

CHAPTER ONE

INTRODUCTION

EXTRACTION AND CHARACTERIZATION OF VEGETABLE OIL USING BREAD FRUIT SEED.

1.1 Vegetable oil

A vegetable oil is a triglyceride extracted from a plant. Such oils have been part of human culture for millennia. The term "vegetable oil" can be narrowly defined as referring only to substances that are liquid at room temperature, or broadly defined without regard to a substance's state of matter at a given temperature. For this reason, vegetable oils that are solid at room temperature are sometimes called vegetable fats. Vegetable oils are composed of triglycerides, as contrasted with waxes which lack glycerin in their structure. Although many plant parts may yield oil, in commercial practice, oil is extracted primarily from seeds.

1.2 Production of Vegetable Oils

To produce vegetable oils, the oil first needs to be removed from the oil-bearing plant components, typically seeds. This can be done via mechanical extraction using an oil mill or chemical extraction using a solvent. The extracted oil can then be purified and, if required, refined or chemically altered.

1.2.1 Mechanical extraction

Oils can also be removed via mechanical extraction, termed "crushing" or "pressing." This method is typically used to produce the more traditional oils (e.g., olive, coconut etc.), and it is preferred by most health food customers in the United States and in Europe. There are several different types of mechanical extraction: expeller-pressing extraction is common, though the screw press, ram press, and Ghani (powered mortar and pestle) are also used. Oil seed presses are commonly used in developing countries, among people for whom other extraction methods would be prohibitively expensive; the Ghani is primarily used in India.

1.2.2 Solvent extraction

The processing of vegetable oil in commercial applications is commonly done by chemical extraction, using solvent extracts, which produces higher yields and is quicker and less expensive. The most common solvent is petroleum-derived hexane. This technique is used for most of the "newer" industrial oils such as soybean and corn oils. Supercritical carbon dioxide can be used as a non-toxic alternative to other solvents.

1.2.3 Sparging

In the processing of edible oils, the oil is heated under vacuum to near the smoke point, and water is introduced at the bottom of the oil. The water immediately is converted to steam, which bubbles through the oil, carrying with it any chemicals which are water-soluble. The steam sparging removes impurities that can impart unwanted flavors and odors to the oil.

1.2.4 Hydrogenation

Oils may be partially hydrogenated to produce various ingredient oils. Lightly hydrogenated oils have very similar physical characteristics to regular soya oil, but are more resistant to becoming rancid. Hardening vegetable oil is done by raising a blend of vegetable oil and a catalyst in near-vacuum to very high temperatures, and introducing hydrogen. This causes the carbon atoms of the oil to break double-bonds with other carbons, each carbon forming a new single-bond with a hydrogen atom. Adding these hydrogen atoms to the oil makes it more solid, raises the smoke point, and makes the oil more stable.

Hydrogenated vegetable oils differ in two major ways from other oils which are equally saturated. During hydrogenation, it is easier for hydrogen to come into contact with the fatty acids on the end of the triglyceride, and less easy for them to come into contact with the center fatty acid. This makes the resulting fat more brittle than a tropical oil; soy margarines are less "spreadable". The other difference is that trans fatty acids (often called trans fat) are formed in the hydrogenation reactor, and may amount to as much as 40 percent by weight of a partially hydrogenated oil. Hydrogenated oils, especially partially hydrogenated oils with their higher amounts of trans fatty acids are increasingly thought to be unhealthy.

1.3 Uses of triglyceride vegetable oil

The following are some of the uses of vegetable oils:

1) Culinary uses: Many vegetable oils are consumed directly, or indirectly as ingredients in food – a role that they share with some animal fats, including butter and ghee;

2) Industrial uses: Vegetable oils are used as an ingredient or component in many manufactured products. Many vegetable oils are used to make soaps, skin products, candles, perfumes and other personal care and cosmetic products. Some oils are particularly suitable as drying oils, and are used in making paints and other wood treatment products. Dammar oil (a mixture of linseed oil and dammar resin), for example, is used almost exclusively in treating the hulls of wooden boats. Vegetable oils are increasingly being used in the electrical industry as insulators .

3) Pet food additive: Vegetable oil is used in production of some pet foods. In some poorer grade pet foods though, the oil is listed only as "vegetable oil", without specifying the particular oil.

4) Fuel: Vegetable oils are also used to make biodiesel, which can be used like conventional diesel. Some vegetable oil blends are used in unmodified vehicles but straight vegetable oil, also known as pure plant oil, needs specially prepared vehicles which have a method of heating the oil to reduce its viscosity. The vegetable oil economy is growing and the availability of biodiesel around the world is increasing. It is believed that the total net greenhouse gas savings when using vegetable oils in place of fossil fuel-based alternatives for fuel production, range from 18 to 100% [10].

1.4 Negative health effects

Hydrogenated oils have been shown to cause what is commonly termed the "double deadly effect", raising the level of low density lipoproteins (LDLs) and decreasing the level of high density lipoproteins (HDLs) in the blood, increasing the risk of blood clotting inside blood vessels.

A high consumption of omega-6 polyunsaturated fatty acids (PUFAs), which are found in most types of vegetable oil (e.g. soyabean oil, corn oil– the most consumed in USA, sunflower oil, etc.) may increase the likelihood that postmenopausal women will develop breast cancer. A similar effect was observed on prostate cancer in mice. Plant based oils high in monounsaturated fatty acids, such as olive oil, peanut oil, and canola oil are relatively low in omega-6 PUFAs and can be used in place of high-polyunsaturated oils.

1.5 Uses/Importance of Vegetable oils

1.5.1 Margarine

Margarine originated with the discovery by French chemist Michel Eugene Chereul in 1813 of margaric acid (itself named after the pearly deposits of the fatty acid from Greek (*margaritēs* / *márgaron*), meaning *pearl-oyster* or *pearl*, or (*margarís*), meaning palm-tree, hence the relevance to palmitic acid). Scientists at the time regarded margaric acid, like oleic acid and stearic acid, as one of the three fatty acids which, in combination, formed most animal fats. In 1853, the German structural chemist Wilhelm Heinrich Heintz analyzed margaric acid as simply a combination of stearic acid and of the previously unknown palmitic acid.

Emperor Louis Napoleon III of France offered a prize to anyone who could make a satisfactory substitute for butter, suitable for use by the armed forces and the lower classes. French chemist Hippolyte Mege-Mouries invented a substance he called oleomargarine, the name of which became shortened to the trade name "margarine". Mège-Mouriès patented the concept in 1869 and expanded his initial manufacturing

operation from France but had little commercial success. In 1871, he sold the patent to the Dutch company Jurgens, now part of Unilever. In the same year the German pharmacist Benedict Klein from Cologne founded the first margarine factory "Benedict Klein Margarinewerke", producing the brands Overstolz and Botteram.

Margarine is a semi-solid emulsion composed mainly of vegetable fats and water. While butter is derived from milk fat, margarine is mainly derived from plant oils and fats and may contain some skimmed milk. In some locales it is colloquially referred to as oleo, short for oleomargarine. Margarine, like butter, consists of a water-in-fat emulsion, with tiny droplets of water dispersed uniformly throughout a fat phase which is in a stable crystalline form. Margarine has a minimum fat content of 80%, the same as butter, but unlike butter reduced-fat varieties of margarine can also be labelled as margarine. Margarine can be used both for spreading or for baking and cooking. It is also commonly used as an ingredient in other food products, such as pastries and cookies, for its wide range of functionalities.

1.5.1.2 Manufacture of Margarine

The basic method of making margarine today consists of emulsifying a blend of hydrogenated vegetable oils with skimmed milk, chilling the mixture to solidify it and working it to improve the texture. Vegetable and animal fats are similar compounds with different melting points. Those fats that are liquid at room temperature are generally known as oils. The melting points are related to the presence of carbon-carbon double bonds in the fatty acids components. Higher number of double bonds give lower melting points.

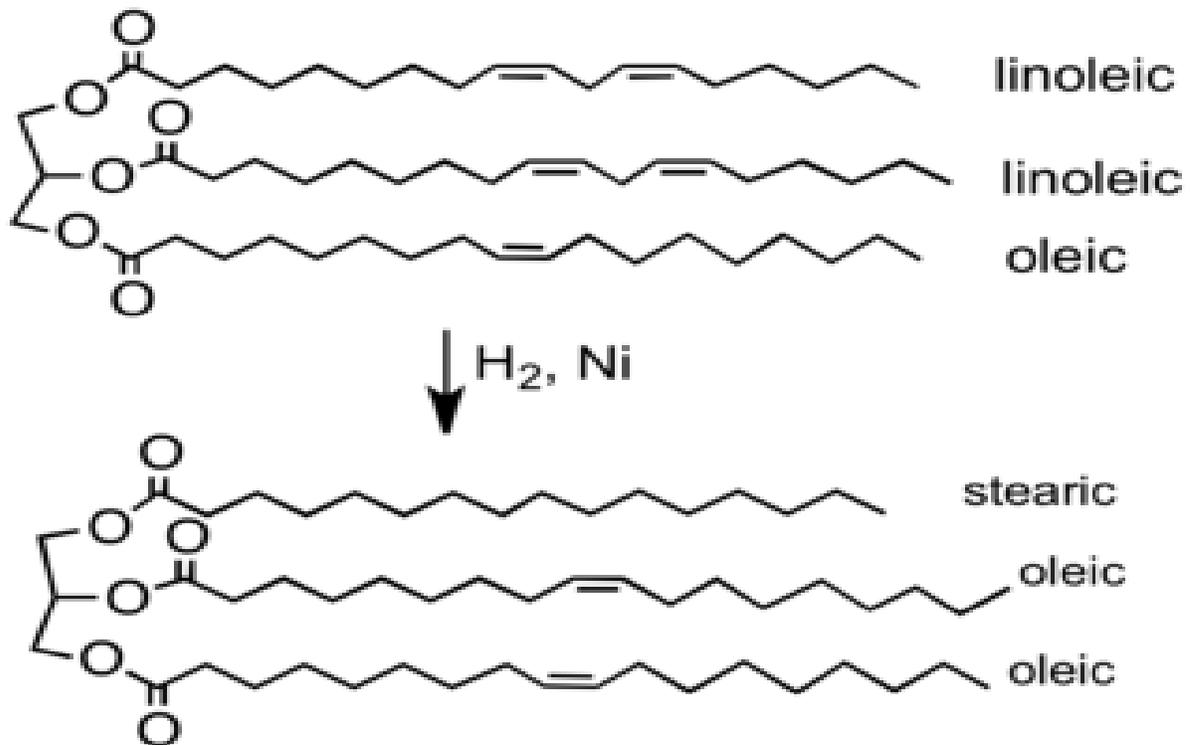


Figure 1: Hydrogenation of vegetable oils

Partial hydrogenation of a typical plant oil to a typical component of margarine, makes most of the C=C double bonds be removed in this process, which elevates the melting point of the product. Commonly, the natural oils are hydrogenated by passing hydrogen through the oil in the presence of a nickel catalyst, under controlled conditions. The addition of hydrogen to the unsaturated bonds (alkenic double C=C bonds) results in saturated C-C bonds, effectively increasing the melting point of the oil and thus "hardening" it. This is due to the increase in van der Waals' forces between the saturated molecules compared with the unsaturated molecules. However, as there are possible health benefits in limiting the amount of saturated fats in the human diet, the process is controlled so that only enough of the bonds are hydrogenated to give the required texture. Margarines manufactured in this way are said to contain hydrogenated fat. This method is used today for some margarines although the process has been developed and sometimes

other metal catalysts are used such as palladium. If hydrogenation is incomplete (partial hardening), the relatively high temperatures used in the hydrogenation process tend to flip some of the carbon-carbon double bonds into the "trans" form. If these particular bonds aren't hydrogenated during the process, they will still be present in the final margarine in molecules of trans fats, the consumption of which has been shown to be a risk factor for cardiovascular disease. For this reason, partially hardened fats are used less and less in the margarine industry. Some tropical oils, such as palm oil and coconut oil, are naturally semi solid and do not require hydrogenation.

Three types of margarine are common:

- Soft vegetable fat spreads, high in mono- or polyunsaturated fats, which are made from safflower, sunflower, soybean, cottonseed, rapeseed or olive oil.
- Margarines in bottle to cook or top dishes
- Hard, generally uncolored margarine for cooking or baking.

1.5.2 Soap

In chemistry, soap is a salt of a fatty acid. Soaps are mainly used as surfactants for washing, bathing, cleaning, in textile spinning and are important components of lubricants. Soaps for cleansing are obtained by treating vegetable or animal oils and fats with a strongly alkaline solution. Fats and oils are composed of triglycerides; three molecules of fatty acids are attached to a single molecule of glycerol. The alkaline solution, which is often called lye, (although the term "lye soap" refers almost exclusively to soaps made with sodium hydroxide) brings about a chemical reaction known as saponification. In saponification, the fats are first hydrolyzed into free fatty acids, which then combine with the alkali to form crude soap. Glycerol (glycerin) is

liberated and is either left in or washed out and recovered as a useful byproduct, depending on the process employed.

When used for cleaning, soap allows otherwise insoluble particles to become soluble in water and then be rinsed away. For example: oil/fat is insoluble in water, but when a couple drops of dish soap are added to the mixture the oil/fat apparently disappears. The insoluble oil/fat molecules become associated inside micelles, tiny spheres formed from soap molecules with polar hydrophilic (water-loving) groups on the outside and encasing a lipophilic (fat-loving) pocket, which shielded the oil/fat molecules from the water making it soluble. Anything that is soluble will be washed away with the water. Synthetic detergents operate by similar mechanisms to soap.

The type of alkali metal used determines the kind of soap produced. Sodium soaps, prepared from sodium hydroxide, are firm, whereas potassium soaps, derived from potassium hydroxide, are softer or often liquid. Historically, potassium hydroxide was extracted from the ashes of bracken or other plants. Lithium soaps also tend to be hard these are used exclusively in greases.

Typical vegetable oils used in soap making are palm oil, coconut oil, olive oil, and laurel oil. Each species offers quite different fatty acid content and, hence, results in soaps of distinct feel. The seed oils give softer but milder soaps. Soap made from pure olive oil is sometimes called Castile/Marseille soap, and is reputed for being extra mild. The term "Castile" is also sometimes applied to soaps from a mixture of oils, but a high percentage of olive oil.

1.5.2.1 Purification and finishing



Figure 2: A generic bar of soap, after purification and finishing

In the fully boiled process on factory scale, the soap is further purified to remove any excess sodium hydroxide, glycerol, and other impurities, colour compounds, etc. These components are removed by boiling the crude soap curds in water and then precipitating the soap with salt. At this stage, the soap still contains too much water, which has to be removed. This was traditionally done on chill rolls, which produced the soap flakes commonly used in the 1940s and 1950s. This process was superseded by spray dryers and then by vacuum dryers. The dry soap (about 6–12% moisture) is then compacted into small pellets or noodles. These pellets or noodles are then ready for soap finishing, the process of converting raw soap pellets into a saleable product, usually bars.

Soap pellets are combined with fragrances and other materials and blended to homogeneity in an amalgamator (mixer). The mass is then discharged from the mixer into a refiner, which, by means of an auger, forces the soap through a fine wire screen. From the refiner, the soap passes over a roller mill (French milling or hard milling) in a manner similar to calendering paper or plastic or to making chocolate liquor. The soap is then

passed through one or more additional refiners to further plasticize the soap mass. Immediately before extrusion, the mass is passed through a vacuum chamber to remove any trapped air. It is then extruded into a long log or blank, cut to convenient lengths, passed through a metal detector, and then stamped into shape in refrigerated tools. The pressed bars are packaged in many ways.

Sand or pumice may be added to produce a scouring soap. The scouring agents serve to remove dead cells from the skin surface being cleaned. This process is called exfoliation. Many newer materials that are effective, yet do not have the sharp edges and poor particle size distribution of pumice, are used for exfoliating soaps.

Nanoscope metals are commonly added to certain soaps specifically for both colouration and antibacterial properties. Titanium dioxide powder is commonly used in extreme "white" soaps for these purposes; nickel, aluminium and silver compounds are less commonly used. These metals exhibit an electron-robbing behaviour when in contact with bacteria, stripping electrons from the organism's surface, thereby disrupting their functioning and killing them. Since some of the metal is left behind on the skin and in the pores, the benefit can also extend beyond the actual time of washing, helping reduce bacterial contamination and reducing potential odours from bacteria on the skin surface.

1.5.3 Biodiesel production

Biodiesel production is the process of producing the biofuel/biodiesel, through the chemical reactions: transesterification and esterification. This involves vegetable or animal fats and oils being reacted with short-chain alcohols (typically methanol or ethanol). The major steps required to synthesize biodiesel are as follows:

- 1. Feedstock pretreatment:** Common feedstock used in biodiesel production include yellow grease (recycled vegetable oil), "virgin" vegetable oil, and tallow. Recycled oil is processed to remove impurities from cooking, storage, and handling, such as dirt, charred food, and water. Virgin oils are refined, but not to a food-grade level. De-gumming to remove phospholipids and other plant matter is common, though refinement processes vary. Regardless of the feedstock, water is removed as its presence during base-catalyzed transesterification causes the triglycerides to hydrolyse, giving salts of the fatty acids (soaps) instead of producing biodiesel.
- 2. Determination and treatment of free fatty acids:** A sample of the cleaned feedstock oil is titrated with a standardized base solution in order to determine the concentration of free fatty acids (carboxylic acids) present in the vegetable oil sample. These acids are then either esterified into biodiesel, esterified into glycerides, or removed, typically through neutralization.
- 3. Reactions:** Base-catalyzed transesterification reacts lipids (fats and oils) with alcohol (typically methanol or ethanol) to produce biodiesel and an impure co-product, glycerol. If the feedstock oil is used or has a high acid content, acid-catalyzed esterification can be used to react fatty acids with alcohol to produce biodiesel. Other methods, such as fixed-bed reactors, supercritical reactors, and ultrasonic reactors, forgo or decrease the use of chemical catalysts.
- 4. Product purification:** Products of the reaction include not only biodiesel, but also byproducts, soap, glycerol, excess alcohol, and trace amounts of water. All of these byproducts must be removed to meet the standards, but the order of removal is process-dependent. The density of glycerol is greater than that of biodiesel, and

this property difference is exploited to separate the bulk of the glycerol co-product. Residual methanol is typically recovered by distillation and reused. Soaps can be removed or converted into acids. Residual water is also removed from the fuel.

1.5.3.1 Reactions

Animal and plant fats and oils are composed of triglycerides, which are esters containing three free fatty acids and the trihydric alcohol, glycerol. In the transesterification process, the alcohol is de-protonated with a base to make it a stronger nucleophile. Commonly, ethanol or methanol are used. As can be seen, the reaction has no other inputs than the triglyceride and the alcohol. Under normal conditions, this reaction will proceed either exceedingly slowly or not at all, so heat, as well as catalysts (acid and/or base) are used to speed up the reaction. It is important to note that the acid or base are not consumed by the transesterification reaction, thus they are not reactants, but catalysts. Common catalysts for transesterification include sodium hydroxide, potassium hydroxide, and sodium methoxide.

Almost all biodiesel is produced from virgin vegetable oils using the base-catalyzed technique as it is the most economical process for treating virgin vegetable oils, requiring only low temperatures and pressures and producing over 98% conversion yield (provided the starting oil is low in moisture and free fatty acids). However, biodiesel produced from other sources or by other methods may require acid catalysis, which is much slower.

The transesterification reaction is base catalyzed. Any strong base capable of deprotonating the alcohol will do (e.g. NaOH, KOH, sodium methoxide, etc.), but the sodium and potassium hydroxides are often chosen for their cost. The presence of water causes undesirable base hydrolysis, so the reaction must be kept dry. In the transesterification mechanism, the carbonyl carbon of the starting ester (RCOOR^1) undergoes nucleophilic attack by the incoming alkoxide (R^2O^-) to give a tetrahedral intermediate, which either reverts to the starting material, or proceeds to the transesterified product (RCOOR^2). The various species exist in equilibrium, and the product distribution depends on the relative energies of the reactant and product.

GENERAL PROPERTIES OF VEGETABLE OILS

1.6 Vegetable oils - General properties

Vegetable oils are obtained from oil containing seeds, fruits, or nuts by different pressing methods, solvent extraction or a combination of these (Bennion, 1995). Crude oils obtained are subjected to a number of refining processes, both physical and chemical. These are detailed in various texts and articles (Bennion, 1995), (Fennema, 1985). There are numerous vegetable oils derived from various sources. These include the popular vegetable oils: the foremost oilseed oils - soybean, cottonseed, peanuts and sunflower oils; and others such as palm oil, palm kernel oil, coconut oil, castor oil, rapeseed oil and others. They also include the less commonly known oils such as rice bran oil, tiger nut oil, patua oil, ko_me oil, niger seed oil, piririma oil and numerous others. Their yields, different compositions and by extension their physical and chemical properties determine their usefulness in various applications aside edible uses.

Cottonseed oil was developed over a century ago as a byproduct of the cotton industry (Bennion, 1995). Its processing includes the use of hydraulic pressing, screw pressing and solvent extraction (Wolf, 1978). It is classified as a polyunsaturated oil, with palmitic acid (C₁₆H₃₂O₂) consisting 20 – 25%, stearic acid (C₁₈H₃₆O₂) 2 – 7%, oleic acid (C₁₈H₃₄O₂) 18 – 30%, and linoleic acid (C₁₈H₃₂O₂) 40 – 55% (Fennema, 1985). Its primary uses are food related – as salad oil, for frying, for margarine manufacture, and for manufacturing shortenings used in cakes and biscuits.

Palm oil, olive oil, cottonseed oil, peanut oil, and sunflower oil amongst others are classed as Oleic – Linoleic acid oils seeing that they contain a relatively high proportion of unsaturated fatty acids, such as the monounsaturated oleic acid and the polyunsaturated linoleic acid (Dunn, 2005; Gertz et al., 2000). They are characterized by a high ratio of polyunsaturated fatty acids to saturated fatty acids. As a consequence of this, they have relatively low melting points and are liquid at room temperature. Iodine values, saponification values, specific compositions and melting points in addition to other physical properties have been determined and are widely available in the literature (Williams, 1966), (Oyedeji et al., 2006).

Other oils fall under various classes such as the erucic acid oils which are like the oleic linoleic acid oils except that their predominant unsaturated fatty acid is erucic acid (C₂₂). Rapeseed and mustard seed oil are important oils in this class. Canola oil is a type of rapeseed oil with reduced erucic acid content (Applewhite, 1978). It is a stable oil used in salad dressings, margarine and shortenings. Soybean oil is an important oil with numerous increasing applications in the modern day world. It is classed as a linolenic acid oil since it contains the more highly unsaturated linolenic acid. Other oils include castor oil (a hydroxy-acid oil) which contains glycerides of ricinoleic acid (Erhan et al.,

2006). Also worthy of note is that coconut oil, which unlike most vegetable oils, is solid at room temperature due to its high proportion of saturated fatty acids (92%) particularly lauric acid. Due to its almost homogenous composition, coconut oil has a fairly sharp melting point (Bennion, 1995).

1.7 Auto oxidation and oxidative stability in vegetable oils

By definition, the oxidative stability of oil is a measure of the length of time taken for oxidative deterioration to commence. On a general level, “the rates of reactions in auto-oxidation schemes are dependent on the hydrocarbon structure, heteroatom concentration, heteroatom speciation, oxygen concentration, and temperature (Ferrari et al., 2004).

If untreated, oils from vegetable origin oxidize during use and polymerize to a plastic like consistency (Honary, 2004). Even when they are not subjected to the intense conditions of industrial applications, fats and oils are liable to rancidity (Eastman Chemical Company, 2001; Morteza- Semnani et al., 2006). This happens more so in fats that contain unsaturated fatty acid radicals (Charley,

1970). Indeed the oxidisability of a vegetable oil is dependent on the level of unsaturation of their olefinic compounds. In general terms, oxidative rancidity in oils occurs when heat, metals or other catalysts cause unsaturated oil molecules to convert to free radicals. These free radicals are easily oxidized to yield hydroperoxides and organic compounds, such as aldehydes, ketones, or acids which give rise to the undesirable odors and flavors characteristic of rancid fats (Eastman Chemical Company, 2001). The role of peroxides is exploited in monitoring oxidative deterioration by measuring peroxide values (POV) (Mochida et al., 2006).

Lipid oxidation occurs via auto oxidation or lipoxygenase catalysis. Auto oxidation refers to a complex set of reactions which result in the incorporation of oxygen in lipid structures. Auto oxidation reactions are seen to progress more rapidly in oils that contain predominantly unsaturated fat molecules; other relevant factors include the presence of light, transition metal ions, oxygen pressure, the presence or absence of antioxidants and pro oxidants, temperature and moisture content. Auto oxidation reactions occur at an increasing rate after the initial induction period. This behavior can be explained by assuming that oxidation proceeds by a sequential free radical chain reaction mechanism. Relatively stable radicals that can abstract hydrogen atoms from the allylic methylene groups in olefinic compounds are formed. Hence auto oxidation is a radical induced chain reaction which proceeds through the traditional stages of initiation, propagation and termination. Detailed proposed mechanisms for these free radical chain reactions are available in literature (Fennema, 1985).

Lipoxygenases are metal proteins with an iron atom as the active center. They catalyze the oxidation of unsaturated fatty acids to hydroperoxides as with auto oxidation. Enzyme activation usually occurs in the presence of hydroperoxides, even though enzyme catalyzed oxidation can occur even in the absence of hydroperoxides (Fennema, 1985). As earlier stated, the more unsaturated the fatty acid involved is, the greater its susceptibility to oxidative rancidity. For instance, the linolenic acid esters present in soybean oil (with twice the unsaturation as monounsaturated esters) is particularly sensitive to even oxidation of the slightest kind, commonly referred to as flavor reversion, resulting in beany, grassy or painty flavors (Wolf, 1978). A highly saturated fatty acid level is confirmed to be of benefit in terms of storage ability when compared to more unsaturated vegetable oils (Ferrari et al., 2004). Indeed, the tendency of an oil to

combine with oxygen of the air and become gummy (known as drying) is measured with the iodine number, which in fact is merely a measure of the level of unsaturation of the oil in question (a higher iodine number will indicate higher unsaturation seeing that iodine is absorbed primarily by the mechanism of addition to the double bonds characteristic of unsaturation) (Gunther, 1971).

Based on studies by Toshiyuki. (1999), the oxidative stability of refined vegetable oils is found to be determined considerably by the fatty acid composition, the tocopherols content and the carbonyl value (Toshiyuki, 1999). When observed at frying temperatures, it is seen that in general, non-refined oils prove to have a better stability than refined oils (Gertz et al., 2000). This could be attributed to the fact that refining steps, in particular deodorization, remove a percentage of the tocopherols, which act as natural anti-oxidants in vegetable oils (Applewhite, 1978). Corn oil has a better stability than soybean oil, while rapeseed oil is seen to give a better performance than olive oil. This can be explained in terms of their compositions (Isbell et al., 1999). When investigated at a temperature of 110°C, vegetable oils still show the trend of increased stability in the unrefined state than when refined. Meadow foam oil is reported as the most stable oil in the study conducted by Isbell et al. (1999). High oleic sunflower oil and crude jojoba oil also had good values of oxidative stability (Isbell et al., 1999). Other studies indicate that the presence of free fatty acids has a pro-oxidant effect on vegetable oils (Frega et al., 1999). Hence refining practices are important, seeing Aluyor and Ori-Jesu 4839 that improper handling and raw material abuse can result in the stimulation of enzymatic activity which could produce free fatty acids (Applewhite, 1978). Further investigations on manufacturing practices also reveal research which indicates the importance of the solvent used in the extraction of vegetable oils. Traditional solvents utilized such as

hexane or petroleum ether have the characteristic of extracting only non-polar species. Isopropanol however, as documented by Oyedeji et al. (2006) would extract some polar and high molecular weight compounds. Among these compounds are the natural antioxidants and pigments in oilseeds which presence lead to extended shelf life and hence better oxidative stability (Oyedeji et al., 2006).

1.8 Antioxidants and stability of vegetable oils

Numerous experimental works have established the positive effect of anti-oxidants on the oxidative stability of vegetable oils for both edible uses and industrial uses. An important class of anti-oxidants consists of the phenolic compounds butylhydroxyanisole (BHA), butylhydroxytoluene (BHT), propyl gallate, and tert-butyl hydroquinone (TBHQ). Their use in vegetable oils meant for domestic and industrial processes is widespread.

Vegetable oils in their natural form possess constituents that function as natural antioxidants. Amongst them are ascorbic acids, α -tocopherole, β -carotene, chlorogenic acids and flavanols (Ullah et al., 2003). Tests conducted to investigate the effectiveness of natural anti-oxidants contained in red pepper oil added to soybean and sunflower oils indicate that they provide variable protection against light induced auto-oxidation.

In the above mentioned study on the inhibitive effect of natural antioxidants contained in red pepper oil, it was additionally observed that the phenolic anti-oxidant butylated hydroxytoluene (BHT) shows more effectiveness generally than natural anti-oxidants (Ullah et al., 2003). In the work done by Robert (2005), the common phenolic anti-oxidants were tested for their effectiveness in improving the oxidative stability of biodiesel obtained from soybean oil. Dunn monitored the oxidative stability by means of

pressurized differential scanning calorimetry (P-DSC). For both static and dynamic conditions, improvements in oxidative stability are observed with the application of antioxidants, which included BHA, BHT, TBHQ, propyl gallate (PrG) and α -tocopherol. The work of (Dunn, 2005) further showed that the relative effectiveness of the different antioxidants differed for static and dynamic conditions, although all showed superior performance when compared with α -tocopherol.

A recent area of interest in antioxidant research is concerned with finding effective replacements for the conventional synthetic antioxidants from among various natural extracts from plant species which are seen to possess antioxidant properties. Such research is in the main prompted by the reported possibility of synthetic antioxidants having adverse health effects on humans exposed to them. Specifically, they are known to contribute to liver enlargement and an increase in microsomal activity (Khanahmadi et al., 2006; Morteza- Semnani et al., 2006). Maduka et al. (2003) investigated the effectiveness of a Nigerian alcoholic beverage additive, *Sacoglottis gabonensis* stem bark extract as an antioxidant for common stored vegetable oils. Inhibition of lipid peroxidation was found to be comparable to inhibitions obtained with treatment with vitamins C and E (Maduka et al., 2003). The *Ferulago angulata* plant indigenous to the west of Iran also has proven antioxidant properties. Experimental studies documented indicate that these plants' essential oils and extract begins to show preservative properties on vegetable oils at a minimum concentration of 0.02%. In fact, it even shows more effectiveness than TBHQ at concentrations of 0.5% (Khanahmadi et al., 2006). When evaluated by measuring reducing power, ability to inhibit linoleic acid peroxidation, and 2,2-diphenyl picrylhydrazyl radical scavenging activities, the alkaloid extracts of *Fumaria capreolata* and *Fumaria bastardii* demonstrated strong total antioxidant

activity, with effectiveness marginally less than that of the common synthetic antioxidant butylated hydroxyanisole, and better than quercetine and caffeine. These species have wide distribution in the Mediterranean region and have a reputation for effectiveness in treating hepatobiliary dysfunction and gastrointestinal disorders via local therapies (Maiza et al., 2007). Methanolic extracts of *Phlomis bruguieri*, *P. herbaventi*, *P. olivieri*, *Stachys byzantine*, *S. inflata*, *S. lavandulifolia* and *S. laxa* were tested in sunflower oil stored at 70°C for antioxidant effectiveness, using peroxide values as a measure. Comparisons included samples containing BHA. Highest effectiveness in stabilizing sunflower was obtained from methanolic extracts of *P. bruguieri*, and *S. laxa*. These tests and their findings suggest strongly the possibility of having in these plants a viable source of natural antioxidants of high performance (Morteza- Semnani et al., 2006).

1.9 Vegetable oils as lubricants, bio-fuels, and transformer coolants

The application of vegetable oils and animal fats for industrial purposes, and specifically lubrication has been in practice for many years. Inherent disadvantages and the availability of inexpensive options have however brought about low utilization of vegetable oils for industrial lubrication (Honary, 2004). When applied in the science of tribology, vegetable oils fall under the class known as fixed oils (Gunther, 1971). They are so named because they do not volatilize without decomposing. Prior to recent developments, vegetable and animal oils in tribology have functioned mainly as additives to mineral lubricating oil formulations, although in some cases they are applied exclusively, or in blends. For instance, tallow (acidless) has been used as an emulsifying agent for steam cylinder oils, while castor, peanut and rapeseed oils have been used in blends with mineral oils to improve lubrication performance. Palm oil has been used in

isolation as a fluxing dip in the tin plating of steel, while olive oil has applications as a yarn lubricant (Gunther, 1971).

Reasons for the use of vegetable oils in the science of lubrication abound. Their superior lubricity and emulsifying characteristics increase their desirability as additives to the cheaper but less effective mineral oil based lubricants. Their superior lubricity in industrial and machinery lubrication sometimes even necessitates the addition of friction materials in tractor transmissions in order to reduce clutch slippage (Honary, 2004).

Other advantages that encourage the use of vegetable oils include their relatively low viscosity-temperature variation; that is their high viscosity indices, which are about twice those of mineral oils (Honary, 2004). Additionally, they have low volatilities as manifested by their high flash points (Honary, 2004). Significantly, they are environmentally friendly: renewable, non toxic and biodegradable (Howell, 2007). In summary, engine lubricants formulated from vegetable oils have the following advantages deriving from their base stock

chemistry: higher Lubricity resulting in lower friction losses, and hence more power and better fuel economy; lower volatility resulting in decreased exhaust emissions; higher viscosity indices; higher shear stability; higher detergency eliminating the need for detergent additives; higher dispersancy; rapid biodegradation hence decreased environmental / toxicological hazards (Erhan et al., 2002).

In a comparison of palm oil and mineral based lubricants, palm oil based lubricants were found to be more effective in reducing the hydrocarbon and carbon monoxide emission levels, among other things (Masjuki et al., 1999).

Vegetable oils have also been identified as having a lot of potential as alternative diesel engine fuels (Kayisoglu et al., 2006). This is supported by an interest in a cleaner environment, as well as the increasing cost of mineral deposit based energy (Howell, 2007). Based on the potential availability to meet demand, soybean, peanut and sunflower oils have been identified as the most promising fuel sources (Kayisoglu et al., 2006). When used as a fuel, the term “biodiesel” is applicable.

Biodiesel is defined strictly as “...*the mono alkyl ester (usually methyl ester) of renewable fats and oils...*” (Howell, 2007). It consists primarily of long chain fatty acid esters, produced by the transesterification reaction of vegetable oils with short chain alcohols. Distinct advantages of biodiesel include a high flash point of over 100°C, excellent lubricity, a BTU content comparable to that of petrol diesel, and virtually no sulfur or aromatic content. Above all, biodiesel is non-toxic and biodegradable (Howell, 2007). Results from investigating performance of vegetable oils in blends with diesel indicate that blending up to 25 percent biodiesel (sunflower) with mineral diesel has no adverse effect on performance (Kayisoglu et al., 2006).

Vegetable oils have also been applied as transformer coolant oils and have been found to conform to all industry standards with performances and cost profiles comparable to the conventional mineral oils applied in transformer cooling (ABB Inc., 2002). Transformer oil products have been produced from soybean oils as well as castor oils (Honary, 2004).

Whether applied for lubrication purposes or as biodiesel or as transformer cooling fluid, one of the major challenges in the utilization of the more environmentally friendly vegetable oils is their poor oxidative stability (Honary, 2004), (Howell, 2007). Combating the issue of oxidative instability in vegetable oils for industrial use is a

continuing research area. In the United States, for instance, three avenues are being pursued. These are (Howell, 2007):

- Genetic modification of oils to give higher mono unsaturated compounds;
- Chemical modification
- The use of various additives and property enhancers

Genetic modification has been made possible by recent advances in biotechnology. DuPont Technology has developed a soybean seed that presents 83% oleic acid as against having the more unsaturated linolenic acid as the major constituent. This new seed provides oils that show about 30 times the oxidative stability and viscosity stability of the conventional oil. High oleic varieties of rapeseed, canola and sunflower seed oils are increasingly being used as base stocks for lubricant formations (Honary, 2004).

Chemical modifications involve the partial hydrogenation of the vegetable oil and a shifting of its fatty acids (Honary, 2004). In one study, epoxidized soybean oil was chemically modified with various alcohols in the presence of sulfuric acid as a catalyst. Better performance was recorded (Hwang et al., 2001).

The use of additives known as antioxidants to control the development of oxidative rancidity has been applied in the US since 1947 (Bennion, 1995). They still remain one of the most efficient and cost effective ways to improve the oxidative stability of oils in both domestic and industrial conditions.

CHAPTER TWO

CHARACTERISATION OF VEGETABLE OILS: REVIEW OF EMPIRICAL STUDIES

2.1 Extraction, Characterization and Modification of Castor Seed Oil

This paper carried out experimental study, through extraction and characterization of both crude and refined castor oil. Normal hexane was used as solvent for the extraction process. The oil produced was refined through degumming, neutralization and bleaching process using local adsorbent (activated clay). The characterization analysis revealed that tested parameters, which include specific gravity, refractive index, acid value, saponification value and iodine value for both crude and refined castor oil produced, were within the ASTM standard specifications. In fact the iodine value obtained (84.8) for the refined oil indicates that the oil could certainly be used as lubricant, hydraulic break fluid and protecting coatings. The oil was modified via sulphation method to produce Turkey – red oil that was tested on wooden material, paper and cloth. The test revealed that the Turkey – red oil produced is suitable to be used as a good dyeing agent and polish.

2.2 Proximate Composition, Extraction, Characterization and Comparative Assessment of Coconut (*Cocos nucifera*) and Melon (*Colocynthis citrullus*) Seeds and Seed Oils

Proximate composition, extraction, characterization and comparative assessment of *Cocos nucifera* and *Colocynthis citrullus* seeds and seed oils were evaluated in this work using standard analytical techniques. The results showed the percentage (%) moisture,

crude fibre, ash, **crude protein**, lipids and total carbohydrate contents of the seeds as 7.51 and 4.27, 7.70 and 5.51, 1.02 and 2.94, 10.57 and 11.67, 47.80 and 50.42 and 32.84 and 29.47 while the calorific values were 553.99 and 567.32 Kcal/100 g for *C. nucifera* and *C. citrullus*, respectively. The two seed oils were odourless and at room temperature (30°C) liquids, with a pale yellow to yellowish colouration. Lipid indices of the seed oils indicated the Acid Values (AV) as 2.06-6.36 mg NaOH g⁻¹ and 2.99-6.17 mg NaOH g⁻¹, Free Fatty Acids (FFA) as 1.03-3.18 and 1.49-3.09%, Saponification Values (SV) as 252.44-257.59 and 196.82-201.03 mg KOH g⁻¹, Iodine Values (IV) as 9.73-10.99 and 110.93-111.46 mg of I₂ g⁻¹ of oil and Peroxide Values (PV) as 0.21-0.21 and 1.53-2.72 mg O₂ kg⁻¹ for soxhlet-mechanical extracted *C. nucifera* and *C. citrullus* seed oils, respectively. The studied characteristics of the oil extracts in most cases compared favourably with most conventional vegetable oils sold in the Nigeria markets; however, there were some observed levels of significant differences in the values at $p \leq 0.05$. These results suggest that the seeds examined may be nutritionally potent and also viable sources of seed oils judging by their oil yield. The data also showed that the seed oils were edible inferring from their low AV and their corresponding low FFA contents. Industrially, the results revealed the seed oils to have great potentials in soap manufacturing industries because of their high SV. They were also shown to be non-drying due to their low IV which also suggested that the oils contain few unsaturated bonds and therefore have low susceptibility to oxidative rancidity and deterioration as confirmed by their low PV which also serves as indicators of the presence or high levels of anti-oxidants in the oils.

2.3 Characterization of a high oleic oil extracted from papaya (*Carica papaya* L.) seeds

The physicochemical characteristics, fatty acid, tocopherol, and carotenoid composition of a crude oil extracted from papaya (*Carica papaya* L.) seeds, *formosa* variety, were investigated. The oil yield from the seeds was 29.16%. The data obtained for the analytical indexes were in agreement with those of other edible oils. The oil obtained had high oxidation resistance (77.97 hours). The major fatty acids in total lipid were oleic (71.30%), palmitic (16.16%), linoleic (6.06%), and stearic (4.73%) acid. The α and δ -tocopherol were the predominant tocopherols with 51.85 and 18.89 mg.kg⁻¹, respectively. The β -cryptoxanthin (4.29 mg.kg⁻¹) and β -carotene (2.76 mg.kg⁻¹) were the carotenoids quantified, and the content of total phenolic compounds was 957.60 mg.kg⁻¹. Therefore, the potential utilization of the papaya seeds for oil production seems favorable. However, toxicological studies need to be carried out before the oil is appropriate for food applications.

2.4 Extraction and characterization of vegetable oils from legume and palmae; using African oil bean (*Pentaclethra macrophylla*) and akwu ojukwu (*Elaeis Guineensis*) respectively

The extraction and characterization of vegetable oils from the seed of *Pentaclethra macrophylla* (oil bean) and *Elaeis guineensis* (Akwu Ojukwu) was done. Extraction was done by solvent extraction method using petroleum spirit as the solvent for Akwu Ojukwu fibre and petroleum ether for oil bean kernel. There was a significant increase ($P \leq 0.05$) in the oil yield of Akwu Ojukwu fibre (48%) when compared to oil bean seed (32%). Some Physico-chemical, vitamin and mineral parameters of both oils were analyzed. Results of the physico-chemical analysis showed no significant difference

($P \leq 0.05$) in the relative density, peroxide value and thiobarbituric acid value of both oils. The melting point and acid value (25°C and 7.35mg respectively) of Akwu Ojukwu oil increased significantly ($P \leq 0.05$) when compared with that of oil bean seed oil (22°C and 3.62mg respectively). On the other hand, the moisture content (0.40%), iodine value (53.25mg) and saponification value (196.38mg) of Akwu Ojukwu oil decreased significantly to that of oil bean oil (8.80% , 121.80mg and 213.10mg respectively). Akwu Ojukwu oil was red while oil bean oil had a light-yellow colour. The vitamin and mineral determination showed that P, Fe and vitamin C content increased significantly ($P \leq 0.05$) in oil bean seed oil. The general result shows that Akwu Ojukwu oil is a non-drying oil, while oil bean oil is a semi-drying oil. Both oils could be used for edible (when processed), medicinal and industrial purposes.

2.5 Characterization of Breadfruit Seed Hull Ash for Potential Utilization in Metal Matrix Composites for Automotive Application

Breadfruit seed hull (seed coat or seed shell) is an agricultural waste. The waste is produced in abundance globally and poses risk to health as well as environment. Thus their effective, conducive and eco-friendly utilization has always been a challenge for scientific applications. This paper mainly deals with characterization of breadfruit seed hull ash obtained from breadfruit seed hull using spectroscopic and microscopic analysis. The characterization of the breadfruit seed husk ash was investigated through scanning electron microscopy (SEM), X-ray diffractometer (XRD), thermogravimetric analysis (TGA/DTA), Fourier transform Infra red spectrometry (FTIR) and X-ray Fluorescent spectrometry (XRF). Density and refractoriness of the breadfruit seed hull ash were also found. The results were compared and it was observed that the ash possesses nearly same chemical phases and other functional groups as reinforcement like

CHAPTER THREE

Materials & Methods

Raw breadfruit was collected from a local market in Enugu Metropolis. The breadfruit was deshelled and the seed were sundried for over 12hours. The samples were crushed in a laboratory mortar, to reduce the surface area, immediately before extraction using n-hexane (M & B, \analR grade). Extraction was carried out using soxhlet extraction unit of 100°C. A solvent-to-solute ratio of 10:1 (vol/wt) was used, with 20g of sample in the soxhlet unit.

The required quantity (20g) of samples was weighed out on an electronic weighing balance. The extraction was carried out to for 5hrs ensure optimum oil extraction. After extraction, the miscella was filtered and extracted oil was also desolventized to determine the yield.

$$\% \text{Yield} = \frac{A}{W} \times 100$$

Where A = Amount of oil after desolventizing(g)

W = Initial weight of sample(g)

2 Relative Density: A clean and dry relative density bottle was weighed (M1). The bottle was filled with distilled water and gently covered with the lid, the outside walls of the bottle was cleaned and weighed (M2). The same procedure was conducted for the oil and relative density calculated thus: $\frac{(M1-M2) \text{ oil}}{(M2-M1) \text{ water}}$

3 Moisture Content: A portion(2g) of the oil was measured into a preweighed porcelain allowed to cool in a desiccators and the weight measured. Moisture content was boat and placed in a laboratory oven at 105 °C for 2 hours. The sample was removed and calculated

$$\text{thus; M.C.} = \frac{(M2-M1)}{W} \times 100$$

Acid Value: A 50ml (1:1) solvent mixture of diethyl ether and ethanol was measured into an erlemeyer flask and 2g of oil was added and shaken. To the solution 1% phenolphthalein solution was added and titrated with 0.1M potassium hydroxide solution.

$$\text{Acid Value} = \frac{\text{weight of sample}}{\text{Titration (ml)} \times 5.61}$$

4 Free fatty Acid (FFA)

1ml of 0.1M KOH = 0.282g oleic acid

5 Peroxide Value: A portion (2.15g) of sample was added to 30ml of ethanoic acid chloroform mixture in the ratio of 3:2 respectively and 5ml of saturated potassium iodide (KI) was added. A 15ml of sodium thiosulphate solution was added until a yellowish colour had almost disappeared then 2ml of starch solution (indication) was added and titration continued to a colourless end point.

$$\text{Peroxide value} = 2V \text{ MEq/kg.}$$

6 Saponification Value: A portion (2g) of the sample was measured into a flask and 25ml of alcoholic potassium hydroxide solution was added to it. A reflux condenser was attached to the flask and the solution heated for one hour with occasional shaking. A 3 drops of phenolphthalein solution was added and titrated with 0.5M HCL. A blank was carried out with water and recorded as

B.(bml).

$$\text{S.V.} = \frac{(b-a) \times 28.05}{\text{Wt.of sample}}$$

Wt.of sample

7 Iodine Value: About 0.5g of the sample was dissolved in 10ml of chloroform in a conical flask. 25ml of the Hanus solution was added to the chloroform and corked. This was kept in dessicator for 30 minus in the dark. A blank (b) was also carried out under the same conditions, when reaction was completed in the flask, which is at the time, 15cm of 10% potassium iodide (KI) solution and 10ml of distilled water was added to each flask and mixed by gentle shaking. The content of both flasks were titrated with 0.1M $\text{Na}_2\text{S}_2\text{O}_3$ to pale. Yellow and 2ml of starch solution indicator was added. Titration was continued until the blue-black colour was completely discharged.

8 Ester value: ester value represents the number of milligrams of potassium Hydroxide required to saponify the ester present in 1g of the oil. It is obtained as the difference between the saponification value and the acid value.

CHAPTER FOUR

RESULTS AND DISCUSSION

%OIL	10.5
Relative density (kg/m ³)	0.94
Moisture content (%)	2.2%
Acid value (mg KOH/g)	7.38
FFA (mg KOH/g)	1.09
Peroxide value (mg/kg)	5.6
Saponification value (mg KOH/g)	42.1
Iodine value (g/2/100g)	42.4
Ester value (mg KOH/g)	34.72

YIELD: The oil content of the breadfruit was 10.5%. This shows that a slight fraction of the fruit is made up of oil. The results of this study are comparable with that of avocado pear 9.1%, extracted with chloroform (Ikhuoria and Maliki; 2007), but is below that of groundnut oil 50% (Mba et al; 1974), cashew nut oil (49.1) (Akiihanmi and Alasie'2008) and (Akpabio et al., 20011) Avocado pear oil 52.4%.

b. Saponification value: The saponification value of the oil was 42.1 mg KOH/g. The result are comparable to that of native pear fruit oil 25.9 (Akpabio et al 2011); but below those of cotton seed oil 194.3 (Eka; 1977), shear butter oil 185.20 (Asuquo et al., 2010), conophor seed oil 194.7 (Eka, 1977), cashew nut oil 137.00 (Akinhanmi and Atasie., 2008). Saponification values of oil serve as important parameter in determining the suitability of oil in soap making. The oil obtained gave a clear solution in water. This type of oil is grouped among those

yielding soaps of soft consistency. Hence, the oil could be used for making shaving creams since it requires oils of soft consistency.

c. Iodine value: The iodine value of breadfruit seed oil was 42.4g I/100g. The values are comparable to that of palm kernel oil 37 (Oyenuga et al., 1975) and cashew seed oil 41.3 (Akinhanmi and Atasie, 2008), but below that of avocado seed oil 62.8 (Akpabio et. al., 2011). Hence breadfruit seed oil will contain lesser percentage of unsaturated fatty acids than avocado seed oil.

However, the iodine value of the oil is in agreement with the standard, hence the oil could be classified as non drying oil, since their iodine value are less than 100 (Asuquo., 2008).

FFA: The FFA value of breadfruit seed oil is 1.09. The FFA of the oil is in agreement with that of native pear oil 2.4% (Akpabio et al., 2011), but below those of avocado seed oil 5.4 % (Akpabio et al., 2011), that rubber seed oil 5.2 (Eka. 1997) and that of cashew nut 5.4% (Akinhanmi and Atasie 2008).

Ester value: ester value represents the number of milligrams of potassium hydroxide required to saponify the ester presents in 1g of the oil. It is obtained as the difference between the saponification and acid value. The ester value for breadfruit seed oil was 34.72mg KOH/g. The value obtained in this study is in line with the native pear 18.8mgKOH/g. (Asuquo et al., 2011) but far below those of avocado pear 172.8mgKOH/g (Asuquo et al., 2011), castor seed oil 174.09mgKOH/g, rubber seed oil 191.93mg KOH/g which are all vegetable oil (Asuquo;2008)

Acid value: The acid value obtained for breadfruit seed oil is 7.38mgKOH/g. the value are high compare to that of butter oil, 1.79mgKOH/g (Asuquo et al., 2010) but are lesser than those of cashew nut .10.7mgKOH/g. (Akinhanmi and Atasie, 2008): avocado seed oil

16.8mgkoH/g (Akpallino et al., 2011), but is in agreement with that of native pear oil 7.1mgkoH/g (Akpahio et al., 2011). The low acid value of breadfruit seed oil (7.38) suggest that the oil may be for advantage for paint making and also that the oil is edible (Akpabio et al., 2011). The lower the acid value of oil, the few fatty acid it contains which makes it less exposed to the phenomenon of rancidity (Roger et al ., 2010).

Peroxide value: The peroxide value obtained for the oil was 5.6mg/kg. The peroxide value helps in determining whether the oil will be easily susceptible to oxidative rancidity (Akpabio et. al., 2012). The value obtained in the analysis is below those reported by Akpabio et al., 2012 28.8mg/kg and 126.4mg/kg for avocado and native pears respectively. This shows that breadfruit oil will not be easily susceptible to rancidity.

CONCLUSION

Acid value is an important index of physicochemical properties of oil which is used to indicate the quality age, edibility, and suitability of oil for use in industries such as paint. Acid value are used to measured the extent to which glycerides in the oil has been decomposed by lipase and other physical factors such as light and heat. Peroxide value is used as a measured of the extent to which rancidity reaction have occurred during storage. Saponification value is an index of average molecular mass of fatty acid in oil sample.

REFERENCES

- Applewhite, T. H. (1978). *Fats and Fatty Oils: Encyclopedia of Chemical Technology*, (3rd Edition). New York: John Wiley and Sons Inc.
- Ascherio, A., Herinekens, C. H., Baring, J. E., Master, C., Stampfer, M. J., & Willett, W. C. (1994). Trans fatty acids intake and risk of myocardial infarction; *Circulation* 89: 94—101.
- Bennion, M. (1995). *Introductory Foods*, (10th Edition). New Jersey: Prentice-Hall Inc.
- Berry, E. M., & Hirsch, E. (1986). Does dietary linolenic acid influence blood pressure? *American Journal of Clinical Nutrition* 44: 336—340.
- Beyers, E. C., & Emken, E. A. (1991). Metabolites of cis, trans. and trans, cis isomers of linoleic acid in mice and incorporation into tissue lipids. *Biochimica et Biophysica Acta* 1082: 275—284.
- Birch, D. G., Birch, E. E., Hoffman, D. R., & Uauy, R. D. (1992). Retinal development in very-low-birth-weight infants fed diets differing in omega-3 fatty acids. *Investigative Ophthalmology and Visual Science* 33 (8): 2365—2376.
- Du, W. (2004). Comparative study on lipase-catalyzed transformation of soybean oil for biodiesel production with different acyl acceptors. *Journal of Molecular Catalysis B: Enzymatic* 30 (3-4): 125—129.
- Dunn, R. O. (2005). Effect of Antioxidants on the Oxidative Stability of Methyl Soyate (Biodiesel). *Fuel Proc. Technol.* 86: 1071—1085.
- Eisenmenger, M., Dunford, N. T., Eller, F., Taylor, S., & Martinez, J. (2006). Pilot- scale supercritical carbon dioxide extraction and fractionation of wheat germ oil. *Journal of the American Oil Chemists' Society* 83 (10): 863-870.
- Emanuel, N. M., & Lyaskovskaya, Y. N. (1967). *The Inhibition of Fat Oxidation Processes*. Oxford, London: Pergamon Press Ltd.
- Emken, E. A. (1984). Nutrition and biochemistry of trans and positional fatty acid isomers in hydrogenated oils. *Annual Reviews of Nutrition* 4: 339—376.
- Enig, M. G., Atal, S., Keeney, M., & Sampugna, J. (1990). Isomeric trans fatty acids in the U.S. diet. *Journal of the American College of Nutrition* 9: 471—486.
- Eunkyakiat, K. (2006). Continuous Production of Biodiesel via Transesterification from Vegetable Oils in Supercritical MethanoP. *Energy and Fuels (American Chemical Society)* 20 (2): 812—817.
- Frega, N., Mozzon, M., Lercker, G. (1999). Effects of Free Fatty Acids on Oxidative Stability of Vegetable Oil. *J. Am. Oil Chemists' Soc.* 76 (3): 325-329.

- Gertz, C., Klostermann, S., & Kochhar, S. P. (2000). Testing and Comparing Oxidative Stability of Vegetable Oils and Fats at Frying Temperature. *Eur. J. Lipid Sci. Technol.* 102(8-9): 543-541.
- Hwang, H., & Erhan, S. Z. (2001). Modification of Epoxidized Soybean Oil. for Lubricant Formulations with Improved Oxidative Stability and Low Pour Point. *J. Am. Oil Chem. Soc.* 78(12): 1179-1184.
- John, U. (1936). A Comparison of Industrial Growth in France and England from 1540 to 1640: III. *The Journal of Political Economy* 44 (5): 643—666.
- Kayisoglu, B., Ulger, P., Akdemir, B., & Aytac, S. (2006). A Research Determining Some Performance Values by Using Proportional Mixture of Vegetable Oils and Diesel Fuel at a Diesel Engine. *J. Tekirdag Agric. Fac.* 3(1)16-24, 115-123.
- Khanahmadi, M., & Janfeshan, K. (2006). Study on Antioxidation Property of *Ferulago angulata* Plant. *Asian J. Plant Sci.* 5(3): 521-526.
- Marchand, C. M. (1982). Positional isomers of trans-octadecenoic acids in margarine. *Canadian Institute of Food Science and Technology Journal* 15: 196—199.
- Mensink, R. P., Zock, P. L., Katan, M. B., & Hornstra, G. (1992). Effect of dietary cis- and trans-fatty acids on serum lipoprotein[a] levels in humans. *Journal of Lipid Research* 33: 1493—1501.
- Mochida, Y., & Nakamura, S. (2006). Determination of Total Hydroperoxides in Oxidized Vegetable Oils Based on Triphenylphosphine Oxidation Using Electron Ionization Mass Spectrometry. *J. Mass Spectrom. Soc. Jpn* 54(6): 235-241.
- Oyedeji, F. O., & Oderinde, R. A. (2006). Characterization of Isopropanol Extracted Vegetable Oils. *J. Appl. Sci.* 6(11): 2510-2513.
- Scott, G. (1965). *Atmospheric Oxidation and Antioxidants*. Amsterdam: Elsevier Publishing Company.
- Sharma, K. B., Perez, J. M., & Erhan, S. Z. (2007). Soybean Oil-Based Lubricants: A Search for Synergistic Antioxidants. *Energy Fuels* 21(4): 2408- 2414.
- Siguel, E. N., & Lerman, R. H. (1993). Trans fatty acid patterns in patients with angiographically documented coronary artery disease. *American Journal of Cardiology* 71: 916—920.
- Sonestedt, E., Ulrika, E., Bo, G., Kerstin, S., Hakan, O., & Wirfalt, E. (2008). Do both heterocyclic amines and omega-6 polyunsaturated fatty acids contribute to the incidence of breast cancer in postmenopausal women of the Malmo diet and cancer cohort? *The International Journal of Cancer* 123 (7): 1637—1643.

- Tautorus, C. L., & McCurdy, A. R. (2006). Effect of Randomization on Oxidative Stability of Vegetable Oils, at Two Different Temperatures. *J. Am. Oil Chem. Soc.* 67(8): 525-530.
- Toshiyuki, C. (1999). Prediction of Oxidative Stability Based in Various Chemical Properties for Refined Vegetable Oils. *J. Japan Oil. Chem. Soc.* 48(8):781-786.
- Troisi, R., Willett, W. C., & Weiss, S. T. (1992). Trans-fatty acid intake in relation to serum lipid concentrations in adult men. *American Journal of Clinical Nutrition* 56: 1019—1024.
- Ullah, J., Hamayoun, M., Ahmad, T., Ayub, M., & Zarafullah, M. (2003). Effect of Light, Natural and Synthetic Antioxidants on Stability of Edible Oil and Fats. *Asian J. Plant Sci.* 2(17-24): 1192-1194.
- Willett, W. C., Stampfer, M. J., Manson, J. E., Colditz, G. A., Speizer, F. E., Rosner, B. A., Sampson, L. A., & Hennekens, C. H. (1993). Intake of trans fatty acids and risk of coronary heart disease among women. *The Lancet* 341: 581—585.
- Williams, K. A. (1966). *Oils, Fats and Fatty Foods — Their Practical Examination*, (4th Edition). New York: American Elsevier Publishing Company Inc.