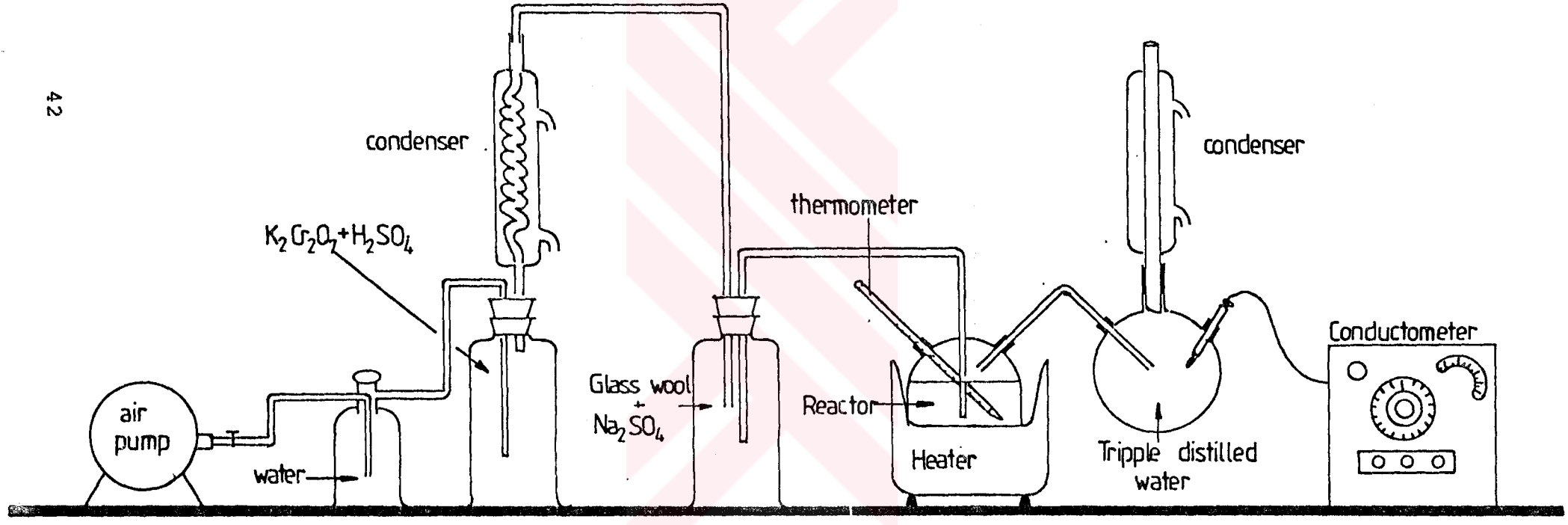


Figure 7. Modified Rancimat Method Apparatus

CHAPTER III

RESULTS

3.1. General

In this chapter, the compositional analysis of margarine samples will be presented in tabular forms. The PV (as meq/kg), FFA values (as % oleic acid) and microbial analysis of margarine stored at 4°C (35 % R.H.) and 14°C (70 % R.H.), the effects of relative humidity on the storage stability (as an indicator of storage quality parameter such as PV, FFA and microbial count) at 14°C and the ASLT results will be presented in tabular and graphical forms. A complete tabulation of the experimental data can also be found in the appendices.

The initial composition of margarine samples was analyzed and the results have been shown in Table 3. It indicates that the fat content of Margarine (B) is higher than (A) while the water content of Margarine (A) is higher than (B).

The a_w values which were determined using PEC method for margarines (A) and (B) were found at 14°C as 0.769 and 0.768 respectively.

Table 3. Compositional analysis of margarine samples.

COMPOSITION	MARGARINE (A)	MARGARINE (B)
Water %	15.2	11.6
Salt %	0.195	0.171
Fat %	84.1	88.1
Iron (mg/kg sample)	19.8	46.2
Cupper (mg/kg sample)	1.39	2.05
Benzoic Acid (mg/kg sample)	619.0	538.0

Before the graphical representation of ASLT results, first, how the ASLT results were treated, to predict the end of shelf-life of margarine will be presented.

3.2. Treatment of ASLT Data

3.2.1. Determination of Induction Time

The induction times were determined by plotting the conductivities against time. Then, the induction time was found by tangent method [20]. In this method, the tangent lines were drawn on both the vertical and horizontal portions of the curve. The extent of intersection of these two lines to x-axis gave the induction time (end of shelf-life) and shown in Fig. 8 as an example.

3.2.2. Treatment of Induction Times by Simple Shelf-Life Plot Approach

Induction time results were treated by simple shelf-life plot approach which based on the Arrhenius model. In Arrhenius model, the temperature effect is incorporated into

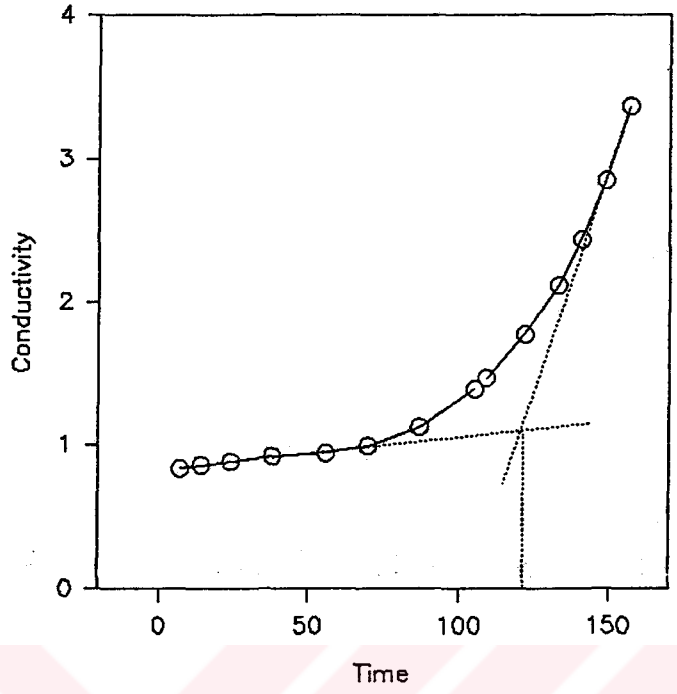


Figure 8. Determination of induction time by tangent method.

an exponential model of the constant described in section 1.4.2.1.

A convenient tool in transforming the Arrhenius plot is to plot induction times rather than rate constants on the y-axis. This is derived simply from mathematical manipulation of the Arrhenius equation for any order mentioned in section 1.4.2.2. Since most ASLT studies are done over a rather narrow temperature range, a simplification can be made in which log (induction time) is plotted directly against T (°C).

Instead of the activation energy concepts, many researcher in the food field have used the Q_{10} approach for

temperature acceleration.

In this chapter, log (induction time) against temperature in °C will be plotted and extrapolated to lower temperatures to determine the shelf-life at normal conditions.

The temperature sensitivity value (Q_{10}) will be determined graphically.

3.3. Margarine (A)

3.3.1. ASLT Results

3.3.1.1. Induction Time (IT)

The conductivity values were plotted against the time and the induction times of margarine studied at various temperatures were determined using tangent method. Such representations were given in Fig. 9, 10, and 11.

The graphs were fitted by the curve fitting method. The induction times of Margarine (A) were determined graphically from above Figures at various temperatures and tabulated in Table 4.

Table 4. Induction times determined at various temperatures for Margarine (A).

TEMPERATURE (°C)	INDUCTION TIME (hour)
85	6.80
94	2.70
107	1.20

3.3.1.2. Simple Shelf-Life Plot

The logarithm of induction times was plotted against temperature on a semilog graph by using linear regression method as shown in Fig. 12. In order to find the induction times at low temperatures (4 and 14°C), the graph was extrapolated to log-axis. The induction times at 4 and 14°C which were determined from graph were 134 and 77 days respectively.The Q_{10} value was also estimated as 2.05 from Fig. 12.

3.3.2. Normal Storage at 4 and 14°C

3.3.2.1. PV Results

The peroxide values were plotted against time. In order to determine induction times, the graphs were fitted by curve fitting method and the induction times were determined for each graph by using tangent method described in section 3.2.1. Such representations were given in Fig. 13 for 4°C and in Fig. 14 for 14°C. The induction times which were found from Fig. 13 and 14 were 137 and 103 days respectively.

3.3.2.2. FFA Results

The FFA values were plotted against time at 4 and 14°C and shown in Fig. 15 and 16. Both graphs show that the hydrolytic rancidity is not a good indicator for determining the shelf-life of a margarine at these conditions. It almost remained constant throughout the period of study.

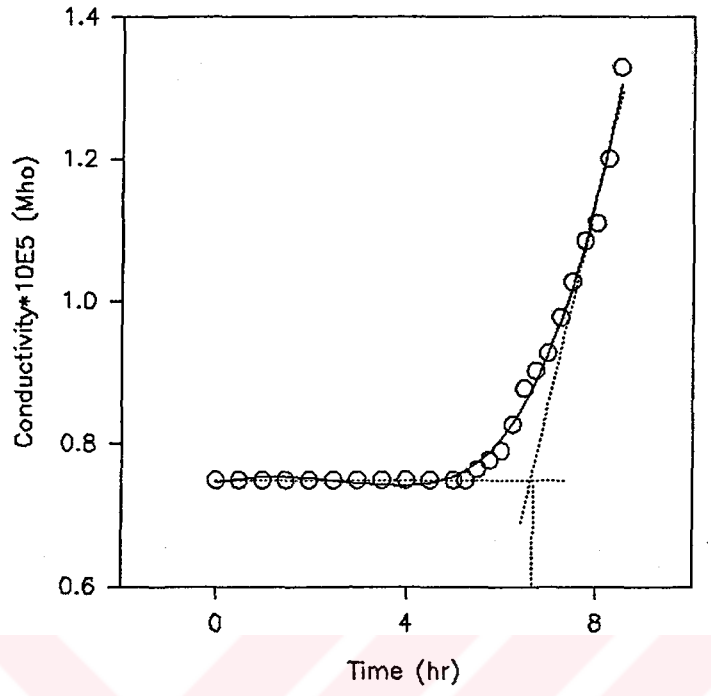


Figure 9. Plot of conductivity against time at 85°C.

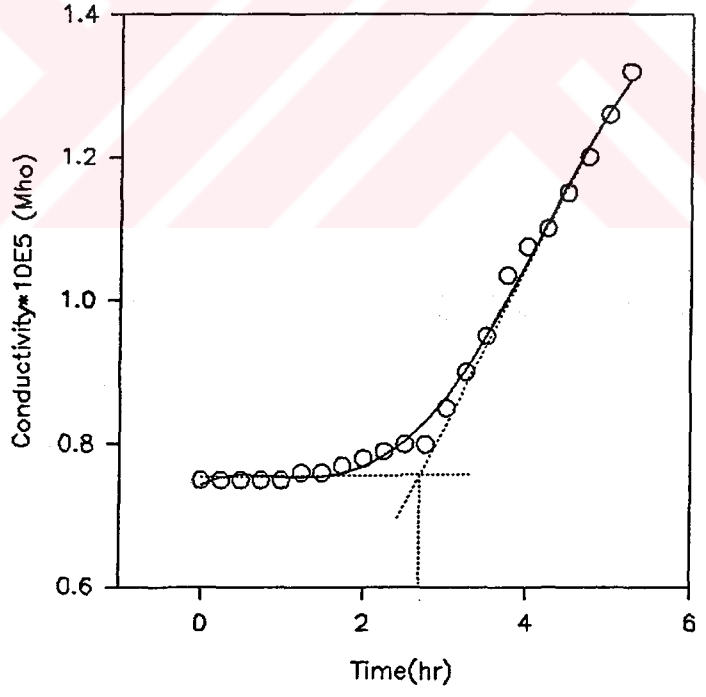


Figure 10. Plot of conductivity against time at 94°C.

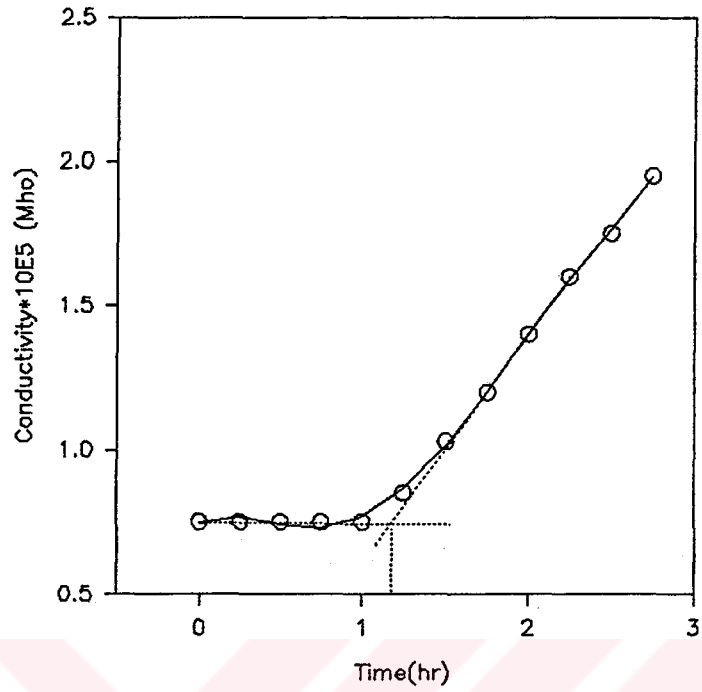


Figure 11. Plot of conductivity against time at 107°C.

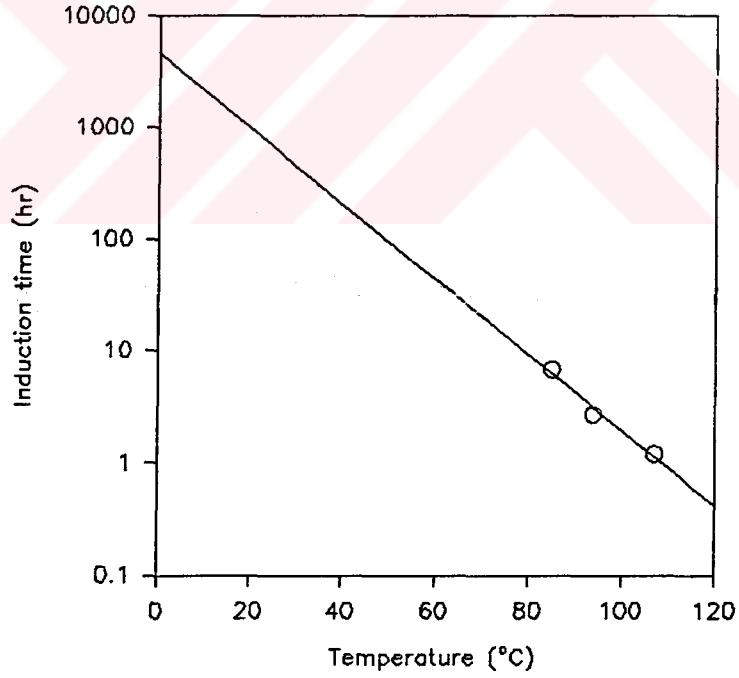


Figure 12. Plot of log(IT) against temperature.

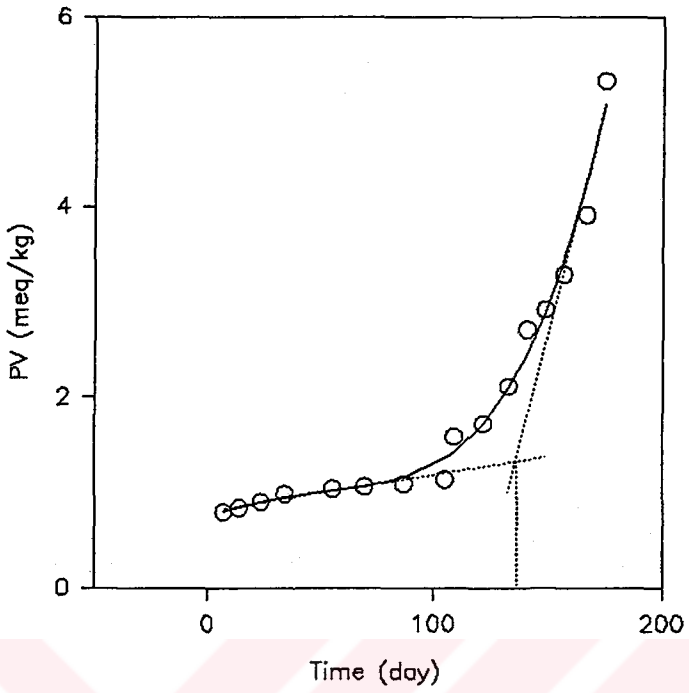


Figure 13. Plot of PV against time at 4°C.

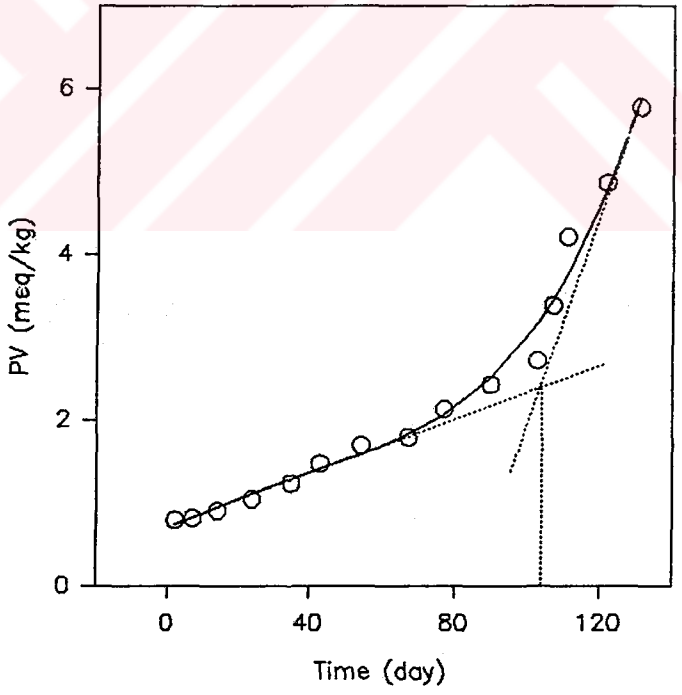


Figure 14. Plot of PV against time at 14°C.

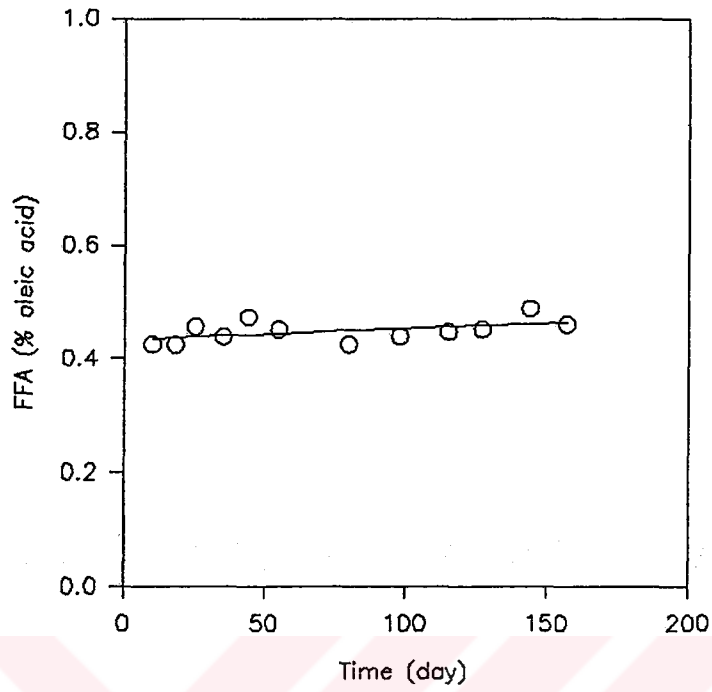


Figure 15. Plot of FFA values against time at 4°C.

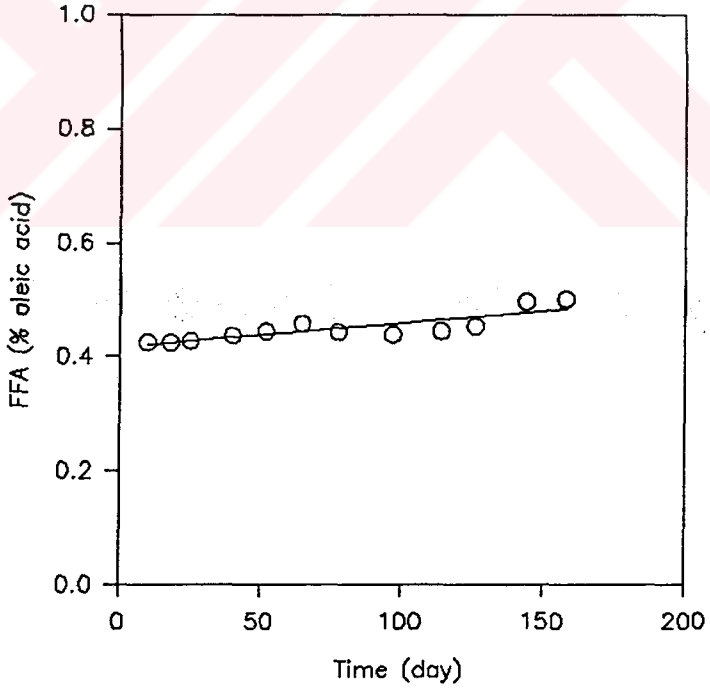


Figure 16. Plot of FFA values against time at 14°C.

3.4. Margarine (B)

3.4.1. ASLT Results

3.4.1.1. Induction Time

The conductivity values were plotted against time and the induction times of margarine studied at various temperatures were determined using tangent method. Such representations were given in Fig. 17, 18, and 19.

The graphs were fitted by the curve fitting method. The induction times of Margarine (B) were determined graphically at various temperatures and tabulated in Table 5.

Table 5. Induction times determined at various temperatures for Margarine (B).

TEMPERATURE (°C)	INDUCTION TIME (hour)
87.0	5.60
98.0	1.69
110.0	0.93

3.4.1.2. Simple Shelf-Life Plot

The logarithm of induction times was plotted against temperature as shown in Fig. 20 and graph was linearized by a linear regression method. In order to find the induction times at low temperatures (4 and 14°C), the graph was extrapolated to log-axis. The induction times determined graphically at 4 and 14°C were 125 and 63 days respectively. Q_{10} value was estimated as 2.1 from Fig. 20.

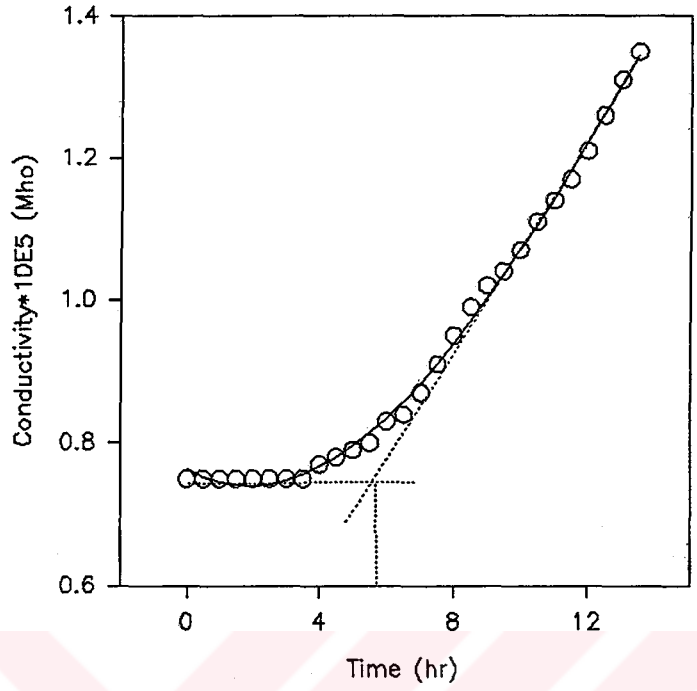


Figure 17. Plot of conductivity against time at 87°C.

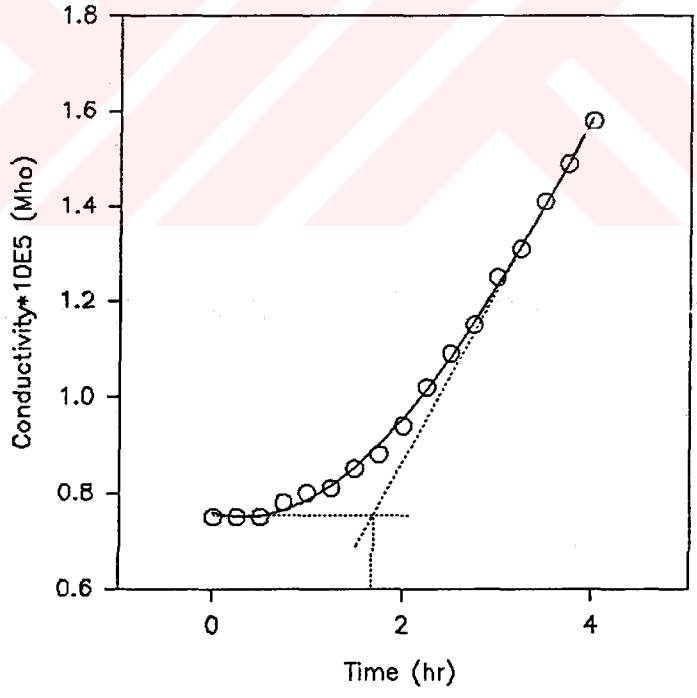


Figure 18. Plot of conductivity against time at 98°C.

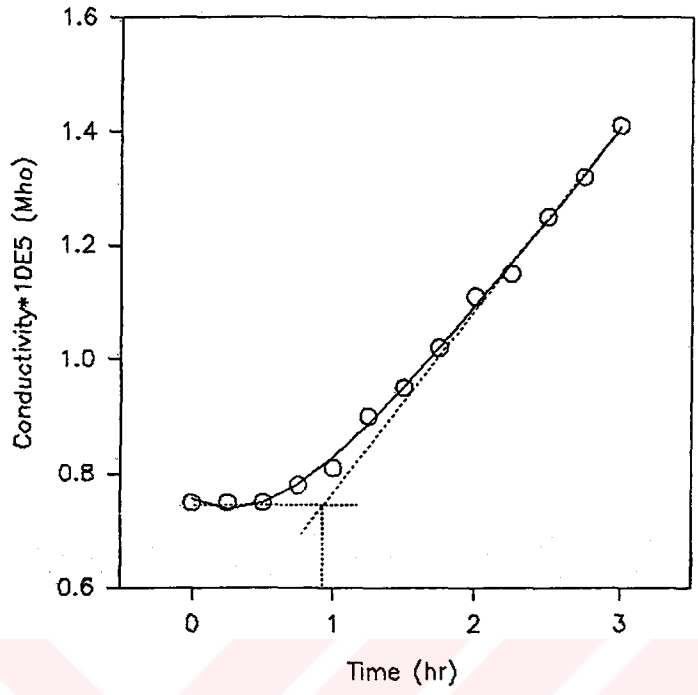


Figure 19. Plot of conductivity against time at 110°C.

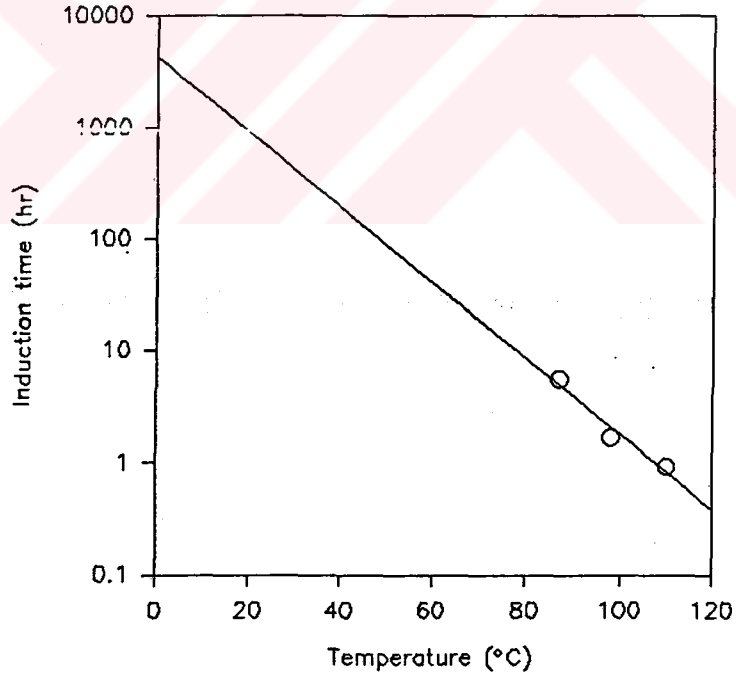


Figure 20. Plot of log(IT) against temperature.

3.4.2. Normal Storage at 4 and 14°C

3.4.2.1. PV Results

The peroxide values were plotted against time. In order to determine induction times, the graphs were fitted by curve fitting method and the induction times were determined for each graph by using tangent method described in section 3.2.1. Such representations were given in Fig. 21 at 4°C and in Fig. 22 at 14°C.

The induction times at 4 and 14°C were determined from graphs as 130 and 97 days respectively.

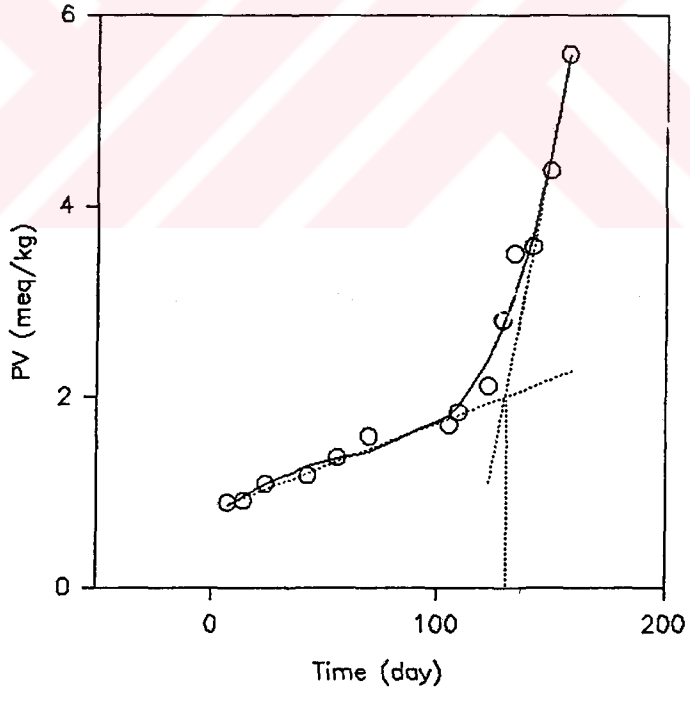


Figure 21. Plot of PV against time at 4°C.

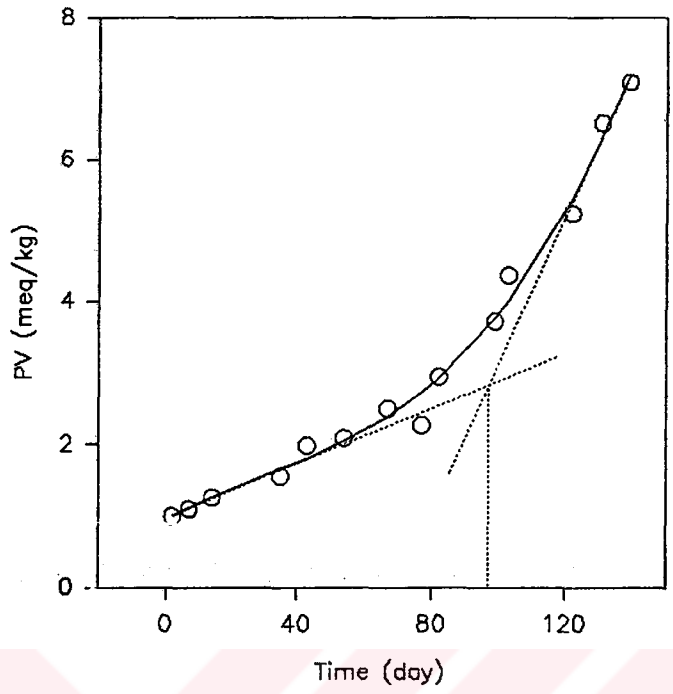


Figure 22. Plot of PV against time at 14°C.

3.4.2.2. FFA Results

The FFA values were plotted against time at 4 and 14°C and shown in Fig. 23 and 24 respectively.

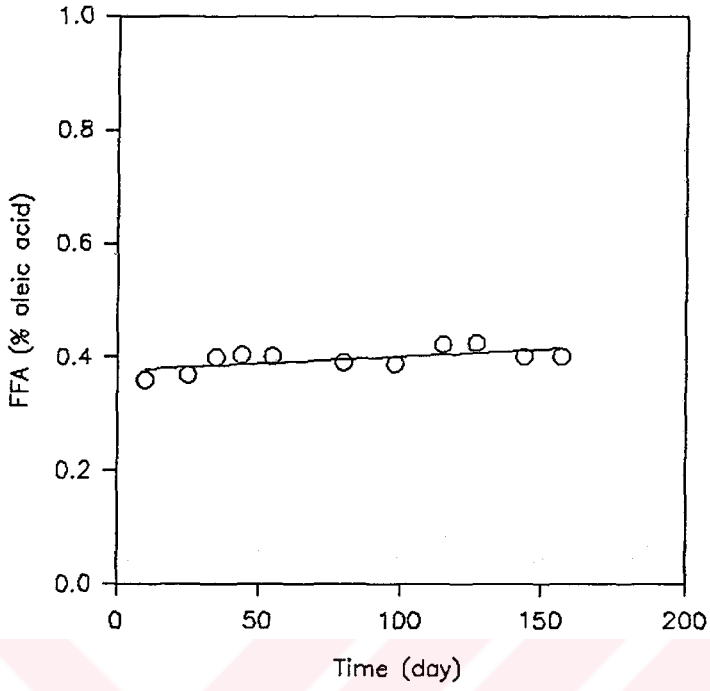


Figure 23. Plot of FFA values against time at 4°C.

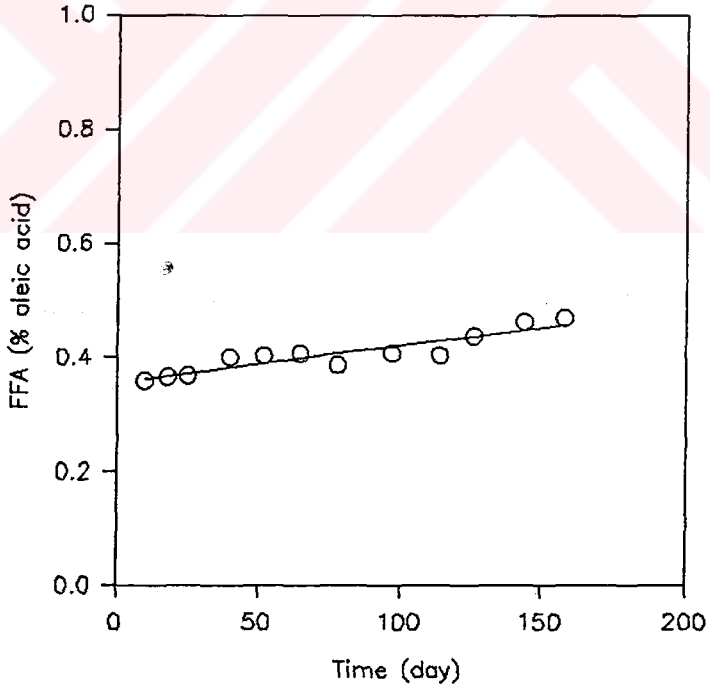


Figure 24. Plot of FFA values against time at 14°C.

3.5. Effect of Relative Humidity on Storage Stability of Margarine (A) and (B) at 14°C

3.5.1. PV and FFA Results

3.5.1.1. Margarine (A) and (B) stored at 85 % R.H.

The PV results of Margarines (A) and (B) were shown in Fig. 25 and 26. At 85 % R.H., the PV of Margarine (A) approximately remained constant up to 90th days. After this time, the mold and yeast growth was observed. The microbial growth at 85 % R.H. was a good indicator to decide the end of shelf life for Margarine (A).

Under the same conditions, no mold and yeast growth was observed while PV increased after 110 days for Margarine (B).

The FFA results were shown for both margarine samples in Fig. 27 and 28. It was seen that FFA values were approximately constant during storage for Margarine (A) and (B).

3.5.1.2. Margarine (A) and (B) Stored at 98 % R.H.

The PV results in Fig. 29 and 30, and FFA results in Fig. 31 and 32 were plotted against time for Margarines (A) and (B) respectively.

The same explanation is valid as described in section 3.5.1.1., but, mold and yeast began to grow after 73 days during the storage of Margarine (A) and the PV of Margarine (B) started to increase sharply after 108 days.

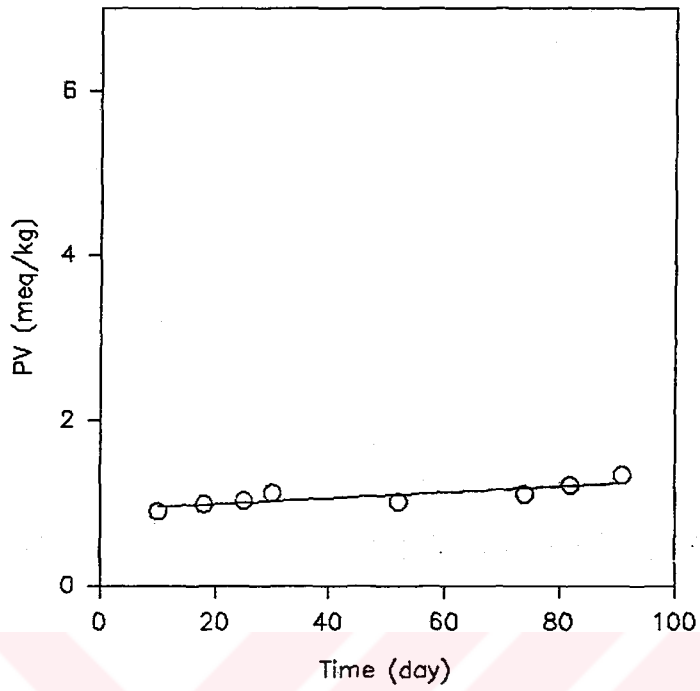


Figure 25. Plot of PV against time at 85 % R.H. for Margarine (A) at 14°C.

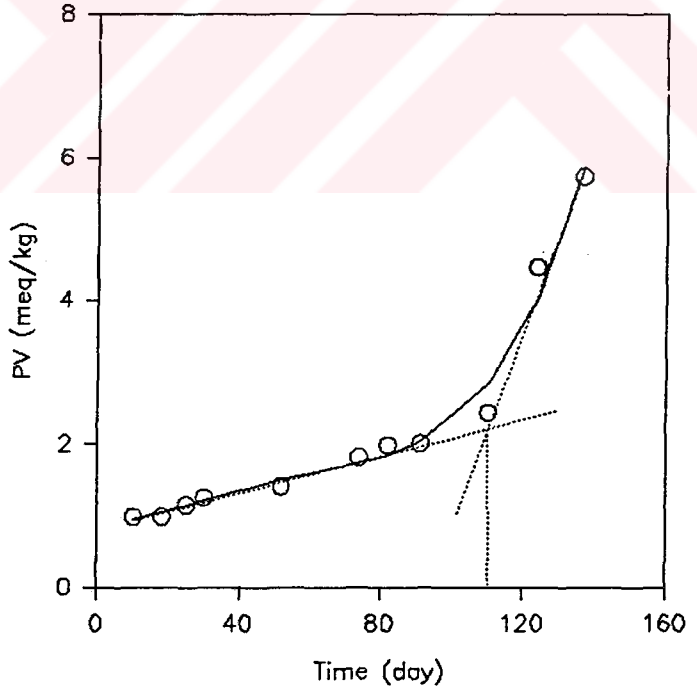


Figure 26. Plot of PV against time at 85 % R.H. for Margarine (B) at 14°C.

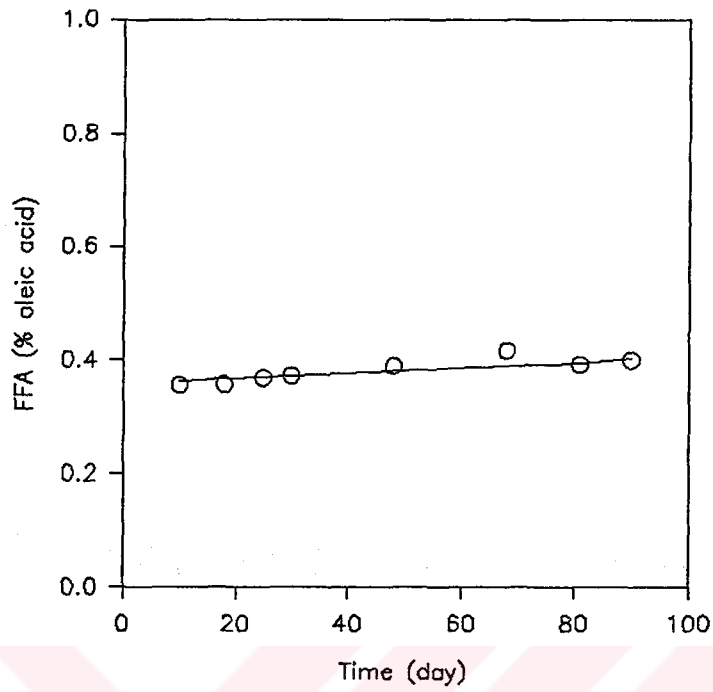


Figure 27. Plot of FFA values against time at 85 % R.H for Margarine (A) at 14°C.

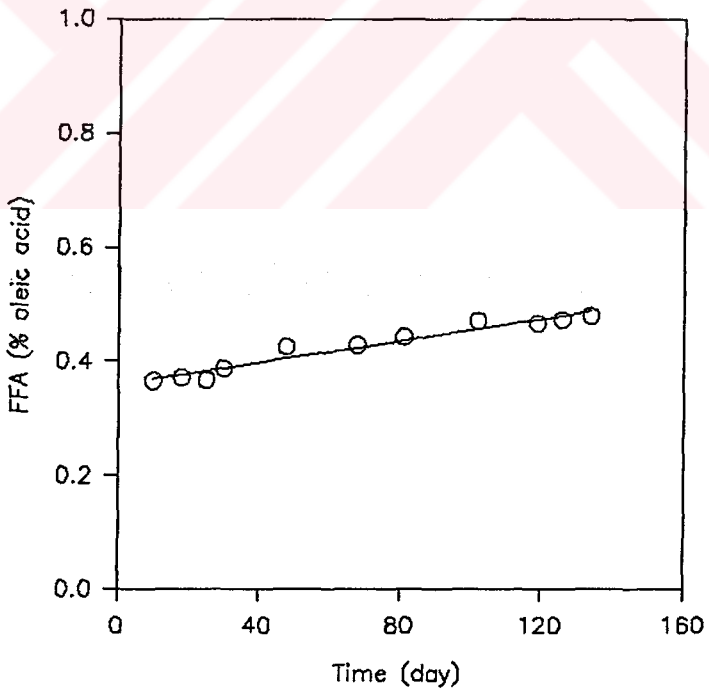


Figure 28. Plot of FFA values against time at 85 % R.H. for Margarine (B) at 14°C.

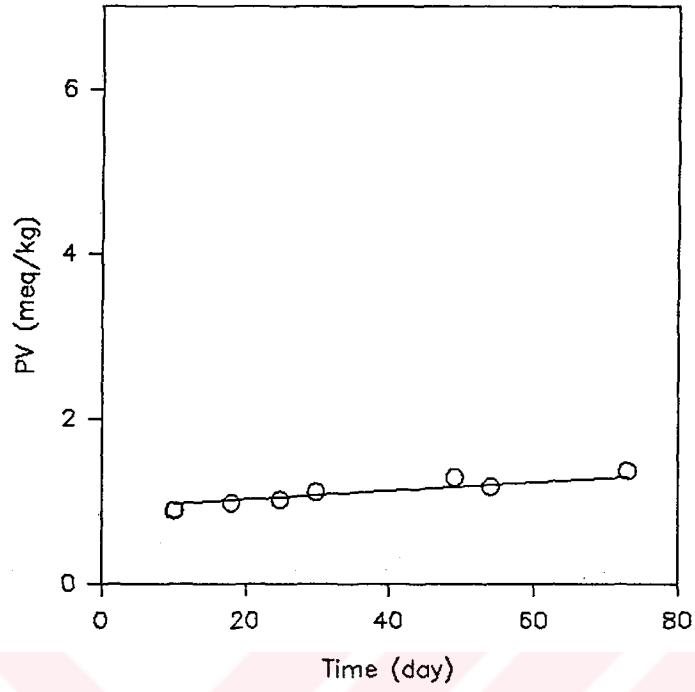


Figure 29. Plot of PV against time at 98 % R.H. for Margarine (A) at 14°C.

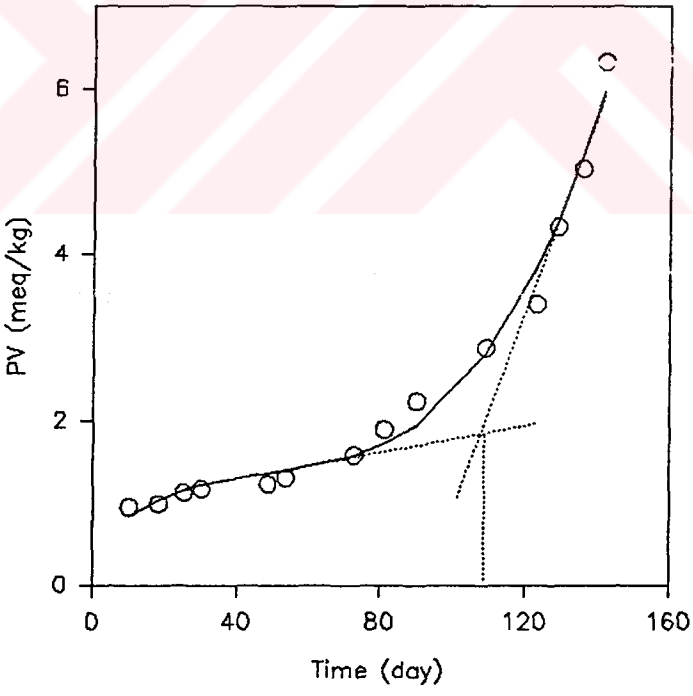


Figure 30. Plot of PV against time at 98 % R.H. for Margarine (B) at 14°C.

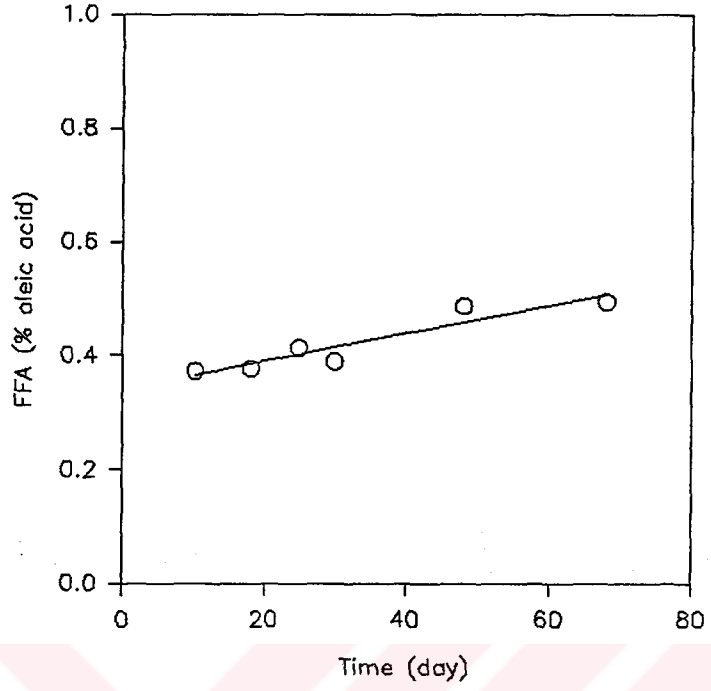


Figure 31. Plot of FFA values against time at 98 % R.H. for Margarine (A) at 14°C.

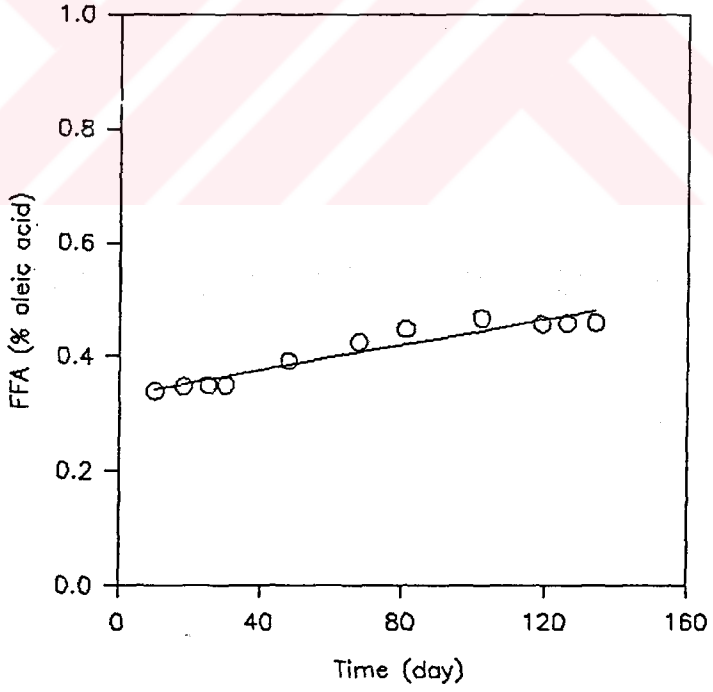


Figure 32. Plot of FFA values against time at 98 % R.H. for Margarine (B) at 14°C.

3.6. Microbial Count Results

Total microbial count (mold and yeast) was performed periodically for all storage conditions and the results were tabulated in Table 6.

Table 6. Microbial growth period for margarine samples.

STORAGE CONDITION	MARGARINE (A)	MARGARINE (B)
4°C (35 % R.H.)	No growth	No growth
14°C (70 % R.H.)	No growth	No growth
14°C (85 % R.H.)	17 counts/gr fat after 90 days	No growth
14°C (98 % R.H.)	22 counts/gr fat after 73 days	No growth

Table 6. indicates that the microbial growth was a criteria for the shelf-life determination of Margarine (A) at 14°C storage condition with the relative humidity above 85 %.

The maximum limiting value has been permitted as 10 counts (mold and yeast)/gr sample by Turkish National Standards for margarines.

3.7. Graphical Comparison of Margarines (A) and (B)

The temperature sensitivity of Margarines (A) and (B) were shown in Fig. 33 and 34 respectively. The Q_{10} value based on the PV was for Margarine (A) as 1.33 and for Margarine (B) as 1.34. It indicates again that Margarine (B) is temperature sensitive than Margarine (A).

Also, the differences between shelf-life depending on the PV at 4 and 14°C were illustrated in Fig. 35 and 36.

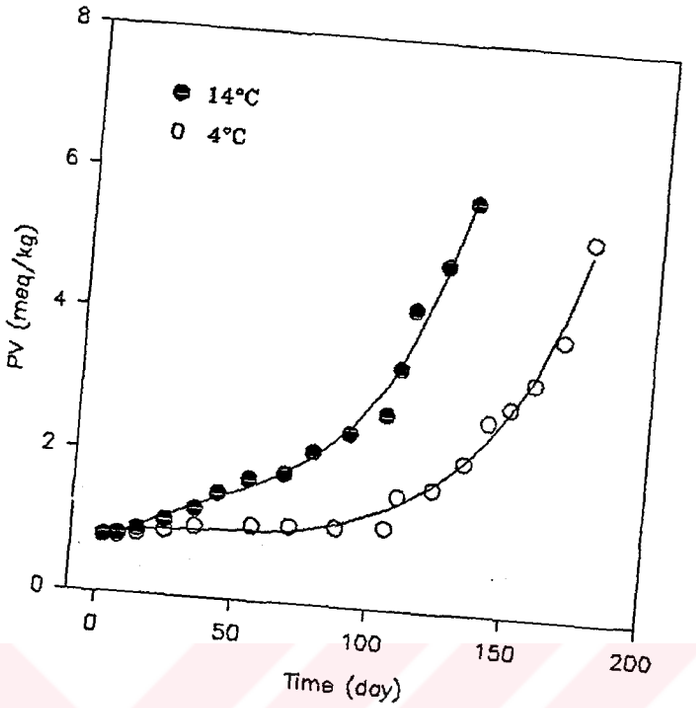


Figure 33. Plot of PV against time at 4 and 14°C for Margarine (A).

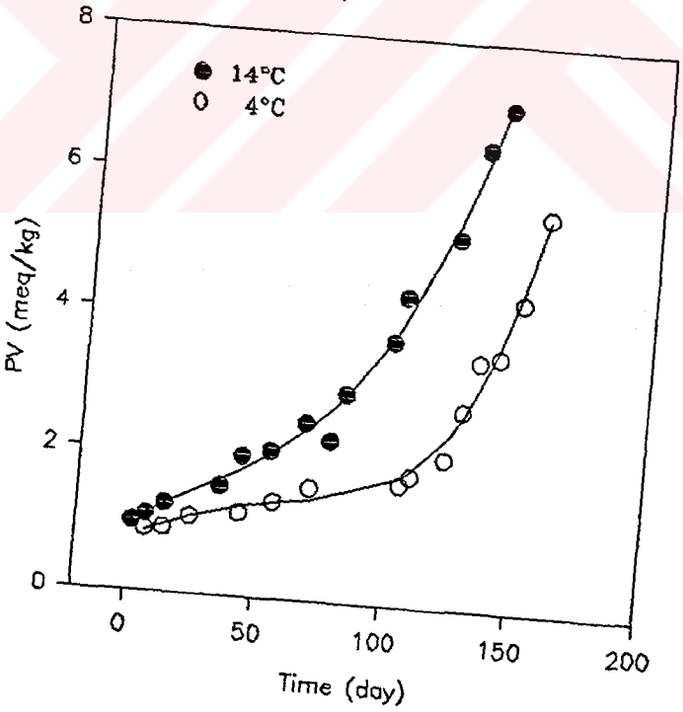


Figure 34. Plot of PV against time at 4 and 14°C for Margarine (B).

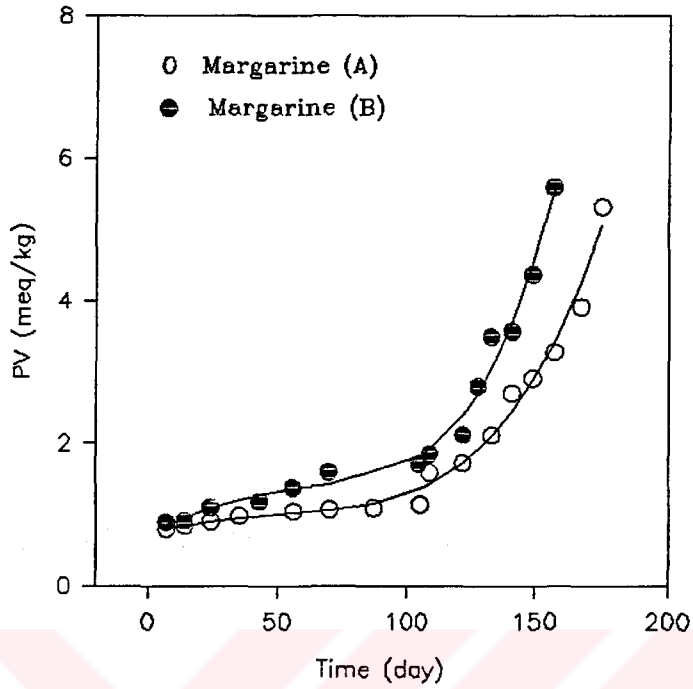


Figure 35. Graphical comparison of PV against time for Margarines (A) and (B) at 4°C.

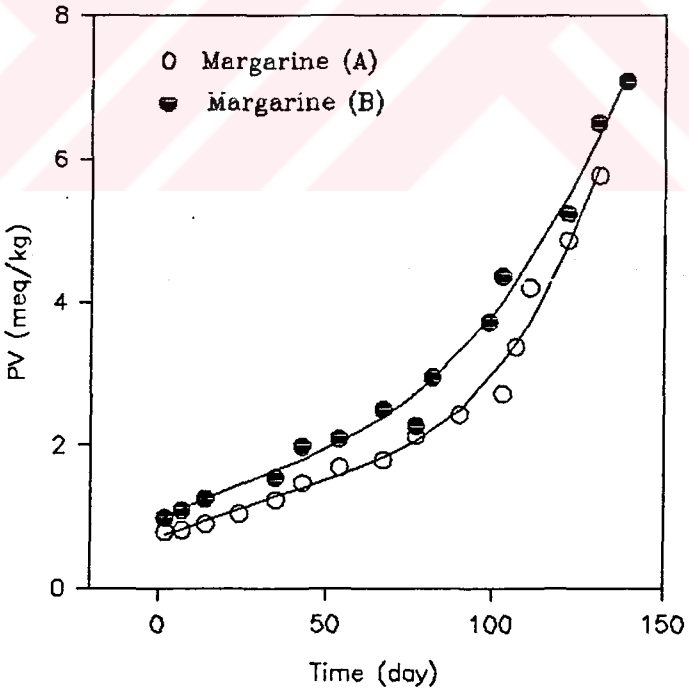


Figure 36. Graphical comparison of PV against time for Margarines (A) and (B) at 14°C.

CHAPTER IV

DISCUSSION

4.1. General

In order to discuss the storage stability of Margarine (A) and Margarine (B), firstly, it will be useful to mention about the compositional analysis of these margarines which has been shown in Table 2.

The compositional analysis showed that the Margarine (B) had lower water and higher fat content than Margarine (A). The low melting point (34°C for Margarine (A) and 30°C for (B)) was also observed for Margarine(B). Having a low melting point depends on the degree of hardening during hydrogenation process of margarine. The degree of hardening for Margarine (B) should be lower than Margarine (A), then it should contain more fat and low water, considering the oil/water balance of emulsion. If it had a higher water content than 11 %, Margarine (B) would become very soft and layered into water and fat phases loosing its crystal structure at market condition.

The storage stability will be shortened due to high fat content and the metal ion contaminated during refining. Since

there is no complete hardening, fat will still contain some unsaturated fatty acids. The presence of unsaturated fatty acids together with some amount of metal and nutrient sources (such as milk) will increase the oxidative rancidity.

4.2. Estimation of Shelf-Life from ASLT Results

The complete tabulation of estimated shelf-life values, which have been found from ASLT results by extrapolation together with 0°C and found from normal storage conditions, are to be shown in Table 7.

Table 7. Estimated shelf-life values from ASLT results for margarine samples.

MARGARINE	TEMPERATURE(°C)	ASLT(days)	NORMAL(days)
(A)	0	192	-
	4	134	137
	14	77	103
(B)	0	171	-
	4	125	130
	14	63	97

It shows that Margarine (B) has shorter shelf-life than Margarine (A). The Q_{10} values also correlates this result. High Q_{10} value indicates that the food is more temperature sensitive and it will easily deteriorate at high temperatures. The estimated Q_{10} values based on the formic acid (ASLT) and peroxide formation (normal storage) were 2.05 and 1.33 for Margarine (A), and 2.10 and 1.34 for Margarine

(B). The literature Q_{10} value based on the 25 % loss of vitamin has been found as 1.91 and in another study it has been found as 2.08 without indicating any criteria [27].

When the shelf-life of both margarines obtained from ASLT studies are compared with shelf-life obtained from normal storage based on the PV, it can be seen that the actual shelf-life obtained from normal storage is longer than the ASLT results. This is probably due to some potential problems. Firstly, it is well known that as temperature rises, phase changes may occur (e.g., fat changing from solid to liquid) which can accelerate the certain reactions [21]. Therefore, the shelf-life which was found by extrapolation to the lower temperature may be shorter than normal storage results. The air flow-rate is not effective on the induction time during conductometric measurement in ASLT and it changes only the shape of the upper portion of the curve [12]. Another important point is the catalytic effect of metal contamination. At low temperatures, metals are found in hydrated forms in crystal structure of margarine and do not responsible for oxidative reaction. In hydrated form, the mobility of metals are limited due to hydration by water molecules. So, the catalytic effect of metals slows down at low temperatures. However, the metals easily initiate the oxidative reaction at high temperatures due to the phase changes occurred between the components of margarine and the high ability of catalyst to mobile.

Given these potential problems, predictions of actual shelf-life from ASLT may be severely limited except in very simple chemical system (such as edible oils). The degree of accuracy is dependent on the purity of the material studied. Edible oils are nearly assumed to be pure. So the shelf-life estimated from ASLT method for oils gives their actual shelf-life. These statements are not valid for margarine at high temperatures but assumed to be valid at lower or refrigerated conditions. Because at the latter conditions, there is no phase changes and mobility of reactants.

4.3. Estimation of Shelf-Life from Normal Storage Temperatures

The estimation of shelf-life from normal storage temperatures based on the some shelf-life determining criteria. These were PV, FFA and microbial count and they were easily monitored during the storage. Fig. 11, 12, 19 and 20 indicate that the PV is only shelf-life limiting parameter at each temperature. There was only slight increase in the FFA results without any sharp increase and no microbial growth during storage at both temperature studied because at these temperatures, the relative humidities were low for the growth of microorganisms. It is known that mold, yeast and bacteria begin to grow gradually above 70 % relative humidities [9].

The shorter shelf-life for Margarine (B) than (A) can be

explained depending on the its high fat content and the low degree of hardening. Since unsaturated fatty acid content is higher than Margarine (A), the shorter shelf-life for Margarine (B) was expected. Also the experimental results proved this assumption.

The PV was main criteria for shelf-life determination of margarine samples at 4 (35 % R.H.) and 14°C (70 % R.H.).

The assumption is based on the energy concept. The energy requirement for hydrolytic rancidity is higher than for oxidative rancidity. Hydrolysis leads to hydrolytic rancidity and involves hydrothermal or enzymic (lipase) hydrolysis breaking the ester linkages to form free fatty acids and the glycerol. The breakage of ester linkages requires much more energy than those of required for the initiation reaction of oxidation. The initiation reaction can easily be started up by the presence of small amounts of metals, oxygen, light. Since these initiating parameters are available either in margarine or in storage atmosphere, they are responsible for the oxidation in the early initiation process even if at lower temperatures without utilizing much more energy.

An additional interesting factor affecting oxidation is whether the lipid is in the solid or liquid state. Several studies have shown that the rate of oxidation of a lipid is much higher than expected when in the solid phase

[9]. This is because in the solid phase the ROO. radical, diffuses slowly and reacts more readily with the substrate continuing the propagation, whereas the radical in the liquid fat can terminate more readily with other radicals.

In the present study, the induction times, generally around 3 meq peroxide/kg fat, from PV results were determined by using tangent method. However, according to Turkish National Standard, the shelf-life is ended when the PV for the margarine reaches to a value of 5 meq/kg. This is not a good criteria because after the induction time PV increases rapidly to a value of 10 or 20 or decreases to a low value due to decomposition of peroxides in a few days if the storage conditions is not controlled [9]. The value indicated by Turkish National Standards can not be followed due to chain reaction of lipid oxidation. For instance, it is desired to examine whether a margarine sample deteriorates or not after a certain storage period. If the PV of the sample is around 5 and the experiment is performed after a few days, the result may be found above or below of this value because of the formation or decomposition of peroxides in a short time.

4.4. Effect of Relative Humidity on the Shelf-Life

The effect of % relative humidities on the storage stability of margarine samples is to be shown in Table 8 at constant storage temperature (14°C).

As explained in section 4.3., the shelf-life determining parameter was PV at 70 % R.H. for both margarine samples. Above this point, the criteria for deciding end of shelf-life of Margarine (A) changed depending on the % relative humidity of storage atmosphere due to microbial growth. However, the criteria remained unchanged for Margarine (B), because no microbial growth and hydrolytic rancidity (increase in FFA value) were observed.

Table 8. Effect of % R.H. on the shelf-life of margarine samples at 14°C.

% R.H.	MARGARINE (A)	MARGARINE (B)
70	103 days (based on PV)	97 days (based on PV)
85	90 days (based on microbial growth)	110 days (based on PV)
98	73 days (based on microbial growth)	108 days (based on PV)

In Fig. 1, the effect of water activity (% R.H./100) on the stability of food was graphically illustrated [9]. It shows that the microorganisms begins to grow above water activity of 0.7. The microbial growth was expected for both margarine samples at 85 % R.H. and 98 % R.H., but it has grown only on during the storage of Margarine (A). Other than high relative humidity, the package type of margarine samples is important, because the microbial growth related directly with the packaging material. Margarine (B) is packaged with

plastic under vacuum and this type of package does not allow the microbial contamination during storage in market conditions. Although, when the margarine packaged with parchment was stored at high relative humidities above 70 %, it would be contaminated by microorganisms due to permeability of the parchment package.

Table 8 also indicates that the longer shelf-life was estimated for Margarine (B) at 85 % R.H.. It should be known that the shelf-life is inversely related with the rate constant or rate of the reaction. It means that the higher rate constant or reaction rate shows shorter shelf-life of a food product.

Fig. 1 also indicates the rate of change for lipid oxidation. Historically, dehydration of foods by removal of water, whereby the water activity is reduced to low values, was a good method to prevent microbiological spoilage. However, it has been found that if foods were dried to too low moisture content (less than 2 to 3 %) they became very susceptible to oxidation. There is an optimum water activity around 0.3 known as monolayer value in which the relative rate of lipid oxidation decreases to minimum values. Above and below this water activity, the lipid oxidation increases rapidly. In the present study, the shelf-life estimated at 70 % relative humidity shows a minimum value when compared with other shelf-life estimated at 85 and 98 % relative humidities for Margarine (B). It can be seen also from Fig. 1, the rate

of lipid oxidation reaches maximum value in water activity range of 0.70-0.75. In this range, the short shelf-life can easily be expected. This assumption was supported by the result found in present study.

The remaining part (rate of lipid oxidation in the range of water activity 0.8-1.0) of Fig. 1 has not been completed yet according to literature survey which has been previously done before the present study started. However, it can be expected that the reaction rate would decrease when the curve has been extended. The estimated shelf-life values in the present study correlates this assumption. Because at 85 and 98 % relative humidities, the estimated shelf-life values are longer in this range, that is rate of lipid oxidation is low.

How water interacted to slow lipid oxidation has been mentioned by Labuza [9]. It has been shown that the basic protective function (lowering the rate of lipid oxidation) that water exhibited when the water activity value increases. It can be accounted by two factors:

(i) Water interacted with metal catalysts making them less effective through changes in their coordination sphere.

(ii) Water hydrogen bonded with hydroperoxides tieing them up so that they no longer were available for decomposition through initiation reactions.

These two factors slow the rate of initiation reaction of lipid oxidation. This shortens the lifetime of radicals readily, possibly by allowing them to migrate out of the

trapped state. The water attached to sites on the food surface, thereby excluding oxygen from the lipid, will also decrease the lipid oxidation.

The solvent and mobilization properties of water become more important at higher water activity ranges. The catalysts present are more easily mobilized and possible swelling of the solid matrix exposes new catalytic sites, making the oxidation rates even higher than in the dry state.

CHAPTER V

CONCLUSIONS

The storage stability of Margarines (A) and (B) was found as a function of temperature and moisture at studied conditions. It was concluded that parchment packaged Margarine (A) is more stable than plastic packaged Margarine (B) at 4°C, 35 % R.H. and 14°C, 70 % R.H. but, unstable at higher relative humidities (85, 98 %) due to mold and yeast growth.

The study of storage stability of Margarine revealed the following facts:

1. The shelf-life of Margarine (A) decreased about 34 and that of (B) 33 days when temperature was increased from 4 to 14°C due to peroxide formation. This is because of the structural change of margarine which affects rate of reactions.

2. Microbial growth was observed at relative humidities below 85 % for Margarine (A), and at all relative humidities for Margarine (B), because, parchment package is water, and mold

and yeast permeable, but, plastic package is not. Increasing % R.H. from 70 to 98, shortened the shelf-life of parchment packaged Margarine (A) about 30 days.

3. Shelf-life of Margarine (B) increased 13 days while % R.H. was increased from 70 to 85, and decreased 2 days while % R.H. was increased from 85 to 98. These results show that plastic package of Margarine (B) absorbed water from the storage atmosphere.

4. The estimated shelf-lives of both samples (at 4 and 14°C) are nearly the same as of 4°C obtained from normal storage conditions, because there is no phase change at low temperatures and free radical formed diffuses slowly due to the hard texture of samples, but, different from those of 14°C due to the change in state of margarine, increase in mobility of catalyst and free radicals, and relatively a rapid increase in rate of oxidation at higher temperatures.

It was concluded that Arrhenius plot is valid for nonhomogeneous fat containing food products (as Margarine) only at low temperatures (i.e., refrigeration temperatures).

5. The estimated Q_{10} values for Margarine (A) and (B) are 2.05 and 2.1 respectively based on formic acid formation, and 1.33 and 1.34 based on PV during normal storage conditions. It was concluded that the rate of deterioration of Margarine

(B) was faster than (A). It misleads if Q_{10} values based on different criteria are compared with each other.

6. Tangent Line Method was found to be more accurate than measuring PV values directly as criteria for shelf-life determination of fat-containing foods. At the end of shelf-life, PV values found to be in the range of 3 to 3.5 meq/kg in contrast to 5 given by Turkish National Standards.

7. It was also concluded that it is not feasible to have a decision on deterioration of any margarine sample by analyzing just one deterioration parameter. Decision should be made after a complete parameter analysis (of PV, FFA and microbial count).

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APPENDICES

Table 1. Conductivity values for Margarine (A) at 85°C.			
Time(hour)	Conductivity*E5	Fitted Values	Parameters
0.00	0.7500	0.7470	0.000280360
0.50	0.7500	0.7508	-0.001467100
1.00	0.7500	0.7531	-0.001037400
1.50	0.7500	0.7537	0.008345500
2.00	0.7500	0.7523	0.747010000
2.50	0.7500	0.7494	
3.00	0.7500	0.7458	
3.50	0.7500	0.7427	
4.00	0.7500	0.7417	
4.50	0.7500	0.7448	
5.00	0.7500	0.7546	
5.25	0.7500	0.7629	
5.50	0.7650	0.7740	
5.75	0.7775	0.7883	
6.00	0.7905	0.8062	
6.25	0.8275	0.8283	
6.50	0.8775	0.8550	
6.75	0.9025	0.8869	
7.00	0.9275	0.9245	
7.25	0.9775	0.9685	
7.50	1.0275	1.0194	
7.75	1.0850	1.0779	
8.00	1.1100	1.1446	
8.25	1.2000	1.2202	
8.50	1.3300	1.3055	

Table 2. Conductivity values for Margarine (A) at 94°C.			
Time(hour)	Conductivity*E5	Fitted Values	Parameters
0.00	0.7500	0.7434	0.000049909
0.25	0.7500	0.7533	-0.003587800
0.50	0.7500	0.7567	0.033625000
0.75	0.7500	0.7563	-0.075003000
1.00	0.7500	0.7545	0.055994000
1.25	0.7600	0.7533	0.743420000
1.50	0.7600	0.7544	
1.75	0.7700	0.7591	
2.00	0.7800	0.7686	
2.25	0.7900	0.7837	
2.50	0.8000	0.8048	
2.75	0.8000	0.8322	
3.00	0.8500	0.8658	
3.25	0.9000	0.9053	
3.50	0.9500	0.9501	
3.75	1.0350	0.9994	
4.00	1.0750	1.0520	
4.25	1.1000	1.1066	
4.50	1.1500	1.1615	
4.75	1.2000	1.2151	
5.00	1.2600	1.2650	
5.25	1.3200	1.3092	

Table 3. Conductivity values for Margarine (A) at 107°C.			
Time(hour)	Conductivity*E5	Fitted Values	Parameters
0.00	0.750	0.7437	0.008485400
0.25	0.750	0.7688	0.000000001
0.50	0.750	0.7419	-0.338090000
0.75	0.750	0.7283	1.191300000
1.00	0.750	0.7648	-1.162400000
1.25	0.850	0.8634	0.321780000
1.50	1.030	1.0166	0.743720000
1.75	1.200	1.2043	
2.00	1.400	1.4015	
2.25	1.600	1.5884	
2.50	1.750	1.7616	
2.75	1.950	1.9466	

Table 4. Conductivity values for Margarine (B) at 87°C.			
Time(hour)	Conductivity*E5	Fitted Values	Parameters
0.00	0.750	0.7629	-0.001966300
0.50	0.750	0.7521	-0.000818100
1.00	0.750	0.7448	0.000116470
1.50	0.750	0.7408	0.000840780
2.00	0.750	0.7399	0.001356400
2.50	0.750	0.7422	
3.00	0.750	0.7474	
3.50	0.750	0.7555	
4.00	0.770	0.7664	
4.50	0.780	0.7799	
5.00	0.790	0.7959	
5.50	0.800	0.8143	
6.00	0.830	0.8350	
6.50	0.840	0.8579	
7.00	0.870	0.8829	
7.50	0.910	0.9099	
8.00	0.950	0.9387	
8.50	0.990	0.9692	
9.00	1.020	1.0014	
9.50	1.040	1.0351	
10.00	1.070	1.0702	
10.50	1.110	1.1065	
11.00	1.140	1.1441	
11.50	1.170	1.1827	
12.00	1.210	1.2223	
12.50	1.260	1.2627	
13.00	1.310	1.3039	
13.50	1.350	1.3456	

Table 5. Conductivity values for Margarine (B) at 98°C.			
Time(hour)	Conductivity*E5	Fitted Values	Parameters
0.00	0.750	0.7584	0.000000000
0.25	0.750	0.7495	-0.005861400
0.50	0.750	0.7512	0.089555000
0.75	0.780	0.7630	-0.057717000
1.00	0.800	0.7844	0.758410000
1.25	0.810	0.8148	
1.50	0.850	0.8536	
1.75	0.880	0.9003	
2.00	0.940	0.9543	
2.25	1.020	1.0152	
2.50	1.090	1.0822	
2.75	1.150	1.1550	
3.00	1.250	1.2330	
3.25	1.310	1.3155	
3.50	1.410	1.4021	
3.75	1.490	1.4922	
4.00	1.580	1.5853	

Table 6. Conductivity values for Margarine (B) at 110°C.			
Time(hour)	Conductivity*E5	Fitted Values	Parameters
0.00	0.750	0.7559	-0.004889000
0.25	0.750	0.7386	0.010135000
0.50	0.750	0.7493	-0.000332000
0.75	0.780	0.7811	-0.001074000
1.00	0.810	0.8285	-0.017433000
1.25	0.900	0.8866	
1.50	0.950	0.9519	
1.75	1.020	1.0215	
2.00	1.110	1.0937	
2.25	1.150	1.1677	
2.50	1.250	1.2438	
2.75	1.320	1.3233	
3.00	1.410	1.4082	

Table 7. PV for Margarine (A) at 4°C.			
Time(day)	PV	Fitted Values	Parameters
7.0	0.7959	0.8067	0.0000000087
14.0	0.8405	0.8481	-0.0000008521
24.0	0.9023	0.9004	-0.0000146030
35.0	0.9823	0.9475	0.0064697000
56.0	1.0393	1.0151	0.7623900000
70.0	1.0629	1.0610	
87.0	1.0889	1.1537	
105.0	1.1350	1.3554	
109.0	1.5846	1.4228	
122.0	1.7197	1.7210	
133.0	2.1124	2.0914	
141.0	2.7080	2.4461	
149.0	2.9182	2.8862	
157.0	3.2834	3.4245	
167.0	3.9121	4.2568	
175.0	5.3192	5.0681	

Table 8. FFA values for Margarine (A) at 4°C.			
Time(day)	FFA	Fitted Values	Parameters
10	0.4255	0.4361	0.0001943
18	0.4244	0.4376	0.4341200
25	0.4570	0.4390	
35	0.4390	0.4409	
44	0.4734	0.4427	
55	0.4512	0.4448	
80	0.4256	0.4497	
98	0.4393	0.4532	
115	0.4481	0.4565	
127	0.4525	0.4588	
144	0.4889	0.4621	
157	0.4611	0.4646	

Table 9. PV for Margarine (A) at 14°C.			
Time(day)	PV	Fitted Values	Parameters
2	0.7903	0.7421	0.0000000286
7	0.8175	0.8239	-0.0000030038
14	0.9000	0.9405	0.0000848710
24	1.0437	1.1055	0.0157670000
35	1.2365	1.2802	0.7102900000
43	1.4704	1.4040	
54	1.6905	1.5791	
67	1.7919	1.8198	
77	2.1306	2.0602	
90	2.4281	2.5009	
103	2.7232	3.1669	
107	3.3800	3.4330	
111	4.2100	3.7337	
122	4.8646	4.7697	
131	5.7728	5.8905	

Table 10. FFA values for Margarine (A) at 14°C.			
Time(day)	FFA	Fitted Values	Parameters
10	0.4255	0.42136	0.0004235
18	0.4244	0.42475	0.4171300
25	0.4278	0.42771	
40	0.4373	0.43407	
52	0.4449	0.43915	
65	0.4586	0.44466	
78	0.4437	0.45016	
97	0.4390	0.45821	
114	0.4457	0.46541	
126	0.4532	0.47049	
144	0.4966	0.47811	
158	0.5016	0.48404	

Table 11. PV for Margarine (B) at 4°C.			
Time(day)	PV	Fitted Values	Parameters
7	0.8928	0.8545	0.000000023
14	0.9135	0.9535	-0.000003278
24	1.0935	1.0843	0.000051417
43	1.1807	1.2730	0.014077000
56	1.3704	1.3551	0.754490000
70	1.5911	1.4210	
105	1.7100	1.8075	
109	1.8418	1.9096	
122	2.1172	2.3932	
128	2.8060	2.7142	
133	3.5046	3.0396	
141	3.5777	3.6870	
149	4.3708	4.5158	
157	5.5950	5.5570	

Table 12. FFA values for Margarine (B) at 4°C.			
Time(day)	FFA	Fitted Values	Parameters
10	0.3600	0.3795	0.000248340
25	0.3691	0.3832	0.376990000
35	0.4003	0.3857	
44	0.4040	0.3879	
55	0.4021	0.3907	
80	0.3916	0.3969	
98	0.3883	0.4013	
115	0.4236	0.4056	
127	0.4259	0.4085	
144	0.4013	0.4128	
157	0.4017	0.4160	

Table 13. PV for Margarine (B) at 14°C.			
Time(day)	PV	Fitted Values	Parameters
2	0.9905	0.99545	-0.004944800
7	1.0935	1.1074	-0.013932000
14	1.2546	1.2513	0.003301900
35	1.5457	1.6427	-0.097024000
43	1.9853	1.7979	0.187460000
54	2.0897	2.0385	
67	2.5032	2.3898	
77	2.2732	2.7297	
82	2.9508	2.9279	
99	3.7244	3.7739	
103	4.3689	4.0173	
122	5.2376	5.4501	
131	6.5108	6.3093	
139	7.0862	7.1832	

Table 14. FFA values for Margarine (B) at 14°C.			
Time(day)	FFA	Fitted Values	Parameters
10	0.3600	0.3632	0.000642750
18	0.3670	0.3684	0.356820000
25	0.3691	0.3729	
40	0.4011	0.3825	
52	0.4035	0.3902	
65	0.4073	0.3986	
78	0.3873	0.4070	
97	0.4078	0.4192	
114	0.4039	0.4301	
126	0.4379	0.4378	
144	0.4628	0.4494	
158	0.4699	0.4584	

Table 15. PV for Margarine (A) at 14°C (85 % R.H.).			
Time(day)	PV	Fitted Values	Parameters
10	0.8950	0.9516	0.000000000
18	0.9860	0.9749	-0.000000155
25	1.0230	0.9974	0.000027221
30	1.1165	1.0143	0.002251100
52	1.0074	1.0953	0.926490000
74	1.1013	1.1792	
82	1.2093	1.2085	
91	1.3332	1.2398	

Table 16. FFA values for Margarine (A) at 14°C (85 % R.H.).

Time(day)	FFA	Fitted Values	Parameters
10	0.3565	0.3633	0.000444330
18	0.3570	0.3669	0.358850000
25	0.3687	0.3700	
30	0.3724	0.3722	
48	0.3897	0.3802	
68	0.4170	0.3891	
81	0.3923	0.3948	
90	0.4000	0.4042	

Table 17. PV for Margarine (A) at 14°C (98 % R.H.).

Time(day)	PV	Fitted Values	Parameters
10	0.8950	0.9712	0.005245400
18	0.9860	1.0132	0.918760000
25	1.0230	1.0499	
30	1.1165	1.0761	
49	1.2960	1.1758	
54	1.1869	1.2020	
73	1.3740	1.3017	

Table 18. FFA values for Margarine (A) at 14°C (98 % R.H.).

Time(day)	FFA	Fitted Values	Parameters
10	0.3724	0.3663	0.002407000
18	0.3765	0.3855	0.342180000
25	0.4132	0.4024	
30	0.3894	0.4144	
48	0.4865	0.4577	
68	0.4941	0.5059	

Table 19. PV for Margarine (B) at 14°C(85 % R.H.).			
Time(day)	PV	Fitted Values	Parameters
10	0.9870	0.9562	0.000000057
18	0.9902	1.0477	-0.000010170
25	1.1506	1.1444	0.000587190
30	1.2546	1.2163	0.000466330
52	1.4102	1.5002	0.902410000
74	1.8204	1.7363	
82	1.9890	1.8523	
91	2.0099	2.0428	
110	2.4385	2.8473	
124	4.4663	4.0415	
137	5.7353	5.8670	

Table 20. FFA for Margarine (B) at 14°C (85 % R.H.).			
Time(day)	FFA	Fitted Values	Paramaters
10	0.3658	0.3689	0.000960610
18	0.3720	0.3766	0.359280000
25	0.3675	0.3833	
30	0.3875	0.3881	
48	0.4259	0.4054	
68	0.4291	0.4246	
81	0.4436	0.4371	
102	0.4712	0.4573	
119	0.4669	0.4736	
126	0.4734	0.4803	
134	0.4802	0.4880	

Table 21. PV for Margarine (B) at 14°C (98 % R.H.).			
Time(day)	PV	Fitted Values	Parameters
10	0.9450	0.8279	0.000005086
18	0.9870	1.0268	-0.000751220
25	1.1250	1.1504	0.042836000
30	1.1703	1.2158	0.469520000
49	1.2266	1.3632	
54	1.3015	1.3930	
73	1.5840	1.5718	
81	1.8897	1.7133	
90	2.2306	1.9475	
109	2.8703	2.7997	
123	3.3994	3.8372	
129	4.3307	4.4121	
136	5.0306	5.1939	
142	6.3288	5.9669	

Table 22. FFA for Margarine (B) at 14°C (98 % R.H.).			
Time(day)	FFA	Fitted Values	Parameters
10	0.3400	0.3436	0.001096200
18	0.3476	0.3524	0.332650000
25	0.3498	0.3601	
30	0.3501	0.3655	
48	0.3922	0.3853	
68	0.4251	0.4072	
81	0.4485	0.4215	
102	0.4673	0.4445	
119	0.4564	0.4631	
126	0.4578	0.4708	
134	0.4587	0.4796	