MUHAMMAD ALHAJI ADAMU

LIQUID-LIQUID EXTRACTION OF CAROTENOIDS FROM PALM OIL (*Elaeis guineensis*)

Dissertation submitted to the Food Science and Technology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

Adviser: Jane Sélia dos Reis Coimbra

Co-advisers: Simone Monteiro e Silva
César Augusto Sodré da Silva
Emille Rocha B. Almeida Prata

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Jane Sélia dos Reis Coimbra
Adviser

To my parents and Brothers

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Alone, we can do so little; together, we can do so much.
Helen Keller

ABSTRACT

MUHAMMAD, Adamu A., M.Sc., Universidade Federal de Viçosa, February 2023. **Liquid-liquid extraction of carotenoids from palm oil (*Elaeis guineensis*)**. Adviser: Jane Sélia dos Reis Coimbra. Co-Advisors: César Augusto Sodré da Silva, Simone Monteiro e Silva and Emille Rocha Bernardino de Almeida Prata.

Palm oil is one of the major fats and oils produced in worldwide. It forms an important ingredient in the diet of many people in the world. The quality of palm oil is mostly determined by the following parameters: Free Fatty Acid (FFA) content, Iodine Value (IV), Peroxide Value (PV), Moisture Content, Saponification Value (SV) and Impurity Content. The crude and degummed palm oil results were, respectively, (a) acid value (mg KOH/g) from 2.79 ± 0.21 and 4.31 ± 0.06, (b) peroxide value (mEq/kg) of 3.82 ± 0.03 and 1.91 ± 0.01, (c) iodine value (g I2/100 g) of 50.01 ± 2.33 and 45.96 ±1.09, (d) saponification value (mg KOH/g) of 199.57 ± 4.20 and 174.85 ± 26.66, (e) reflective index (40°C) of 1.4578 ± 0.00 and 1.4588 ± 0.00, and (f) total carotenoids (µg/g) of 668.81 ± 0.01 and 638.14 ± 0.03. Crude palm oil presented an ash content of 0.03 ± 0.00% and humidity of 0.28 ± 0.04% (m/m). The ash and humidity contents of degummed palm oil were not determined. Density and rheological behavior were evaluated for both crude and degummed palm oil. All the parameters investigated had values within the standards. The liquid-liquid extraction at temperatures of 40, 45, and 50 °C. First, studies were conducted to evaluate the phase-forming capability and extraction performance of low-toxicity, low-cost, and environmentally friendly organic solvents. According to the screening results and the good performance of palm carotene extraction with high distribution coefficients, ethanol and dimethyl sulfoxide were the best solvents for extraction, followed by acetic acid with less effective extraction. The density-based solvation behavior effectively explained the affinity of carotene for the solvent mixture and the tendency of biphasic system formation. The total carotenoid extraction increased with increasing temperature, with 50 °C having the highest extraction, followed by 45 and 40 °C. Equilibrium data for a system composed of palm oil + anhydrous ethanol + DMSO at temperatures ranging from 40, 45, and 50 °C. Using the method developed by Merchuk et al. (1998). Cloud point determination was used to generate experimental binodal curves, showing a decrease in the biphasic region with increasing temperature. The rise in temperature

within the experimental ranges enhanced oil solvent miscibility in both stages, according to the findings.

Keywords: Palm oil. Liquid- liquid equilibrium. Carotenoids. Solvent.

RESUMO

MUHAMMAD, Adamu A., M.Sc., Universidade Federal de Viçosa, fevereiro de 2023. **Extração líquido-líquido de carotenoides do óleo de palma (*Elaeis guineensis*).** Orientadora: Jane Sélia dos Reis Coimbra. Coorientadores: César Augusto Sodré da Silva, Simone Monteiro e Silva e Emille Rocha Bernardino de Almeida Prata.

O óleo de palma é um dos principais óleos produzidos em todo o mundo, sendo um ingrediente importante na dieta de muitas pessoas no mundo. A qualidade do óleo de palma é determinada principalmente pelos seguintes parâmetros: Índice de ácidos graxos livres (FFA), índice de iodo (IV), índice de peróxido (PV), teor de umidade, índice de saponificação (SV) e teor de impurezas. Os resultados do óleo de palma bruto e degomado foram, respectivamente, (a) valor de acidez (mg KOH/g) de 2,79 ± 0,21 e 4,31 ± 0,06, (b) índice de peróxido (mEq/kg) de 3,82 ± 0,03 e 1,91 ± 0,01, (c) índice de iodo (g I2/100 g) de 50,01 ± 2,33 e 45,96 ±1,09, (d) índice de saponificação (mg KOH/g) de 199,57 ± 4,20 e 174,85 ± 26,66, (e) índice de reflexão (40°C) de 1,4578 ± 0,00 e 1,4588 ± 0,00, e (f) carotenoides totais (µg/g) de 668,81 ± 0,01 e 638,14 ± 0,03. O óleo de palma bruto apresentou teor de cinzas de 0,03 ± 0,00% e umidade de 0,28 ± 0,04% (m/m). Os teores de cinzas e umidade do óleo de palma degomado não foram determinados. A densidade e o comportamento reológico foram avaliados para os óleos de palma bruto e degomado. Todos os parâmetros investigados apresentaram valores dentro dos padrões. A extração líquido-líquido, foi realizada nas temperaturas de 40, 45 e 50 °C. Primeiro, foram conduzidos estudos para avaliar a capacidade de formação de fases e o desempenho de extração em solventes orgânicos de baixa toxicidade, baixo custo e ambientalmente corretos. De acordo com os resultados da triagem e o bom desempenho da extração de caroteno de palma com altos coeficientes de distribuição, o etanol e o dimetilsulfóxido foram os melhores solventes para extração, seguidos do ácido acético com extração menos efetiva. O comportamento de solvatação baseado na densidade explicou efetivamente a afinidade do caroteno pela mistura de solventes e a tendência de formação do sistema bifásico. A extração de carotenoides totais aumentou com o aumento da temperatura, sendo a maior extração realizada à 50 °C, seguida de 45 e 40 °C. Dados de equilíbrio foram obtidos para um sistema composto de óleo de palma + etanol anidro + DMSO em

temperaturas variando entre 40, 45 e 50 °C, usando o método desenvolvido por Merchuk et al. (1998). A determinação do ponto de nuvem foi usada para gerar curvas bimodais experimentais, mostrando uma diminuição na região bifásica com o aumento da temperatura. O aumento da temperatura dentro das faixas experimentais aumentou a miscibilidade entre solvente e óleo em ambas as etapas, de acordo com os resultados.

Palavras-chave: Óleo de palma. Equilíbrio líquido-líquido. Carotenoides. Solventes.

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LIST OF ACRONYMS AND ABBREVIATIONS

DMSO – Dimethylsulfoxide

LLE – Liquid-liquid Extraction

FAME – Fatty acid methyl esters

TAG – Triacylglycerol

DAG – Diacylglycerol

MAG – Monoacylglycerol

FFA – Free fatty acid

CPO – Crude palm oil

SON – Standard Organization of Nigeria

FFB – Fresh fruit bunch

PLs – Phospholipids

POF – Palm oil fruit

RBD – Refined bleached and deodorized

LIST OF SYMBOLS

≥ - Greater than or equal to

% - Percentage

β - Beta

α - Alpha

± - Plus - minus

Ki - Distribution coefficient of compound *i*

SUMÁRIO

1. INTRODUCTION

Palm oil fruit (POF) is a drupe fruit from the palm tree (Elaeis guineensis). It is reddish and grows in bunches. Each fruit comprises an oily, fleshy outer layer (the mesocarp) with a single seed (the palm kernel). The oil extracted from the fruit mesocarp is known as crude palm oil (CPO), and the oil extracted from the kernel is called palm kernel oil (Edem, 2002; Lin, 2011). It is among the most economically important plant sources for edible and industrial oil.

Furthermore, one of the major oils traded in the global edible oil market is found in every ten food products worldwide. According to the European Palm Oil Alliance (EPOA, 2016), palm oil production worldwide grew from 15.2 million tons to 62.6 million between 1995 and 2015. Among all vegetable oils, palm oil exhibits the highest production volume and exceeds the oilseed crop (second largest production) by more than 10 million tons.

2. GOALS

2.1 Main goal

Extraction of carotenoids from palm oil using liquid-liquid extraction at different solvents and extraction temperatures.

2.2 Specific objectives

1. Physicochemical characterization palm oil.
2. Equilibrium data determination for systems composed of palm oil, carotenoids, and solvents (ethanol, DMSO, and acetic acid) at 40 °C, 5°C, and 50°C.
3. Evaluation of the partition coefficient (K), selectivity (S), and the extraction efficiency of carotenoids from palm oil using ethanol, DMSO, and acetic acid at 40°C, 45°C, and 50°C.
4. Define operating parameters (temperature, type of solvent, solvent: oil ratio) that enable greater extractive yield, requiring smaller amounts of solvent.

CHAPTER 1 - LITERATURE REVIEW

ABSTRACT

Palm oil is one of the major fats and oils produced in worldwide. It forms an important ingredient in the diet of many people in the world. The quality of palm oil is mostly determined by the following parameters: Free Fatty Acid (FFA) content, Iodine Value (IV), Peroxide Value (PV), Moisture Content, Saponification Value (SV) and Impurity Content. Palm oil is cultivated across all countries, its fruits present high potentials for functional food production because of the presence of bioactive compounds in its compositions and high nutritive value with considerable amount of carotene and as well as provides an important source of vitamin A. In view of this dissemination of works already performed with this fruit, strategically relevant, can stimulate new line of research to cons0lidate this field for food, Pharmaceutical and cosmetic industries.

Keywords: Palm oil, carotenoids, nutritional composition, and Fatty acids.

1.1 Palm

The palm fruit (Elaeis guineensis), also known as the African oil palm, is a member of the Arecaceae family, including date and coconut palms (Verheye, 2010). It is also commonly known as oil palm and is among the most economically important palms in the world. A common consensus among researchers is that the plant is native to west and southwest Africa, specifically in Angola and the Gambia. The species name guineensis refers to the name for the Guinea area (FAO, 2002) that was domesticated in its native range, probably in Nigeria, and moved throughout tropical Africa by humans practiced shifting agriculture at least 5000 years ago (Soyebo et al., 2005). The oil palm may grow to a height of approximately 10 m after approximately 25-30 years (Basri et al., 2010). The palm produces its fruit in bunches, often referred to as fresh fruit bunches, as shown in Figure 1.1 and 1.2.

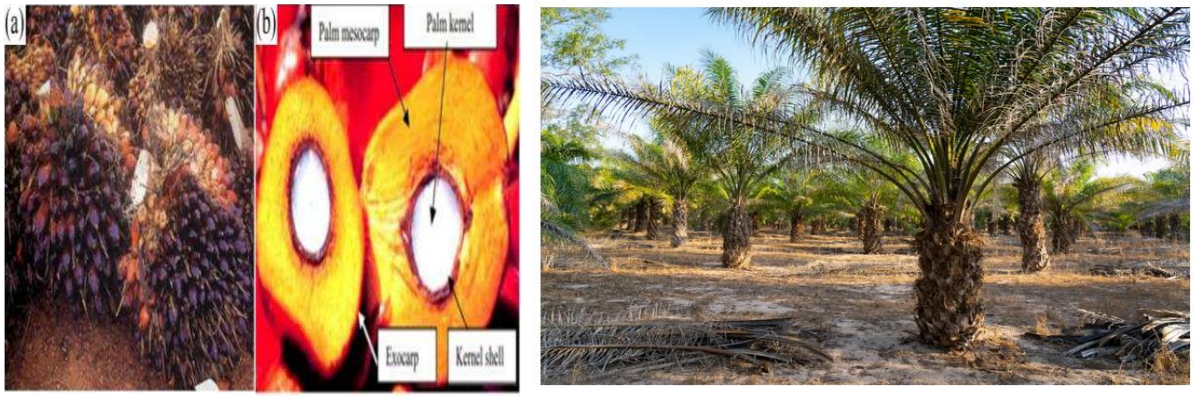


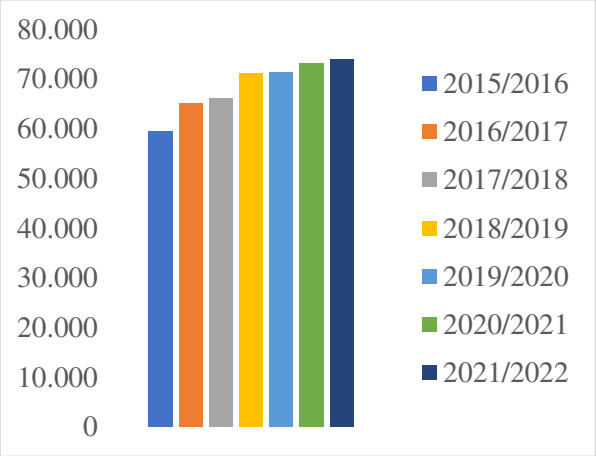
Figure 1.1: Fresh fruit bunch (a). Anatomy of the palm oil fruit (b). **Figure 1.2**: Palm oil tree

Adapted from MPOC (2007) and MPOB (2007)

1.2 Palm oil

The palm oil tree is a multipurpose plant whose parts are very useful and of economic value. It is an economically important crop in the tropics representing approximately 63% of the global vegetable oil export (Ayodele, 2010). Oil from the palm tree is extracted by heating and pressing the fruit's pulp. The refining process is applied to purify crude palm oil, providing a highly versatile oil with significant functional properties. Subsequently, palm oil can be separated into different fractions, liquid (oil) and solid (fats), which can then be processed and mixed according to

specific purposes to provide exclusive taste and texture in food products. Palm oil can be used for a variety of purposes. At the household level, palm oil is used for cooking, and at the industrial level, the oil has been applied in soap making, metal plating making, glycerin, margarine, and lubricants. The kernel cake has been applicable in animal feed production and organic fertilizer as a substrate for much room production. The midribs and rachis are applicable in making local roofing material (Samuel et al., 2018). According to the FAO, Malaysia and Indonesia became the leading countries producing palm oil, surpassing Africa in 1966, as shown in Figure 1.3. The economic importance of palm oil as a high-yielding source of edible and technical oils boosts its plantation in most countries with high rainfall in tropical climates within 10° of the equators, as shown in Figure 1.4.



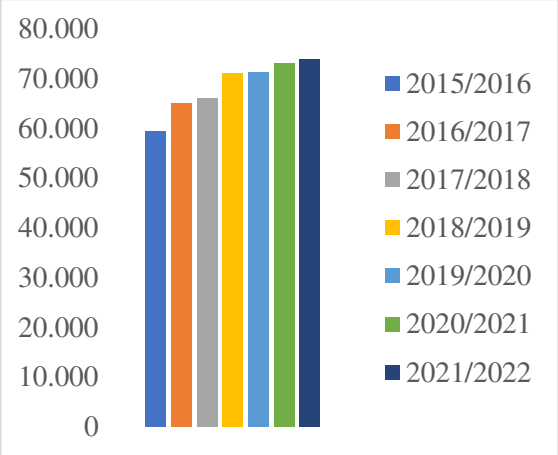


Figure 1.3: Palm oil consumption worldwide producers 2021/2022. (In 1,000 metric tons).

Figure 1.4: Palm oil top global

Source: Adapted from Shahbandeh (2022).

1.2.1 Processing and refining palm oil

When completely developed, the mesocarp of palm fruits contains approximately 56-70% (w/w) edible oil. There are several ways to obtain this oil. These approaches are divided into four groups based on their complexity and throughput. Traditional techniques, tiny mechanical units, medium-scale mills, and massive industrial mills are among them (Mba et al., 2015). Fruit sterilization, fruit loosening/stripping, digestion, oil extraction, and clarification are some fundamental unit processes in palm oil manufacturing. Fruit sterilization refers to moisture

absorption and heat rendering. The fruit mesocarp lipolytic enzymes are inactivated. The two main separation techniques are solvent extraction and mechanical pressing, as shown in Figure 1.5.

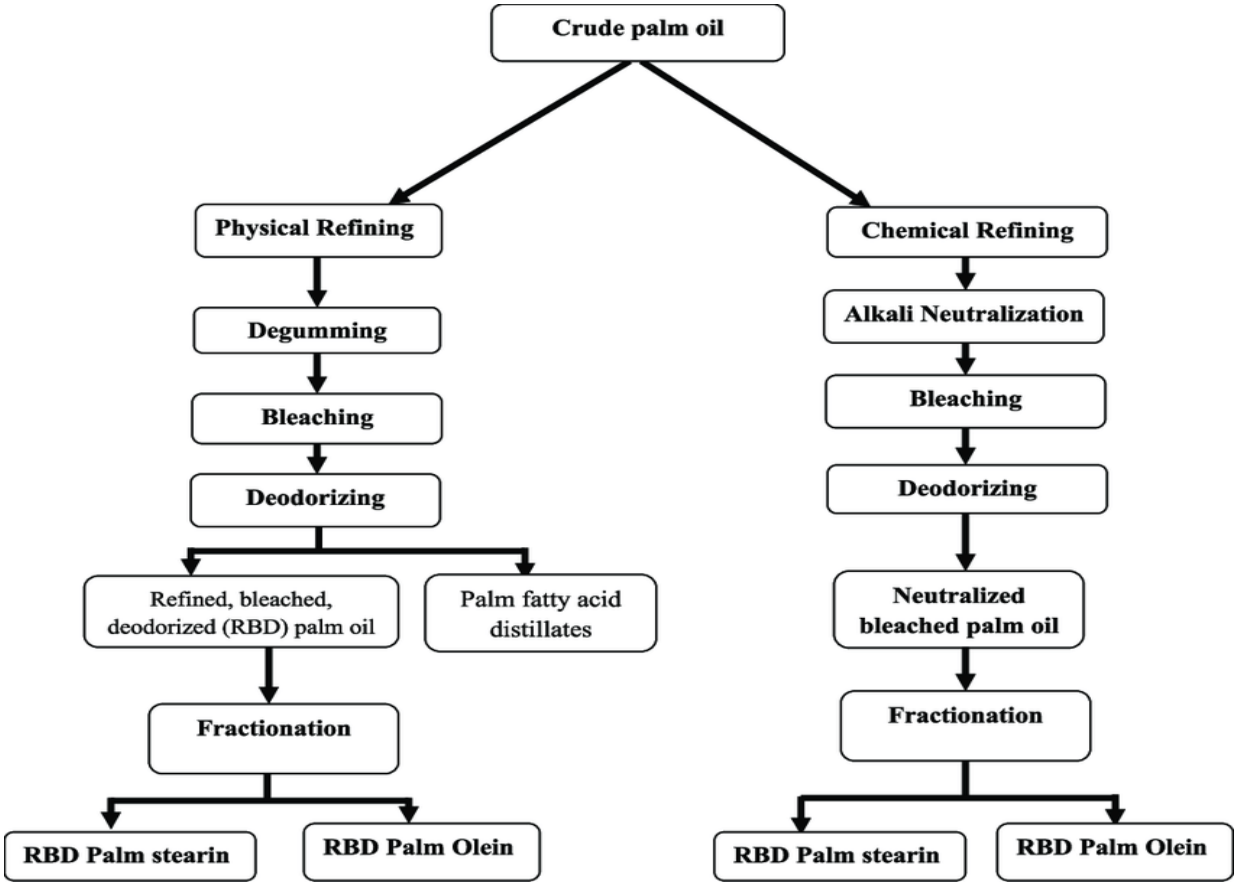


Figure 1.5: Palm oil refining process.

An oil extraction efficiency range of 75– 90% has been reported for mechanical screw presses (Owolarafe et al., 2002). Both favorable and unwanted chemicals are present in CPO (Crude Palm Oil) that either solvent extraction or mechanical pressing has produced.

Triacylglycerols (TAGs) (neutral lipids), carotenoids, phytosterols, and vitamin E (tocopherols and tocotrienols) are desirable oil substances because their chemical functions as nutrients and antioxidants benefit health. The most harmful substances for the oil quality are oxidation products from lipids, free fatty acids (FFAs), phospholipids (PLs) and gums. In terms of sensory perception, the contaminants are objectionable (Dunford, 2012). The impurities are removed during the oil refining, and

the wet or dry processes are the two most popular ways to extract palm oil. In the wet process, the oil from the milled palm fruits is extracted using a liquid, typically water. Oil is extracted from ruptured oily cells of palm fruits using hot water or steam. In addition to coagulating proteins, the hot water treatment hydrolyzes any starch, glue, or gum that may be present. During frying, the gums and resins cause the oil to foam. During oil clarification, the hydrolyzed and coagulated byproducts are eliminated. After the moisture has evaporated, the extracted oil is recovered (Obibuzor et al., 2012; Poku, 2002). The dry method employs a hydraulic press, a screw press, or centrifugation. The screw press is generally more applicable in continuous extraction systems, while the hydraulic press is commonly used in batch or semi-batch extraction systems (Poku, 2002). After pressing, crude palm oil drains from the fibrous mesocarp, leaving behind fiber components that still contain approximately 5-6% (w/w) oil.

Pressure is typically decreased to prevent palm kernel breaking, and oil retention rises to 10-12% (Corley & Tinker, 2003; Obibuzor et al., 2012). The resulting press liquid contains different amounts of water, oil, dirt, and fruit fragments. To increase oil yield and lower the CPO's (crude palm oil) moisture content to ≤ 10%, the liquor is treated further (Poku, 2002). In addition to being essential to crude palm oil quality, this procedure causes oil loss and environmental contamination. Palm oil mill effluent (POME), which remains after the majority of the oil has been collected, is produced. When food-grade solvents such as hexane and petroleum ether are utilized for the oil extraction, more oil may be extracted from POME (Obibuzor et al., 2012). According to Poku (2002), the initial oil and moisture contents, operation temperature, heating time, and applied pressure all affect the yields and quality of the extracted oil. Crude oil is often washed with sodium hydroxide or sodium carbonate solutions to lower the FFA level and remove the PLs and other polar lipids in the alkali refining process. Alkali refining alone cannot eliminate all potentially present undesirable chemicals (Čmolík & Pokorný, 2000). The grades and reward amount depend on the quality of the extracted palm oil. Generally, high-grade palm oil has low FFA and moisture contents, low levels of contaminants, and an excellent bleachability index.

Palm oil has been used for domestic cooking in Southeast Asia and tropical Africa for centuries. The food industry has adopted palm oil in its refined form in

recent decades because of its functional benefits, versatility, and widespread availability. Its main advantages are as follows:

- High stability over time: Palm oil helps to maintain the product taste throughout its whole shelf life because of its higher stability to oxidation compared to other vegetable oils (Sulieman et al., 2006).

- Neutral taste and smell: deodorized palm oil can be used in many different foods without affecting their taste; due to its neutral taste, it does not mask the flavor of other ingredients, such as milk, cocoa, and hazelnuts (Matthäus, 2007).

- Palm oil is a versatile vegetable fat due to the possibility of fractionation into different parts: liquid and solid. The possible mixes of these fractions make it suitable for different requirements of texture and flavor in the final product (Dian et al., 2017).

- Smooth and creamy texture: food products with palm oil have an excellent mouth feel with specific characteristics for each product. For example, palm oil contributes to chocolate spreads' smooth and creamy texture and spreadability (Dian et al., 2017).

- Alternative to trans-fat: palm oil is a suitable replacement for partially hydrogenated fat containing trans-fatty acids (Wang et al., 2016).

A high percentage of the products on sale in supermarkets use palm oil in their formulation. These products include food products such as margarine, confectionery, ready-to-eat meals, food snacks, chocolate, ice cream, bakery products, and nonfood products such as soap, candles, and cosmetics.

1.2.2 Crude palm oil

The oil extracted from the mesocarp of the palm oil fruit is known as crude palm oil (CPO). Fresh fruit bunches are shown in Figure 1.6, where various processes are used to create CPOs. Further processing of the crude palm oil (CPO) produced either red or bleached cooking oil or detergents.

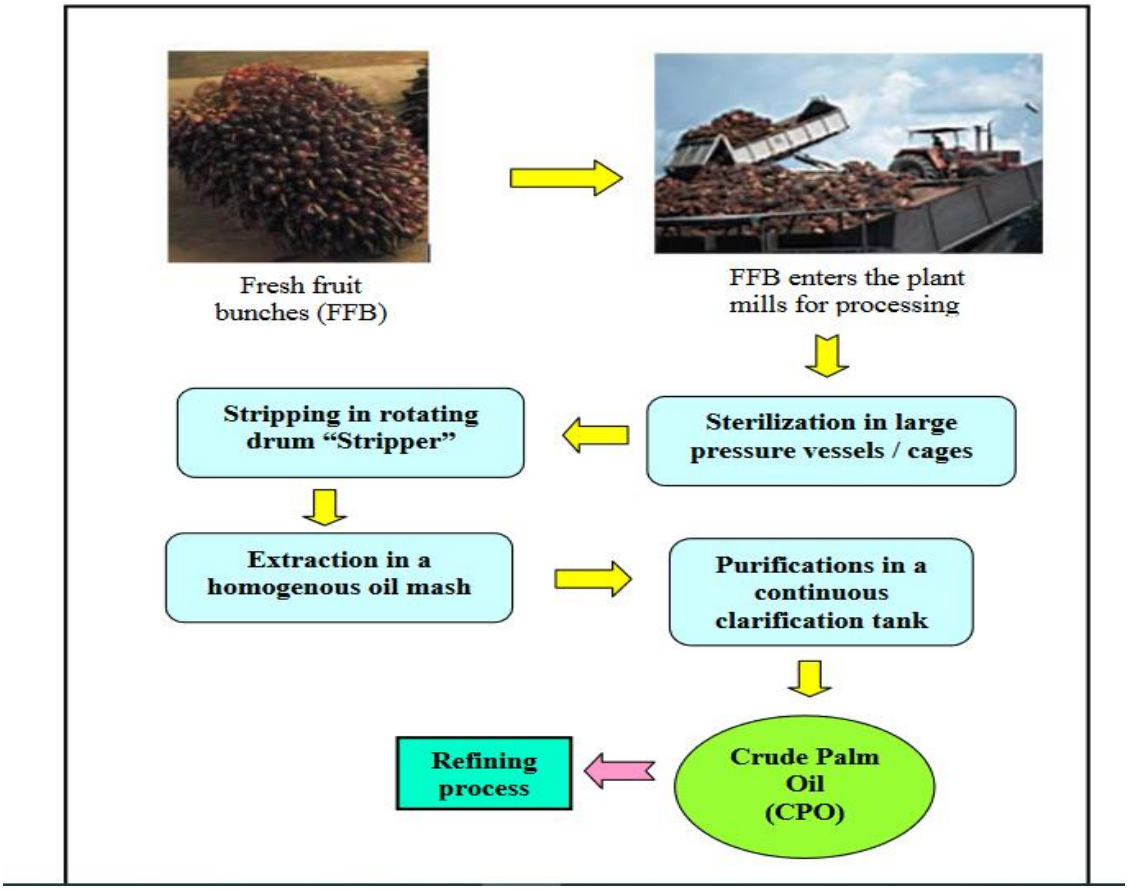


Figure 1.6: Flowchart of crude palm oil (CPO) production.

1.2.3 Composition of CPO

CPO is naturally yellowish-red in color because of its high β-carotene content. (Rui Li, 2012) reported that the carotene content of CPO is between 700-800 ppm, and 90% is β- and α-carotene. It is a vegetable oil with high saturated fat; hence, it is semisolid at tropical room temperature. According to (Kenechi et al., 2017), CPO contains several saturated and unsaturated fats in the forms of glyceryl laurate (0.1% saturated), myristate (0.1% saturated) palmitate (44% saturated), stearate (5% saturated), oleate (39% monosaturated), linoleate (10% polyunsaturated) and linolenate (0.3% polyunsaturated). However, the main composition of CPO is TAG, along with minor components such as FFA, MAG, DAG, metals, phospholipids, peroxides, chlorophylls, carotenoids, phenolic compounds, and tocopherols/tocotrienols (Lin, 2011). (Abdul Azis, 2000) classified the compositions of CPO into a mixture of 5 main chemical groups, as shown in Table 1.1.

Table 1.1: General composition of CPO.

Group	Components in the group
Oil	TAG, DAG, MAG Phospholipids, glycolipids, lipoprotein FFA
Oxidized products	Peroxides, aldehydes, ketones, furfurals (from sugars)
Nonoil (but oil soluble)	Carotene Tocopherols Squalene Sterols
Impurities	Metal particles Metal ions Metal complexes
Water soluble	Water (moisture) Glycerol Chlorophyll pigments Phenols Sugars (soluble carbohydrates)

Figures 1.7 and 1.8 show the composition of palm oil fruit and palm oil mesocarp. The composition of mesocarp is with oil making up 39% of the total composition. After undergoing many procedures, such as sterilization, stripping, extraction, and purification, crude palm oil (CPO) is derived from the mesocarp portion of the palm oil fruit.

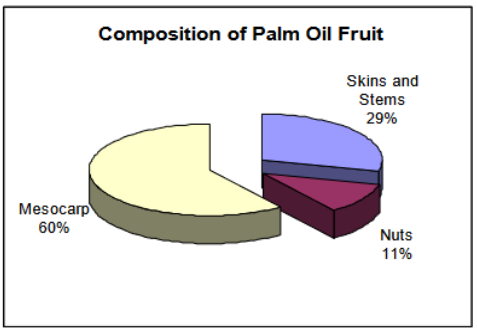


Figure 1.7: Composition of palm oil fruit. Source: Bockish (1993).

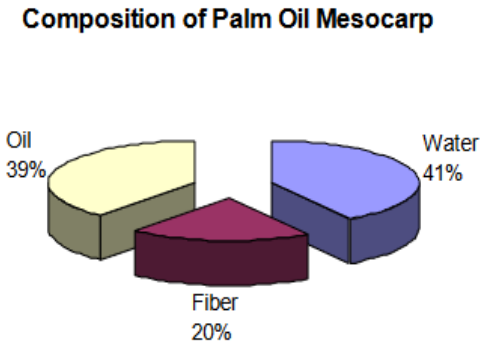


Figure 1.8: Composition of palm oil mesocarp. Source: Bockish (1993).

1.2.4 Palm oil uses

Palm oil yields two types of oils: palm oil from the fibrous mesocarp and palm kernel oil from the palm kernel. There are several uses for palm oil and palm kernel oil; approximately 80% of them are for food purposes, and the remaining 20% are utilized as feedstock for various nonfood applications (Salmiah. 2000). Refined, bleached, and deodorized (RBD) olein is mainly utilized in food products such cooking and frying oils, shortenings, and margarine. In contrast, RBD stearin is used to make margarine and shortenings. RBD palm oil, unfractionated palm oil, is used to make ice cream, margarine, shortening, vanaspati (vegetable ghee), frying fats, and ice cream (Salmiah. 2000).

1.2.5 Carotenoids

The major carotenoids in palm oil are β-carotene and α-carotene, which account for 90% (w/w) of the total carotenoids (Goh et al., 1985). Carotenoids are one of the most valuable constituents of palm oil. These are fat-soluble pigments responsible for the orange color of the oil, with antioxidant properties that have proven benefits for human health, which makes palm oil employable in the prevention of vision problems, cardiovascular disease, and cancer (Alves et al., 2017). Carotenoids have also been reported in some vegetable oils, but their amounts are generally much lower, usually less than 100 ppm (Choo, 1995). Carotenoids are classified into carotenes or xanthophylls according to their chemical structure. Carotenoids are the precursors of vitamin A, with β-carotene having the highest provitamin A (retinol) activity.

Carotenoids have a linear or cyclic hydrocarbon chain at one or both ends of the molecule, as shown in Figure 1.9. They are soluble in preferentially nonpolar solvents; xanthophylls consist of oxygenated carotene derivatives, whose groups are hydroxyl (β-cryptoxanthin), keto (canthaxanthin), epoxide (violaxanthin), and aldehyde (β-citraurine), which are soluble in polar solvents (Rodriguez-Amaya et al., 2008). Palm oil is more than 15 times more retinol equivalent than carrots and 300 times more retinol equivalent than tomato fruit (Choo 1994). Furthermore, carotenes are very sensitive to light and oxygen.

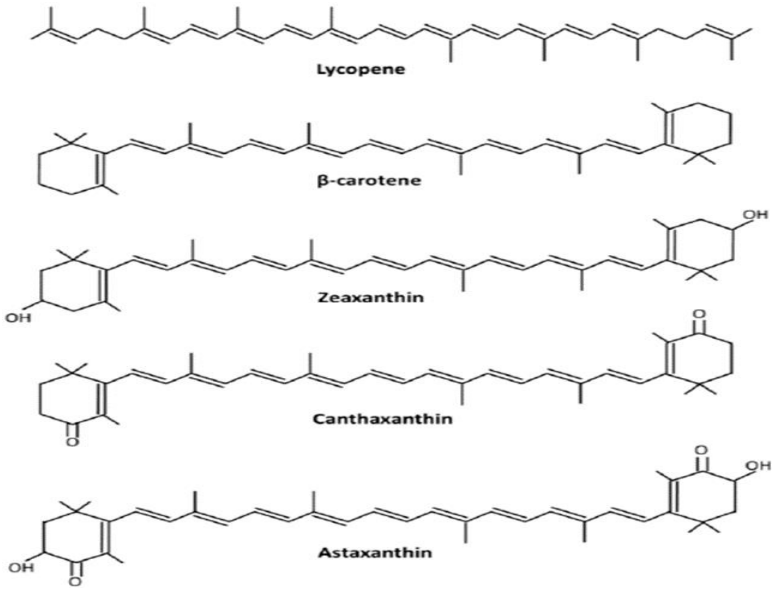


Figure. 1.9: Structural representation of some carotenoids.
Source**:** Adapted from Mesquita (2017).

For the extraction of carotenoids, several methods are described in the literature, including saponification, solvent extraction, enzyme-assisted extraction, ultrasound and microwave-assisted extraction, supercritical fluid extraction, and others. However, in cases where the component adheres to a liquid matrix, such as palm oil, the most applicable method is extraction with liquid due to the simplicity of execution in the laboratory and on a small scale and due to the efficiency and low energy cost (Liu et al., 2021).

Liquid-liquid extraction (LLE) is a unitary operation that uses a liquid or solvent to extract a specific solute in a liquid mixture called the feed. The solvent, insoluble in the feed, promotes solute separation due to the difference in the solute distribution between the immiscible liquids. In this way, two phases are formed, one composed of the solvent enriched with the solute, called the extract, and the other containing the solute-depleted remaining liquid called the raffinate (Mandowara & Bhattacharya, 2011). Compared to other separation methods, such as evaporation and distillation, liquid-liquid extraction presents operational and economic advantages since the procedures can be carried out at room temperature and atmospheric pressure. Furthermore, the extraction step can be carried out in single or multiple stages to reach the desired efficiency. It is essential to choose an appropriate solvent because it influences the process efficiency e and the stability of the solute, as well as the

toxicity of the final product (Jiménez-González & Guerrero-Beltrán, 2021). In this choice, some characteristics of the initial mixture and of the solvent must be analyzed, including the selectivity, miscibility, and density, ease of recovery, viscosity, reactivity, toxicity and cost.

Liquid-liquid extraction of carotenoids and other bioactive compounds is advantageous because they can be carried out at low temperatures, avoiding thermal degradation. In general, the separation of natural pigments in fruits and other natural products has been increasing; however, the extraction of these compounds in vegetable oils is still scarce, although they are widely produced, which restricts their potential for use. As described, the conventional pigment extraction process uses mainly organic solvents such as acetone, hexane, chloroform, and methanol, which, although efficient, are pollutants and toxic, compromising safety and adding environmental problems to the process (Perrier et al., 2017). To overcome this obstacle, extraction with more environmentally friendly solvents has been carried out, among which dimethyl sulfoxide (DMSO) and ethanol stand out due to their lower cost and affinity with hydrophobic compounds, being able to extract both polar pigments (xanthophylls) and nonpolar pigments (carotenes) efficiently (Yang et al., 2015). In this sense, these and other renewable or less toxic compounds with similar characteristics can be used pure or combined with other solvents to make the extraction of carotenoids more economical and sustainable (Yara-Varón et al., 2017). Research has been performed on the extraction of carotenoids and pigments from pequi oil, pulp, and almond oil to increase the availability of these compounds in the market (Mesquita, 2017).

1.3 Liquid-liquid equilibrium data

The representation of the liquid-liquid equilibrium is made in triangular or rectangular diagrams, which describe, in a given temperature range, the existence of one or two liquid phases for the system under study (Tadini, 2016). In triangular diagrams, as shown in Figures 1.10 and 1.11, each triangle vertices presents the composition of the pure components, while the sides represent the binary mixture, and the points located in the interior region indicate a ternary mixture of the components.

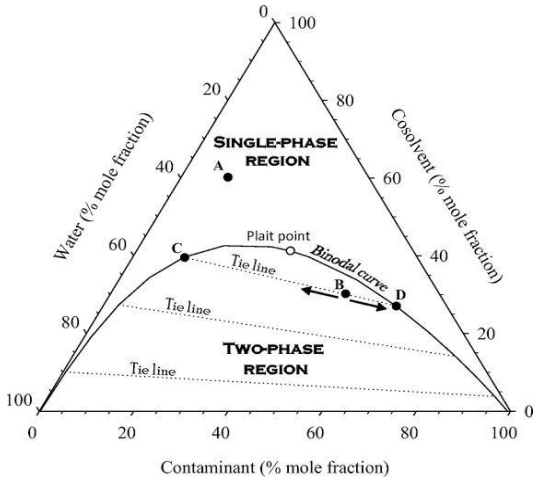


Figure 1.10: Liquid-liquid diagram of a ternary system. Source: Modified from (Tadini, 2016).

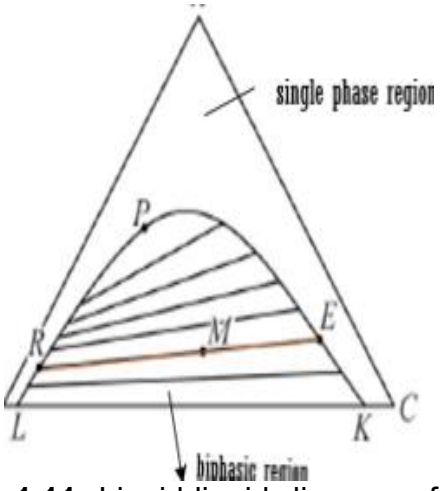


Figure 1.11. Liquid-liquid diagram of a ternary system. Source: Modified from (Tadini, 2016).

The system miscibility delimitation is symbolized by the binodal curve (LRPEK). Thus, any mixing point on the outside of this curve will be a homogenous solution formed by a single phase, while any mixing point on the inside of the binodal curve will be a mixture of two partially miscible liquid phases. The tie-line-like line (RME) is located inside the binodal curve and relates the composition of the extract (rich in component A) and raffinate (rich in component B) phases in equilibrium. The P point, in which these compositions are equal, is called the critical point (plait point), and it is impossible to carry out phase separation at this point(Silva, 2011). In this context, to estimate the efficiency achieved in the extraction process, it is necessary to know the partition or distribution coefficient, mathematically described by the ratio between the solute concentration in the extract and the raffinate phase:

$$Ki = \frac{yi}{xi} \qquad (1)$$

The determination of this parameter can be done from equilibrium data, being of great importance to know the degree of separation of the extractive process. Thus, partition coefficient values above unity are desirable since this indicates ease of migration of the solute present in the initial solution to the phase rich in the solvent incorporated into the process, which allows for a smaller number of equilibrium

stages, and a smaller number of solvent volumes can be used to extract a particular component of interest efficiently. Bessa et al., (2015) studied the β-carotene solubility in ethanol between 10 and 60°C, observing that increasing the temperature increased the carotenoid solubility in the solvent. However, Ribeiro et al., (2012) warn that it is more feasible to perform extraction at lower temperatures due to the thermosensitivity of the pigment (Ribeiro et al., 2012).

In this context, some studies also used water as a cosolvent for the extraction of carotenoids (Goiris et al., 2012); for example, they carried out the extraction of pigment from 32 species of microalgae using different solvents: ethanol-water (3:1) ethyl acetate, hexane, and water, concluding that the ethanol-water mixture allowed greater extraction efficiency in most species. Chen et al. (2016) reported the lutein extraction from the microalgae Chlorella sorokiniana using ethanol, tetrahydrofuran, acetone, and hexane as solvents. Ethanol provided a better extraction yield under a pressure of 450 mbar, 35 °C, and 40 min of extraction time. This study also showed a significant degradation in extraction conducted at temperatures above 55 °C.

1.3.1 Effect of temperature on the liquid-liquid extraction process

Liquid-liquid extraction presents operational and economic advantages since the procedures can be carried out at room temperature and atmospheric pressure. Rostami et al. (2013) reported that solubility in ternary mixtures increased with increasing temperature, and thus, the size of the two-phase region shrank. Batista et al. (2009) presented the liquid-liquid equilibrium for the system containing refined canola oil + commercial oleic acid + short-chain alcohols at different temperatures. For systems with anhydrous methanol and anhydrous ethanol, the heterogeneous region decreases with increasing temperature from 293 to 303 K, and only a slight change in the distribution coefficient of oleic acid is observed. The increasing mutual solubility of canola oil and anhydrous methanol or anhydrous ethanol with almost no impact on the slope of tie lines causes a decrease in the selectivity of the solvents with increasing temperature. Oliveira et al. (2012) also reported that increasing the temperature of the extraction process ensures that similar oil recovery levels can be achieved with fewer theoretical stages. Dias et al. (2015) also presented liquid–liquid equilibrium data for pseudo-ternary systems containing vegetable oil + ethylic biodiesel of vegetable oil + anhydrous ethanol obtained at different temperatures.

Liquid-liquid equilibrium data for the pseudo-ternary systems containing vegetable oil + ethylic biodiesel of vegetable oil + anhydrous ethanol were obtained at different temperatures. From the results obtained, it was found that the solubilities of the systems were affected by the temperature and concentration of biodiesels. Moreover, the solubility of the oil-rich phase and the ethanol-rich phase was enhanced by increasing the temperature in the systems studied at different temperatures.

1.3.2 Liquid-liquid extraction of carotenoids from oil

Liquid-liquid extraction is a tried-and-true technique for extracting phytochemicals, and its appeal is due to its high scalability, low cost of operation, and straightforward extraction procedure. Carotenoids and tocols were simultaneously extracted from crude palm olein using green solvents. Effective extraction from vegetable oils requires knowledge of the solubilities of various vegetable oils in the proposed solvent to design an efficient process; thus, published data on the solubilities of oil in solvents are scant. Hoe et al. (2022) studied the direct recovery of palm carotene by LLE and revealed the phase-forming capacity of solvents. Gonçalves et al. (2016) reported successful LLE for some vegetable oils, such as palm oil and fatty acid. LLE of carotene from palm oil is also appealing from an economic standpoint due to its low startup costs and the opportunity to keep the CPO after the extraction procedure.

1.4. Final Consideration

In industries extraction process plays a key role in separations of desired components from its mixture using a suitable solvent. Extraction has great application in almost all industries such as Pharmaceuticals, Chemical, effluent treatment, Petrochemical etc. In liquid-liquid the solvent should be well miscible with the liquid to be extracted.Howerver, the boiling point of the solvent should be low enough (well below the melting point of the solute), viscosity, and flammability. The solvent should not be toxic or corrosive as it can harm the extraction instruments.

CHAPTER 2 - PHYSICOCHEMICAL CHARACTERIZATION OF PALM OIL

Abstract

Crude and degummed palm oil quality are defined mainly by the contents of free fatty acids, total carotenoids, ash, and moisture and the acid, peroxide, reflective, saponification, and iodine indices. The gas chromatography findings showed that the dominant fatty acids were palmitic acid (43.60%) and oleic acid (40.03%). The crude and degummed palm oil results were, respectively, (a) acid value (mg KOH/g) from 2.79 ± 0.21 and 4.31 ± 0.06, (b) peroxide value (mEq/kg) of 3.82 ± 0.03 and 1.91 ± 0.01, (c) iodine value (g I2/100 g) of 50.01 ± 2.33 and 45.96 ±1.09, (d) saponification value (mg KOH/g) of 199.57 ± 4.20 and 174.85 ± 26.66, (e) reflective index (40°C) of 1.4578 ± 0.00 and 1.4588 ± 0.00, and (f) total carotenoids (μg/g) of 668.81 ± 0.01 and 638.14 ± 0.03. Crude palm oil presented an ash content of 0.03 ± 0.00% and humidity of 0.28 ± 0.04% (m/m). The ash and humidity contents of degummed palm oil were not determined. Density and rheological behavior were evaluated for both crude and degummed palm oil. All the parameters investigated had values within the standards.

1. Introduction

Palm oil fruit (POF) is a drupe fruit from the palm tree *(Elaeis guineensis)*. It is reddish and grows in bunches. Each fruit comprises an oily, fleshy outer layer (the mesocarp) with a single seed (the palm kernel). Crude palm oil (CPO) is the oil obtained from the mesocarp part of palm oil fruit, and the oil from the kernel is called palm kernel oil (Edem, 2002; Lin, 2011). It is among the most economically valuable plant sources for edible and industrial oil.

This production volume is the highest among all vegetable oils, exceeding the second-largest oilseed crop by more than 10 million. The main reasons behind this growth could be attributed to the (a) high productivity nature of the palm oil fruit, (b) the discovery and development of new applications beyond traditional food use, (c) population growth in emerging markets, (d) expansion by the food processing industry, (e) replacement of hydrogenated oils by a trans-fat-free, and (f) utilization for biofuel or biodiesel production. Additionally, there is a demand for palm oil consumption to keep growing (Norizzah et al., 2018; Pirker et al., 2016).

More than 90% of the oil produced worldwide is used for food. This has made it necessary to adequately illustrate the nutritional, physical, and chemical qualities of its fractions. Additionally, since margarine is one of many food applications for which palm oil and its by-products have favorable physical and chemical properties. This indicates that all goods made from palm oil fruit have practical uses, the bulk of them are in the production of edible foods. Because of its distinctive composition, palm oil can be used in a variety of businesses, including those that produce food as well as chemicals, cosmetics, and pharmaceuticals. The oil is rich in palmitic acid, β-carotene and vitamin E. (O. I. Mba, 2015). It has been shown that Crude palm oil is the richest natural source of carotenoid. It contains about 15 times more carotenoid than carrot. Red palm oil is a deacidified and deodorized oil that retains 80% of the original carotenoids, making it a remarkable source of vitamin A. it is therefore important to examine the physico-chemical properties of palm oil.

2. Materials and methods

2.1 Chemicals

Crude palm oil was acquired from Ayasco (Fagge LGA, Kano State, Nigeria). Potassium iodine (99 % purity), natrium hydroxide (99%), and hexane (HPLC/GC grade) were purchased from Sigma Aldrich (USA). Phosphoric acid (85.0%), hydrochloric acid (36.5%), phenolphthalein, sodium thiosulphate (99%), and chloroform (99%) were acquired from Sigma Aldrich (USA). Deionized water (Milli-Q) was used to prepare all solutions. All other chemicals used were of analytical grade.

2.2 Methods

2.2.1 Production of degummed palm oil

Depending on the extraction technique by which vegetable oils are obtained, they may have a high percentage of impurities, such as phospholipids, phosphatides, and fatty acids, substances that can interfere with the quality and stability of the product. Thus, acid degumming of palm oil was carried out to remove the impurities. First, 100 mL of oil was weighed in a 125 mL Erlenmeyer flask, then placed on a hot plate, heated at 70°C, and magnetically stirred for one hour. During heating, 4% phosphoric acid and 4% water were slowly added to the previously weighed oil mass. After that, the mixture was placed in a separatory funnel for phase separation. Then, the upper apolar phase obtained was centrifuged (Heraeus Multifuge X3R, Thermo Scientific, USA) at 5000 × g for 5 min. After centrifugation, the mixture was redirected to the separation funnel, where washing was done by spraying it with hot water until neutral pH. Finally, the degummed oil was collected, fractionated in tubes, and stored for physicochemical analyses.

2.2.2 Physicochemical characterization of palm oil

Palm oils were analyzed in triplicate for total acidity, density, viscosity water content, ash, peroxide, iodine, saponification, and refraction. Such analyses were carried out according to the norms of the Adolfo Lutz Institute (1985) and official methodologies (AOCS, 1990, 1995). The quantification of total carotenoids in the oil was carried out according to the Rodriquez-Amaya methodology (1999).

2.2.2.1 *Total acidity*

The acid number corresponds to the NaOH amount (mg) needed to neutralize 1 g of the sample. It was determined by weighing 2 g of the sample in a 25 mL Erlenmeyer flask and then adding 25 mL of ether: ethanol solution (2:1) and two drops of phenolphthalein indicator. After these additions, titration was performed with NaOH solution (0.01 M) until the pink color appeared. The acidity index was then calculated using Equation 1 (Akinola et al., 2010).

$$\text{Acid index (mg KOH/g)} = \frac{5.61 \cdot \text{V} \cdot \text{f}}{\text{m}} \quad (1)$$

Where: V is the volume of NaOH solution (0.1 M) used in the titration (mL), f is the correction factor (0.1 N sodium hydroxide solution), and m is the sample mass (g).

2.2.2.2 Peroxide index

This analysis determines all substances in terms of milliequivalents of peroxide per 1000 g of sample, which oxidizes potassium iodine (KI) under the test conditions. This analysis was performed by weighing 5 g of the sample in a 125 mL Erlenmeyer flask, in which 30 mL of acetic acid: chloroform solution (3:2) was added. Stirring was carried out until the sample was completely dissolved. After that, 0.5 mL of a saturated solution of KI was added, leaving it to rest in the dark for precisely 1 min. Soon after, 30 mL of water was added and titrated with sodium thiosulfate solution (0.1 N) under constant stirring until the yellow color disappeared. Then, 0.5 mL of indicator starch solution was added, and the titration continued until the blue color disappeared. A blank test was prepared and titrated under the same conditions described for this analysis. The peroxide index was calculated using Equation 2 (AOCS, 1990).

$$\text{Peroxide index (mEq/kg)} = \frac{1000 \cdot (\text{Va} - \text{Vb}) \cdot \text{N} \cdot \text{f}}{m}$$

Where A = volume of sodium thiosulfate solution (0.1 or 0.01 N) spent on sample titration (mL), B = volume of sodium thiosulfate solution (0.1 N) used in the blank titration (mL), N = normality of the sodium thiosulfate solution, f is the correction factor, and m is the sample mass (g).

2.2.2.3 Index of refraction

The refractive index is a parameter related to the saturation degree of bonds but is affected by other factors, such as free fatty acid content, oxidation, and heat treatment. Each type of oil has a characteristic refractive index within certain limits. The refractive index determination was performed using a refractometer, which must be clean, dry, and calibrated. Thus, the measurement compartment was first opened, and the refractometer was calibrated with distilled water (RI = 1.33 at 20°C). Then, three drops of the oil previously heated in a water bath at 40°C were placed in the refractometer prism; the measurement compartment was closed. After 15 s, the refractive index readings (40°C) were taken on the equipment display. The equipment was calibrated after each measurement (Onwuka 2005).

2.2.2.4 Saponification index

The saponification index represents the amount of potassium hydroxide (mg) needed to saponify 1 g of oil sample. The procedure for determining this parameter was started by weighing 5 g of the sample in a 250 mL Erlenmeyer flask and adding 50 mL of an alcoholic potassium hydroxide solution (KOH). A condenser was connected to the flask, and the sample was subjected to gentle boiling until complete saponification took approximately one h. After that, the condenser was disconnected, and the sample was allowed to cool. Then, 1 mL of phenolphthalein indicator was added, and titration was performed with a hydrochloric acid solution (0.5 M) until the pink color disappeared. A blank was prepared and analyzed simultaneously with the sample. The saponification index was calculated using Equation 3 (AOCS, 1990).

$$\text{Saponification index (mg KOH/g)} = \frac{28.06 \cdot (\text{Vb} - \text{Va}) \cdot \text{f}}{\text{m}} \quad (3)$$

Where: Va is the volume spent on sample titration (mL), Vb is the volume spent on blank titration (mL), f is the factor of 0.5 M HCl solution, and m is the sample mass (g).

2.2.2.5 Iodine index

The iodine index of oil measures its degree of unsaturation and is expressed in terms of the iodine mass (centigrams) absorbed per sample mass (g) (% iodine absorbed). First, 0.25 g of the sample was weighed in a 500 mL Erlenmeyer flask.

After that, 25 mL of Wijs solution was transferred with a burette to this container, which was capped, carefully shaken with a rotation movement to ensure perfect homogenization, and left to rest in the dark and at room temperature for 30 min. Subsequently, 10 mL of a KI solution (15%) and 100 mL of freshly boiled and cold water were added. Then, titration was performed with sodium thiosulfate solution (0.1 M) until the appearance of a soft yellow color. After that, 2 mL of 1% starch indicator solution was added, and the titration was continued until the blue color completely disappeared. A blank was prepared, and the same analytical procedure described was performed. The iodine content was calculated by applying Equation 4 (AOCS, 1995).

$$\text{Iodine index (g I2/100g)} = \frac{12.68 \cdot (\text{Vb} - \text{Va}) \cdot \text{M}}{\text{m}} \qquad (4)$$

Where: Va is the volume spent on sample titration (mL), Vb is the volume spent on blank titration (mL), M is the $Na_2S_2O_3$ solution molarity (mol/L), and m is the sample mass (g).

2.2.2.6 Lipid profile

Palm oil fatty acid composition was determined using gas chromatography. First, a sample quantity between 10 and 50 mg was transferred to a derivatization bottle. Then, 1 to 2 mL of a sulfuric acid solution (3%) in methanol was added to the sample. The sample tubes were closed and incubated at 90°C in a dry bath for 90 min under agitation at 200 rpm on a shaker table. After incubation, the sample was cooled on a bench, 1 mL of deionized water and 2 mL of hexane were added to the tube, and the mixture was vortexed. A 1 mL aliquot of the supernatant was taken and transferred to an Eppendorf tube containing 0.05 g of anhydrous sodium sulfate. Then, the material was vortexed and centrifuged at 5000 × g for 5 min at 25°C. A 200 µL aliquot of the liquid sample in hexane was withdrawn and transferred to a 2000 µL vial. Then, 800 µL of hexane was added to the vial, and the sample was analyzed in a gas chromatograph (GC 2010, Shimadzu, Japan) to obtain the fatty profile. Direct injection of 1 µL of the sample, the linear heating ramp from 100°C to 270°C at 20°C/min, and high linear speed for better peak resolution were the chromatographic conditions used (Ichihara & Fukubayashi, 2010).

2.2.2.7 Ash content

Ash analysis determines the residue quantity remaining after incineration under specific test conditions. It is based on the weight loss of the product incinerated at 550°C, with the destruction of organic matter without appreciable decomposition of the mineral residue constituents or loss by volatilization. The procedure started with heating the sample in a muffle furnace at 550°C for one hour. Next, the sample was cooled in a desiccator to room temperature and weighed on filter paper, which was submerged in ether solvent in a 50 mL porcelain capsule or 100 mL platinum capsule. The sample was then carbonized in a Bünsen burner with a low flame and incinerated in a muffle furnace at 550°C. After that, the samples were cooled in a desiccator to room temperature and weighed. Heating and cooling operations were repeated until a constant mass was reached (AOCS, 1990).

$$\text{Ash}\ (\%, \text{m/m}) = \frac{100 \cdot \text{p}}{m} \qquad (5)$$

Where: p is the solid residue mass (g), and m is the sample mass (g).

2.2.2.8 Moisture and volatile matter

The analysis of moisture and volatile matter corresponds to the mass loss of a given product heated under conditions in which water and other volatile substances are removed. In fats and oils, sample direct heating to 105°C provided moisture and volatile matter contents that are legal parameters for quality assessment.

The analytical procedures to determine the samples' moisture and volatile matter contents were performed by weighing 2 g of the sample in a previously tared porcelain capsule, heating in an oven at 105°C for three h, and cooling in a desiccator to room temperature. After that, the sample was weighed, and the heating and cooling operations were repeated until constant mass. The humidity was determined by Equation 6 (AOCS, 1996)

$$\text{U}\ (\%) = \frac{100 - \text{N}}{m} \qquad (6)$$

Where: U is the humidity or volatile substances at 105°C (%), N is the moisture mass (g), and m is the sample mass (g).

2.2.2.9 Total carotenoids

Palm oil carotenoids were quantified using ultraviolet-visible (UV-Vis) spectroscopy. Thus, 0.1 g of oil was weighed and diluted in 25 mL hexane solvent. The measurement was performed at a wavelength of 446 nm, and the concentration of total carotenoids ((μg/g) was calculated using Equation 7.

$$\text{Carotenoid content } ((\mu g/g) = 25\, x\, \frac{383}{100W}\, x\, (a_s - a_b) \quad (7)$$

Where: 25 is the volume of solvent used in the analysis (mL), a_s is the absorbance of the sample, a_b is the absorbance of the blank (hexane) and W is the mass of the sample (g).

2.2.2.10 Water and sediment contents

Water and sediment contents were determined (ASTM D2709, 2022), after centrifugation (Heraeus Multifuge X3R, Thermo Scientific, USA) of 20 g of oil in a 50 mL tube at 5000 × g for 5 min. Thus, the nonpolar phase was separated and removed, and the tube was weighed to quantify the residual mass. Water and sediment contents were determined through the ratio between the residual phase mass and the total sample mass.

2.2.3 Rheological characterization

2.2.3.1 Viscosity

Oil viscosity was measured in a rheometer (model RN 4.1, Rheotest, German). This equipment has a sample holder compartment coupled with a thermostatic bath. The spindle rotated in the oil for 2 min, awaiting the meter monitor's reading to be stable between 40 and 90 °C.

2.2.3.2 Density

Density analysis was performed using a bench top digital densimeter (EDM model, Schmidt Haensch, China). Density measurements of crude and degummed palm oils in the temperature range from 10 to 90 °C were made after calibrating the equipment with distilled water at 25 °C.

2.2.3.3 Pour point

The pour point is the lowest temperature at which the oil can maintain its fluidity characteristics, indicating the cold oil temperature properties. This characteristic is of paramount importance when, among other applications, oil is used as an automotive lubricant or liquid insulator in environments with low temperatures (Moosasait, 2021). The sample was cooled in a glass tube under the prescribed conditions. The system was inspected at 3 °C intervals until no movement was observed, even when the surface place was held vertically for 65 s. Thus, the pour point was taken as 3 °C above the temperature of the stopping flow (ASTM D2709, 2022).

3. Results and discussion

3.1 Physicochemical properties of crude and degummed palm oils

Crude palm oil is a complex mixture of different types and compositions of glycerides. Triglycerides are the major component, whereas mono- and diglycerides are in small amounts. The minor components are mostly carotenoids, vitamin E (tocopherol and tocotrienols), phospholipids, phenolic acids, alcohols, sterols, and flavonoids. Red palm oil is produced from crude palm oil through a milder process that enables the retention of most carotenes and vitamins in the refined oil (Alyas, 2006). Thus, red palm oil is considered one of the richest plant sources of carotenes, a precursor of vitamins A and E (Nagendran, 2000). Physicochemical parameters, such as moisture content and acidity, peroxide, iodine, and saponification indices, are essential for evaluating the quality of vegetable oils since they provide information mainly related to the product conservation state. Analogously, the carotenoid content points to foods with bioactive properties, expanding their consumption possibilities. Table 2.1 presents the physicochemical results of crude and degummed palm oil.

Table 2.1: Physicochemical analyses for crude and degummed palm oil.

Characteristics	Crude palm oil	Degummed palm oil
Acid value (mg KOH/g)	2.79 ± 0.21	4.31 ± 0.06

Peroxide value (mEq/kg)	3.82 ± 0.03	1.91 ± 0.01
Iodine value (g I2/100 g)	50.01 ± 2.33	45.96 ± 1.09
Saponification value (mg KOH/g)	199.57 ± 4.20	174.85 ± 26.66
Reflective index (40 °C)	1.4578 ± 0.00	1.4588 ± 0.00
Ash content (%)	0.03 ± 0.00	Nd
Humidity (%)	0.28 ± 0.04	Nd
Total carotenoids (µg/g)	668.81 ± 0.01	638.14 ± 0.03

Nd = Not determined

Vegetable oil tends to degrade when obtained at high temperatures or stored in the presence of light. The acidity index is a parameter that can measure oil degradation because degradation leads to glyceride decomposition and forms free fatty acids by fungi and microorganisms (Houria et al., 2002; Okechalu et al., 2011). The acid index of crude palm oil observed (2.79 ± 0.21 mg KOH/g) was lower than the maximum value of 3.5 mg KOH/g (SON, 2000). The high acid value may lead to higher free fatty acids, decreasing the oil quality and reducing its industrial applications and nutritional value (Akinyeyea et al., 2011). According to the literature, the oil acid index (in terms of oleic acid) for food applications should not exceed 0.4% of the free fatty acid composition (Amoo, 2004), indicating that the observed acid index falls outside the nutritional limit. The acid index of degummed palm oil observed (4.31 ± 0.06 mg KOH/g) was slightly higher than the crude acid value found. The fact that the palm oil in this work was degummed but not neutralized accounts for the high acidity index score. Therefore, a procedure should be used to neutralize the current free fatty acids.

The peroxide index is the parameter used to indicate the level of lipid peroxidation or oxidative degradation, measuring a temporary oxidation product. A low value may represent early or advanced oxidation, and the peroxide value increases with the oil samples' temperature, storage duration, and contact with air (Ekwu et al., 2004). The peroxide index of crude palm oil observed (3.82 ± 0.03 mEq/kg) was lower than the standard values (10 mEq/kg) specified by SON (2000) and NIS (1992). Agbaire et al. (2012) reported a much lower peroxide index than the

standard value. Thus, it is inferred that the palm oil analyzed in the present work did not present an accelerated lipid oxidation process, and the result fell within the standard 0.00 – 10.40 mEq/kg specified by SON (2000) and NIS (1992). Degummed palm oil presented a peroxide index (1.91 ± 0.01 mEq/kg) approximately twice as low as that of crude oil. Accordingly, removing gums and phospholipids during the degumming process helps preserve the integrity and quality of the product, and adding antioxidants may be a good way to prolong the oil stability.

Iodine indices of 50.01 ± 2.33 g I2/100 g and 45 ±1.09 g I2/100 g were determined for crude and degummed palm oil, respectively, as shown in Table 1. These low values suggest that the oil has a low level of unsaturation and might not be susceptible to oxidation since the iodine value measures the degree of unsaturation (C=C) or fat. Thus, it can also be used to detect oil or fat adulteration. Our results were in the same range as those reported by Ekwenye & Ijeoma (2005), Udensi & Iroegbu (2007), and Okechanalu et al. (2011). A similar finding was also reported by Enyoh et al. (2018). The difference in these values can be attributed to the fatty acid composition in crude palm oil. These low values suggest that the oil has a low level of unsaturation and might not be susceptible to oxidation.

The saponification index is related to the molecular mass of oil triglycerides and the oil decomposition level (Neagu et al., 2013). It provides information on the character of the oil's fatty acids, particularly concerning the solubility of their soap in water. A higher saponification index (SI) indicates a high proportion of low fatty acids since saponification is inversely proportional to the average molecular mass or length of fatty acids (Muhammad et al., 2011). Oils with low SI values can produce soap, candles, and raw materials for lubricants. The SI of 199.57 ± 4.20 mg KOH/mg for crude palm oil is within the recommended range from 195 to 205 mg KOH/mg (SON, 2000; NIS, 1992). The SI for degummed palm oil was 174.85 ± 26.66 mg KOH/mg.

Palm oil's high refractive index (RI) can be attributed to the high number of carbon atoms in its fatty acid composition (Falade et al., 2008). The RI results (40°C) were found to be 1.4578 ± 0.00 °Bx for crude palm oil and 1.4588 ± 0.00 °Bx for degummed palm oil. These values are close to the value of 1.4600°Bx obtained by Akinyeyea et al. (2011). The refractive index measures how much light bends through oil (Esmaeili & Rahimpour, 2017). Ash refers to the organic residue remaining after the water and organic matter have been removed by ignition or

complete oxidation of organic matter in a food sample. The inorganic residue consists mainly of the minerals present in the food sample. The ash content of 0.03 ± 0.00 % for crude palm oil indicates the presence of minerals in the sample before oil degumming. The minerals were probably removed during degumming; thus, they were not detected in our assays.

The oil moisture content enables assessing the oil quality because it indicates any food water activity (a_w) (Fraziar & Westoff, 1978), ease of spoilage and rancidity, and short shelf life. The moisture contents of palm oil are directly affected by the final extraction efficiency and clarification processes (Wolves, 1969; Johansson & Pehlergard, 1977; Poku, 2002; Orji, 2006; Mbata & Orji, 2008). The moisture of crude palm oil observed in our work was 0.28 ± 0.04 %, near the recommended standard of 0.29 % (SON, 2000; NIS, 1992). Enyoh et al. (2017) reported a higher moisture content than our palm oil value. Low moisture values will benefit palm oil stability during storage.

Palm oil is the world's richest source of natural plant carotenoids in terms of equivalent retinol (provitamin A). Carotenoids are responsible for the color variations in palm oil and other vegetable oils, and the dark red color of oils is due to a high carotene content (500 - 1000 mg/kg), which may provide oxidative stability to the oil, and the major carotenoids are β-carotene and α-carotene (Rodriguez-Amaya, 1999; Edem, 2002; PORAM, 2013). The carotenoid content of palm oil indicates its freshness and is influenced by many factors, such as species, variety, or hybrid plants. The total carotene content, which decreased with storage time, was determined to be 668.81 ± 0.01 µg/g for crude palm oil and 638.14±0.03 µg/g for degummed oil. The reduction in carotene content in degumming was due to phosphoric acid's removal of the minor components. Degumming caused a reduction of 30.67 µg/g of total carotene content of 668.81 µg/g of carotene concentration in crude palm oil (CPO). Such results were within the values (500-2000 (mg/kg) recommended by SON (2000) and NIS (1992).

The results of these analyses are shown in Table 2.2. Crude palm oil was further described in terms of centesimal composition, expressed in lipids and total polar compounds, as well as in water and sediment content.

Table 2.2: Presented lipid contents, total polar compounds, and sediments of palm oil.

Parameters	Content (% m/m)
Lipids	86.92 ± 0.006
Total polar compounds	12.08 ± 0.01
Water and sediment	1.0 ± 0.00

When calculating the lipid content using the Bligh & Dyer technique (1959), it was found that palm oil contained 86.92 ± 0.01% soluble apolar lipids in the upper phase (chloroform), while polar lipids and other chemicals were solubilized in the lower phase (water-methanol). When compared to the sediment content of refined sunflower and canola oils (0.2%), castor oil (0.74%), and other oils, the water and sediment content (1.0± 0.00%) was reported by (Evangelista et al., 2006). However, the higher water and sediment contents were higher in the extraction process of oil done by hand without the use of adequate machinery to optimize steps such as decanting, evaporation, and filtration, which are necessary to reduce the water content and buildup of fiber and fruit residues in the finished product.

Figure 2.1 shows the fatty acid profile of palm oil as determined by gas chromatography, and Table 2.3 lists the relative amounts of the discovered fatty acids.

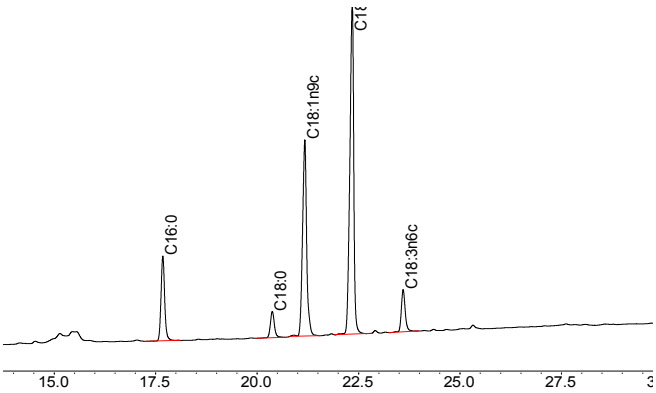


Figure 2.1: Profile chromatogram of FAMES palm oil

Table 2.3: Fatty acids in palm oil and their ratios

Fatty acids	Percent %

C16: 0 (Palmitic)	43.60
C16: 1(Palmitoleic)	-
C18:0 (Stearic)	5.58
C18:1n9c (Oleic)	40.03
C18:2n6c (Linoleic)	10.30
C18:3n6	-
Saturated fatty acids	49.18
Monounsaturated fatty acids	40.03
Polyunsaturated fatty acids	10.77

The free fatty acid (FFA) composition of palm oil was palmitic (43.6 %), stearic (5.58 %), oleic (40.03 %), and linoleic (10.30 %), as shown in Table 2.3. In this context, the fatty acid profile determined in this work is similar to those determined by Japir et al. (2017), who determined the physicochemical characteristics of high free fatty acid crude palm oil. However, it was discovered that 50.8% of palm oil's total fatty acid composition is composed of unsaturated fatty acids, which is why the product has caught the attention of the functional food market. It is also widely used in folk medicine to treat other cardiovascular issues and lower LDL cholesterol (Pessoa et al., 2015).

The pour point recorded for palm oil was 24 °C. Oil flow in the tube was no longer visible because the oil hardened at approximately 25 °C, and the first fat agglomerates developed. Verma et al. (2016) reported similar results. This behavior can be attributed to the oil's high proportion of unsaturated fatty acids, which allows it to remain entirely or partially liquid at temperatures below room temperature (25 °C).

3.2 Rheological properties

Vegetable oils are susceptible to thermal processes during manufacturing, storage, and consumption that may change one or more of their physicochemical properties. In this regard, it is crucial to research rheological behavior as a function of temperature to gather knowledge that can be used to maintain the quality of the final product and properly dimension the unit activities that make up its manufacturing process.

A measure of the mass of a substance per unit volume, density is a significant and distinctive physical property of a given substance. The density of crude and

degummed palm oils was assessed in the temperature range of 10 to 90 °C, considering that the value of this property can change as a function of temperature. The results are displayed in Figure 2.2.

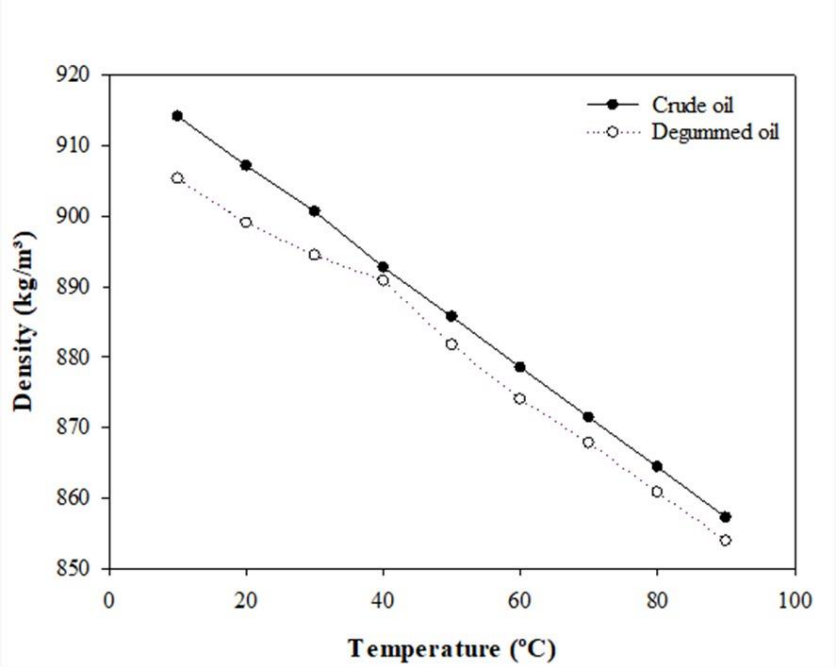


Figure 2.2: Density of crude and degumming palm oil

Because of the degumming process, which eliminates gums, phospholipids, and suspended chemicals that have a density greater than the density of oil and thus increases the value of this parameter, crude palm oil has a higher density than degummed palm oil at all temperatures examined. This finding is in the same agreement with (Narváez et al., 2008). However, both samples had similar behavior as a function of temperature, with the increase in temperature leading to a drop in density, which according to (Esteban et al., 2012), is expected in the case of vegetable oils.

Viscosity is a physical characteristic that describes a fluid's resistance to flowing at a specific temperature. As a result, such a fluid's flow grows slower as its viscosity increases, typical when the operation is carried out at low temperatures. Thus, in the case of vegetable oils and other foods, it is crucial to understand how viscosity changes with temperature to visualize how the product behaves under various processing circumstances and, from there, take action to improve its

performance in response to market demands. Therefore, the crude and degummed palm oil viscosities were evaluated from 40 to 90°C, as shown in Figures 2.3 to 2.6.

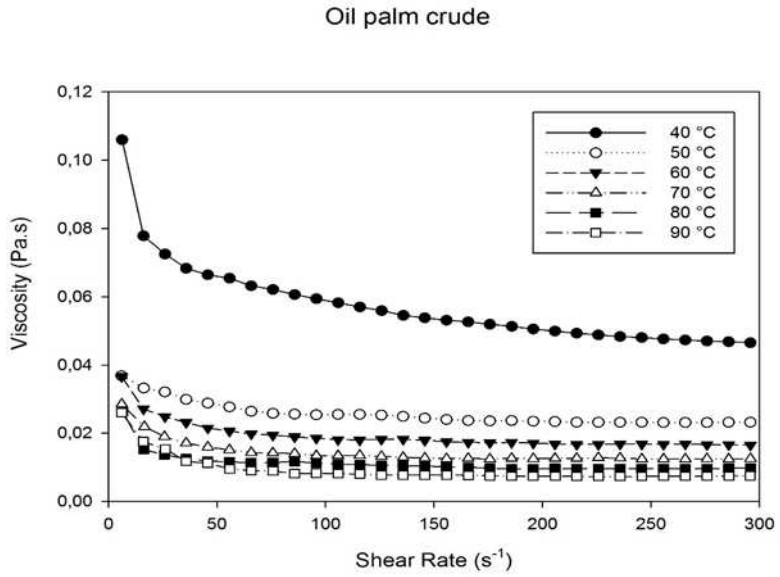


Figure 2.3: Viscosity versus shear rate of crude palm oil

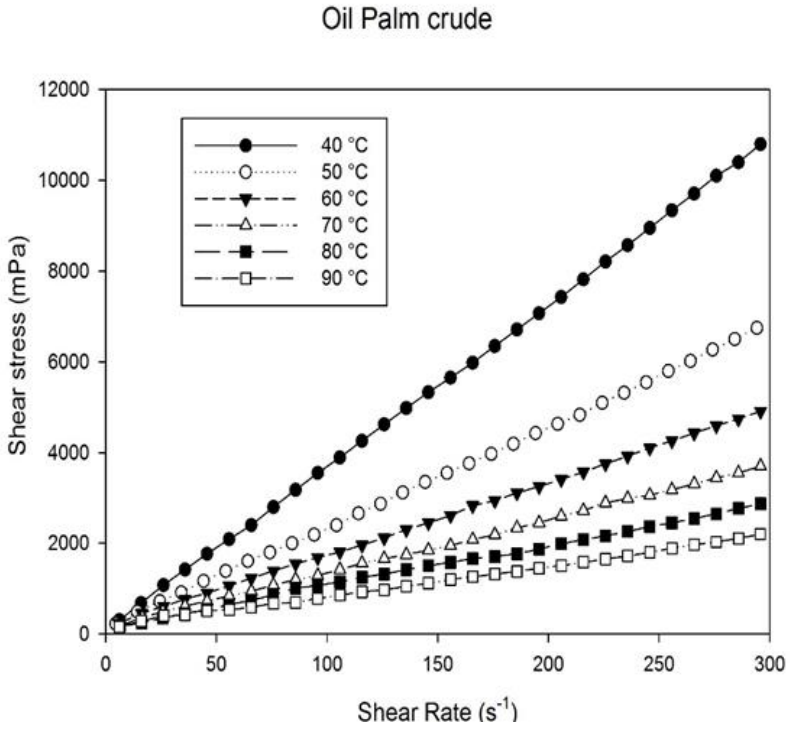


Figure 2.4: Shear Stress versus Shear Rate of crude palm - Degummed palm oil

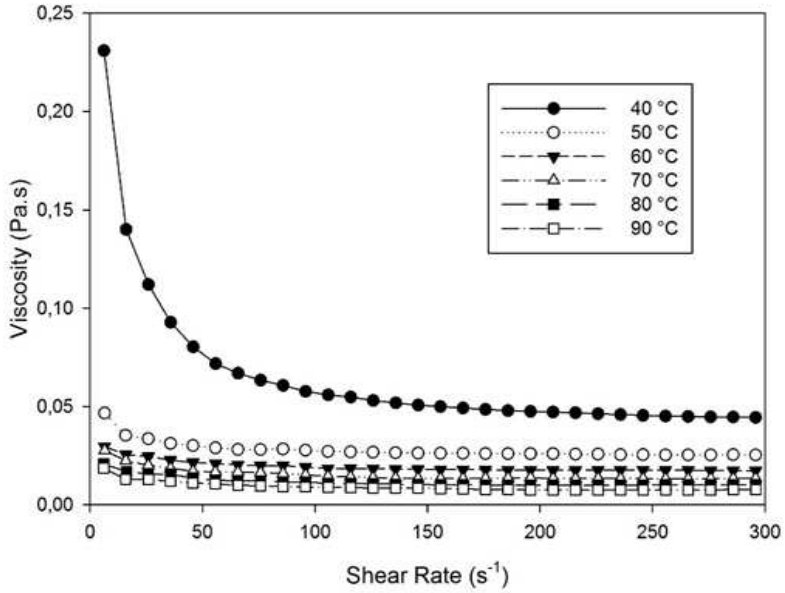


Figure 2.5: Viscosity versus shear rate of degummed palm oil

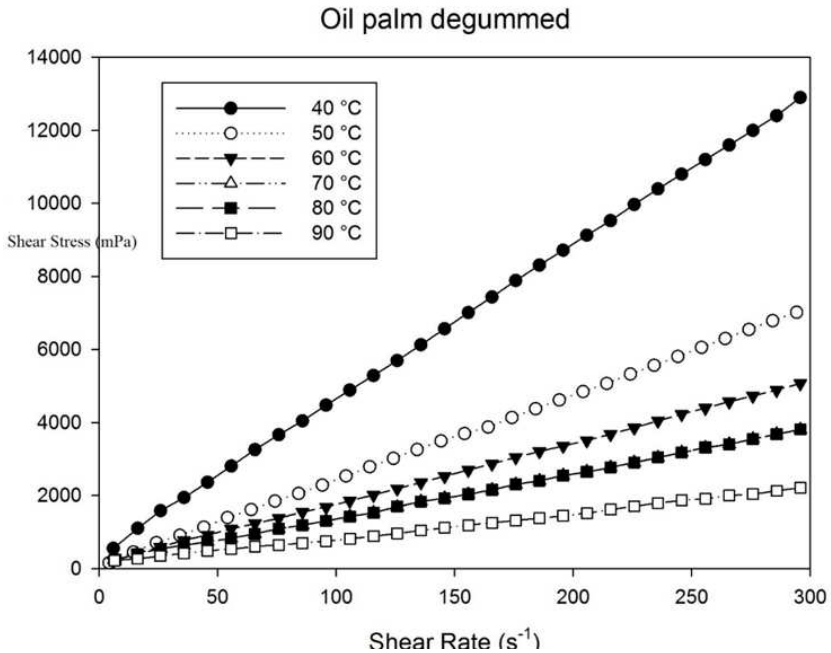


Figure 2.6: Shear Stress versus Shear Rate of Degummed palm oil

Figures 2.3 and 2.5 illustrate that crude palm oil and degummed palm oil have a higher viscosity (approximately 0.06 Pa.s and 0.05 Pa.s, respectively) at a temperature of 40 °C and that the viscosity decreased with increasing temperature. The molecular interactions increased as the temperature rose, causing molecules to move more quickly. The molecular exchange that took place in liquids is comparable

to that seen in gases, with the exception that, in contrast to gases, there was a significant increase in cohesive forces between the molecules of a liquid. The result of raising a liquid's temperature was a decrease in cohesive forces and an increase in the rate of molecular interchange. This behavior is similar to the findings of Esteban et al. (2012). Impurities in crude oil often precipitate on the tube walls at low temperatures. Some solid particles in the mass stream increase the oil's viscosity, which raises the pipeline's pressure drop. The results in the viscosity increase and the flow properties of the oil are characteristics of non-Newtonian behavior (Sathivel et al., 2003)

Figures 2.4 and 2.6 for crude and degummed palm oils show the temperature influence on the rheological oil behavior. As the temperature increased, there was a decrease in viscosity for all oils, which was also observed for crude palm oil by Freitas et al. (1998). The viscosity of edible vegetable oils decreases with increasing temperature because the molecules move more thermally, which lowers the viscosity (Kahn et al., 1990; Forster & Ferrier, 1979). Goodrum & Geller (2000) reported for vegetable oils and analogous triglycerides that the viscosity decreased by at least 25 % for every 20 °C increase in the temperature range from 25 to 80 °C. Wang & Briggs (2002) found similar behavior of viscosity decrease with temperature increase for soybean oils with modified fatty acid compositions. The degree of viscosity reduction depended on the oil's fatty acid composition. Kim et al. (2010) also verified a viscosity decrease with the temperature increase for some vegetable oils. In general, in these studies, an increase in the behavior index and a decrease in the consistency index parameter were observed with increasing temperature, indicating that the pulps are less viscous as the temperature increases. Only a fluid that can withstand certain operating temperatures is chosen; otherwise, the purpose of using such a fluid is defeated(Esteban et al., 2012).

4. Conclusion

The Nigerian palm oil evaluated presents quality within the recommended values, and the degumming step promoted the removal of nearly all phospholipids, which is crucial for the final oil quality. The processing and storage methods employed to handle crude and degummed palm oils do not alter the oil characteristics; thus, both oils are excellent materials for formulations or nonfood applications. The use of phosphoric acid in degummed palm oil during the

degumming process, when compared to crude oil, has a high retention of up to 99 % of significant components and safeguards oil quality. The visual aspect of degumming palm oil is more attractive than that of crude palm oil because the heating process more efficiently reduces carotene during the degumming step. However, from this study, temperature affects the viscosity of crude and degummed palm oil oils at temperatures between 40 to 90 °C, with viscosity decreasing exponentially and linearly with increasing temperature. Additionally, temperature dependencies of density were measured for crude and degummed palm oil. Therefore, it is reasonable to conclude that the palm oil samples studied were not adulterated and that the processing and storage methods employed were adequate.

CHAPTER 3 - EFFECT OF TEMPERATURE AND TYPE OF SOLVENT AND EQUILIBRIUM DATA ON THE EXTRACTION OF CAROTENOIDS FROM PALM OIL

Abstract

Crude palm oil contains the most known carotenoids among crops. The scalable liquid extraction technique can potentially be applied to the direct recovery of palm carotene. Thus, this work reports the carotenoid extraction from palm oil using liquid-liquid extraction at temperatures of 40, 45, and 50 °C. First, studies were conducted to evaluate the phase-forming capability and extraction performance of low-toxicity, low-cost, and environmentally friendly organic solvents. According to the screening results and the good performance of palm carotene extraction with high distribution coefficients, ethanol and dimethyl sulfoxide were the best solvents for extraction, followed by acetic acid with less effective extraction. The density-based solvation behavior effectively explained the affinity of carotene for the solvent mixture and the tendency of biphasic system formation. The total carotenoid extraction increased with increasing temperature, with 50 °C having the highest extraction, followed by 45 and 40 °C. Equilibrium data for a system composed of palm oil + anhydrous ethanol + DMSO at temperatures ranging from 40, 45, and 50 °C. Using the method developed by Merchuk et al. (1998). Cloud point determination was used to generate experimental binodal curves, showing a decrease in the biphasic region with increasing temperature. The rise in temperature within the experimental ranges enhanced oil solvent miscibility in both stages, according to the findings.

1. Introduction

Crude palm oil is also called red palm oil because of its high content of carotenoids. The extraction and recovery of carotenoids from palm oil would gain significant value to the palm oil industry. Tocopherols and carotenoids, which give palm oil its distinctive orange-red color, support the stability and nutritional value of palm oil. Thus, palm oil is an alternative source to supply natural carotene for several uses in the food and pharmaceutical industries (Imoisi et al., 2015). The increasing application of carotenoids in medical and cosmetic industries also contributes to global carotenoids market. Various methods for carotenoid extraction are documented in the literature, including saponification, solvent extraction, enzyme-assisted extraction, extraction assisted by ultrasound and microwave, supercritical fluid extraction, Soxhlet extraction, and atmospheric liquid extraction with maceration (Saini & Keum, 2018).

Carotenoid extraction with organic solvents provides good extraction yields without sophisticated instruments because it is straightforward to carry out in a laboratory setting and on a small scale and is effective and energy-efficient (Liu et al., 2021). Time, type and volume of solvent, oil dosage, and temperature are the variables that most affect the yield of solvent-based extraction processes. The liquid-liquid extraction procedure can be completed at room temperature and air pressure, and offers operational and financial advantages (Santana et al., 2012).

Solvent extraction is typically carried out at a temperature close to the solvent's boiling point to ensure the effectiveness of the operation. This temperature condition lowers the oil's viscosity and increases the dissolution ability of the solvent (Egbuna et al., 2019). Thus, the extraction of carotenoids can benefit from the synergistic usage of a wide range of solvent combinations. Understanding how different vegetable oils react with the solvents is necessary to build an effective procedure for extracting carotenoids. Therefore, a critical action for successfully extracting carotenoids is the selection of an appropriate solvent or solvent combination. Knowledge of the solvent and vegetable oil's mutual solubility is crucial for better comprehension of the procedure, an improvement in reaction rates, and the subsequent stage for industrial processing. Therefore, from an industrial point of view, solvent extraction has been always the first option because of its simplicity and low costs (Strati & Oreopoulou, 2011).

Dimethyl sulfoxide (DMSO) and ethanol stand out among the more environmentally friendly extraction solvents because of their affinity for hydrophobic compounds and lower cost, making it possible for them to effectively extract both polar pigments (xanthophylls) and nonpolar pigments (carotenes) (Yang et al., 2015). Therefore, this study aims to evaluate the effect of temperature and type of solvent on carotenoid extraction from palm oil. There have been focused efforts over the past few decades to improve carotenoid extraction techniques. However, recovery from complex food matrices is still poor because the food matrix contains a number of physical and chemical barriers that prevent the mass transfer of carotenoids during extraction. It is particularly challenging to extract multiple carotenoids simultaneously since different groups have different degrees of polarity. The ability of carotenoids to oxidize also restricts their exposure to extremes in temperature, light, acidity, and extraction durations.

Carotenoids are traditionally extracted using organic solvents due to their hydrophobic nature. Typically, polar solvents such as acetone, ethanol, and ethyl acetate are better suited for the extraction of polar carotenoids, whereas nonpolar solvents such as hexane, petroleum ether, or tetrahydrofuran (THF) are ideal choices for nonpolar carotenes or esterified xanthophylls. Extraction by organic solvents is one of the most commonly used methods in the separation of carotenoids from palm oil (Tang & Ho Row, 2020). A method such as this uses less energy because it is performed at ambient temperature and pressure (Teixeira Barcelos et al. 2018). Finding solvents with the ability to perform the desired separation and having low toxicity, reactivity, and flammability characteristics that result in little oil loss and exhibit a significant density difference between the system's components is a crucial step in making liquid–liquid extraction feasible. Due to their low toxicity, strong polarity, and limited solubility in oils, DMSO and ethanol are potential solvents to be explored in research on the liquid extraction of carotenoids from palm oil. The US Food and Drug Administration classifies DMSO as a low-hazard potential solvent for humans (FDA). It is regarded as a green solvent because it is one of the least harmful organic compounds apart from other green solvents discovered. Therefore, DMSO and ethanol are solvents that are favorable to the environment (Soroko et al., 2011). The ensuing purification procedures for the extraction of carotenoids, as well as a better comprehension of the process and an improvement in reaction rates,

depend greatly on one's knowledge of the mutual solubility between vegetable oils and ethanol and DMSO. Additionally, it should be noted that phase equilibrium information for ternary mixture systems is required for the development of liquid–liquid extraction procedures for the extraction of carotenoids. Thus, the aim of the present work was to investigate the phase equilibrium of palm oil + ethanol + DMSO at temperatures ranging from 40, 45, and 50 °C. The vegetable oils chosen for this study were palm oils. Such solvents are already used in the extraction process and are considered solvents with low toxicity.

2. Materials and methods

2.1. Materials

The palm oil was purchased from Ayasco (Kano, Nigeria) and was shielded from light for the total use time. The chemicals used were ethanol (>99.4 % purity, Sigma Aldrich, USA), DMSO (99.9 % purity, Perfyl Tech, Brazil), acetic acid (99.5% purity, C.R. Product, Brazil), and n-hexane (95 % purity, Química Moderna, Brazil). The equipment needed to carry out the experiments was as follows: liquid–liquid cell, Marconi Ma 085 magnetic stirrer, balance analytical Shimadzu AUY 220Ultra-thermostatic bath Brand and Model -TE-184, Country -Brazil and digital densimeter EDM 4000 Schmidt Haensch.

2.2 Methods

Apparatus and Procedures. Determination of Liquid -Liquid Equilibrium using the glass equilibrium cell model proposed by Silva et al. (1997). Palm oils were mixed for the experiment. Ethanol, DMSO, or acetic acid in oil/solvent mass ratio of 1:1. Each component was weighed on an analytical balance (AUY 220, Shimadzu, Japan), accurate to ± 0.0001 g. The mixtures were prepared inside the cell and then vigorously agitated for 15 min with a magnetic stirrer (Magnetic stirrer BS-2H, Brazil), and the temperature was controlled with a thermostatic bath (thermostatic bath TE-184, Brazil), accurate to ± 0.01 K. After a clear and well-defined interface was formed (approximately 24 h later), samples of both phases were collected separately using syringes. The solvent was evaporated in an oven (Marconi model, MA03213, Brazil) for 3 h, sufficient conditions for the remaining mixture to achieve a constant mass. Once the amount of solvent has been established, the amount of oil is subsequently calculated by computing the difference. All measures in this study were carried out in duplicate.

2.2.1 Determination of carotene content

The carotene content of palm oil was quantified using ultraviolet-visible (UV–Vis) spectroscopy (SKU MTH 5100, Kasuaki, Shanghai-China). A sample mass of 0.1 g was mixed with 25 mL of hexane. The diluted sample was transferred to a cuvette with a path length of 1 cm, followed by wavelength measurement at 446 nm. The carotene content in the sample was calculated using Equation 1 (Lau et al.,

2008).

$$\text{Carotenoid content } ((\mu g/g) = 25\ x\ \frac{383}{100W}\ x\ (a_s - a_b) \quad (7)$$

Where: 25 is the volume of solvent used in the analysis (mL), a_s is the absorbance of the sample, a_b is the absorbance of the blank (hexane) and W is the mass of the sample (g).

2.2.2 Partition coefficient (k)

The partition coefficient (k) is a measure of the distribution of carotenoids in the two phases that make up the system, and is expressed as described in equation 3.4, by the ratio between the masses of carotenoids in the extract and raffinate phase, obtained through the concentration analysis carried out by spectrophotometry and mass balance of the systems under study.

Partition coefficients of palm carotene in the extraction system were calculated using Eq. 8.

$$Partition\ coeficient\ (k) = \frac{mass\ of\ carotenoid\ in\ extract\ phase\ (\mu g)}{mass\ of\ carotenoid\ in\ raffinate\ phase\ (\mu g)} \quad (8)$$

2.2.3 Construction of the binodal curve

The binodal curve for the palm oil + DMSO + Ethanol pseudoternary systems at temperatures of 40, 45 and 50°C was performed using the turbidimetric method. First, the cell temperature was adjusted and 2 ml of oil were added. After temperature stabilization, 100 µL of solvent was added to the oil, under vigorous stirring. Each solvent was added separately. Thus, solvent titration was carried out until the oil inside the cell became visibly cloudy. When this condition was observed, the amount of solvent used was recorded to determine a point on the binodal curve. After that, 50 µL of solvent were added until the mixture became completely homogeneous. The described titration procedure was repeated until a sufficient number of points was obtained to construct the curve. Thus, from the elaborated binodal curves, it was possible to delimit the biphasic region of the palm oil + solvents mixture to proceed with the thermodynamic study of the systems and separation of carotenoids present in the oil.

After experimentally obtaining these points, mathematical models described by equations (4.1), (4.2) and (4.3) were adjusted to the data to predict the binodal curves. Equation (9), originally proposed by Merchuk, Andrews and Asenjo (1998), presents 3 adjustable parameters. Aiming to increase the accuracy of the adjustment, other collaborators proposed empirical equations (10) and (11) that also describe the curve binodal, but with 4 adjustable parameters (ALVAREZ-GUERRA et al., 2016; HAN et al., 2012; LI et al., 2014; MERCHUK; ANDREWS; ASENJO, 1998).

$$w_1 = a \cdot \exp\left(b \cdot w_2^{0,5} - c \cdot w_2^3\right) \quad (4.1)$$

$$w_1 = a + b w_2^{0,5} + c w_2 + d w_2^2 \quad (4.2)$$

$$w_1 = \exp\left(a + b w_2^{0,5} + c w_2 + d w_2^2\right) \quad (4.3)$$

Where: w_1 is the mass fraction of palm oil; w_2 is the solvent mass fraction and a, b, c and d are the model fit parameters.

2.2.4 Construction of mooring lines

With the construction of the binodal curve, it was possible to delimit the phase separation region and choose global mixing points to determine the tie lines for the Palm oil + DMSO + Ethanol system. The mooring lines were obtained using the gravimetric method described by Merchuk et al. (1998). Thus, points in the biphasic region were selected and mixtures (palm oil + solvents) were prepared, with identified global compositions, at temperatures of 40, 45 and 50 °C.

The components of the mixture were then weighed, added into 15 mL Falcon tubes, and subjected to vigorous agitation for approximately 30 minutes. Then, the tubes were kept closed and at rest for 12 hours, in a thermostatic bath with controlled temperature to separate the phases and to establish equilibrium. After that, the two phases (extract and raffinate) were separated and weighed on an analytical balance.

The composition analysis was performed using the gravimetric method, based on mass balance equations, with no need for chemical analyzes (MERCHUK; ANDREWS; ASENJO, 1998). Thus, in a region far from the interface, samples of the two phases (extract and refined) were collected using disposable syringes, for

weighing on a scale analytics. To ensure the reliability of the results, analyzes were performed in duplicate.

The mass balance equations proposed by Merchuk et al. (1998), for composition analysis, are presented below as a system of equations with the oil and solvent compositions of each phase as unknowns.

$$w_1^t = a \cdot \exp\left(b \cdot w_2^{t\,0,5} - c \cdot w_2^{t\,3}\right) \quad (4.4)$$

$$w_1^b = a \cdot \exp\left(b \cdot w_2^{b\,0,5} - c \cdot w_2^{b\,3}\right) \quad (4.5)$$

$$w_1^t = \frac{w_1^m}{\propto} - \left[\frac{1-\propto}{\propto}\right] \cdot w_1^b \quad (4.6)$$

$$w_2^t = \frac{w_2^m}{\propto} - \left[\frac{1-\propto}{\propto}\right] \cdot w_2^b \quad (4.7)$$

$$\propto = \frac{m\ topo}{m\ total} \quad (4.8)$$

Where: w_1^t and w_2^t are the oil and solvent compositions in the top phase respectively, w_1^b and w_2^b are the oil and solvent compositions in the top phase bottom respectively, w_1^m and w_2^m are the overall mixture compositions in the system, m top is the total mass of the top phase and m total is the total mass of the system.

3. Results and discussion

For each solvent, the extraction temperatures were 40, 45, and 50°C.

3.1 Pseudo-binary systems

Table 3.1 presents the experimental data to determine the pseudo-binary systems composed of palm oil + ethanol at atmospheric pressure.

Table 3.1: Liquid-liquid equilibrium data for palm oil (w_1) + anhydrous ethanol (w_2) systems.

Temperature	Overall composition		Solvent-rich phase		Oil-rich phase	
	100 w_1	100 w_2	100 w_1	100 w_2	100 w_1	100 w_2
	49.87	50.13	10.58	89.42	87.64	12.36
40	38.22	61.78	10.36	89.64	87.59	12.41
	35.26.	64.74	10.54	89.46	87.61	12.39
	30.01	69.99	10.56	89.44	87.51	12.49
	49.73	50.27	12.45	87.55	85.24	14.76
45	37.66	62.34	12.21	87.79	85.51	14.49
	34.94	65.06	12.49	87.51	85.36	14.64
	29.69	70.31	12.45	87.55	85.31	14.69
	49.75	50.25	14.44	85.56	81.84	18.16
50	38.11	61.89	14.54	85.46	81.53	18.47
	35.00	65.00	14.13	85.87	81.81	18.19
	34.72	65.28	14.34	85.66	81.66	18.34

Figure 3.1 show the equilibrium diagrams at 40, 45, and 50°C for palm oil + ethanol obtained experimentally through graphical representation.The solubility of anhydrous ethanol with palm oil increased with increasing temperature. Thus, an increase in the mutual solubility of palm oil and ethanol was noticed with temperature increase, despite the component's different polarities and sizes. The oil-solvent miscibility dependence on the temperature agrees with the studies of Da Silva et al. (2010) and Rodrigues & Oliveira (2010) concerning mutual solubility for systems composed of vegetable oil + ethanol + water at different temperatures. According to Arnold & Choudhury (1962), ethanol is an appropriate solvent for vegetable oil extraction, and Macías-Sánchez et al. (2008) reported that acquiring carotenoids with ethanol provides a high-speed process.

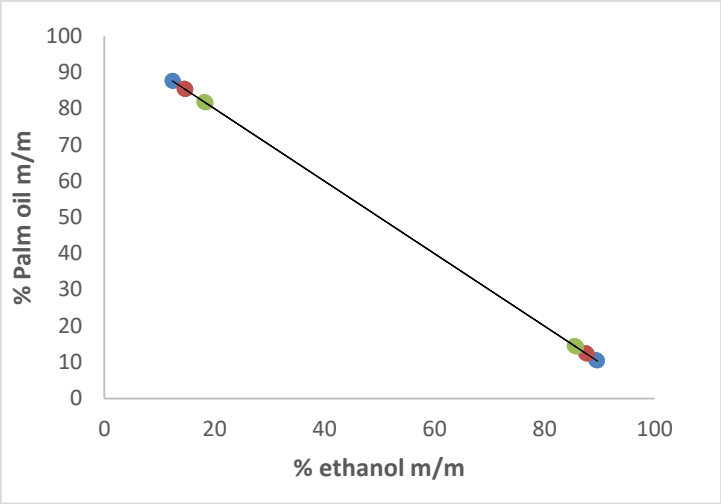


Figure 3.1: Equilibrium system contains Palm oil + ethanol at different temperatures

Table 3.2 and Figure 3.2 present the distribution coefficients (K) for palm oil + ethanol systems obtained at different temperatures and oil: solvent ratios. The 70/30 ratio was a good for the distribution coefficients at all temperatures for carotene extraction, followed by 65/35, 60/40, and 50/50. The temperature of 50 °C was best for all the extraction processes. These findings are in agreement with Oliveira et al. (2012).

Table 3.2: Distribution coefficient (K) at different temperatures for ethanol: palm oil ratios

Temperature °C	**K in Different Proportions**			
	50/50	**60/40**	**65/35**	**70/30**
40	0.30	0.34	0.41	0.47
45	0.33	0.40	0.42	0.50
50	0.40	0.52	0.58	0.67

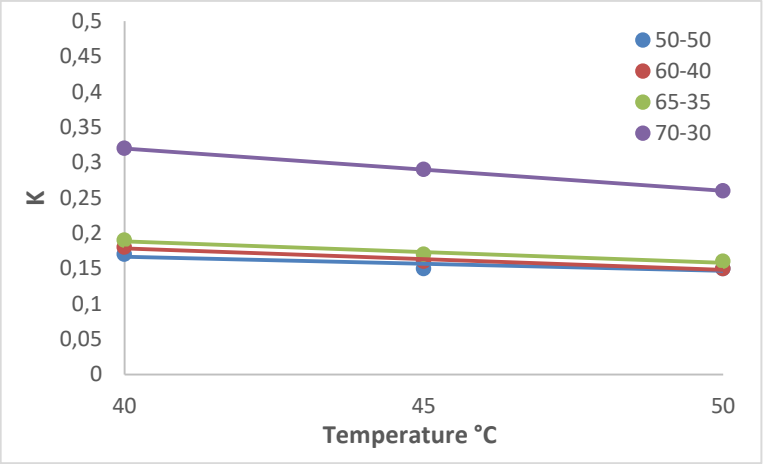


Figure 3.2: Distribution coefficient (K) at different temperatures and compositions using ethanol.

The distribution coefficient (k) of the carotene extracted from the oil-rich phase and solvent-rich phase was found to be desirable in the 70/30 composition of solvent and oil, which means that more solvent has a significantly higher increase in the extraction efficiency, which is in agreement with the findings of (Bou Orm et al., 2020) using ethanol solvent. Similar agreement was also reported by (Kua et al., 2018).

Table 3.3: Liquid–liquid equilibrium data obtained from systems containing palm oil (w_1) + dimethyl sulfoxide (w_2) at different temperatures.

Temperature	***Overall composition***		***Solvent-rich phase***		***Oil-rich phase***	
40	100 w_1	100 w_2	100 w_1	100 w_2	100 w_1	100 w_2
	49.94	50.06	4.18	95.82	88.56	11.44
	40.06	59.94	4.12	95.88	88.27	11.73
	34.96	65.04	4.02	95.98	88.19	11.81
	29.85	70.15	3.95	96.05	87.73	12.27
45	50.17	49.83	6.05	93.95	86.59	13.41
	40.11	59.89	5.92	94.08	86.42	13.68
	35.77	64.23	5.83	94.17	86.23	13.77
	30.14	69.86	5.63	94.37	86.16	13.84
50	49.65	50.35	7.04	92.96	83.64	16.36
	39.71	60.29	6.94	93.06	83.45	16.55

34.87	65.03	6.84	93.16	83.42	16.58
30.11	69.89	6.72	93.28	83.29	16.71

The only solvent mixture that was substantially denser than palm oil was discovered to be the DMSO mixture. Conditions for LLE at various temperatures show that the temperature has little effect on how effectively LLE extracts. Since the solvent mixture had good phase-forming and extraction capabilities, it was used in LLE. In comparison, the extraction with ethanol was found to be better than that with DMSO because DMSO is a polar solvent resulting in the poor recovery of palm carotene from palm oil, which is nonpolar and tends to have high hydrophobicity. This is in agreement with (Hoe et al., 2022). Below are the diagrams for palm oil + DMSO obtained experimentally through graphical representation, as shown in Figure 3.3.

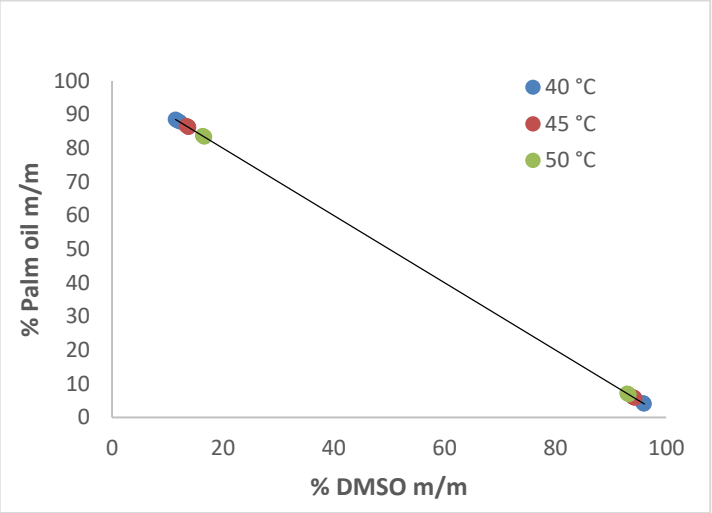


Figure 3.3: Equilibrium system contains Palm oil + DMSO at different temperatures

Distribution coefficient (K) at different temperatures and compositions from data obtained in Table 3.4 using equilibrium data from Table 3.3, as shown below.

Table 3.4: Distribution coefficient (K) at different temperatures and compositions

Temperature °C	**K in Different Proportions**			
	50/50	**60/40**	**65/35**	**70/30**
40	0.25	0.27	0.30	0.33
45	0.36	0.40	0.43	0.45

50	0.44	0.45	0.49	0.52

The distribution coefficient (k) of the carotene extracted from the oil-rich phase and solvent-rich phase was found to be desirable in the 70/30 composition of solvent and oil, followed by 65/35, 60/40, and 50/50, which shows that as the proportion of solvent increases the distribution coefficient increases as well, and the extraction was good at a temperature of 50 °C. (Lau et al., 2006) investigated the extraction of palm oil from mesocarp using supercritical carbon dioxide and reported that the carotene content increased gradually when increasing the operating temperature. Below is the graphical representation of the distribution coefficient, as shown in Figure 3.4.

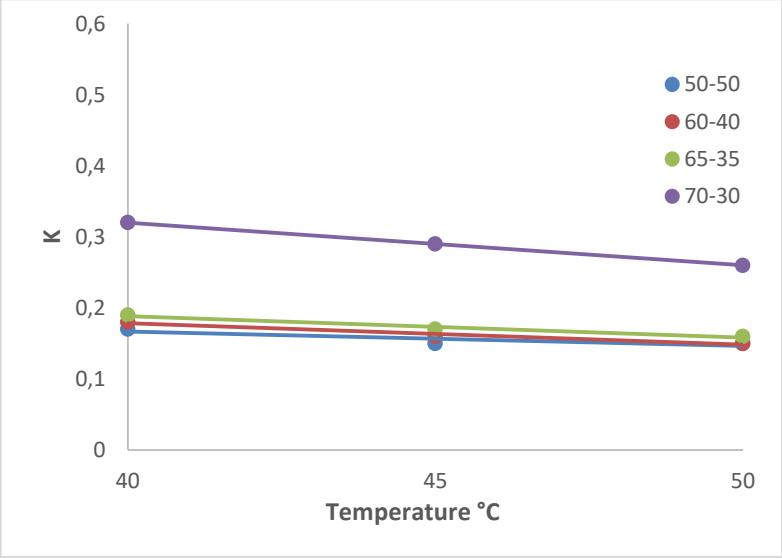


Figure 3.4: Distribution coefficient (K) at different temperatures and compositions using DMSO

Table 3.5: Liquid–liquid equilibrium data obtained from systems containing palm oil (w_1) + acetic acid (w_2) at different temperatures.

Temperature	***Overall composition***		***Solvent-rich phase***		***Oil-rich phase***	
40	100 w_1	100 w_2	100 w_1	100 w_2	100 w_1	100 w_2
	50.03	49.97	4.18	92.82	73.43	26.57
	40.19	59.81	8.01	92.82	73.06	26.94
	34.96	65.04	7.43	92.57	72.51	27.49
	30.08	69.92	7.72	92.28	72.99	27.01

45	50.07	49.93	10.44	89.56	71.89	28.11
	39.99	60.01	10.47	89.53	71.94	28.06
	34.97	65.03	10.14	89.86	71.85	28.15
	29.99	70.01	10.87	89.13	71.92	28.08
50	50.01	49.99	13.41	86.59	69.24	30.75
	39.68	60.32	13.38	86.62	69.36	30.64
	35.06	64.94	13.19	86.81	69.17	30.83
	30.08	69.92	13.38	86.61	69.34	30.66

From liquid–liquid equilibrium data obtained from systems containing palm oil (w_1) + acetic acid (w_2) at different temperatures, the oil phase and solvent phase extraction were investigated at temperatures ranging from 40, 45, and 50 °C. The results of the present study indicated that the mutual solubility of the oil decreases with an increase in the solvent and an increase in the temperature of the solution. The results reveal the formation of a biphasic system consisting of an oil phase enriched in nonpolar compounds. This trend is in agreement with results reported by (Rasrendra et al., 2011)

Figures 3.5 show the graphical presentation of the data obtained in Table 3.4.

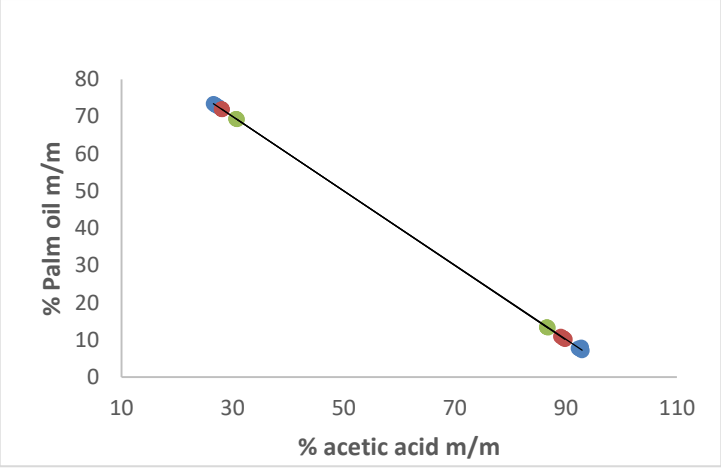


Figure 3.5: Equilibrium system contains Palm oil + acetic acid at different temperatures.

The distribution coefficients (K) at different temperatures and compositions are shown in Table 3.6.

Table 3.6: Distribution coefficient (K) at different temperatures and compositions

Temperature °C	**K in Different Proportions**			
	50/50	**60/40**	**65/35**	**70/30**
40	0.17	0.18	0.19	0.32
45	0.15	0.16	0.17	0.29
50	0.15	0.15	0.16	0.26

Below is the graphical representation of the distribution coefficient, as shown in Figure 3.6.

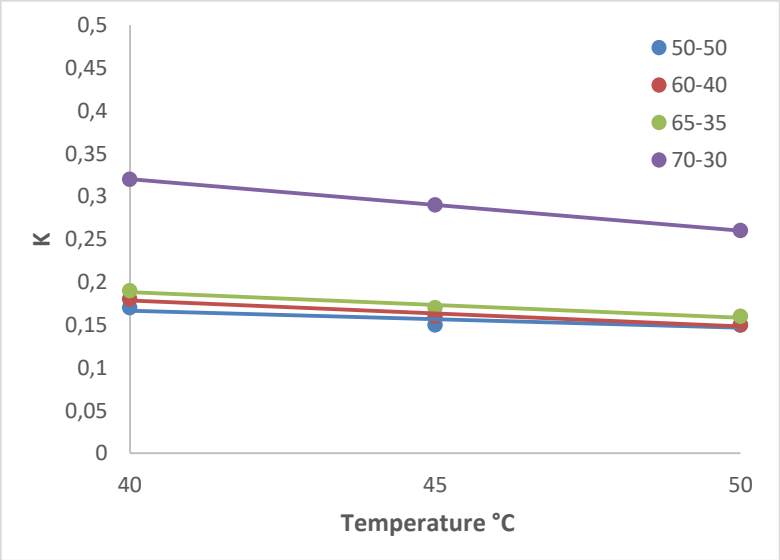


Figure 3.6: Distribution coefficient (K) at different temperatures and compositions using acetic acid.

From the results above, the extraction at proportions of 70 solvent and 30 oil was found to be good proportion and temperature for carotene extraction, followed by 65/35 60/40, and 50/50. 40 °C was the best temperature for all the extraction processes.

Dimethyl sulfoxide (DMSO), acetic acid, and ethanol are the three solvents utilized in the extraction. A comparison of the listed solvents in these studies of palm carotene liquid–liquid extraction from palm was made. The distribution coefficients and the selectivity factors of solvents for the extraction of carotenoids from oil palm oil at 40, 45, and 50 °C were investigated. Among the three extraction solvents,

ethanol was found to be the best solvent for extraction because of the biphasic system formation and affinity of carotenoids toward the solvent, followed by DMSO and acetic acid. The lower extraction efficiency with DMSO may be due to the polarity, which may lead to poor extraction and a high density difference between palm oils. Extraction of carotenoids using acid solvent was proven to be possible, but distribution coefficients were not as high as expected.

For the Palm Oil + DMSO + Ethanol system, two binodal curves were obtained. The experimental data for their construction, as well as the adjustment parameters for the empirical equations proposed by Merchuk, Andrews and Asenjo (1998), Alvarez-guerra et al. (2016), Han et al. (2012) and Li et al. (2014), are tabulated below.

3.2 For the left binodal curve

Table 3.7: Experimental data for the construction of the binodal curve (on the left) of the system: Palm Oil (1), DMSO (2) and Ethanol (3).

40°C		45°C		50°C	
100w1	**100w2**	**100w1**	**100w2**	**100w1**	**100w2**
0.0122	0.9829	0.0187	0.9783	0.0227	0.9639
0.0144	0.9533	0.0194	0.9607	0.02446	0.9378
0.0181	0.9342	0.0209	09433	0.02643	0.8693
0.0197	0.9056	0.0224	0.9126	0.02847	0.8344
0.0218	0.8804	0.0249	0.8752	0.03067	0.8025
0.0242	0.8495	0.0263	0.8398	0.03581	0.7527
0.0254	0.8208	0.0287	0.8165	0.03767	0.7287
0.0271	0.7813	0.0308	0.7904	0.04126	0.6814
0.0307	0.7658	0.0317	0.7658	0.04353	0.6553
0.0314	0.7375	0.0331	0.7375	0.04492	0.6219
0.0321	0.7081	0.0364	0.6925	0.05123	0.5938
0.0342	0.6806	0.0381	0.6732	0.0539	0.5425
0.0358	0.6542	0.0406	0.6438	0.05583	0.5284
0.0379	0.6214	0.0435	0.6021	0.05935	0.4691
0.0410	0.5782	0.0463	0.5682	0.06268	0.4193

0.0432	0.5457	0.0492	0.5371	0.06598	0.3839
0.0455	0.5249	0.0552	0.47832	0.07032	0.3522
0.0471	0.4907	0.0598	0.4256	0.0753	0.2871
0.0493	0.4682	0.0633	0.3975	0.0778	0.2417
0.0529	0.4237	0.0675	0.3641	0.0792	0.2065
0.0554	0.4025	0.0683	0.3379	0.0863	0.1638
0.0575	0.3833	0.0721	0.3025	0.0896	0.1239
0.0583	0.3647	0.0739	0.2668	0.0963	0.0743
0.0601	0.3345	0.0785	0.2192		
0.0622	0.2976	0.0795	0.1827		
0.0632	0.2576	0.0873	0.1526		
0.0667	0.2261	0.0902	0.0983		
0.0693	0.1983				
0.0714	0.1538				
0.0783	0.1145				

Table 3.8: Parameters for adjusting the binodal curves by equation 4.1 for the Palm Oil + DMSO + Ethanol system.

Temperature °C	***a***	***b***	***c***	***r²***	***100sd****
40	0.1059	-0.9356	1.1054	0.9957	0.1183
45	0.1299	-1.0712	1.021	0.9955	0.1480
50	0.1248	-0.9327	1.0023	0.9964	0.1314

*Standard error of estimate

Table 3.9: Parameters for adjusting the binodal curves by equation 4.2 for the Palm Oil + DMSO + Ethanol system.

Temperature °C	***a***	***b***	***c***	***d***	***r²***	***100sd****
40	0.0953	-0.0457	-0.0252	-0.0120	0.9962	0.1196
45	0.0858	0.0717	-0.1949	0.0549	0.9978	0.0962
50	0.1054	-0.0156	-0.0808	0.0095	0.9956	0.1172

*Standard error of estimate

Table 3.9.1: Parameters for adjusting the binodal curves using equation 4.3 for the Palm Oil + DMSO + Ethanol system.

Temperature °C	*a*	*b*	*c*	*d*	*r²*	*100sd**
40	-1.8912	-2.9181	3.1039	-2.5558	0.9955	0.1196
45	-2.2922	-0.1678	-0.5299	-1.0424	0.9980	0.0962
50	-1.9979	-1.6053	1.4181	-1.7709	0.9969	0.1172

*Standard error of estimate

3.3 For the right binodal curve:

Table 3.9.2: Experimental data for the construction of the binodal curve (on the right) of the system: Palm Oil (1), DMSO (2) and Ethanol (3).

40°C		**45°C**		**50°C**	
100w1	**100w2**	**100w1**	**100w2**	**100w1**	**100w2**
0.9032	0.0968	0.8761	0.1239	0.8356	0.1279
0.8953	0.0668	0.8700	0.1016	0.8340	0.1660
0.8861	0.0472	0.8525	0.0734	0.8265	0.1012
0.8645	0.0258	0.8407	0.0569	0.8133	0.0623
0.8219	0.0000	0.830	0.0357	0.7657	0.0000
		0.7930	0.0000		

Table 3.9.3: Parameters for adjusting the binodal curves by equation 4.1 for the Palm Oil + DMSO + Ethanol system.

Temperature °C	*a*	*b*	*c*	*r²*	*100sd**
40	0.8210	0.3467	13.2155	0.9919	0.29
45	0.7922	0.2564	-6.7796	0.9884	0.33
50	0.7652	0.2569	3.7350	0.9902	0.29

*Standard error of estimate

Table 3.9.4: Parameters for adjusting the binodal curves by equation 4.2 for the Palm Oil + DMSO + Ethanol system.

Temperature °C	*a*	*b*	*c*	*d*	*r²*	*100sd**
40	0.8219	0.1214	1.0858	-6.5928	0.9971	0.18
45	0.7931	0.0976	0.5285	-1.0156	0.9909	0.29
50	0.7657	0.0309	0.8082	-2.8306	0.9916	0.26

*Standard error of estimate

Table 3.9.5: Parameters for adjusting the binodal curves using equation 4.3 for the Palm Oil + DMSO + Ethanol system.

Temperature °C	*a*	*b*	*c*	*D*	*r²*	*100sd**
40	-0.1962	0.1626	1.1609	-7.3483	0.9969	0.18
45	-0.2318	0.1257	0.6267	-1.3649	0.9910	0.29
50	-0.2669	0.0566	0.9420	-3.3920	0.9918	0.26

*Standard error of estimate

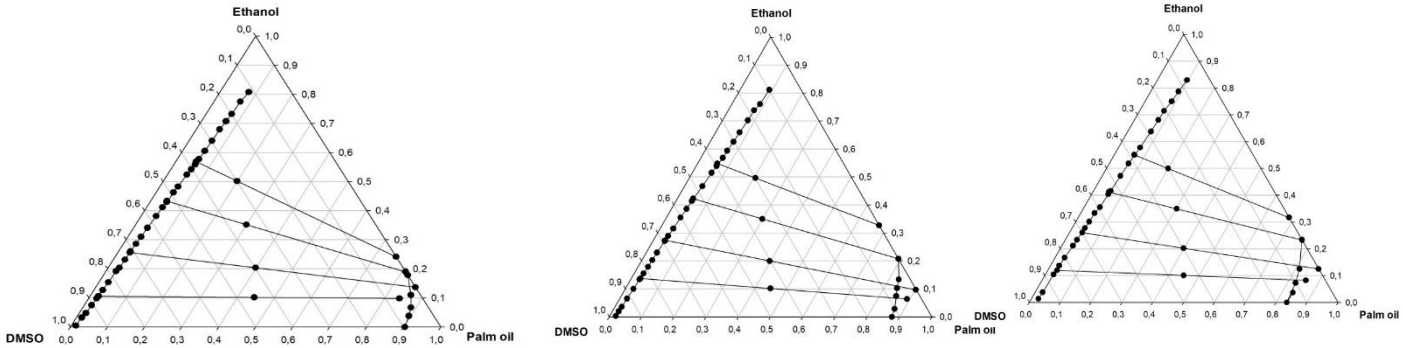
As shown in the table, the experimental data fit well with the described models, since the determination coefficients for the three equations were also satisfactory (> 0.9). In this case, model 3 better described the binodal curves at all temperatures (40°C, 45°C and 50°C), and for this reason was selected for the construction of the mooring lines for these systems.

Table 3.9.6: Equilibrium data for the system (1) Palm Oil, (2) DMSO and (3) Ethanol.

System total		**Extract phase**		**Raffinate Phase**	
100w1	**100w2**	**100w1**	**100w2**	**100w1**	**100w2**
		40°C			
0.3984	0.3983	0.0328	0.7111	0.8633	0.0005
0.2995	0.3488	0.0441	0.5231	0.8106	0.0000
0.1994	0.2994	0.0547	0.3768	0.7590	0.0000
0.4456	0.4528	0.0231	0.8711	0.8399	0.0625
		45°C			
0.3969	0.4034	0.0375	0.6895	0.9014	0.0018
0.2994	0.3506	0.0488	0.5287	0.7925	0.0003

0.2050	0.2989	0.0604	0.3912	0.6731	0.0002
0.4487	0.4499	0.0281	0.8346	0.8909	0.0454
		50°C			
0.3984	0.3994	0.0411	0.6984	0.8745	0.0009
0.3025	0.3484	0.0521	0.5360	0.7675	0.0001
0.2002	0.3004	0.0640	0.3851	0.6832	0.0001
0.4497	0.4498	0.0302	0.8501	0.8550	0.0630

Figures 3.7a, 3.7b and 3.7c show the equilibrium diagrams for the Palm oil + DMSO + Ethanol system obtained experimentally.



a) **b)** **c)**

Figure:3.7a, 37b, and 3.7c: Binodal curve for the Palm Oil + DMSO + Ethanol system at 40 ,45 and 50 °C

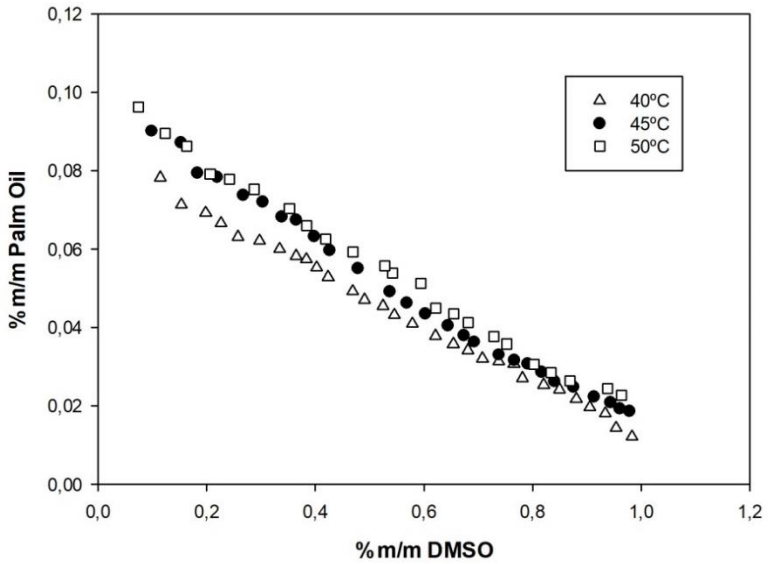


Figure 3.8: Influence of temperature on binodal curves

As shown, temperature influences the size of the biphasic region. However, above 40°C there is a decrease in the two-phase region, which can be explained by the fact that the increase in temperature provides greater solubility between the components of the system.

Table 3.9.7 shows the partition coefficients found in the respective extractions performed using different oil-solvent ratios.

Table 3.9.7: Partition coefficients found in the extraction of carotenoids with a mixture of DMSO and Ethanol solvents.

Temperature °C	**Proportion Oil-DMSO- Ethanol**	**K**
40	45-45-10	0.35
	40-40-20	0.41
	30-35-35	0.45
	20-30-50	0.53
45	45-45-10	0.48
	40-40-20	0.52
	30-35-35	0.58
	20-30-50	0.64
50	45-45-10	0.43
	40-40-20	0.47
	30-35-35	0.53
	20-30-50	0.60

As exemplified in the previous ternary diagrams, from the use of the Palm Oil + DMSO + Ethanol system, which presents a large biphasic region, a greater partitioning of carotenoids in the extract phase was observed, when compared to the exclusive use of DMSO as solvent of extraction. Thus, as shown in table X, higher partition coefficients (K) were found in mixtures at 45 and 50 ºC, composed of mixtures with higher proportions of ethanol and, consequently, lower proportions of

DMSO and oil. In this context, aiming at an extraction with better results from the qualitative and economic points of view, it is recommended to use a temperature of 45ºC due to the possible degradation of the dyes when exposed to high temperatures.

4. Conclusions

This work presents experimental results of palm oil extraction using liquid–liquid extraction. Ethanol, DMSO and acetic acid are solvents used at different temperatures and operation conditions. Solvent mixture was adopted in LLE due to its good phase-forming ability and extraction performance. Toxicity, vapor pressure, density, chemical reactivity, and solvent viscosity are factors to consider when choosing a solvent. A temperature of 50 °C was found to be the best temperature for extraction in all solvents used. The distribution coefficient was used to evaluate the effectiveness of the extraction of palm carotene. The 70 solvents and 30 oils showed the highest values for both solvents used, which are :ethanol and DMSO, and acetic acid. The Liquid–liquid experimental data of the palm oil + ethanol+ DMSO system were experimentally determined at temperatures ranging from 40, 45, and 50°C. Binodal curves were presented and demonstrated the influence of temperature on the size of the biphasic region. (40 °C, 45 °C and 50 °C), are all temperatures selected for the construction of binodal curves. The mutual solubility of the reaction system depended on the oil type and was sensitive to temperature changes. All the experimental data fitted well to the models applied in both the left and right binodal curves. The coefficients of determination for the three equations were satisfactory (> 0.99). All models were suitable for the construction of mooring lines and for the determination of the critical points of this system.

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