PHYSICO-CHEMICAL PROPERTIES AND CHARACTERISTICS OF PALM OIL

By

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of Master of Science of the University of Nairobi.

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DECLARATION

The contents of this thesis are my original work and has not been submitted for a degree in this or any other University.

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DEDICATION

To my family and parents

ABSTRACT

A survey of the literature revealed that most palm oil imports carried the exporter's specifications which created undue disputes that took long periods to settle while the oil remained stored under varying conditions at the point of destination; that such imports were likely to be affected by certain conditions of storage, transportation and handling; and that no comprehensive studies had been carried out to establish critical parameters that could be used to characterise these imports and hence provide a correct assessment of their market value and appropriate tax valuation in Kenya.

Palm oil samples from eight ex-Malaysia and Indonesia shipments were collected from twenty-three storage tanks at Mombasa port over a period of ten months. The objective was to investigate the characteristics of the oil in relation to possible effects of handling, storage and transportation.

Mean density and refractive index when determined at 50 ± 0.1°C were found to be 0.8905 g/ml and 1.4548, respectively. The iodine value and softening point were found to be 52.2 and 36.8°C, respectively; while saponification value and unsaponifiable matter content fluctuated around 197 mgKOH/g and 0.43%, respectively. Further investigations with unsaturated fatty acids using the Wij's iodine addition method revealed that the iodine value (Y) for varying amounts of palm oil (X) can be corrected using the equation: $Y = -0.6203X + 52.666$, thus enabling an optimum standardization of the weight range ($0.31 < X < 1.21$ g) of the oil for testing. The content of carotenoids (expressed as beta-carotene) was found to be 496 ± 21 ppm; while the free fatty acid content (as palmitic acid equivalent and volatile matter were 3.47% and 0.12%, respectively.

The values of density, softening point, saponification value and refractive index were generally higher than those reported from palm oil surveys in Malaysia; while refractive index, iodine value, free fatty acid content, unsaponifiable matter, carotenoids content and volatile matter were lower. A study of the variation of viscosity of palm oil, measured at temperatures between 25 and 90°C yielded data which gave two regression lines that intersected at 48.1°C, a value taken as the critical temperature for viscous flow of palm oil.

These results can be used as reference properties for the grading of palm oil imports as well as correct tax valuation; and for categorizing those imports that may require further processing in

this country. Strategies and priorities have also been proposed for further investigations concentrating on the iodine value, carotenoids content and the viscosity-temperature relationship, as critical parameters that can be used to assess palm oil imports. In this way constraints on revenue collection by the relevant tax agencies will be identified and alleviated.

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CHAPTER 1

INTRODUCTION

The world consumption of all types of fats and oils has been increasing gradually owing to population growth and industrialization [1, 2]. In Kenya, agricultural production of these products cannot meet the demands for food and feedstock and therefore, a large proportion is imported [3]. During 1994, for example, Kenya imported 205,357 tonnes of fats and oils. Crude palm oil (mainly from Malaysia and Indonesia) valued at Kenya Shillings 4.0 billion (US$ 71.4 million) accounted for 72% of these imports, making it the most important raw material in the fats and oils category [4]. World production figures (Table 1.1) show that an increase of 115% was realised in the 1984-93 period, making palm oil second to soya bean oil in production [5].

Most palm oil producing countries encourage the export of the commodity in the processed form due to economic reasons [6, 7]. However, the majority of the importers prefer the crude form owing to lower import taxes levied on it. The tariff rates imposed depend on the level of processing the oil has undergone, with highly processed products attracting higher rates [8, 9]. Proper identification of palm oil is therefore, required for proper assessment of the appropriate taxes due to the government.

Table 1.1. Trends in Sources and World Production of Fats and Oils (In tonnes).

	1984	1993
Soya bean	13,276,000	17,200,000
Palm	6,280,000	13,512,000
Rapeseed	5,226,000	9,144,000
Sunflower	5,877,000	7,504,000
Tallow	6,372,000	6,771,000
Butter fat	6,290,000	5,925,000
Lard	4,878,000	5,476,000

Source: Oil World magazines (1985 and 1994).

Palm oil is used mainly for the production of cooking oil. Non-edible applications of the oil include manufacture of soap, lubricants, and fatty acids and their derivatives (oleochemicals) [10]. However, the chemical and physical properties that determine the types of processes or uses of the oil also depend on the conditions prevailing at the time of harvesting and during processing of the fruits; as well as handling, storage and shipment of the oil [11]. In view of such changes, the palm oil consumer needs information about the characteristics and physico-chemical properties of that oil to facilitate the selection of suitable processing conditions for the production of the desired products [12].

Palm oil is one of the richest sources of natural pro-vitamin A carotenoids and Vitamin E compounds associated with protection against various types of cancer and other health disorders [13]. This has led to a new demand for an edible oil that has been lightly refined to retain some of these minor components in the oil [14].

Because of its semi-solid nature at ambient temperature, a relatively high temperature is unavoidable in handling the oil. It has to be transferred when it is both homogeneous and viscous enough to be pumped through pipelines [15]. Therefore, the relationship between temperature and viscosity is fundamental in the design of pumping, mixing and transferring systems, and selection of handling parameters which reduce damage to the oil. Information on the heat stability is required if preservation of the carotenoids in palm oil is to be ensured.

In the work undertaken, density, softening (slip-melting) point, refractive index, iodine and saponification values; unsaponifiable matter, carotenoids and free fatty acid contents, and volatile matter of typical consignments of palm oil from Malaysia and Indonesia were studied; and the results compared with data available from Malaysia on the same. Also, the stability of palm oil carotenes at 50°C, viscosity and the energy of activation for viscous flow of palm oil, properties and behaviour of the heated oil, the interactions between solid and liquid fractions of palm oil, and the variation of iodine value with the test sample weight were investigated. The main objective of these investigations was to establish the identity characteristics of imported crude palm oil, at storage installations, which can be used as reference properties for correct tax evaluation and collection of revenue.

CHAPTER 2

LITERATURE REVIEW

2.1. CHARACTERIZATION OF PALM OIL.

The advances in oil palm breeding and selection, have demanded continuous research with greater need for more scientific understanding of the physico-chemical properties of palm oil. This has culminated in reports on variations between the content and characteristics of palm oil of different species and varieties of the genus Elaies grown in various parts of the world [16, 17, 18].

The neutral lipids of the dura and pisifera varieties have been reported to contain 87.7% and 89.8% of triacylglycerols, respectively [19], while the fatty acid composition of the oil from various origins has shown a higher stearic acid content for Ivory Coast oil and higher palmitic acid content for Sumatra oil [20]. In addition, eleven types of carotenoids have been identified in oils from different oil palm species [21]. For the oil extracted from the Tenera variety of Elaies guineensis species, the carotenoids concentration ranges from 500 to 700 ppm [22]. However, no systematic variation of practical significance was reported in a study of the variations in characteristics and composition of palm oil throughout a seasonal year, from mills and bulking installations, in Malaysia. These results have been used to describe the physical and chemical characteristics of palm oil from the species Elaeis guineensis [23].

2.2. STABILITY OF PALM OIL.

Investigations of possible changes in palm oil at various stages of transfer during a shipping period indicated a rise in free fatty acid (FFA) content, peroxide value and iron content [15]. The alteration of the oil during storage or transport has been attributed to the occurance of autocatalysis and biochemical reactions [24]. The rate of lipolytic reaction has been shown to be dependent on the initial level of FFA, moisture content, temperature of the oil and period of storage or shipment [25]. A significant increase in the FFA content after sea voyage has been related to heating of the oil before discharge [26], and is largely responsible for the refining loss in crude palm oil [27].

When kept at 60 and 80°C, molecular absorption of crude palm oil decreases in the carotenoid wavelength range while peroxide and carbonyl levels rise [28]. At 55°C, the oil shows increased oxidation with storage period [29] depending on the type of container. In one study, oil packaged in plastic and clear glass bottles gave higher total oxidation values than lacquired metal cans and amber or green glass bottles. The rate of oxidation was faster in direct sunlight than in diffused light [30]. Factors which promote oxidation of crude palm oil during production, storage or shipment have also been found to affect its bleachability as the coloured compounds generated during the process are difficult to remove [31].

Tocopherol and tocotrienols have been reported to be effective antioxidants in crude palm oil [32]. In thermal oxidation of fats, the stability of tocopherols has been found to be strongly affected by the degree of unsaturation of the triglycerides [33], with greater tocopherol loss in saturated oils [34]. When kept at 180°C for 50 hours palm oil liquid (olein) fraction, mainly used for cooking, showed an increase in free fatty acid content and a decrease in iodine value [35]. At higher temperatures above 300°C, pyrolytic reactions of fatty acid esters produce fatty acid and acrolein [36].

2.3. PHYSICO-CHEMICAL PROPERTIES OF PALM OIL FATS.

The properties of fatty oils have been described by equations which relate viscosity to iodine and saponification values [37]. The viscosity of palm oil investigated between 20 and 45°C showed non-newtonian flow below 35°C due to the presence of fat crystals [38]. However, for simple triglycerides the viscosity between the melting points of alpha and beta polymorphic forms showed increase in the activation energy for viscous flow because of enhanced molecular aggregation of the supercooled melts [39]. For palm oil, the nucleation from the supercooled melts showed a discontinuity at 297K (24°C) indicating the critical temperature for the occurance of beta-polymorphic phase crystals [40].

The crystallization behaviour [41], and fractionation of palm oil into liquid and solid components [42, 43] show that the properties of solid fractions obtained from various separation methods differ due to the amount of mother liquor or liquid oil trapped in the fat crystals [44]. Iodine value therefore, is a more sensitive parameter for the characterization of palm oil fractions. The iodine value of the solid (stearin) and liquid (olein) oil fractions range from 21.6 to 49.4, and 56.1 to 60.6, respectively [45]. In addition, the insolubility of endogenous carotenes in the solid fraction shows a good correlation of the carotenoids concentration with palm oil solid fat content, and has been applied in determination of solid and liquid oil components of palm oil [46].

The slip-melting point of various compositions of palm oil and its solid (stearin) fraction shows a curve which deviates from the theoretically expected linear logarithmic plot [38]. In the case of interaction of palm and palm kernel oils, a eutectic mixture formed with minimum slip-melting point showed improved cold stability [47]. But a Differential Scanning Calorimetry (DSC) study on palm oil liquid fraction (olein) blended with tripalmitin indicated a new peak which has not been elucidated on the crystallization thermograms [48].

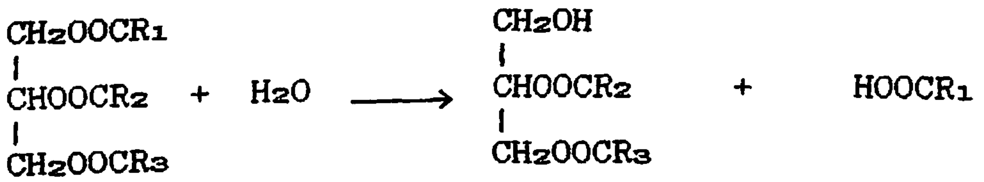
2.4. NUTRITIONAL BENEFITS OF PALM OIL.

Triacylglycerols, which make up the bulk of dietary fats and oils provide twice as much energy per gram as carbohydrates. Fats and oils also contribute a number of important fat-soluble vitamins such as Vitamins A, D, E and K [49]. Palm oil is particularly rich in pro-vitamin A carotenoids and contains about 800 ppm vitamin E compounds rich in tocopherols and tocotrienols [50]

Palm oil has shown beneficial nutritional effects due to antioxidant properties of carotenes, tocopherols and tocotrienols. These compounds act as scavengers for free radicals arising from biological metabolism or from the action of toxic chemicals and environmental pollutants. Free radicals have been implicated in aging, chronic degenerative diseases such as atherosclerosis and arthritis, and carcinogenesis [51, 52, 53].

2.5. CHEMISTRY OF FATS AND OILS.

Fats and oils are mainly esters of different types of fatty acids and glycerol [54]. The minor components contain a wide variety of chemical compounds, some of which are removed in the refining process [55]. Most reactions of fats involve the ester group or the carbon-carbon double bond [36]. Triglyceride esters undergo partial or complete hydrolysis in presence of water and acid catalyst to give constituent fatty acids and glycerol. Figure 2.1 shows the hydrolysis reaction in fats where R_1, R_2, and R_3 may represent the same or different hydrocarbon chains of fatty acid radicals [49]. Hydrolysis of triglycerides represents a loss in yield of the refined fat [11].



$$\begin{array}{l} CH_2OOCR_1 \\ | \\ CHOOCR_2 \\ | \\ CH_2OOCR_3 \end{array} + H_2O \longrightarrow \begin{array}{l} CH_2OH \\ | \\ CHOOCR_2 \\ | \\ CH_2OOCR_3 \end{array} + HOOCR_1$$

Triglyceride Diglyceride Fatty acid

Figure 2.1. Structure and partial hydrolysis reaction of triglycerides.

The kinds of fatty acids present in a triglyceride have marked effects upon its physical and chemical behaviour [56]. Lauric, palmitic, stearic, oleic and linoleic acids are the most common fatty acid residues in natural fats and oils. They differ in chain length and the number of double bonds as shown in Figure 2.2 [54]. The double bonds are potential centres of chemical reaction, where the alpha methylene group is notably reactive. The reactions that are influenced by the presence of unsaturated bonds are very important in understanding the chemistry and utilization of fats. Oxidation of unsaturated fats by atmospheric oxygen is an important part of the series of reactions that occur during the drying of paint oils, and rancidity in fats and oils [36].

Lauric acid : $CH_3(CH_2)_{10}COOH$

Palmitic acid : $CH_3(CH_2)_{14}COOH$

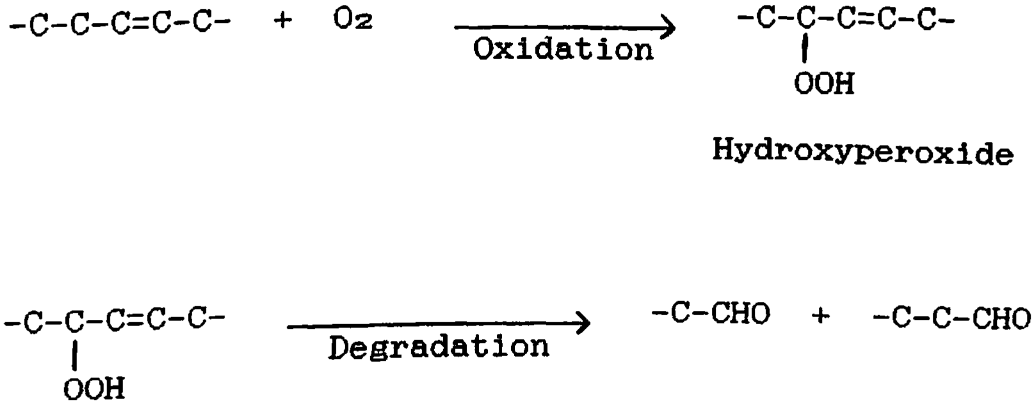
Stearic acid : $CH_3(CH_2)_{16}COOH$

Oleic acid : $CH_3(CH_2)_7.HC{=}CH.(CH_2)_7COOH$

Linoleic acid : $CH_3(CH_2)_4.HC{=}CH.CH_2.HC{=}CH(CH_3)_7COOH$

Figure 2.2. Structural formulae for the common natural fatty acids.

The oxidation of unsaturated fats proceeds by autoxidation or photo-oxidation processes. Quite distinct from autoxidation which relies on the free radical mechanism for its propagation, photo-oxidation is the direct attack of singlet oxygen molecules [11]. The activation energy for the autoxidation is derived from heat or light. However, photo-oxidation depends upon excitation of oxygen to the singlet state by the action of light energy through an intermediary sensitizer such as chlorophyll. The oxidation process is usually faster in free fatty acids than their esters [57], and is catalyzed by transition metals to yield hydroperoxides (Figure 2.3). At ambient temperatures hydroperoxides decompose into diverse compounds, some of which are responsible for rancidity of fats and oils, and include hydrocarbons, aldehydes, ketones or alcohols [11, 36].



$$\text{-C-C-C=C-C-} + O_2 \xrightarrow[\text{Oxidation}]{} \text{-C-}\underset{\text{OOH}}{\underset{|}{\text{C}}}\text{-C=C-C-}$$

Hydroxyperoxide

$$\text{-C-}\underset{\text{OOH}}{\underset{|}{\text{C}}}\text{-C=C-C-} \xrightarrow[\text{Degradation}]{} \text{-C-CHO} + \text{-C-C-CHO}$$

Figure 2.3. Oxidative breakdown of fats.

2.6. FATTY ACID DISTRIBUTION IN TRIGLYCERIDES.

The proportions of various fatty acids in natural glycerides vary from fat to fat. Each fat has its characteristic composition, which does not differ very much from sample to sample [58]. But the differences in composition depend on the source of the fat especially the family, genus, or type of plant species [36]. Many different naturally occuring fatty acids give rise to a number of possible permutations and combinations of triglycerides [57]. However, a study on the structure of naturally occurring fats and oils shows non-random distribution of fatty acid radicals. The triglycerides have been found to vary considerably in their general tendency to assume configurations which are specific with respect to the relative positions of the saturated (S) and unsaturated (U) fatty acid radicals [59]. In vegetable oils and fats, the saturated radicals have a tendency to take up 1-position in preferance to 2-position, with S-U-S and S-U-U as the dominant configurations [60].

The sequence in which the different fatty acids are arranged over the carbon atoms in glycerol determine the crystal structure and the polymorphic phases of the triglyceride molecules, and influences the melting point and crystallisation behaviour of triglycerides [39]. The difference in melting point ranges of mixed triglycerides is used to isolate solid and liquid components of fats and oils by fractional crystallization [56, 61]. Variation of the fatty acid composition or their distribution in the triglyceride molecules by interesterification provides ways of modifying the physical properties of natural fats and oils [49].

2.7. BIOSYNTHESIS OF FATTY ACIDS.

Most of the natural fatty acids are straight chain alkanoic or alkenoic acids with even numbers of carbon atoms. Usually, the unsaturated acids have cis-double bond arrangement due to the biosynthesis of fatty acids in biological systems [54]. The carbon chain is built stepwise, by addition of two carbon atoms at a time, from acetate units in steps that closely resemble malonic ester synthesis in organic chemistry. The process is termed the acylpolymalonate pathway [58]. The carbon atoms from acetic acid units are carried as acetylcoenzyme-A ($CH_3CO\text{-}S\text{-}CoA$). In presence of carbon dioxide, acetylcoenzyme-A is converted to malonylcoenzyme-A, $HOOCCH_2CO\text{-}S\text{-}CoA$, with Adenosine triphosphate (ATP) as a source of energy:

$$CH_3CO\text{-}S\text{-}CoA + CO_2 + ATP \rightleftharpoons HOOCCH_2CO\text{-}S\text{-}CoA + ADP + P_i.$$

where P_i represents inorganic phosphate.

Transesterification of malonylcoenzyme-A with an acyl carrier protein, ACP-SH, gives a thiol ester which may condense with acetylcoenzyme-A with loss of carbon dioxide, to form acetoacetyl-S-ACP:

$$HOOCCH_2CO\text{-}S\text{-}ACP + CH_3CO\text{-}S\text{-}ACP \rightleftharpoons CH_3COCH_2CO\text{-}S\text{-}ACP + CO_2 + ACP\text{-}S\text{-}H.$$

The carbonyl group of $CH_3COCH_2CO\text{-}S\text{-}ACP$ can be reduced by nicotinamide adenine dinucleotide phosphate (NADPH) to an alcohol. The unsaturated double bond formed by dehydration of the alcohol may be hydrogenated, with NADPH as the reducing agent. These reactions are stereospecific and each step is catalyzed by a particular enzyme. The reaction sequence can repeat itself with acetlycoenzyme-A as a carrier of the acetate units and stops after some cycles with the formation of straight, saturated hydrocarbon chains as in palmitic and stearic acids. However, during some of the reaction cycles, the cis-double bonds formed are not hydrogenated and form unsaturated fatty acids such as oleic acid [54, 58].

2.8. BIOSYNTHESIS OF TRIGLYCERIDES.

Fats and oils are the main constituents of fat stored in cells of animals and plants and are therefore, widely distributed in nature. The number of triglycerides formed in a given natural fat depends on the specificity of the enzyme systems involved in the particular fat synthesis reactions [54]. There is evidence that the glycerol moiety utilized in triglyceride biosynthesis is derived mainly from the Laevo-optical isomer of alpha-glycerophosphate.

The biosynthesis of triglycerides involves a number of acyltransferases which use acetylcoenzyme-A as a carrier of acetyl radicals [62]. In plants, the pathway is via phosphatidic acid (Figure 2.4) and is identical to that in animals. Glycerol-3-phosphate (G3P) is esterified at 1-position carbon, by enzyme 3GP-acyltransferase (3GP AT), to lysophosphatidic acid (LPA). The next acylation is catalysed by 1-acyl glycerol-3-phosphate acyltransferase (AG PAT) and forms phosphatidic acid (PA) which is then dephosphorylated and acylated at the third carbon by diacylglycerol acyltransferase (DG AT) [63]. Some of the reactions may lead to formation of saturated acyl groups at the 2-position in plant triglycerides [54, 62, 63].

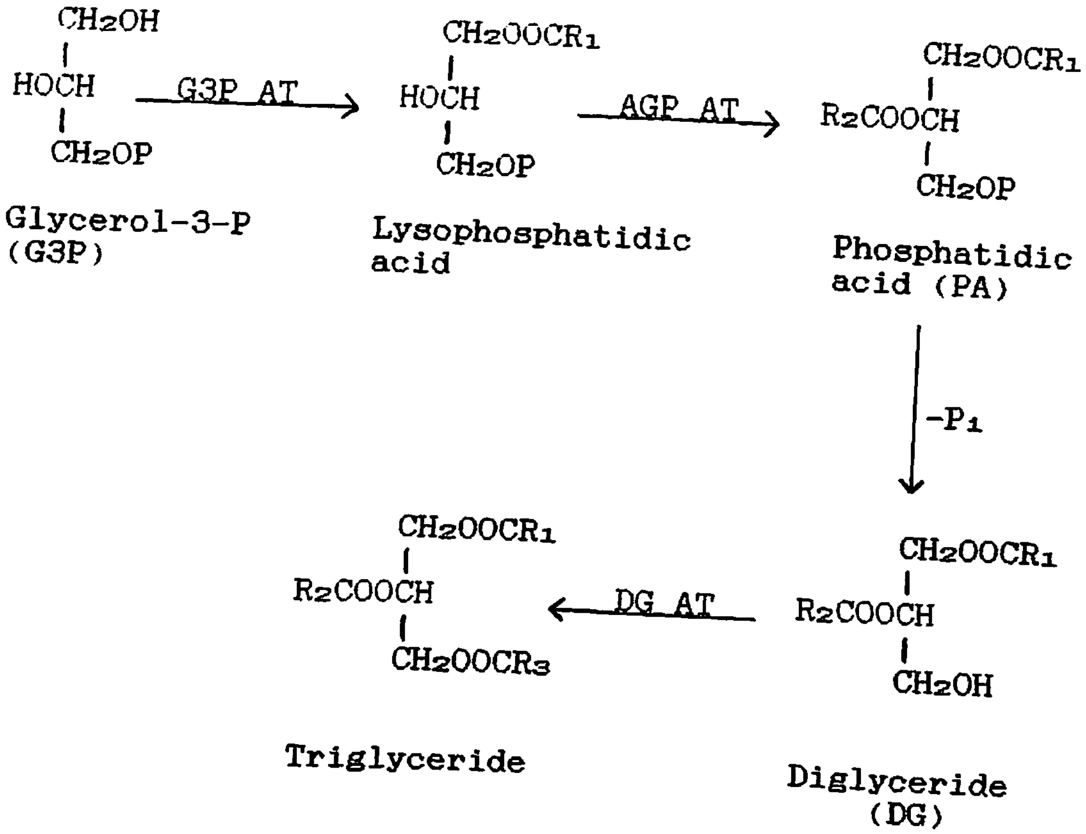


Figure 2.4. Reactions for triglyceride synthesis in plants.

2.9. EXTRACTION OF PALM OIL FROM OIL PALM FRUIT.

Palm oil is the type of oil extracted from the mesocarp pulp of the ripe fruit of various Elaeis oil palm species [18]. Before the advent of machinery in Africa, palm oil from the indigenous oil palm grooves was extracted by a simple process where ripe fruits picked from oil palm bunches were first boiled in pots. The softened fruits were pounded in a wooden mortar, with the use of a pestle to give a homogeneous mixture of pericarp pulp and loosened palm kernel nuts. The mixture was immersed in hot water and stirred to extract the oil, which then floated on the surface and was skimmed off. The crude oil was purified by repeated stirring in hot water to separate the fruit fibres by gravity. The floating oil layer was then skimmed off and heated in a shallow pot to evaporate the water prior to storing in a suitable container [16, 36].

Today, the extraction of palm oil from ripe oil palm fruits uses sophisticated methods (but based on the same principle originally used in West Africa) which involve varying degrees of mechanization designed for various production capacities [64]. In general, the extraction involves sterilisation of the fruits by steam treatment, stripping of the fruits from bunches, extraction of the oil from the fruit mash homogenate, and clarification and purification of the oil [65]. Commercially, the oil is stored in bulk using large tanks. In order to avoid heat damage, it is usually cooled to about 45 to 50°C before being discharged into the storage tanks [15].

2.10. HANDLING, STORAGE AND TRANSPORT OF PALM OIL.

Before crude palm oil reaches the processing or refining factories it undergoes various stages of storage and shipment, some of which involve heating and pumping of the oil. Palm oil from oil mill storage tanks is delivered by tankers to the designated oil bulking and storage installations. From these facilities, the oil is loaded into maritime vessels for transport in bulk by sea. On arrival at the point of destination, it is discharged into on-shore storage tanks for delivery to the processing or refining factories [66].

Because improper handling of oils and fats during the process of bulk storage and transportation can introduce contamination by other materials or cause deterioration by chemical reactions [11], the palm oil industry maintains liaison with the handling agencies for the control of damage to the oil during loading, carriage and discharge. Therefore, samples taken at points of discharge are useful in assessing the quality of the oil [31].

2.11. TECHNIQUES OF SAMPLING PALM OIL.

Crude palm oil is commercially stored in vertical and cylindrical mild steel bulk storage tanks of capacities ranging from about 500 to 3000 tonnes. Various techniques [67, 68] have been devised for drawing representative samples of the liquid oil from such tanks. There are two types of sampling apparatus: continuous and zone samplers. When a continuous sampler is allowed to fall through a liquid, the rate of entry of the oil into the apparatus is adjusted to allow continuous drawing of the oil from top to the bottom of a tank. In stratified sampling, a zone sampler is used to obtain samples from any specified section of a tank.

The valve sampling cylinder is an example of a zone sampling apparatus and uses the property of the fluid's resistance when compacted. It consists of an open-headed cylinder with a bottom valve which is forced open by the liquid pressure as the sampler falls through the liquid. This allows an even flow of the oil through the cylinder. The valve closes when the lowering is stopped such that the oil in the cylinder represents the oil at the depth reached by the sampler. This method of sampling depends on the homogeneity of the oil, which is a function of temperature [67, 68].

2.12. THEORETICAL BACKGROUND OF EXPERIMENTAL METHODS.

2.12.1. DENSITY.

The weight of matter contained in a unit volume is a quantity characteristic of that substance at the specified temperature and pressure. This is defined as density and is a fundamental property of a compound that reflects the type and arrangement of the molecules in a macroscopic sample. It also affords a means of identifying organic substances. In general, density increases with molecular compaction [69], and for triglycerides, it increases with both saponification and iodine values. Because fatty acids have lower density than triglycerides, crude oils containing high free fatty acid content have lower density than refined oils [70].

The densities of oils are usually determined in liquid state at 25°C by use of density bottle. However, for fats and oils not completely liquid at this temperature the determination is performed at 50°C. Temperature control to within 1°C is required because the coefficient of cubical expansion increases with temperature [69].

2.12.2. SOFTENING (SLIP-MELTING) POINT.

A solid melts when the molecules in a crystal lattice are separated by thermal forces into a liquid state. This is usually accompanied by an increase in entropy, heat content and volume. The temperature at which solid crystals of a substance are in equilibrium with the liquid phase under its own vapour pressure is called the melting point of that particular substance. Usually, the effect of vapour pressure upon the equilibrium temperature is negligible and, the melting point is taken with the equilibrium mixture in contact with air [71].

The existence of alternative crystal structures with different melting points is a characteristic property of lipids known as polymorphism. The temperature at which two different crystalline forms are in equilibrium at constant pressure is called transition point [72]. A substance is enantiotrophic when the transition temperature is below the melting temperature of both polymorphs. However, it is monotropic if another form is obtained by melting one form. When heated slowly an enantiotropic substance undergoes transformation from alpha to beta-prime form which on further heating, melts by transformation to the beta form as shown in Figure 2.5 [36]. Therefore, when heated,

Alpha phase $\rightleftharpoons$ Beta prime phase $\rightleftharpoons$ Beta phase

Figure 2.5. Phase changes of heated solid fat.

natural fats pass through a stage of gradual softening, over a range of temperatures, before becoming completely liquid. Softening or slip-melting point is the temperature at which a column of fat becomes sufficiently fluid to slip and start rising in an open capillary tube under the influence of hydrostatic pressure [73].

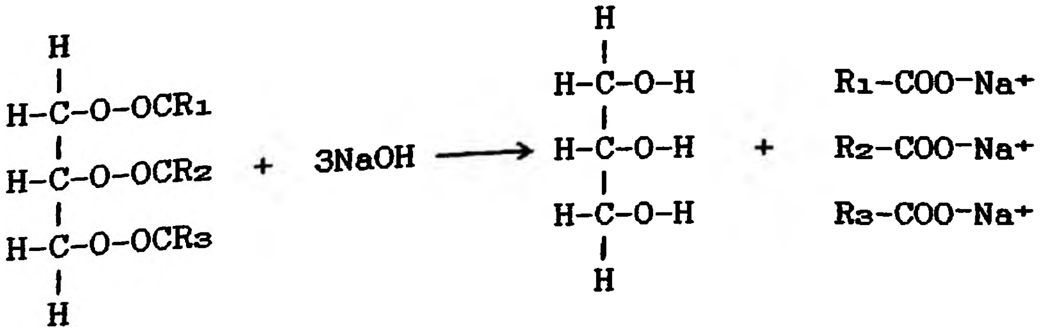
2.12.3. REFRACTIVE INDEX.

The velocity of light depends upon the medium through which it travels. When a ray of homogeneous light passes at an angle from one isotropic medium into another of different density, it undergoes a change in wave velocity so that its direction changes. This phenomenon is called refraction and is defined by Snell's law [74] as the ratio of velocity of light in reference medium to its velocity in the sample medium. Refractive index of a fat or an oil is usually given on the basis of air as reference medium and is related to the molecular structure and unsaturation of fats and oils: The higher the molecular weight and degree of unsaturation the higher the refractive index. Refractive index can therefore be influenced by the oxidation damage to the oil or fat [55].

The dispersion of white light by a prism shows the dependence of refractive index on wavelength: It increases with decreasing wavelength, and for a given wavelength it decreases with temperature. The refractive indices of liquids usually refer to light of wavelength 589.3 nm or the sodium D line (in the emission spectrum of sodium) at a specified temperature, usually $25^{\circ}C$. However, by use of ordinary light the measurement of the critical angle using the Abbe refractometer gives values of refractive index from a scale calibrated directlty in units of the sodium D-line [75, 76].

2.12.4. SAPONIFICATION VALUE.

In presence of alkali, fats and oils are hydrolysed to glycerol and fatty acid-alkali metal salts in a reaction process, called saponification. Figure 2.6 gives a general saponification reaction where R_1, R_2 and R_3 may be the same or different fatty acid chains (Section 2.5) and sodium hydroxide is the alkali:



$$\begin{array}{c} H \\ | \\ H\text{-}C\text{-}O\text{-}OCR_1 \\ | \\ H\text{-}C\text{-}O\text{-}OCR_2 \\ | \\ H\text{-}C\text{-}O\text{-}OCR_3 \\ | \\ H \end{array} + 3NaOH \longrightarrow \begin{array}{c} H \\ | \\ H\text{-}C\text{-}O\text{-}H \\ | \\ H\text{-}C\text{-}O\text{-}H \\ | \\ H\text{-}C\text{-}O\text{-}H \\ | \\ H \end{array} + \begin{array}{c} R_1\text{-}COO^-Na^+ \\ R_2\text{-}COO^-Na^+ \\ R_3\text{-}COO^-Na^+ \end{array}$$

Figure 2.6. Saponification reaction of fats.

The rate of reaction is directly proportional to the concentration of both the ester and alkali:

The rate of reaction = $k[\text{Ester}]^n[\text{Alkali}]^m$,

where k is the rate constant for the reaction; n and m are orders of reaction with respect to Ester and Alkali.

The reaction is usually carried out by refluxing the fat in an ethanolic solution of potassium hydroxide. The number of milligrams of potassium hydroxide required to neutralize and saponify 1 gram of fat is called the saponification value. It is a measure of the number of ester groups present in the fat. Saponification equivalent is another way of expressing the results of saponification value determined, and for a pure fatty acid residue or ester it is equal to its equivalent weight [36, 75].

2.12.5. HALOGENATION AND IODINE VALUE.

Like most alkenes, the unsaturated double bonds in fats and oils can undergo addition as well as substitution reactions [58]. The rate of addition depends upon the structure of the fatty acid, the kind of halogen, reaction time and temperature, excess of the reagent over that required for the saturation of the sample, and the types of solvents and catalysts used. It is very slow for double bonds located at the carbon atom adjacent to the carboxyl group. However, fatty acids with double bonds in the alpha-position rarely occur in natural fats and oils. The addition to conjugated double bonds is much slower than for those in isolated positions [36].

Halogen molecules can be polarized under the influence of solvents or catalysts used so that halogenation reaction proceeds by ionic mechanism. In acetic acid solution, the addition reaction is relatively high for chlorine and slow for iodine. However, the reactivity is moderate for iodine monochloride when used as the halogenating agent in the Wij´s method [68, 77]. After the reaction is complete, the excess potassium iodide added is oxidised by unreacted halogen (X_2) to liberate an equivalent amount of iodine:

$$X_2 + 2I^- \longrightarrow I_2 + 2X^-$$

The liberated iodine can be reduced by use of a strong reducing agent such as sodium thiosulphate:

$$I_2 + 2S_2O_3^{2-} \longrightarrow 2I^- + S_4O_6^{2-}$$

This reaction is used in the quantitative determination of iodine by iodometric titration [75].

The content of double bonds capable of halogenation is expressed as per cent by weight of iodine absorbed by the oil or fat [36].

2.12.6. UNSAPONIFIABLE MATTER.

Natural fats and oils contain mainly fatty acid triglycerides with their hydrolytic products and a minor proportion of various types of fat soluble organic compounds. The minor components include tocopherols, sterols, squalene, long chain and triterpene alcohols, and pigments. Because of their chemical structures they remain unsaponifiable in the saponified fat [36]. The amount of these compounds is expressed as per cent by weight of unsaponifiable material present in a fat. The determination also serves to establish whether damage to the oil by oxidation and or contamination by unsaponifiable foreign materials such as mineral oil has occurred [76].

2.12.7. SPECTROSCOPY AND ABSORPTION CAROTENE.

The internal energy of a molecule can be regarded as the sum of electronic, vibrational and rotational energy components. The behaviour of an electron in a molecular bond can be deduced by use of the fundamental eigenvalue equation of quantum mechanics:

$$H\psi_i = E\psi_i$$

where the Hamiltonian operator (H) operates on the allowed wavefunction ψ_i to give the allowed energy, E. The equation can be used to get an acceptable solution to the Schrodinger's wave equation for a particle in a stationary state by use of the one-dimensional box model:

$$E_n = \frac{n^2h^2}{8md^2} \qquad (2\text{-}1).$$

where n is an integer, E_n is the allowed energy level of the particle of mass m, and d is the dimension of the model [78].

A molecule can only absorb a quantum of incident electromagnetic radiation if it undergoes a change in energy (ΔE) from one transition state to another of magnitude equivalent to the product of radiation frequency (v) and the planck's constant (h). From the solution for Schrodinger equation, ΔE increases with decrease in both the mass of the particle and space in which the particle is confined. Thus the delocalised electrons in molecules with conjugated polyene chain can bring the absorption of radiation into the long wavelength. For example, beta-carotene (Figure 2.7) is coloured orange since it absorbs light at 451 nm [79, 80].

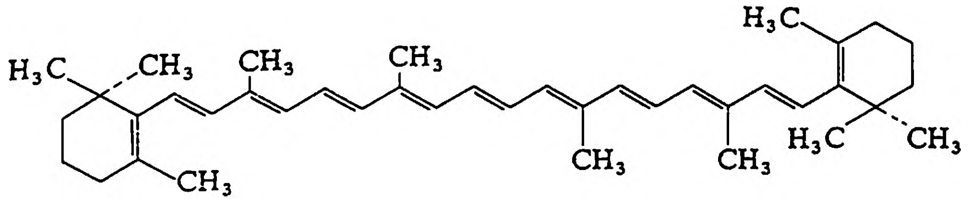


Figure 2.7. Molecular structure of beta-carotene.

The amount of monochromatic radiation absorbed by an absorbing medium results in decrease of intensity (I) of the transmitted radiation. The decrease, -dI, in intensity of an incident beam (I_o) after it has passed through an absorbing medium, is directly proportional to the incident intensity, the path length, b, and concentration, C, of the absorbing species through a proportionality constant k, called the absorption coefficient.

$$-dI = kI_oCb \qquad (2\text{-}2).$$

Rearrangement of the equation and expression in the logarithmic form gives the Beer-Lambert relationship [78]:

$$\log\frac{I_o}{I} = abC = A \qquad (2\text{-}3).$$

where A is the absorbance. a = k/2.303, is called absorptivity and is independent of concentration, path length and the intensity of the incident radiation. However, it depends upon temperature and molecular structure, and is characteristic of a particular combination of solute and solvent for a given wavelength of radiation. The absorbance at any given wavelength can be measured by use of a spectrophotometer [75, 79].

2.12.8. FREE FATTY ACID CONTENT.

Enzyme lipolysis and water hydrolysis of glycerides generate free fatty acids in fats and oils [11]. Carboxylic acids tend to release hydrogen ions more readily than hydroxyl groups of alcohols because of the resonance stabilization of carbanions generated by the neutralization reaction [58]. The extent of hydrolysis of glyceride molecules in fats and oils is measured by the determination of acidity. The number of milligrams of potassium hydroxide required to neutralise the free fatty acids in 1 gram of fat or oil is expressed as acid value. It is also expressed as per cent by weight of the major fatty acid component present in the fat or oil [68].

2.12.9. VOLATILE MATTER CONTENT.

In certain liquid pairs, the mutual solubility is so small that they are considered to be immiscible. Therefore, each liquid exerts its own vapour pressure corresponding to that of pure state at the same temperature. The vapour composition would then be richer in the more volatile constituent. By removing the vapour, the liquid mixture becomes richer in less volatile component [81]. Because water and fat or oil are immiscible, water dispersed in the oil can be separated by evaporation conducted at atmospheric pressure. However, minor volatile components contained in natural fats and oils are also evaporated at the same temperature [36].

2.12.10. DISTILLATION.

At constant pressure, the temperature of a boiling liquid varies as its composition changes due to removal of condensed vapour during distillation [81, 82]. Non-volatile compounds like fats and oils can undergo thermochemical reactions to yield distillates of varying boiling points [36]. The variation of properties of the distillates with the extent of distillation can be used to study the dynamic aspect of heated fats and oils.

2.12.11. VISCOSITY.

The molecules of a liquid are held together by intermolecular forces, but their kinetic energies are comparable to their potential energies. As a result, the whole structure is of high mobility [83]. The flow of a liquid is a rate process similar to the reaction of molecules to form products. Eyring's transition state theory of reaction rates [84] has been applied in explaining the flow of liquid molecules. In order to move, a molecule in a liquid must escape from its neighbours. This resistance to flow in liquids is assumed to be due to difficulty of the molecules to move past their close packed neighbours. Thus, they must attain the minimum energy (Ea) to overcome this resistance (energy barrier) before the elementary flow process can occur [85].

The fraction of molecules that have energy in excess of Ea can be explained by a Boltzmann type of expression. In this relationship, the probability that molecules can acquire the minimum energy (Ea) is proportional to $e^{Ea/RT}$, where R is the gas constant, and T is the Kelvin temperature [82, 83]. Therefore, fluidity, ϕ, of a liquid is proportional to the fraction of molecules that have energy greater than Ea:

$$\phi \qquad \propto \qquad e^{-Ea/RT}. \qquad (2-4).$$

When an incompressible fluid moves through a tube, of circular cross-section of radius R and length L, with velocity v at any distance r from the axis of the tube, one layer of the fluid moves relatively with a velocity gradient, dv/dr. According to the Newton's law [86], the frictional force (f) resisting the relative motion of any two adjacent layers is proportional to the surface area (equal $2\pi rl$) of the interface between them , and to the velocity gradient:

$$f = n(2\pi rl)\frac{dv}{dt} \qquad (2\text{-}5).$$

where η (eta) is a proportionality constant termed coefficient of viscosity, and is inversely proportional to mobility of the molecules [82, 83]:

$$\eta \propto e^{Ea/RT} \qquad (2\text{-}6).$$

Therefore, viscosity of liquids decreases exponentially with temperature, so that:

$$\eta = Ae^{Ea/RT} \qquad (2\text{-}7).$$

where A and Ea are constants characteristic of each liquid. The quantity A is related to the collision frequency of the molecules, whereas Ea corresponds to the energy required by one mole of liquid molecules to escape from its neighbours to a position farther along a tube [82]. Equation (2-7) can be expressed in a more useful form by transforming into the natural and common logarithmic forms:

$$\ln \eta = \ln A + \frac{Ea}{RT} \qquad (2\text{-}8).$$

$$\log \eta = \log A + \frac{Ea}{2.303RT} \qquad (2\text{-}9).$$

A plot of Log η against the reciprocal of the absolute temperature (1/T) should be linear with a slope equal to $\frac{Ea}{2.303R}$ to confirm the validity of equation (2-7).

The viscosity of liquids can be determined by a method based on the Poiseuille equation [82, 84]:

$$\eta = \frac{(\pi R^4)\,Pt}{8LV} \qquad (2\text{-}10).$$

where t is the time taken by a fixed volume (V) of a liquid to fall through a capillary under pressure (P). But since the difference in pressure between the two ends of the tube is proportional to the density (d) of the liquid, it can be shown [85] that for a given volume of liquid:

$$\eta = k.d.t. \qquad (2\text{-}11).$$

where k is a constant that depends only on the dimensions of the apparatus, and is obtained from standard liquids (of known viscosity and density) at the experimental temperature by measuring the time taken by the liquid to flow through the fixed marks on a viscometer [84]. The coefficient of viscosity of liquids is expressed in the units of kg per metre per second or in terms of the unit Poise, where 1 Poise equals 0.1 $kgm^{-1}s^{-1}$ [82].

2.12.12. FRACTIONAL CRYSTALLIZATION.

Compounds which are capable of forming different polymorphic structures can occur in various forms having different physical properties. When mixed with non-polymorphic substances, they show temperatures of spontaneous crystallization. The effect of various solvents on the crystal modifications is largely determined by the electrical properties of the solvent. In general, non-polar solvents promote crystallization of the higher melting point polymorph.

However, the size of crystals formed varies with the rate of cooling and agitation. Large crystals occlude considerable amount of liquid oil, but this can be isolated by multicycle solvent recrystallization of the solid fat crystals [87].

2.12.13. PHASE EQUILIBRIA OF CONDENSED SYSTEMS.

Different solids having the same or different chemical composition with different crystal structures constitute different phases. A system having C components (solutes) distributed throughout P phases is related to the number of degrees of freedom (F) by the Gibb's phase rule equation [81]:

$$F = C - P + 2 \qquad (2\text{-}12).$$

A system with solid-liquid equilibria having relatively small or no gas phase is a condensed system. Due to the small effect of pressure in condensed systems, experiments carried out at constant (atmospheric) pressure have two degrees of freedom [81]:

$$F = 2 - 2 + 2 = 2 \qquad (2\text{-}13).$$

Therefore, temperature and composition are the only variables in a system of two components.

For ideal systems, addition of a solid to a liquid decreases the vapour pressure of the liquid and therefore, lowers or depresses the melting point of the liquid according to Raoult's law [82]. However, non-ideal systems do not obey Raoult's law and solidify to form either eutectic mixtures, solid solutions or inclusion complexes. The relationship between freezing or melting point and composition for these systems can be studied by use of the temperature-composition diagrams, sometimes called phase diagrams [81].

CHAPTER 3

MATERIALS AND METHODS

3.1. SAMPLING.

Twenty-three crude palm oil samples were obtained from eight shipments in storage tanks at Shimanzi oil installations in Mombasa, during a period of ten months. The oil was collected, within four days after discharge from the shipping vessels, when it was still homogeneous at about 50°C.

A clean valve sampling cylinder tied to a long polypropylene cord was allowed to fall through the palm oil in each tank [67, 68]. It was then withdrawn to about half a meter from the bottom of the tank and released. This was repeated three times and the oil-filled apparatus was withdrawn from the tank. Equal portions (300 ml) of the oil from the bottom, middle and top zones of the same tank were combined in a one litre container.

A reference crude palm oil sample was obtained from the Palm Oil Research Institute of Malaysia (PORIM) based in Kuala Lumpur. All the samples were stored before use at 11 ± 1°C.

3.2. APPARATUS.

(1) A Philips model PU 8700 and a Perkin Elmer model 555 uv/visible spectrophotometers

were used to obtain all colorimetric measurements.

(2) Schott-Gerate Ubbelohde suspended level Viscometers (type Ia and IIa) were used to obtain the viscosity measurements.

(3) A Carl Zeiss Abbe refractometer was used to measure refractive indices.

(4) A BHG Hermle Z320 centrifuge was used to purify the palm oil samples and to separate solvent soluble and insoluble palm oil fractions.

(5) A Haake F3 Digital bath circulator was used to thermostat the water-bath at various temperatures for the determination of viscosity.

(6) A Thermomix 1420 immersion circulator was used to maintain water-bath temperature at 50 ± 0.1°C during density measurements.

(7) A Buch Rotavapor R110 was used to extract solvents from the solutions of unsaponifiable matter and soluble fractions of palm oil.

(8) An Ikamag RH magnetic stirrer hotplate was used to heat and stir the water-bath during softening (slip-melting) point measurements.

(9) A 300 ml capacity stainless steel valve sampling cylinder was used to draw all palm oil samples from the storage tanks.

(10) Precision thermometers calibrated to 0.1°C divisions, and with ranges of -15 to 52°C and -4 to 102°C, were used to monitor the temperature during density, viscosity, refractive index and softening point determinations.

(11) Capillary tubes, having a length of 75 mm and 1.1 mm internal diameter were used for determination of softening point.

3.3. CHEMICALS AND REAGENTS.

All chemicals and reagents were of analytical grade or equivalent and were obtained from reputable laboratory supply houses.

Hexane and sodium hydroxide were supplied by Prolabo, France. Acetone, 32% hydrochloric acid, phenolphthalein, potassium dichromate, potassium hydrogen phthalate, sodium thiosulphate and soluble starch were obtained from Merck, Germany, while propan-1-ol was from Riedel-de-Haen AG, Germany.

Carbon tetrachloride, ethanol, iodine, iodine trichloride and potassium hydroxide were from BDH, U.K, while glycerol, methanol, propan-2-ol and toluene were from Koch-Light, U.K. Ethyl acetate and sodium carbonate were purchased from May & Baker, U.K, while diethylether, oleic acid and potassium iodide were from Park Scientific, U.K. All-trans beta-carotene was from Sigma Chemical Company, U.S.A.

Before use, potassium dichromate and sodium carbonate were recrystallized twice from distilled water and dried at 160°C to constant weight [88]. Beta-carotene was recrystallized twice from toluene by addition of methanol, and then dried in a vacuum desiccator to constant weight [89]. Potassium hydrogen phthalate was heated at 120°C to a constant weight [88]. 50% carbonate-free sodium hydroxide solution was prepared in freshly redistilled water [88, 90], while propan-2-ol was neutralized with dilute sodium hydroxide solution [68]. Glycerol was dehydrated by heating at 180°C [91]. Acetone, carbon tetrachloride, ethanol, hexane, methanol and toluene were distilled through a fractionating column of 80 cm length.

3.4. GLASSWARE.

The glassware used was washed with a detergent solution, rinsed several times with distilled water and then dried in an oven thermostated at 103°C. The density bottles and viscometers were filled with a saturated solution of chromic oxide (CrO_3) in sulphuric acid and allowed to stand overnight. Each was then rinsed several times with distilled water and dried at 103°C to constant weight.

3.5. CALIBRATION OF APPARATUS.

3.5.1. Density bottles.

The capacity of any container definitely changes with temperature. The volume of each density bottle used was therefore determined at various experimental temperatures. Each density bottle was weighed to the nearest 0.1 mg and then filled with re-distilled water cooled to 20°C. It was allowed to stand in a water-bath thermostated at 50 ± 0.1°C for 1 hour. The stopper was then inserted and the filled bottle was allowed to cool to ambient temperature. The outer surface of the bottle was wiped with a clean cloth and weighed. The standard density of water at 50°C [92] and the weight obtained were then used to calculate the capacity of the density bottle at 50°C. The same method was also used to calibrate the density bottles used at various temperatures between 25 and 90°C.

3.5.2. Viscometers.

The constants that depend on the dimensions of the Viscometers used were determined using standard liquids [91]. Oleic acid of known absolute viscosity (27.64 cP) at 25°C was transferred to the Viscometer Ia mounted in a water-bath thermostated at 25 ± 0.1°C. It was then allowed to stand for 30 minutes and the time taken by the leading edge of the liquid meniscus to descend between the timimg marks on the Viscometer was taken to the nearest 0.1s. The density of oleic acid was then determined at the same temperature according to the procedure described in Section 3.8.1. The average efflux time and density found were then used to calculate the constant for the Viscometer using equation (2-11). The same procedure was used to get the constant for the Viscometer IIa using glycerol having absolute viscosity of 587 cP at 30°C. The results obtained are presented in Appendix I.

3.6. PREPARATION OF THE REAGENT SOLUTIONS.

3.6.1. INDICATOR SOLUTIONS.

Methyl orange solution (0.2%).

0.10 g methyl orange was dissolved and diluted to 50 ml with ethanol [88].

Phenolphthalein solution (1%).

0.50 g phenolphthalein was dissolved and diluted to 50 ml using ethanol [77].

Starch solution (1%).

0.50 g of soluble starch was mixed with 50 ml of distilled water. The dispersion obtained was then boiled for 3 minutes before cooling to room temperature [68].

3.6.2. STANDARD SOLUTIONS.

Potassium dichromate solution (0.1N).

4.9032 g potassium dichromate was taken and quantitatively transferred to a 1 liter volumetric flask and dissolved using distilled water. The solution was then diluted to the mark.

Beta-carotene solution (10 ppm).

0.0500 g beta-carotene was weighed and transferred quantitatively to a 500 ml volumetric flask. It was then dissolved and diluted to the mark with hexane. A 20 ml aliquot of the solution was diluted ten times using hexane. The spectrophotometric absorbance of a series of solutions (0.5, 1.0, 2.0, 3.0, and 4.0 ppm) at 446 nm [68] was then used to obtain a standard beta-carotene calibration curve (Appendix II).

Sodium thiosulphate solution (0.1M).

An aqueous solution of 24.82 g sodium thiosulphate pentahydrate was diluted to 1 litre with freshly distilled water. 5 ml concentrated hydrochloric acid (32%) and 10 ml of 10%(w/v) aqueous potassium iodide were transferred to a 250 ml erlenmeyer flask containing 25 ml of standard 0.1N potassium dichromate solution. The contents of the flask were mixed and allowed to stand for 5 minutes. 100 ml of distilled water was added and the solution titrated with the sodium thiosulphate solution using starch solution as indicator [68]. The actual concentration of the thiosulphate solution was then calculated.

Hydrochloric acid solution (0.5M).

90 ml of 32% hydrochloric acid solution was diluted to make 1000 ml. A 50 ml aqueous solution of 0.20 g sodium carbonate was then titrated with the hydrochloric acid solution using methyl orange solution as indicator [88]. The actual concentration of the hydrochloric acid solution was then calculated.

Sodium hydroxide solution (0.1M).

8 ± 0.01 g of the carbonate-free 50% sodium hydroxide solution was diluted to 1000 ml with freshly distilled water. A 50 ml aliquot of aqueous potassium hydrogen phthalate (0.80%) was titrated with the sodium hydroxide solution using phenolphthalein solution as indicator [88, 90], to obtain the actual concentration of the sodium hydroxide solution.

3.6.3. WORKING SOLUTIONS.

Ethanolic potassium hydroxide solution.

One liter of ethanol containing 4.0 g potassium hydroxide was refluxed for 1 hour and then distilled. 20 g potassium hydroxide was dissolved in 500 ml of the distillate maintained at a temperature below 15°C using an ice-bath. The solution obtained was allowed to stand overnight at ambient temperature and then decanted into a plastic bottle [93].

Wij's iodine reagent solution.

Iodine trichloride (9 ± 0.01 g) was dissolved in a mixture of 700 ml of glacial acetic acid and 300 ml of carbon tetrachloride in an amber glass bottle. Iodine (10 g) was then added and the solution obtained was stored in a dark and cool place [77].

3.7. SAMPLE PREPARATION.

3.7.1. PREPARATION OF PALM OIL SAMPLES.

Each palm oil sample collected was allowed to melt in a water-bath thermostated at 60°C [68], and the homogeneous oil obtained was transferred into a 100 ml polypropylene bottle for the determination of volatile matter content. For the determination of density, softening point, refractive index, saponification and iodine values, unsaponifiable matter and free fatty acid contents, 50 ml aliquots of the oil sample at 60°C were first centrifuged at 4000 r.p.m. for five minutes and the supernatant decanted into a 100 ml bottle. The bottles filled with the oil were stored in a refrigerator thermostated at 10 ± 1°C, while another 100 ml of the oil was stored in a deep-freezer thermostated at -30°C for use in the determination of the carotenoids content.

300 ml portions taken from each of the first five palm oil samples to be collected were combined and then centrifuged as described above. The supernatant was stored at 10 ± 1°C for use in the preparation of palm oil fractions, determination of the stability of palm oil carotenes, the viscosity and energy of activation for viscous flow of palm oil, properties and behaviour of the heated oil, and variation of iodine value with the weight of test sample. Before use, all the samples were allowed to melt at 60°C.

3.7.2. PREPARATION OF PALM OIL FRACTIONS.

3.7.2.1. SEARCH FOR A SUITABLE SOLVENT FOR FRACTIONAL CRYSTALLIZATION.

The solubility of palm oil in the available solvents was used to get the most suitable solvent for the isolation of high and low melting components of palm oil. An aliquot of the oil (3 g) in a 100 ml conical flask was kept for 1 hour in a water-bath maintained at 25 ± 1°C. The solvent was then added gently from a burette until the oil just dissolved. However, the addition was discontinued when the oil remained insoluble in 10% (w/v) of the mixture.

The insoluble fat in the resulting oil-dissolved mixture was separated by centrifugation at 4000 r.p.m for 5 minutes. The supernatant was decanted into a tared 250 ml round bottom flask and the solvent expelled with a rotatory evaporator. The flask was then kept in an oven at 103°C for the fraction to dry to constant weight.

The different solvents tested were acetone, carbon tetrachloride, diethyl ether, ethanol, ethyl acetate, hexane, methanol, propan-1-ol and toluene. The most volatile solvent in which the oil showed partial solubility at 10%(w/v) was then selected for the fractional crystallization of palm oil [56, 87]. The data (given in Appendix III) show acetone as the most suitable solvent, and was subsequently used.

3.7.2.2. FRACTIONAL CRYSTALLIZATION OF PALM OIL.

An aliquot (6 g) of the oil sample was weighed into a centrifuge tube and allowed to melt at 60°C. It was then dissolved by stirring in 18 ml acetone and then kept in an ice-bath for 1 hour, to allow recrystallization to proceed to completion, before centrifuging at 4000 r.p.m. for 5 minutes. The supernatant was decanted into a 250 ml round bottom flask and recrystallization repeated until the colour of the mother liquor becomes colourless [44].

The solvent in the combined supernatants was expelled using a rotatory evaporator. The oil fractions obtained were then held in an oven maintained at 55 ± 1°C until the respective weights remained constant. The fractions from 10 samples (Appendix IV) were used for the study of the interaction between solid and liquid fractions of palm oil.

3.8. ANALYTICAL PROCEDURES.

3.8.1. DETERMINATION OF DENSITY.

The density of each palm oil sample was determined according to the procedure described by BAUER and LEWIN [69]. The density bottle calibrated at 50°C was weighed and then filled with the oil sample cooled to 48°C. It was allowed to stand for 1 hour in a water-bath thermostated at 50 ± 0.1°C. The outer surface of the oil-filled bottle was then cleaned using hexane and allowed to dry before weighing. The ratio of the mass of the oil to the volume of the bottle at the same temperature was expressed as the density of the palm oil at 50°C.

3.8.2. DETERMINATION OF SOFTENING POINT.

The softening (slip-melting) point was determined as the temperature at which a column of fat started rising in the capillary tube under the hydrostatic pressure [68, 73]. Two clean capillary tubes were dipped into a homogeneous palm oil sample such that a 10.0 mm column of the oil was formed in each tube. The fat was solidified by pressing each tube against a piece of ice.

The tubes were then kept for 16 hours in a refrigerator thermostated at 10 ± 1°C and then attached to the thermometer by use of a rubber band, with their lower position close to mercury bulb of the thermometer. The tubes were immersed in water, and the temperature of the stirred water was raised gradually at the rate of 1°C per minute. The rate was then decreased to 0.5°C as the softening point was approached.

3.8.3. DETERMINATION OF REFRACTIVE INDEX.

The refractive index was determined as the value corresponding to the critical angle of refraction [74]. The refractometer was positioned to face the window under diffused sunlight and connected to thermostated circulating water to maintain the prisms at 50 ± 0.1°C. The surfaces of the prisms were cleaned using ethanol and allowed to dry. A few drops of the oil were placed between the two prisms and allowed to stand for 10 minutes for the oil to reach the temperature of the tightly closed prisms. The value obtained on the viewed scale was then recorded as the the refractive index of palm oil at 50°C.

3.8.4. DETERMINATION OF SAPONIFICATION VALUE.

The saponification value was determined by hydrolysis in alkaline medium [68, 77]. An aliquot of the palm oil sample (2g) was weighed into a 100 ml round bottom flask and 25.0 ml of ethanolic potassium hydroxide solution added. The mixture was refluxed on a steam bath for 1 hour and the solution titrated with standardized hydrochloric acid solution (0.5M) using phenolphthalein solution as indicator. The titre was corrected for the blank prepared similarly using water instead of the test sample, and the saponification value of the sample then calculated using the formula:

$$\text{Saponification value} = \frac{56.1\,M(b - s)}{W}$$

where M = the accurate molarity of the hydrochloric acid solution used in the titration;

b = volume, in ml, of the hydrochloric acid solution used in the blank titration;

s = volume, in ml, of the hydrochloric acid solution used in titration of test sample;

W = actual mass, in g, of the test portion.

3.8.5. DETERMINATION OF IODINE VALUE.

The proportion of unsaturated constituents in palm oil was measured by the Wij's iodine addition method [68, 77]. An aliquot of the test sample (0.4g) was weighed into a 250 ml glass bottle and dissolved in 15 ml of carbon tetrachloride. 25.0 ml of the Wij's reagent was added and the contents of the stoppered bottle mixed by swirling. The bottle was then placed in a dark place at ambient temperature for 60 minutes. 20 ml of 10% aqueous potassium iodide solution and 100 ml of distilled water were added to the contents of the bottle. The mixture was then titrated with the standardized sodium thiosulphate solution (0.1M) using starch solution as indicator. A blank titration was carried out to enable the accurate determination of iodine value of the sample using the formula:

$$\text{Iodine value} = \frac{12.69 \times 2 \times M(b - s)}{W}$$

where M = the actual molarity of sodium thiosulphate solution used;

b = volume, in ml, of the sodium thiosulphate solution used in the blank titration;

s = volume, in ml, of the sodium thiosulphate solution used in test sample;

W = mass, in g, of the aliquot under test.

3.8.6. DETERMINATION OF UNSAPONIFIABLE MATTER.

The amount of organic extract residue in the saponified fat was used to determine the content of the unsaponifiable matter [68, 73]. An aliquot of a palm oil sample (5 g) was weighed into a 250 ml round bottom flask. 50 ml of ethanol and 5 ml of 50%(w/v) aqueous potassium hydroxide solution were then added to the flask and the mixture refluxed on a steam bath for 1 hour. The contents were transferred to a separatory funnel using, first, 10 ml portion of ethanol and then 20 ml warm distilled water, 20 ml cold distilled water and 10 ml of petroleum ether. The mixture was then shaken with an additional 50 ml of petroleum ether and the upper layer retained. The extraction of the aqueous layer was then repeated five times.

The combined organic extracts was washed by shaking with 25 ml of 10% ethanol followed by 25-ml portions of distilled water until the aqueous layer gave a neutral reaction to phenolphtalein indicator. The ether extract was then quantitatively transferred to a tared 250 ml round bottom flask and the solvent expelled using a rotatory evaporator. The residue was dried at 103°C to constant weight. The amount of unsaponifiable residue in the sample was then calculated and expressed as %(w/w).

3.8.7. DETERMINATION OF CAROTENE CONTENT.

The palm oil carotenoids concentration was determined by spectrophotometry [68, 94]. An aliquot (0.2 g) of the oil sample was dissolved and diluted to 50.0 ml using hexane. The absorbance of the solution was measured at 446 nm using hexane as reference. The carotenoids content was then calculated (as beta-carotene equivalent) from the beta-carotene calibration curve (Appendix II).

3.8.8. DETERMINATION OF FREE FATTY ACID CONTENT.

The content of free-fatty acid (FFA) was determined by the acid-base titrimetry [68, 77]. A 10 g portion of palm oil sample in a 250 ml erlenmeyer flask was mixed with 50 ml of neutral propan-2-ol. The mixture was kept for ten minutes in a water-bath maintained at 40°C, and then titrated with the standardized sodium hydroxide solution (0.1M) using phenolphthalein solution as indicator. The free fatty acid (FFA) content was expressed as acid value, or as palmitic acid equivalent using the following formula:

$$\text{FFA (as \% palmitic acid equivalent)} = \frac{25.6 \text{ X } M \text{ X } t}{W}$$

where M = actual molarity of sodium hydroxide solution;

t = volume, in ml, of sodium hydroxide solution used;

W = mass, in g, of the sample portion.

3.8.9. DETERMINATION OF VOLATILE MATTER CONTENT.

The volatile matter was determined as the loss in weight of the oil when heated at 103°C [68]. 10 g of palm oil sample in a petri-dish (6.5 cm diameter) was heated in an oven for 2.5 hours and re-weighed after cooling in a desiccator. The content of volatile matter was then calculated.

3.8.10. DETERMINATION OF THE STABILITY OF PALM OIL CAROTENES.

The carotenoids contents of palm oil samples stored at 50°C for various number of days were determined according to the procedure described in Section 3.8.7. The test sample was transferred to fill twenty-two glass vials of 30 ml capacity each. Twenty of the stoppered sample vials were then kept in a closed (dark) oven thermostated at 50 ± 1°C, while the remaining two control samples were kept in a deep-freezer at -30°C. Two of the heated samples were transferred to the deep-freezer after every seven days and finally brought to a melt at 55°C in the dark. The variation of palm oil carotenoids content with the period of storage was used to determine the rate of disappearance of the carotenoids at 50°C. The absorbance of both the control and heated samples was then measured from 200 to 800 nm using hexane as reference.

3.8.11. DETERMINATION OF THE PROPERTIES AND BEHAVIOUR OF HEATED PALM OIL.

The acid, saponification and iodine values of fractions obtained by ordinary distillation [81] of three palm oil samples were determined according to the procedures described above. Each sample (100 g) was weighed into a 250 ml round bottom flask containing some boiling chips. The flask was then connected to the distillation apparatus and the oil brought to a boil using an electromantle. The condenser was jacketed to enable collection of distillate in liquid state at 60°C. The distillate was collected in fractions of 10 g. The data were used to study the variation of ester, acid and iodine values with the extent of distillation.

3.8.12. DETERMINATION OF VISCOSITY OF PALM OIL AT VARIOUS TEMPERATURES AND ENERGY OF ACTIVATION FOR VISCOUS FLOW.

Viscosity was determined according to the procedure described by SWINDELS, ULLMAN and MARK [84]. The palm oil sample at ambient temperature was stirred thorougly and transferred to the Viscometer IIa mounted vertically in a water-bath thermostated at 25 ± 0.1°C. The viscosity was determined after keeping the contents in the bath for 30 minutes. The density of the oil was also determined at same temperature. The determinations were repeated at 30 and 40°C, and then at various temperatures between 42 and 90°C using Viscometer Ia. The data was used to obtain the absolute viscosity using equation (2-11). A plot of the logarithm of viscosity versus reciprocal of absolute temperature was used to calculate the energy of activation for viscous flow of palm oil.

3.8.13. DETERMINATION OF THE INTERACTIONS BETWEEN THE SOLID AND LIQUID FRACTIONS OF PALM OIL.

The softening (slip-melting) points of various compositions of solid and liquid fractions of palm oil obtained in Section 3.7.2.2 were determined according to the procedure described above (Section

3.8.2). The liquid fraction was brought to a melt at 65°C and an aliquot (0.05 g) of the oil was then mixed with 0.95 g of the solid fraction in a glass vial. The process was repeated to obtain test samples with increasing composition of the liquid oil fraction. The mixtures were then allowed to melt at 65°C and mixed thoroughly by stirring. The data obtained was then used to study the behaviour of temperature-composition (liquidus) curve of the solid-liquid system of the palm oil fractions.

3.8.14. DETERMINATION OF VARIATION OF IODINE VALUE WITH TEST SAMPLE WEIGHT.

The iodine values of increasing amounts of palm oil test samples were determined according to the procedure described above (Section 3.8.5). The test was stopped when the reaction mixture failed to give the dark-brown colour (of liberated iodine) on addition of 10%(w/v) aqueous potassium iodide solution. The plot of the data obtained was used to investigate the effect of sample weight on the iodine value determination.

3.9. ASSESSMENT OF DATA.

The reliability of the data from the experimental measurements was calculated with the aid of the Student´s (or Gosset) distribution, and the confidence limits for the mean were expressed at 95% ($P < 0.05$) level. All the values obtained were included in the confidence intervals and showed no systematic error. The method of least squares was used to plot the regression lines through the graph points. The significance of the dispersion of the points with respect to the plots obtained was examined with aid of the Student´s t test.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. RESULTS.

The results of all the parameters determined in the palm oil samples collected from Mombasa port are presented in Table 4.1.

4.2. DENSITY.

The twenty-three palm oil samples studied had a mean density of 0.8905 g/ml at 50°C with a standard deviation of 0.0001 g/ml (Table 4.1) which demonstrates good homogeneity of the test samples. The value obtained compares favourably with the mean value of 0.889 g/ml at 50°C reported for palm oil in Malaysia [23], as well as the theoretical value of 0.890 g/ml calculated as a function of both iodine and saponification values, and temperature [70].

4.3. SOFTENING (SLIP-MELTING) POINT.

The softening point of the palm oil samples collected from Mombasa port ranged from 31.5 to 42.5°C with an average value of 36.8°C and a standard deviation of 2.3°C (Table 4.1) whereas a mean value of 34.2°C has been reported for crude palm oil collected from mills and port installations in Malaysia [23]. The slight difference observed may

Table 4.1. Results of various parameters determined for palm oil.

SAMPLE NUMBER	DENSITY at 50°C (g/ml)	SOFTENING POINT (°C)	REFRACTIVE INDEX (at 50°C)	SAPONIFICATION VALUE (mgKOH/g)	IODINE VALUE (%)	UNSAPONIFIABLE MATTER (%)	CAROTENE CONTENT as beta-carotene (ppm)	FFA (%)	VOLATILE MATTER (%)
1	0.8908	37.5	1.4551	193.75	53.93	0.4768	-	2.82	0.1279
2	0.8907	33.3	1.4550	196.66	52.74	0.4280	-	3.02	0.1261
3	0.8906	33.5	1.4551	194.75	52.45	0.4575	-	3.04	0.1222
4	0.8908	31.5	1.4551	195.71	53.87	0.4589	-	3.01	0.0998
5	0.8907	34.0	1.4550	194.61	52.57	0.4427	-	3.06	0.1279
6	0.8905	37.7	1.4549	196.42	51.96	0.3800	492.112	3.25	0.1247
7	0.8905	36.7	1.4550	196.80	52.09	0.3802	531.269	3.24	0.0822
8	0.8904	38.2	1.4549	197.57	52.15	0.4591	542.218	3.55	0.1442
9	0.8904	36.6	1.4550	197.30	51.33	0.4677	526.381	3.68	0.1169
10	0.8905	35.5	1.4549	198.70	52.49	0.3691	413.050	4.77	0.1329
11	0.8905	34.3	1.4549	196.52	52.12	0.4415	505.111	3.58	0.1025
12	0.8905	37.3	1.4549	199.45	51.03	0.3850	438.678	4.41	0.1479
13	0.8905	37.1	1.4548	196.38	52.00	0.4554	470.931	3.83	0.1767
14	0.8904	37.2	1.4548	198.14	52.33	-	504.302	3.40	0.1344
15	0.8904	39.0	1.4548	197.75	51.34	-	470.112	3.30	0.0929
16	0.8905	42.5	1.4547	198.14	51.44	-	445.239	3.72	0.1305
17	0.8905	37.8	1.4548	197.76	51.42	-	513.629	3.35	0.0768
18	0.8904	36.8	1.4548	198.33	52.77	-	582.450	2.90	0.0738
19	0.8906	37.5	1.4548	197.66	51.83	-	453.459	3.72	0.0833
20	0.8905	39.6	1.4546	196.31	51.69	-	506.904	3.38	0.0798
21	0.8904	37.8	1.4546	197.27	51.98	-	502.039	3.40	0.1159
22	0.8904	37.5	1.4549	198.72	52.47	-	511.530	3.63	0.1529
23	0.8903	37.1	1.4548	198.98	52.17	-	519.503	3.70	0.1315
Mean	0.8905	36.8	1.4548	197.12	52.18	0.4309	496.051	3.47	0.1175
S.D.	0.00013	2.3	0.00001	1.45	0.72	0.0384	41.341	0.46	0.0271

S.D. is standard deviation.
FFA is free fatty acid (as palmitic acid).

be attributed to changes in the fatty acid distribution of palm oil glycerides which influence the melting characteristics of the oil [49].

4.4. REFRACTIVE INDEX.

The twenty-three palm oil samples had a mean refractive index of 1.4548 at 50°C with a standard deviation of 1 x 10^{-5} (Table 4.1). This indicated good reproducibility of the method used as well as good homogeneity of the samples tested. The index is in agreement to within 0.03% of the value (1.4553) reported for crude palm oil in Malaysia [23]. The deviation observed may be attributed to changes such as oxidation [11, 36] of palm oil fatty acid double bonds. This reaction can lower the molecular mass and degree of unsaturation of the oil molecules.

4.5. SAPONIFICATION VALUE.

The mean saponification value obtained for the twenty-three oil samples was 197.1 mgKOH/g (Table 4.1). This value is slightly higher than the value of 195.7 mgKOH/g observed for palm oil at the time of shipment in Malaysia [23]. The difference may be attributed to changes such as oxidative breakdown of the unsaturated fatty acid chain [36], which tends to lower the molecular weight of the triglycerides. Evaporation of the volatile and unsaponifiable decomposition products can increase the concentration of the saponifiable molecules in the oil.

4.6. IODINE VALUE.

The iodine values of the twenty-three palm oil samples ranged from 51.03 to 53.93%I with an average value of 52.2%I, and a standard deviation of 0.72%I (Table 4.1). This mean value is slightly lower than 52.9%I (with a standard deviation of 0.89%I) reported for crude palm oil in Malaysia [23, 95]. The slight difference may be attributed to changes in the degree of unsaturation due to oxidation of the oil [11, 36] during handling, storage and shipment.

4.7. UNSAPONIFIABLE MATTER.

Thirteen of the samples tested gave 0.43% unsaponifiable matter content with a standard deviation of 0.038% (Table 4.1). The average value obtained is about 16% lower than the mean value of 0.51% (ranging from 0.15 to 0.99%) reported for crude palm oil in Malaysia [23]. The difference observed may be attributed to the presence of lower levels of non-volatile and unsaponifiable compounds derived from oxidation [36] of the oil.

4.8. CAROTENE CONTENT.

Eighteen of the palm oil samples collected had the carotene content (as beta-carotene) ranging from 413 to 582 ppm with a mean of 496 ppm and a standard deviation of 41.3 ppm (8%) (Table 4.1). The control sample of crude palm oil obtained from the Palm Oil Research Institute of Malaysia gave 617 ppm. The mean concentration in the Mombasa samples is lower than the mean values of 669 and 640 ppm reported in Malaysia for crude palm oil at the oil mills and at the time of shipment, respectively [96], and falls slightly below the minimum level (500 ppm) recommended for crude palm oil [95]. It is also much lower than 673 ppm reported for the oil from the tenera variety of the species Elaies quineensis [21], which is the main source of palm oil in Malaysia. The notably low levels of the carotenoids obtained in this study may be attributed to the degradation of the polyene chain of the carotene molecules [11, 12] during handling, storage and shipment of the oil.

4.9. FREE FATTY ACID CONTENT.

The free fatty acid (FFA) content (equivalent to palmitic acid) in the twenty-three palm oil samples ranged from 2.82 to 4.77 % with a mean value of 3.47%, and a standard deviation of 0.46% (Table 4.1). This value is lower than 3.93 and 4.24% reported for crude palm oil in Malaysia at the time of production and prior to shipment, respectively [96]. The difference may be attributed to consignments of crude palm oil (special quality) having free fatty acid content below 2%w/w at the time of shipment in Malaysia [97].

4.10. VOLATILE MATTER CONTENT.

The volatile matter content of the twenty-three samples varied between 0.0738 and 0.1769% with a mean of 0.12%, and a standard deviation of 0.027% (Table 4.1). The value found is lower than the mean value of 0.144% reported in Malaysia for crude palm oil at dispatch [96]. The slight difference observed may be attributed to the heating loss during storage, handling and shipment of the oil [31].

4.11. STABILITY OF PALM OIL CAROTENES.

Table 4.2 gives the average carotenoids content found in palm oil samples heated for various periods at 50°C. A plot of the data (Figure 4.1) gave a straight line of negative slope. Application of the method of least squares gave a regression line of the form: $Y = -6.3X + 442.226$, indicating that the carotenoids content decreases sharply with the storage time. The rate of disappearance of the carotenes was observed to be constant. The rate constant was found to be 6.3 ppm/day.

Table 4.2. Amount of carotenoids found at different periods of storage of palm oil at 50°C.

Time taken (Days)	Average B-carotene content (for n=2), (ppm)
0	465
7	399
14	357
21	300
28	259
35	213
42	169
49	125
54	82
63	48
70	35

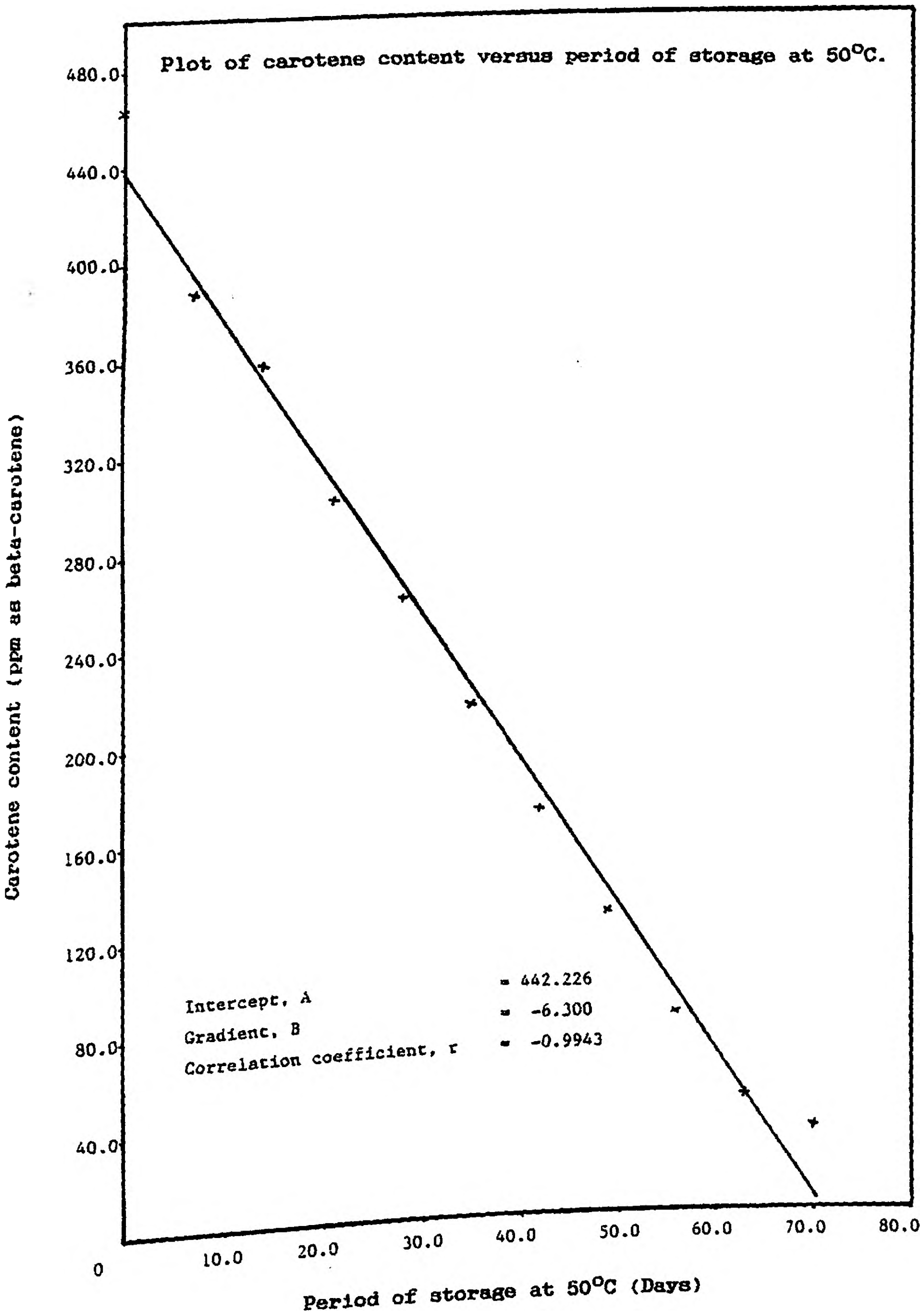


Figure 4.1. Variation of palm oil carotene content with storage period at 50°C.

The linear plot obtained suggests that the process leading to the decrease in palm oil carotenes at 50°C is of zero order kinetics [81]: the rate of decrease of the carotenoids is independent of the amount remaining. The maximum absorbance of the oil at 440 nm decreases by 94% after 70 days with a rise in the absorbance at 270 nm (Figure 4.2). This suggests an increase in the transition energy, ΔE (Section 2.12.7). This change can be attributed to decrease in the space in which the delocalised electrons are confined in the carotene molecules [79] due to degradation of the polyisoprene structure [80]. Because the structural specificity required for the carotene molecules to show vitamin A activity is very narrow [98], the degradation process can reduce the availability of the carotenoids used in the biosynthesis of vitamin A in higher animals.

Studies have shown that at 80°C the carotene content in palm oil declines to the lowest value in about four days [28]. This seems to suggest that the rate of decrease also increases with temperature, thus necessitating the need for handling, storage and transport of crude palm oil at low temperature in order to preserve the pro-vitamin A.

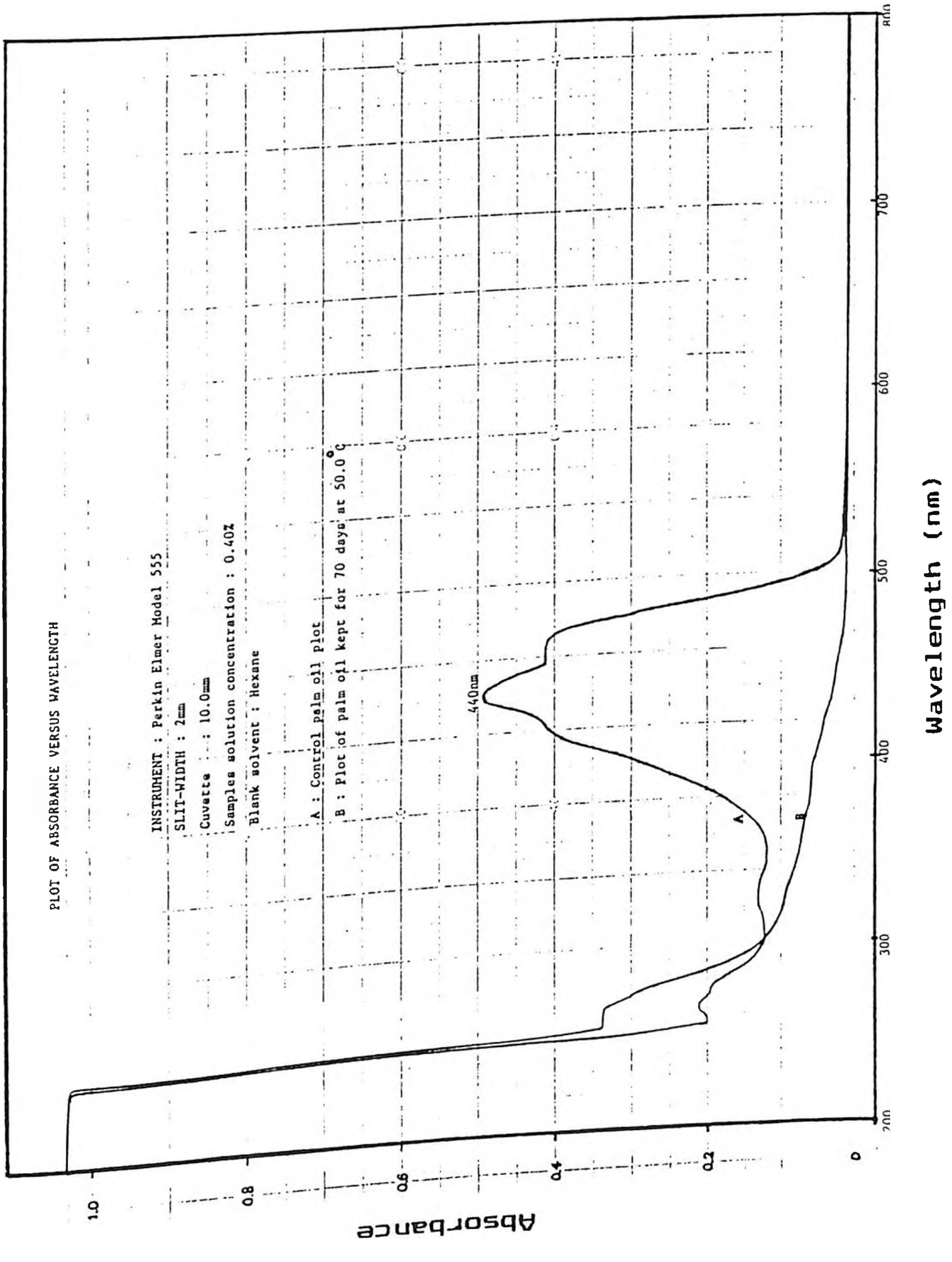


Figure 4.2. UV-Visible spectra of control and a palm oil sample kept for 70 days at 50°C.

4.12. PROPERTIES AND BEHAVIOUR OF HEATED PALM OIL.

Studies on the changes in the properties and behaviour of palm oil distillates were used to explain what happens to the oil when heated. The three samples used started to boil at an average temperature of 358°C, characterized by the release of lachrymatory and extremely pungent fumes. On average, 70.88% of the initial amount of palm oil was collected. A dark residue which accounted for 21.85% may be attributed to cyclic monomers [99], and triglyceride dimers and trimers formed by both carbon-carbon and carbon-oxygen linkages [100]. A weight loss of 7.27% may be attributed to volatile compounds such as n-alkanes, 1-alkenes, alkanals, 2-alkanones and 2-alkenals [101] that could not condense below 60°C. Table 4.3 gives the composition of the distillate fractions collected in terms of acid, ester and iodine values. These parameters were plotted with the extent of distillation (Figure 4.3).

The curves obtained indicate that the oil becomes quite dynamic on attainment of the initial distillation temperature. Compared to palm oil, it clearly shows that the ester value of the distillate is very low at the commencement of distillation and decreases gradually with time. Initially, the high acid value and low ester value found for the

Table 4.3. Parameters of palm oil distillates.

Average amount distilled (%)	5.0	15.0	25.0	35.0	45.0	55.0	65.0
Saponification value (mgKOH/g)	194.57	197.66	192.55	185.75	172.10	134.51	54.88
Standard deviation	2.09	1.14	1.95	0.61	3.64	10.18	8.40
Acid value (mgKOH/g)	170.88	178.45	175.84	170.21	157.19	122.34	49.05
Standard deviation	2.36	2.06	3.07	2.69	1.06	8.08	6.19
Ester value (mgKOH/g)	23.69	19.25	16.62	15.53	14.91	12.17	5.82
Standard deviation	1.30	3.07	1.36	2.07	2.99	2.82	2.50
Iodine value	51.59	41.61	39.86	40.11	45.81	60.34	87.11
Standard deviation	1.47	0.50	0.59	0.58	0.42	2.76	2.52

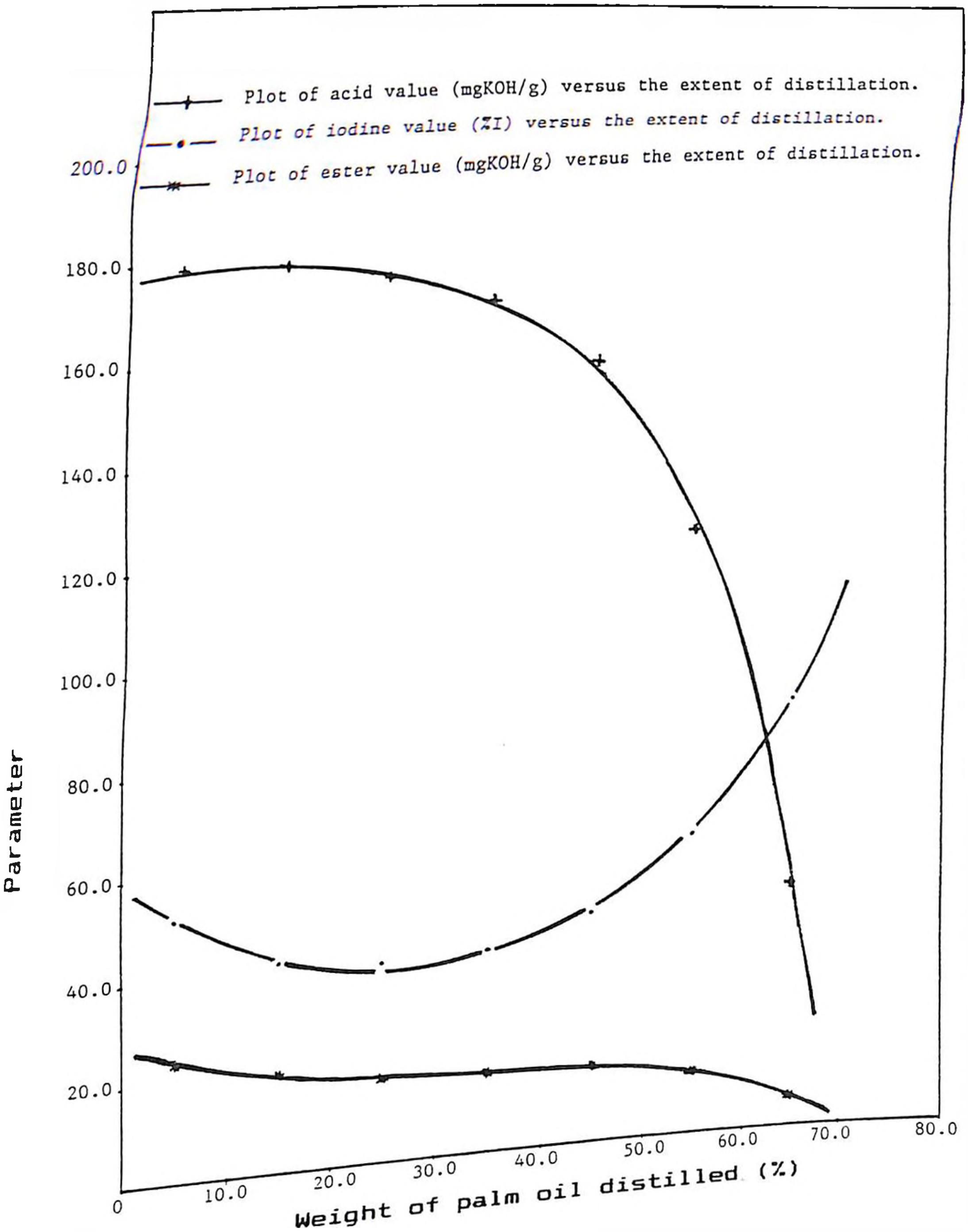


Figure 4.3. Plots of parameters indicating the effect of heat on palm oil.

fractions collected may be attributed to the cleavage of the oil glycerides with the formation of fatty acids of low iodine value (mainly saturated). The process can therefore be made to obtain good yields of useful fatty acid derivatives [102].

Towards the end of the distillation, the distillates collected show a rise in iodine value with diminishing ester and acid values. This implies presence of unsaturated carbon chain compounds with reduced proportion of molecular oxygen due to likely decomposition of the fatty acids to aldehydes, ketones, alcohols, and long chain alkanes and alkenes [102, 103]. The fact that pungent fumes preceed the distillation seems to suggest production of acrolein and other alkenals derived from oxidation of palm oil unsaturated fatty acids [103]; and by removal of elements of water from glycerol [104]:

$$\underset{\text{Glycerol}}{CH_2(OH).CH(OH).CH_2(OH)} \longrightarrow \underset{\text{Acrolein}}{CH_2{=}CH.CHO} + 2H_2O$$

These observations indicate that in the ordinary use of palm oil, conditions should be controlled such that the above changes are avoided as much as possible.

4.13. VISCOSITY OF PALM OIL AT VARIOUS TEMPERATURES AND ENERGY OF ACTIVATION FOR VISCOUS FLOW.

Data of Table 4.4 show viscosity values decrease with rise in temperature of palm oil. A plot of the logarithm of viscosity (Y) (range: 0.349 to 0.008 kg/m.sec) against the reciprocal temperature (X) (range: 298 to 363°K), and application of the method of least squares yields two regression lines (Figure 4.4) described by the equations:

$$\text{Line a:} \quad Y = 4796.2219X - 16.5228.$$

$$\text{Line b:} \quad Y = 1368.1902X - 5.8517.$$

When solved simultaneously, these equations gave the point of intersection of the two linear plots at the critical temperature of 48.1°C. The linear plots also suggest that the viscosity of palm oil does not change exponentially with temperature as implied by the empirical relation given in equation (2-7).

The fact that there is an observed change in gradient at the critical temperature implies that there is some energy to be overcome [105]. The observed large difference (65.64 kJ/mole) in the energies of activation suggests changes in molecular aggregation [39, 83] of the oil at 48.1°C. However, below 35°C palm oil shows non-Newtonian flow due to

Table 4.4. Absolute viscosity of palm oil at various temperatures.

Temperature (T, oK)	$\frac{1}{T}$	Average (n=3) viscosity (kg/m.sec)	Log(Viscosity)
298.15	0.003354	0.34909	-0.4570
303.15	0.003299	0.20181	-0.6950
313.15	0.003193	0.06934	-1.1590
315.15	0.003173	0.05031	-1.2983
317.15	0.003153	0.04469	-1.3497
318.15	0.003143	0.03149	-1.5018
319.15	0.003133	0.02869	-1.5422
321.15	0.003114	0.02640	-1.5783
323.15	0.003094	0.02440	-1.6126
333.15	0.003002	0.01782	-1.7490
343.15	0.002914	0.01353	-1.8687
353.15	0.002832	0.01047	-1.9800
363.15	0.002754	0.00833	-2.0793

the presence of fat crystals [38]. This suggests that the observed critical temperature indicates that the liquid state of all the palm oil triglycerides has been reached, and may signify the beginning of viscous flow of the oil.

Above 48.1oC, the value of energy of activation for viscous flow of 26.1968 kJ/mole may be used to characterize palm oil. However, the relationship between viscosity of the oil and temperature needs to be investigated to ascertain the proper design of pumping, mixing and transferring systems. Using this information, the selection of handling parameters which reduce damage to palm oil triglycerides and carotenes can then be made.

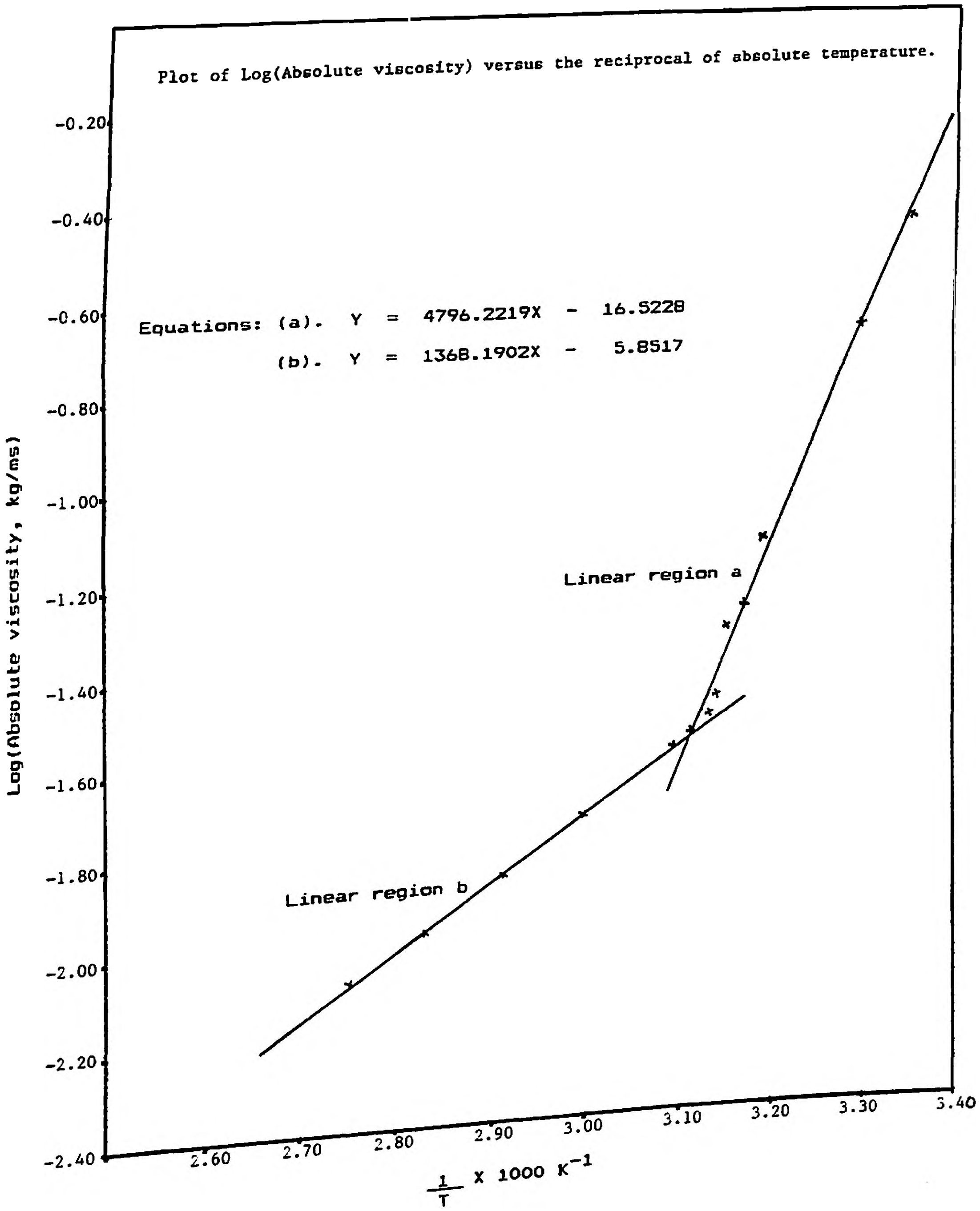


Figure 4.4. Variation of log(Viscosity) with the reciprocal of absolute temperature.

4.14. INTERACTIONS BETWEEN SOLID AND LIQUID FRACTIONS OF PALM OIL.

The softening point of the palm oil liquid fraction was found to increase with the content of the solid fraction (Table 4.5). A plot of the data gave a temperature-composition curve (Figure 4.5) which showed a sharp change in gradient at 8%, resulting in two curves of different slopes.

Table 4.5. Softening (slip-melting) point of mixtures of palm oil fractions.

Solid fraction (%)	Average (n=2) softening point (°C)
0.0	21.33
1.0	24.66
2.0	27.76
4.0	31.63
5.0	33.90
6.0	35.63
7.0	36.83
8.0	38.63
9.0	43.96
10.0	43.86
11.0	45.43
12.0	46.46
15.0	47.00
20.0	48.73
25.0	50.43
30.0	51.56
40.0	53.43
60.0	56.30
80.0	58.40
90.0	59.46
100.0	60.20

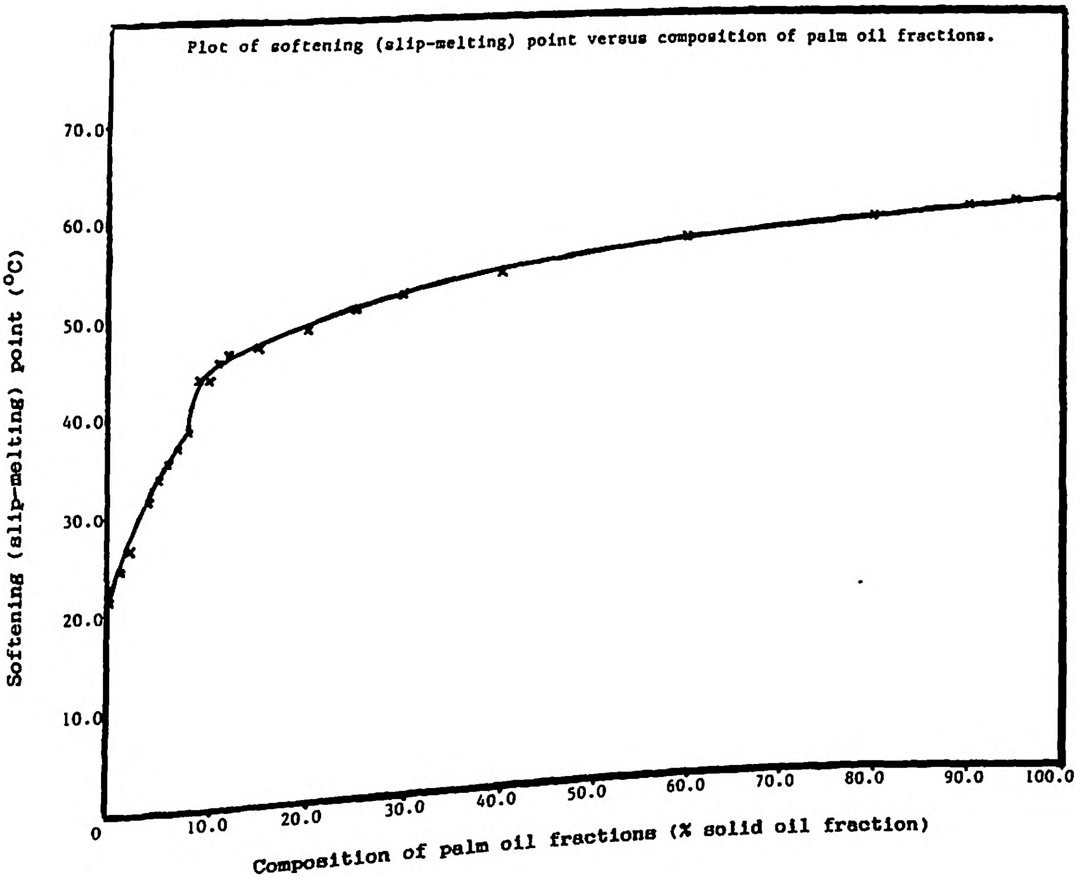


Figure 4.5. Variation of softening point with composition of palm oil fractions.

The two curves may be attributed to different types of interactions between the two palm oil fractions. Since palm oil is a mixture of mixed-chain triglycerides [60] having various polymorphic forms [36], the melting point (liquidus) curve presented may not represent true equilibrium status according to phase rule [106]. However, the variable properties of palm oil solid fractions (stearins) depend on the amount of liquid oil component (olein) trapped in the fat crystals of the solid-liquid system [44]. This suggests formation of complexes which involve geometric or physical entrapment of the liquid fraction [44, 72]. The transition point observed at 8.0% may be attributed to saturation of the solid fat crystal structure by the liquid oil.

The abundance of the liquid oil possibly lowers the softening point very fast by dissolution of the fat crystals. But this needs to be confirmed from studies of the physico-chemical properties and possible colloidal properties of the system formed by the composition at the transition point.

4.15. VARIATION OF IODINE VALUE WITH TEST SAMPLE WEIGHT.

The iodine values of palm oil test samples were found to be dependent on the amount of palm oil used (Table 4.6). For the weight range:$0.31<X<1.21$ g, the variation (shown in Figure 4.6) of iodine value (Y) was found to be described by the equation:

$$Y = -0.6203X + 52.666.$$

The samples investigated include the recommended quantity (0.3-0.40 g) of palm oil which gives between 100 and 150% of the excess halogen based on the amount absorbed [93]. A sudden decrease in iodine value with increased sample size above 1.21 g may be attributed to complete absorption of iodine by an excess of unsaturated palm oil fatty

Table 4.6. Iodine value of various amounts of palm oil used.

Weight taken (gm)	Iodine value (I%)
0.0976	53.52
0.0979	53.40
0.2034	52.93
0.2119	52.91
0.3111	52.01
0.3143	52.70
0.3983	52.46
0.4086	52.38
0.4998	52.51
0.6083	52.15
0.6186	52.52
0.7109	52.33
0.7207	52.15
0.8018	52.12
0.9125	52.22
1.0017	52.02
1.0232	52.05
1.1013	51.94
1.2148	51.81
1.3020	50.88
1.5116	44.42
1.6116	41.66
1.8211	36.87
2.0148	33.32

acids. The equation obtained may be used to determine the factor for correcting deviations in iodine value determined in different amounts of palm oil used.

Although the fatty acid composition provide more detailed information on unsaturation, iodine value still offers a simple means of characterisation of palm oil and its fractions [45] suitable for trading purposes. However, hazardous solvent (carbon

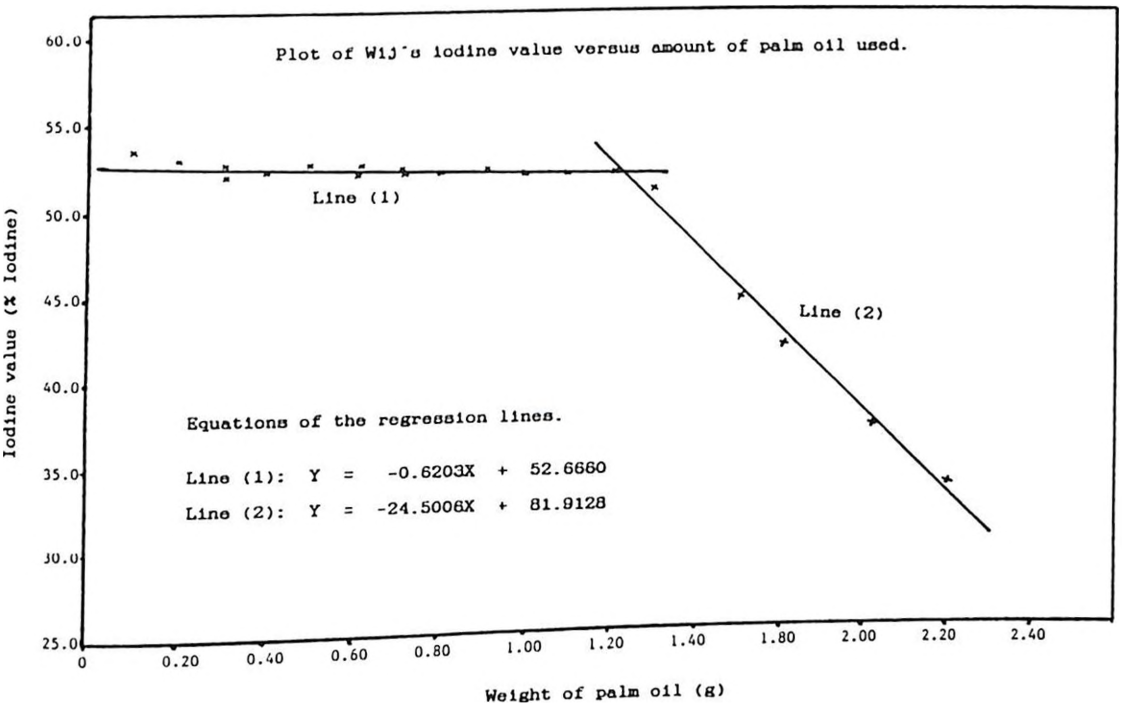


Figure 4.6. Variation of iodine value with the weight of palm oil test sample.

tetrachloride) is used in the Wij´s method. The correction factor for the deviation of iodine value in various amounts of palm oil used can therefore, be applied in correlation and calculation of iodine value from the fatty acid composition [107].

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1. CONCLUSIONS.

In this work, the physico-chemical characteristics of crude palm oil imported into Kenya were determined. A comparison of the results with the parameters of palm oil in Malaysia, prior to shipment, shows some slight variations. The decrease in the free fatty acid content is attributed to the quality of crude palm oil having low free fatty acid content. But the mean carotenoids content falls below the level recommended for crude palm oil, and indicates possible oxidation of the oil during handling, storage or transport. The slight difference in the other parameters investigated is probably due to the conditions prevailing during handling, storage or transport of the oil.

The correction of the variation in iodine value obtained by the Wij´s iodine addition method for the weight range $0.31 < X < 1.21$ g is expressed by the equation: $Y = -0.6203X + 52.666$.

The decrease in the viscosity of palm oil with temperature is not exponential as empirically expected, and shows a critical value at 48.1°C. This is taken as the beginning of viscous flow of the oil at the melting temperature of the fat crystals. Above this value, the energy of activation for viscous flow of the oil is 26.197 kJ/mole.

Although the oil has to be handled when it is viscous enough for transfer through pipe-lines, the normal handling temperature of 50°C lowers the level of palm oil carotenoids. The decrease shows zero order kinetics with a rate constant of 6.3 ppm per day. Moroever, when heated above ordinary cooking temperatures, the physico-chemical properties of the distillates indicate that the oil is quite dynamic following the attainment of the initial distillation temperature.

The interaction of palm oil solid and liquid fractions having the softening point of 60.2°C and 21.3°C, respectively, gives a temperature-composition curve which shows a transition point at 8% solid fraction. This is considered as the saturation limit of the cage-like interstices of the solid fat crystals with the liquid oil fraction.

5.2. RECOMMENDATIONS.

In view of the deterioration of carotenoids observed for the oil investigated in this work, there is need to improve the quality of crude palm oil as received by the refiners in this country, for economical bleaching of the oil to products of low residual colour desired by the consumer.

There is also need to do further work to show the relationship between viscosity of palm oil and temperature which can be used in the design of pumping, mixing and transfer systems, and selection of handling parameters which can reduce damage to the palm oil triglycerides as well as carotenes.

Crude palm oil is a rich natural source of the pro-vitamin A carotenes. However, structural specificity required for a molecule to show pro-vitamin A activity is usually very narrow. Further work is recommended to show the variation in the activity of palm oil pro-vitamin A at cooking temperatures.

Globally, the human diets vary according to traditions and food sources which are a function of geographical or climatic factors. There is need to study the nutritional implications of the edible fats and oils used in this country with respect to the fatty acid composition, positional distribution of triglyceride fatty acids and the trans-fatty acids, so as to avoid those which have been found to promote onset of various types of diseases such as cancer.

Iodine value obtained by the Wij's addition method is widely used for trading purposes of palm oil and its products. But the carbon tetrachloride used in this method has been found to be hepatotoxic. Therefore, there is need to use calculated iodine value from fatty acid composition which takes into consideration the correction factor obtained in this study.

Finally, further research should be done to determine the properties and possible colloidal behaviour of the system formed at the transition point observed in this work for the solid and liquid fractions of palm oil. The composition of the fractions obtained should also be further investigated.

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APPENDIX I

CALIBRATION OF THE VISCOMETERS USED

Table A.1. Density of the liquids used to calibrate the Viscometers.

Test number	Density of the liquids (g/ml). Oleic acid (at 25°C)	 Glycerol (at 30°C)
1	0.8880	1.2550
2	0.8879	1.2551
3	0.8881	1.2550
Mean	0.8880	1.2550
Standard deviation	0.0001	0.00006

Table A.2. Determination of the constant for Viscometer Ia using oleic acid at 25°C.

Test number	Flow-time (Seconds)	Viscometer constant (cm^2/sec^2)
1	622.8	0.04998
2	622.6	0.04999
3	622.9	0.04997
4	622.6	0.04999
Mean viscometer constant		0.04998
Standard deviation		0.00001

(Viscosity of oleic acid at 25°C = 27.64 cP) [89].

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Mean viscometer constant		0.04998
Standard deviation		0.00001

(Viscosity of oleic acid at 25°C = 27.64 cP) [89].

Table A.3. Determination of the constant for Viscometer IIa using glycerol at 30°C.

Test number	Flow-time (Seconds)	Viscometer constant (cm^2/sec^2)
1	919.5	0.50867
2	918.9	0.50901
3	918.2	0.50939
Mean viscometer constant		0.50902
Standard deviation		0.00036

(Viscosity of glycerol at 30°C = 587 cP) [89].

APPENDIX II

Beta-CAROTENE CALIBRATION CURVE

Table A.4. Absorbance of standard solutions of beta-Carotene.

Beta-carotene concentration (ppm)	Absorbance readings at 446 nm (1)	(2)	(3)	(4)	Mean Absorbance	Standard deviation
0.5000	0.125	0.125	0.122	0.127	0.125	0.0020
1.0000	0.257	0.244	0.241	0.251	0.248	0.0072
2.0000	0.482	0.482	0.475	0.493	0.483	0.0074
3.0000	0.725	0.732	0.713	0.735	0.726	0.0098
4.0000	0.967	0.961	0.944	0.978	0.962	0.0142
5.0000	1.185	1.202	1.179	1.211	1.194	0.0148

(Hexane used as blank).

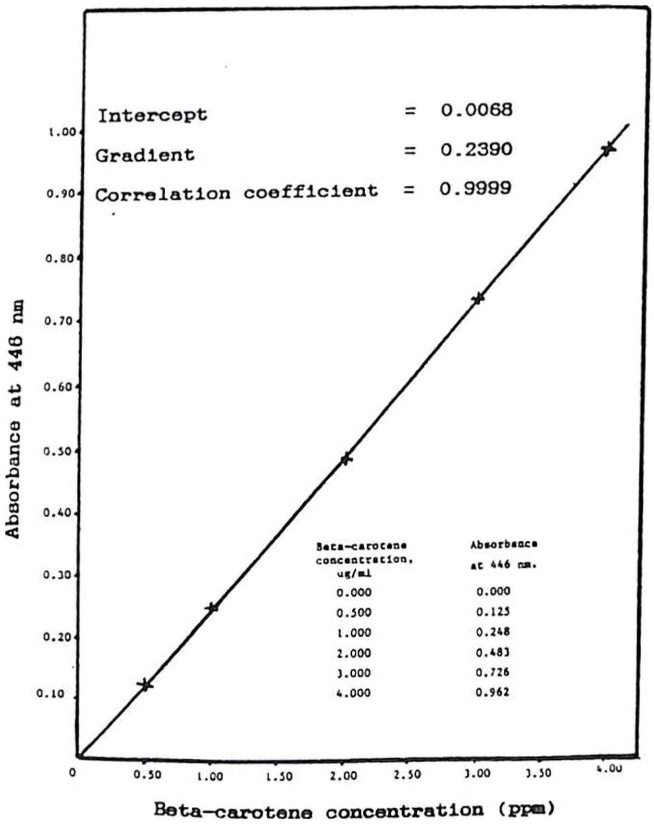

Figure A.1. Plot of absorbance of beta-carotene at 446 nm versus concentration.

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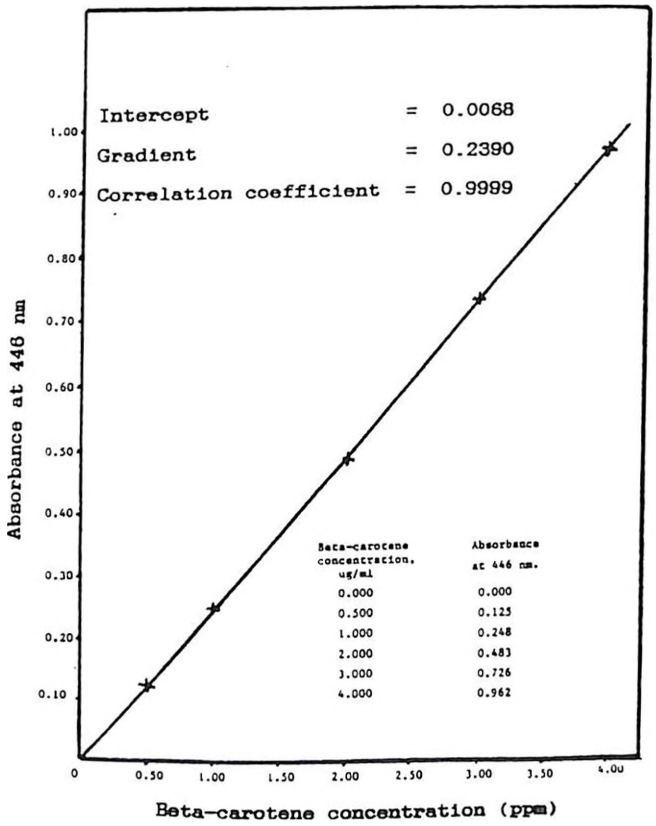

Figure A.1. Plot of absorbance of beta-carotene at 446 nm versus concentration.

APPENDIX III

SEARCH FOR A SUITABLE FRACTIONATION SOLVENT

Table A.5. Solubility of palm oil at 25°C in the available solvents used.

Solvent (Boiling point, °C)	Average solubility (n=3), (g/ml).
Toluene (110-111)	0.92
Carbon tetrachloride (76-77)	0.45
Diethyl ether (34-36)	0.24
Hexane (67-70)	0.16
Ethyl acetate (76-77)	0.10*
Acetone (55-56)	0.09*
Propan-1-ol (96-98)	0.08*
Ethanol (77-79)	0.01*
Methanol (64-65)	0.005*

(*: indicates partial solubility).

APPENDIX IV

FRACTIONAL CRYSTALLIZATION OF PALM OIL

Table A.6. Solid and liquid fractions obtained from fractional crstallization of palm oil using acetone as solvent.

Test number	Solid fraction (%)	Liquid fraction (%)
1	7.2554	92.7080
2	7.1221	92.8828
3	7.1608	92.9411
4	6.9036	93.2160
5	6.9036	92.2160
6	7.8903	92.2918
7	7.6184	92.0138
8	7.9410	92.0423
9	7.9715	92.0151
10	7.0652	92.9613
Mean	7.3837	92.6308
Standard deviation	0.4294	0.4949