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Review

Chromatographic analysis of vegetable oils: A review

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Vegetable oils has become more attractive recently because of its economical benefits as they are used as components in many manufactured products and the fact that it is made from renewable resources. The determination of the minor components is of great importance in establishing the oil quality and their genuineness. The qualitative and quantitative determination of the constituents is done by gas chromatography of compounds or their derivatives. High precision in the gas chromatographic analysis of fatty acid methyl esters is possible with careful attention to detail during sample preparation, injection, chromatographic and data collection. This paper shows the applications of solid-phase extraction for the isolation of certain lipid classes prior to chromatographic analysis and also the most commonly used chromatographic techniques that can be applied for accuracy.

Key words: Chromatography, component, fatty acid methyl esters, sterol.

INTRODUCTION

Vegetable oils are mainly constituted by triacylglycerol (95 - 98%) and complex mixtures of minor compounds (2 - 5%) of a wide range of chemical nature. These minor constituents show a broad qualitative and quantitative composition depending on the vegetable species from which they are obtained. Agronomic and climatic conditions, fruit or seed quality, oil extraction system and refining procedures can cause variation in the content and composition of the constituents of vegetable oil (Cert et al., 2000).

The minor components include mono- and diglycerides, free fatty acids, phosphatides (or phospholipids), sterols, protein fragments, various resinous and mucilaginous materials and oxidative products (International Conference on Palm and Palm Products, 1989). A triglyceride is a chemical compound formed from one molecule of glycerol and three fatty acids.

Fatty acid chains may contain one or more double bonds at specific positions (unsaturated and polyun-saturated), or they may be fully saturated. The physical and chemical properties of a fat depend on the composition of the fatty acid mixture. Fats from plant sources contain a higher proportion of unsaturated acids and are often liquids at room temperature due to hydrogen bonding. Polyunsaturated fats are usually of vegetable origin. Crisco is an example of a vegetable derived, unsaturated fatty acid that has been hydrogenated to form a solid material (Greenlief, 2004).

Fats are used in cooking because they are compounds with very high boiling points. Their high boiling points therefore make these classes of compounds ill suited for analysis by the gas chromatography. However, the glycerol ester can be chemically decomposed into methyl esters of each individual fatty acid (Greenlief, 2004). To analyze the minor component of vegetable oils, a preliminary qualitative and quantitative isolation step from the triacylglycerol matrix is required. Three basic procedures are normally used: saponification, liquid-liquid partition and chromatographic techniques. The saponification (heating with alcoholic solution of potassium hydroxide) transforms the glyceridic compounds in polar soaps allowing the extraction of unsaponified matters with hexane or diethyl ester.

Nevertheless, this procedure is not appropriate for wax

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esters, sterol esters, phenols, pigments, minor glyceridic compound and phospholipids, since they are altered during the saponification. Liquid-liquid partition with polar solvents (methanol-water, hexane-acetonitrile, etc.) is suitable for the isolation of phenols, polycyclic aromatic hydrocarbons and chlorophylls. Recently, supercritical fluids extraction has gained importance in separation techniques, due to the possibility of modifying product solubility through alteration of pressure and or temperature, or adding modifiers, substituting a wide variety of liquid solvent. Column chromatography is widely used to separate fractions having constituents of similar polarities. Actually, the latter is being substituted by solid-phase extraction (SPE), as it is a quicker technique and saves solvent volumes. The isolation of volatile compounds is achieved by gas stripping and distillation. The isolation fractions are still complex and require further fractioning, usually by means of thin-layer chromatography (TLC) or preparative high-performance liquid chromatography (HPLC) (Cert et al., 2000).

Gas chromatography (GC) and primarily high performance liquid chromatography (HPLC) are the two techniques widely applied for the analysis of major (triacylglycerols) and minor constituents of edible oils and fats (Christie, 1987; Cert et al., 2000). The qualitative and quantitative determination of the constituent is often done by capillary gas chromatography (GC) of the compounds or their derivatives. In general, GC assumes that the compound injected are volatile at the temperature of analysis and that they do not decompose at either the temperature of injection or analysis (Cert et al., 2000).

The history of the gas-liquid chromatograph of fatty acids dates back to 1952, when James et al. published a description of separations of free fatty acids from 1 to 12 carbon atoms. Later, they discovered that a previous methylation of fatty acids improved their volatility and their separation at temperatures below 250°C (James et al., 1956). A major advance was the introduction by Orr et al. (1958) of a liquid polyester phase as stationary phase able to conveniently separate saturated and unsaturated fatty acids. Later, the use of capillary columns with very films of various polar phases enabled to profoundly improve fatty acid analyses (Horning et al., 1963).

In the early days of GC, losses of esters of higher molecular weight were obtained because of chemical interactions with polyester liquid phases in packed columns. These problems were eliminated as catalytic-free polyesters available together with improve solid support materials. Perhaps the best objective assessment of the standard of fatty acid analysis possible with this older technology came in collaborative study of IUPAC method in 1979 (Firestone et al., 1979). For a range of fats and oils of commercial interest typical coefficients of variation (%) where quoted as 15 (2% level), 8.5 (5% level), 7 (10% level) and 3 (50% level).

Poorer data where obtain with difficult sample like milk fat. In a comparable study with modern equipment, the results were only a little better (Beare-Rogers et al., 1990).

Preparation of fatty acids

The ability of an analytical method to characterize a vegetable oil is based on the identification and quantification of those compounds that are expected to be in connection with their origin and quality attributes (Cert et al., 2000).

Before analysis of vegetable oil samples can be commenced by any chromatographic procedure, it is first necessary to extract them from their tissue matrices in a relatively pure state (Christie, 1989). The analysis of fatty acids containing compound requires previously their hydrolysis or saponification, separation of the non-acidic constituent (when necessary) and the liberation of the acids from the mixture of soaps (James et al., 1963; Cert et al., 2000).

Adequate sample preparation techniques help to ensure an accurate and precise estimation of the true value of the food components.

Solid phase extraction (SPE)

Solid phase extraction (SPE) is a convenient approach for sample preparation in the Chromatographic analysis of edible fats as it requires a small amount of the test sample, low volumes of organic solvents, whereas the treatment is accomplished in a greatly shorter period of time than in other techniques.

SPE typically involves commercially available prepacked polymer or glass mini disposable columns (cartridges) or disks that can be used to remove contaminants or to further fractionate the analyses prior to analysis. The technique is highly selective and versatile due to the large number of phases introduced in the last ten years. New formats (96-well plates, micro fibers for solid phase micro extraction), automation and on-line use are the important advantages for the current and future applications of SPE.

APPLICATION OF CHROMATOGRAPHIC ANALYSIS ON VEGETABLE OILS

Sterols: Sterols free are esterified with fatty acids, and are found in small concentrations (0.1 - 5.6%) in edible fats. Sterols, such as cholesterols are alcohols with the cyclopentanophenanthrene ring system (atom 1 through 17 as in Figure 1). This substructure is also found in steroid hormones such as testosterone, progesterone, and cortisol. Cholesterol is found in animal fats and at relatively low amounts in certain plant lipids (e.g. palm oil). Cholesterol is classified as an alcohol because it has a

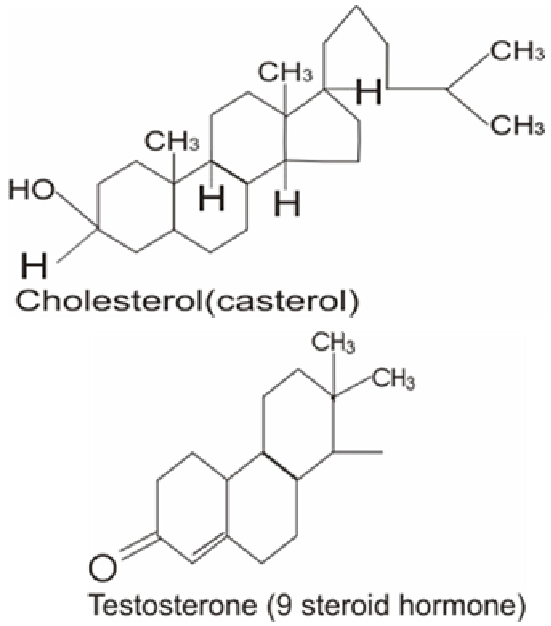

Figure 1. Structural diagrams of some Sterols. (Zamora, 2005).

Table 1. Extraction of sterol fraction according to Toivo et al., (1998)

Step	Action
A	Saponification of sample [0.25-0.50g oil + 5ml 0.5 mM ethanolic KOH, 20min reflux, 80^0C]
B	Addition of betulin as internal standard [1 mg betulin in 5ml $CHCI_3$]
C	PH adjustment, filtration [few drops 5M HCl, 0.45 mm nylon membrane filter]
D	Application to C_{18} cartridge

a hydroxyl group (-OH) in position 3 of the ring system. Cholesterol is produced by the liver and is found in all body tissues where it helps to organize cell membranes and control their permeability. Cholesterol derivates in the skin are converted to vitamin D when the skin is exposed to sunlight. Vitamin D_3 mediates intestinal calcium absorption and bone calcium metabolism. A high level of cholesterol in the blood is considered to be a risk factor for cardiovascular diseases. Cholesterol and lipoprotein levels can be normalized through exercise and reduced calorie diets that eliminate hydrogenated fats and add sources of polyunsaturated fatty acids such as grape oil (Zamora, 2005).

Phytosterols, the plant sterols, are rather simple mixtures of closely related organic compounds. Some of them always accompany each other (e.g. β-sitosterol and campesterol). The analysis of sterols is important for detecting oil adulteration, e.g. of butter fat with vegetable oils or of virgin olive oil with cheaper oils. More recent is the interest in the nutritional value of sterols and the monitoring and accurate quantification of oxysterols. The latter, especially cholesterol oxidation products, have been examined thoroughly due to their potential health risk (Bosinger et al., 1993).

Capillary Gas Chromatography (CGC) is mainly used for the analysis of sterols. The sterol fraction is obtained from the unsaponified matter by Thin Layer Chromatography (TLC) prior to analysis. This tedious procedure, part of the official ones adopted for the control of edible oils (EC, 1991; IURJ\C, 1992; AOAC, 1999) has a little potential for automation and limits the number of samples that can be handled daily. Alternative sample preparation procedures involve the derivation of the unsaponified (Slover et al., 1983), automatic removal of the interfering triacylglycerols by continuous on-line trans-esterification with potassium methylate in methanol (Ballesteros et al., 1996), fractionation on Column Chromatography (CC) (Homberg, 1987) or on-line LC-GC (Grob et al., 1989). Using these alternative procedures, tocopherols, hydrocarbons and related compounds are also determined in the subsequent analysis. A procedure was applied to crude, extra virgin, refined and crude pomace olive oils. Toivo et al. (1998) extended the efforts made by Tsui (1989) on C18 packing material to clean up the unsaponifiables according to the scheme presented in Table1 above. The merits of HPLC procedures have also been investigated in the literature for their potential, either for sample treatment, as direct analytical means or in conjunction with other chromatographic techniques (Holen, 1985; Cert et al., 2000). Thus, there is an increasing interest in developing rapid methods for sterol analysis (Alonso et al., 1997). SPE procedures in the analysis of sterols in edible fats are rather limited though more information can be found in the case of biological fluids.

In all methods 500 mg of milk fat spiked with 30 mg of COPs was applied to the columns. A combination of silica SPE and NH2 cartridges given in detail in Table 2 (below) allowed the optimum removal of interfering components and the reliable separation (free of spurious peaks) and quantification of COPs.

Wax esters: The wax ester formed by the reaction of alcohols (aliphatic, triterpenic, methylsterols and sterols) and free fatty acids are present in seed and fruit. During the oil extraction process, a fraction of these esters is transferred into the oil depending on the oil extraction system. So solvent extracted oils contain higher concentration of wax esters compared with cold-pressed and centrifuged ones. $C_{40,}$ $C_{42,}$ C_{44} and C_{46} waxes, deriving from straight chain alcohols are very abundant in olive pomace oils while low concentrations are found in olive

Table 2. Combined elution of COPs using silica and NH_2 SPE columns (Lai et al. 1995; Ulberth and Rössler 1998)

Step	Action
A	LC-Si SPE column (300mg/3rnl); Conditioning with 3 ml hexane
B	Sample application
C	Elution with 10 ml n-hexane/ethyl ether (95:5,v/v); 25 ml n-hexane/ethyl ether [90 10.15 ml n-hexane/ethyl ether (80:20. v/v), vacuum 29 kPa, solvent flow rate 0.6 ml/mm v/v]
D	Elution of COPs with 5 ml acetone
E	Concentration of the eluate to 1 ml of n-hexane/ethyl ether (90:10, v/v)
F	Application to NH_2-SPE column (500 mg)
G	Elution with 15 ml of n-hexane/ethyl ether (90:10, v/v)
H	Elution of purified COPs with 10 ml acetone

oils. In addition, great concentration of waxes yields turbidness during refining or storage. Consequently, the determination of waxes is important to evaluate the quality and genuineness of some vegetable oils (Cert et al., 2000).

Other alcohols and related derivatives: Waxes are esters of long chain alcohols with fatty acids. They are found in vegetable oils and their levels depend on the technology of extraction from fruits and seeds. The analysis of waxes is important in the quality control of dewaxing and winterization processes (Mariani et al., 1987). The composition of waxes varies in the various oils so that information can be obtained for the presence of inferior quality oils as it is established in the case of virgin olive oil (EC, 1993). The wax esters are obtained from the oil after saponification and then analyzed by capillary GC. Simplification of the sample preparation step has been attempted by several authors (Grob et al., 1989 and 1990; Amelio et al., 1993). Reiter et al. (1999), who applied for the first time SPE to isolate waxes from fennel and caraway seed oils, claimed that the procedure is applicable to other vegetable oils. The oil (30 mg / 500 µl n-hexane) containing the internal standard was applied to silica cartridges (500 mg / 3 ml, Bakerbond, J.T. Baker) preconditioned with the same solvent. The sample was drawn through the column at a flow rate of 2.5 ml/min (controlled by a vacuum manifold processor) and the waxes were then eluted with n-hexane: diethyl ether (99:1, v/v) (10 ml). Recovery studies were very satisfactory. Yields from the capillary gas chromatographic analysis for stearic acid palmityl ester and behenic acid behenyl ester (40 mg/ml, each) ranged between 95 - 98% (SD %< 5). Nota et al. (1999) simplified the method of isolation of waxes of olive oil in an attempt to improve the existing official method for their determination. The separation was carried out on SPE cartridges (1 g Chromabond, Macherey-Nagel) rinsed first with carbon tetrachloride (6 ml). Oil (20 mg) plus the internal standard was transferred onto the column with a minimum amount of carbon tetrachloride (3 x 100 µl) and the waxes were eluted with the same solvent (6 ml). The speed of elution ranged between 0.5 - 1.5 ml/min. High levels of free acidity (up to 10%) did not lower the accuracy of the method.

Phenolic compounds: Phenolic compounds are transferred from fruits and seeds to raw oils during processing. Refining eliminates the content of phenolic compounds; therefore, their presence is mainly of interest in virgin olive oil and to a certain extent in cold-pressed seed oils, e.g. cold -pressed canola oil. The importance of phenolic compounds in the sensory quality and the stability of virgin olive oil and their effect to human health has been discussed (Tsimidou, 1998). The major phenolics found in virgin olive oil are hydroxytyrosol, tyrosol, aldehydic secoiridoids, flavonoids and lignans (acetoxypinoresinol, pinoresinol) (Brenes et al., 2000). The content of these natural antioxidants depends on the olive oil processing (two-phase extractors yield in general oils with more phenols) and storage conditions. Except from the colorimetric procedures using the Folin-Ciocalteu reagent for the determination of total phenols, gas chromatographic methods and, basically, HPLC procedures have been used for the analysis of the oil fraction containing the phenolic compounds (Ryan and Robards, 1998).

Liquid-liquid extraction and more recently solid-phase extraction have been used for the isolation of the so-called "polar fraction". The solvent system usually applied is aqueous methanol in various proportions. Tsimidou (1999) has recently reviewed the advances in extraction and analysis of virgin olive oil polyphenols. Perez-Camino (2000) presented also applications of solid -phase extraction of phenolic compounds of virgin olive oil. The first systematic study was carried out by Papadopoulos and Tsimidou (1992), who, in a comparative study of two SPE systems and a liquid-liquid one, found, using HPLC, that polyvinylopyrrolidone (PVP) traps selectively the phenolic

compounds. C_{18} cartridges of the same bed volume and with the same solvent system did not retain the bound forms of phenolic compounds. Recovery studies using hydroxyltyrosol and tyrosol on an oil matrix (40 mg/kg each) gave yields of 50 and 90%, respectively. In 2001, Liberatore et al., observed the non-homogeneous and even contradictory results concerning the recovery of phenolics using different methods. They reported recovery data for eight representative olive oil phenols (tyrosol, p-hydroxybenzoic, vanillic, protoca-tecnuic, syringic, p-coumaric, ferulic and caffeic acids) at two levels of addition (50 and 500 mg/kg) using three different extraction procedures. The efficiency of two SPE procedures (C_{18} and C_8 end capped) was compared with that of a liquid-liquid extraction previously reported by Tsimidou et al. (1992). End capped reversed-phase was proved less efficient than C_{18}. Suppression of the residual polar groups of silica had an adverse effect on the release mechanism of the analysts. Still, the recovery data were not consistent at the two levels of addition to permit a better understanding of retention of components of similar molecular weight and polarity.

Partial glycerides and free fatty acids: The analysis of partial glycerides, mono-and di-glycerides is usually carried out by capillary gas chromatography. HPLC may only give information on the total amounts of different classes of partial glycerides (e.g. 1, 2- and 1, 3- diglycerides). These compounds, intermediates in the biosynthesis of triglycerides, are also markers of oil freshness or of employed technological treatments. Perez-Camino et al., (1996) reported on alterations on di-glyceride composition using different types of SPE columns (di-ol, silica, amino). They found that only the di-ol phase did not cause isomerization during the extraction. In their recent review (Perez -Camino, 2000), the authors commending on these and other literature data stated that "a lot of care must be taken before choosing a support until more explanation and importance is given to the absorbent employed". Similar observations were also made by Conte et al. (1997).

Free fatty acid content is not a problem in the analysis of vegetable fats that are consumed refined with the exception of virgin olive oil. Hopia et al. (1992), describeing the analysis of lipid classes by solid-phase extraction gave information for the elution of free fatty acids from edible oils and fats. Procedures related to this group of lipids is found for other categories of food products, where the level and composition of free fatty acids, in particular short chain ones, affect the organoleptic characteristics of the finished products (e.g. cheese) (Innocente et al., 2000).

Phospholipids / Phosphatides: Phospholipids or phosphatides are natural surfactants and emulsifiers consisting of an alcohol such as glycerol, one or two molecules of fatty acids and a phosphoric acid compound. They are found in all plants and animals and include such substances are lecithin, cephalin, and sphingamyelin. Lecithin is a significant constituent of brain and nervous tissue consisting of a mixture of diglycerides of stearic, palmitic, and oleic acid linked to the choline ester of phosphoric acid (Zamora, 2005).

Phospholipids contribute to the stability and quality of edible oils, fats and fatty foods through their antioxidative activity or contribution to the texture (Singleton, 1993). On the other hand, they are responsible for oil discoloration during deodorization and steam distillation so that their determination is necessary to evaluate the efficiency of degumming (Mounts and Nash, 1990). Some oils contain substantial amounts of phospholipids, e.g. soybean oil, sunflower oil and canola oil or peanut oil (Przybyski and Eskin, 1991). The most important members of this class of lipids found in edible fats are phosphatidylethanolamine, phosphatidylinositol, phosphatedylserine and phosphatidyl choline. Egg yolk and milk fat also contain significant amount of sphingomyelin. The fatty acid composition of the various phospholipids depends on the fat origin (Lendrath et al., 1991). Fish oils contain similar mammalian oil phospholipids. After refining oils and fats of vegetable origin have rather low amounts so that a concentration step is needed prior to HPLC, which is the preferred method that replaced thin layer chromatography (Singleton, 1993).

SPE has been employed in certain cases for the preconcentration of phospholipids. Nash and Frankel (1986) fractionated degummed, crude soybean oils using silicic acid columns (Sep-Pak, Waters Assoc.) prior to HPLC analysis. Oil, 1 g of a 10% solution in petroleum ethendiethyl ether (95:5, v/v), was applied, the non-polar lipids were eluted with the same system (10 ml), the intermediate polarity lipids were eluted with diethyl ether (20 ml) and finally the phospholipids were obtained with methanol (10 ml). The applicability of the system was checked by thin layer chromatography of the various fractions. Caboni et al., (1996) separated phospholipids from total lipids that had been extracted from food samples (egg powder, chicken meat, ripened cheese and salami) by the Folch method. The lipid extract was purified by using normal phase (silica) ion exchange (aminopropyl) and reversed-phase (C_{18} and C_8) SPE cartridges (500 mg, BondElut, Varian). The purified fractions were analyzed by HPLC coupled with an evaporative light-scattering detector. Details of the four SPE elution systems are given in Table 3.

Based on phosphorous determination in the various fractions, the authors reported that phospholipids eluted in the third fraction from polar phases (silica and amino) and in the first fraction derived from the reversed-phase bonded materials. Though precision data were satisfactory in all four procedures, accuracy data varied significantly. C_8 packing was proved more efficient for individual and

Table 3. SPE Separation of phospholipids from fatty foods according to Caboni et al. (1996)

SPE mode *	Lipid sample	Conditioning solvent system	Elution system (v/v/v)
Silica	200mg/0.5ml $CHCl_3$	3 ml n-hexane	4 ml n-hexane:diethyl ether (8:2); 4 ml n-hexane:diethyl ether (1:1); 4 ml methanol or 4ml 1-5% CH_3COOH in methanol
Amino-propyl	200mg/0.5ml $CHCl_3$	3 ml n-hexane	2x2.5 ml $CHCl_3$:isopropanol (2:1); 2x2.5 ml 2% CH_3 COOH in diethyl ether; 4x1ml methanol
C_{18}	200mg/0.5ml CHC l_3:Methanol (2:1, v/v)	3m/methanol	4ml methanol/water (4:1); 4ml methanol; 4ml methanol; $CHCl_3$ (4:1);
C_8	200gm/0.5ml $CHCl_{3+}$: methanol (2:1, v/v)	3ml/methanol	4 ml methanol; 5 ml $CHCl_3$: methanol (3:2) 5 ml $CHCl_3$

*Column regeneration with 3 ml CHCb; 3 ml CHCIs: methanol (2:1); 5 ml methanol.

total phospholipid recoveries. Carelli et al. (1997) enriched sunflower oil samples with phospholipids using SPE cartridges and subsequently performed HPLC analysis based on the IUPAC standard method for soybean lecithin and ultraviolet detection. The bonded di-ol cartridges (500 mg, J. T. Baker) were preconditioned with methanol (2 ml), chloroform (2 ml) and hexane (4 ml). From the oil dissolved in chloroform (50 - 150 mg) triglycerides were removed with chloroform (2.5 ml). Phospholipids were eluted with 7 ml methanol that contained 0.5 ml / 100 mlof a 25% ammonia solution. Accuracy and precision data reported were quite satisfactory. Phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol were sequentially eluted from amino-propyl bonded silica with acetonitrile/n-propanol (2:1, v/v), methanol, isopropanol/methanolic HCI (4:1, v/v) and methanol/methanolic HC! (9:1, v/v). The recoveries were excellent (Pietsch and Lorenz, 1993).

Conclusion

The qualitative and quantitative determination of the major and minor constituents of vegetable oils are done by gas chromatography and primarily high performance liquid chromatography which are the two techniques widely applied for the analysis of edible oils and fats.

Solid phase extraction (SPE) is a simple approach for sample preparation in the Chromatographic analysis of edible fats as it requires small amounts of the test samples, low volumes of organic solvents, whereas the treatment is accomplished in a greatly shorter period of time than in other techniques. In the course of this work the merits of HPLC procedures have been shown for their potential, either for sample treatment, as direct analytical means or in conjunction with other chromatographic techniques.

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Full Length Research Paper

Effect of refining on the quality and composition of groundnut oil

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Crude groundnut oil was obtained by solvent extraction from groundnut seeds and refined. The crude and refined oils were then analyzed. The results obtained show that the acid value decreased from 2.890 to 0.420 mg/KOH. Free fatty acid decrease after refining from 2.82 to 2.02%; phospholipids decreased from 23.11 to 5.53%; copper content decreased from 0.37 to 0.0361% and iron decreased from 3.98 to 0.31%. These can be viewed as an improvement in the quality of the oil after refining. Refining did not have much effect on the fatty acids composition except for slight inconsistent decrease in saturated and unsaturated fatty acids.

Key words: Free fatty acids, phospholipids, crude, refined, gas chromatography.

INTRODUCTION

Arachis oil (peanut oil; groundnut oil) is derived from groundnuts (seed of *Arachis hypogaea* Linn). Groundnut oil is a vegetable oil which contains only a small proportion of non-glyceride constituents. Its fatty acid composition is complex including saturated fatty acids covering a wide range of molecular weights. Groundnut oil is excellent food oil, with good flavor and high quality with its low free fatty acid value. Vegetable oils generally primarily consist of triglycerides but several other compounds are also present. Some of these additional compounds such as diglyceride, tocopherols, sterols and sterols ester need not necessarily be removed during processing. Compounds such as phosphatide, free fatty acids, odiferous volatiles, colorant, waxes and metal compounds negatively affect taste, smell, appearance and storage stability of the refined oil and hence must be removed. Carefully separated, however, some of these additional compounds, particularly the phosphatides are valuable raw materials (Belcher, 2008).

Although there are many methods that are described in the literature that may be used to analyze fatty acids of vegetable oils, high precision in gas chromatographic analysis of fatty acids is possible with careful attention to details during sample preparation, injection, and chromatograph and data collection. Small theoretical correction factors only should be necessary in most circumstance (Christie, 1993). Also the qualitative and quantitative determination of the constitution is done by gas chromatography of the compound or their derivatives (Cert et al., 2000).

A lot of works have been done in the past and many are still on by lipid analysts to explore the potentials of vegetable oils. The importance of analyzing vegetable oils cannot be overemphasized because in analyzing vegetable oil, the major feature influencing the physical and chemical properties, their application and uses are got. The analysis of phospholipids is important because they contribute to the stability and quality of edible oils, fats and fatty foods through their anti-oxidative activities. They are also responsible for oil discoloration during deodorization and steam distillation so that their determination is necessary to evaluate the efficiency of degumming (Mounts and Nash, 1990; Monte et al., 1992; Nzai and Proctor, 1998). Metals catalyze oxidation and they are responsible for both an increase rate of oxidation and colour fixation. With the analysis of phospholipids, Cu and Fe, the adverse effect of product stability and colour can be estimated. With the analysis of free fatty acids (FFA), suitability of vegetable oils for edible

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purposes can be determined (Esuoso and Odetokun, 1995). This research was to ascertain how the refining of groundnut oil affects its quality characteristics and its constituent

EXPERIMENTATION

Seed oil exraction

Groundnut oil seed was purchased from Lagos State, South–West, Nigeria. The groundnut seedS were separated from the shaft by hand picking method. The seedS that were freed of the dirt were collected into a separate pre- cleaned beaker. The samples were weighed and kept for pulverization. The grinding machine of make JANKE and KUNKEL (IKA-LABORTECHNIC) was used for grinding until the samples were finely grinded. The funnel and the hollow parts of the machine were cleaned before another sample was grinded to avoid cross contamination.

The pulverized samples were then extracted using the soxhlet extractor arrangement equipped with thimble. Hexane was used as the solvent. The duration of the extraction was 6 h. The process was repeated twice to ensure that most of the oil in the seed was removed using fresh solvent. The crude oil with solvent extracted was collected into the pre-cleaned beaker for distillation.

The extract was poured into the round bottom flask of the rotary evaporator arrangement. It separated by driving the hexane off the extract. Then the extracted oil was dried of water by using an anhydrous sodium sulphate.

Steps used in refining the groundnut oil

The crude groundnut oil has to be refined before it can be used for different applications either in the edible or industrial application. The crude oils contain small amount of naturally occurring materials such as proteinaceous free fatty acids and phospholipids.

The method used for the removal of the FFA was by reacting it with sodium hydroxide solution (alkali) in a read stoichiometric ratio to ensure complete reaction and to make sure that no alkali was left over in the oil. The intermediate then reacted with phospholipids. These reacted products and the proteinaceous materials were then removed by centrifuge. Following the alkali refining, the oil was washed with water to remove residual soap caused by saponification of small amount of the triglycerides oil. Colour producing substances within oil, for example carotenoids and chlorophyll, were removed by bleaching process. Acid activated alumina was employed for bleaching by allowing some contact time with the oil. The refined oil was then filtered and kept for usage.

Esterification procedure

The oil sample was air dried by blowing the air gently on it. 1.0 g of oil was weighed into the beaker. The oil sample was heated in a borosilicate beaker container at 140°F with pump running to allow homogeneity of the sample. Some of drops of the acid were added to the oil in the container in a fairly fast manner of proper distribution. The samples are homogenized while the acid was being added with the aid of the mixer. About 3 ml of the methanol was put in a pre-cleaned beaker. The heater and the pump were off to allow the methanol to be added to the mixture. The methanol was added in a fast way. The mixture was mixed properly and fan was allowed to blow the fumes away. The mixture was covered properly and temperature was allowed to drop to ambient temperature (65°F). The pump and fan were on occasionally for about 4 h. After cooling, the sweet fragrance ester was decanted in to a clean borosilicate container before injecting into the gas chromatography.

Fatty acids methyl ester gas chromatogaphy analysis condition

GC: HP 6890 Powered with HP ChemStation Rev. A 09.01 [1206] Software.
Initial temperature: 60°C for 3.0 min.
First rate: 8°C/min to 140°C for 10.0 min, constant at 140°C for 5.0 min.
Second rate: 10°C/min to 250°C for 11 min, constant at 250°C for 10.0 min.
Detection Temp: 275°C.
Injection Temp: 230°C.
Detector: FID.
Carrier Gas: Nitrogen.
Column: HP-INNowax (Cross-Linked PEG).
Column Length: 30.0 m.
Column I.D: 0.32 mm.
Film Thickness: 0.50 µm.
Nitrogen Pressure: 30.0 psi.
Hydrogen Pressure: 22 psi.
Comp. Air Pressure: 28 psi.

Condition of analysis for phospholipids

Column used: ZB-5 column.
Column length:30 m.
Column ID: 0.25 mm.
Column Film: 0.25 µm.
Detector Temp: 300°C.
Detector: PFPD (Pulse Flame Photometric Detector).
Initial Temp: 30°C for 3 min.
First Rate: 4°C/min for 20 min and maintained for 1 min
Second Rate: 15°C/min for 10 min and maintained for 1 min
Mobile phase or carrier:Hydrogen.
Hydrogen column pressure:25 psi.
Hydrogen Pressure: 22 psi.
Compressed oil pressure: 28 psi.

Atomic absorption analysis of the sample

Sample was sub sampled into the conical flask. The content of the flask was treated with 5% nitric acid. The samples were prepared for different metal analysis. The solution was allowed to cool at room temperature. Standard Iron (Fe) and copper (Cu solutions of 0.20, 0.40, 0.60, 0.80 and 1.00 mg/l) were made from each of the standard heavy metals. The sets of standard solutions and filtrate of the sample were analysed by AAS. The detection limits of the heavy metals in the sample were 0.0001 mg/l by means of UNICAM 929 London, atomic absorption spectrophotometer powered by SOLAAR software. Iron and Copper cathode lamps were used for the analysis of Fe and Cu ions, respectively. Air- acetylene gas mixture was used in the generating flame.

Determination of physico-chemical properties

Saponification value of oil sample was determined by dissolving 1 g of the oil in 12.5 ml of 0.5% ethanolic KOH and the mixture of refluxed for 30 min. 1 ml of the phenolphthalein indicator was added

Table 1. Physico-chemical properties of crude and refined groundnut oil.

Parameters	Groundnut oil	
	Crude	Refined
Specific gravity at 20°C	0.916	0.915
Refractive index at 40°C	1.462	1.460
Saponification value (mgKOH/g)	192.00	188.00
Acid value (mgKOH/g)	2.890	0.420
Iodine value (mg/100g)	91.00	86.00
Odour	Agreeable	Agreeable

and the hot soap solution titrated with 0.5 N HCL. A blank determination was also carried out under the same condition and saponification value determined using the equation:

To ascertain the iodine value, 0.1 g of the oil was weighed into the 200 ml bottle capacity. 5 ml of carbon tetrachloride was added and the 2 ml of Wij's solution stood for an hour in the dark before the analysis was carried out. The blank was prepared as the sample. 5 ml of the 10% potassium iodide solution and 50 ml of water to each bottle and titrated against the 0.1 N sodium thiosulphate using starch as the indicator until colour change to permanent pale yellow.

To find the acid value 0.1 g of the dissolved in 2.5 ml of 1:1 v/v ethanol: diethyl ether solvent and titrating with 0.1N sodium hydroxide while swirling using phenolphthalein as indicator.

The Specific Gravity was measured by means of a cleaned pycnometer which was weighed. The pycnometer was filled with cooled distilled water and was stoppered. It was kept in the water bath for 30 min. It was removed and the drops of water were wiped properly and the weight was measured.

The water was thrown away, oven dried and the pycnometer was filled with oil that has been previously dried over sodium sulphate. It was stoppered and kept in the water bath for 30 min. It was then removed, water wiped properly and the weighted was measured.

To find the refractive Index, the surface of the prisms was cleaned up with ether. 2 drops of the oil was applied at the lower prism and the prisms were closed up. Water was passed through the jacket at 45^0C. The jacket was adjusted for reading to be taken.

RESULTS AND DISCUSSION

The physico-chemical properties of crude and refined groundnut oil obtained are shown in Table 1. The specific gravity of groundnut oil decreases from 0.916 to 0.915 after refining. The decrease was not significant hence refining does not have significant effect on the specific gravity. However, the decrease may be due to the removal of gumming materials and some coloring matters which affected the weight of the oil after refining. According to Paul and Palmer (1972), the specific gravity of different refining oils varies with their molecular weights which are affected by refining process involved.

The refractive index of groundnut oil decreased from 1.462 to 1.460 after refining. The decrease was not significant hence refining does not affect the refractive index significantly. In addition, the oil did not differ significantly in refractive index both in crude and at the refined forms. The decrease could be as a result of continuous removal of impurities during the refining process. According to Pearson (1991), the amount of impurities that are contained in the oil affects the degree of reflection caused by a ray of light during refractive index determination of the oil.

The odour in the sample of oils is agreeable. This means that they all have no offensive smell. This shows that refining does not affect the odour of the oil. This could be as a result of the low free fatty acids present (groundnut oil: crude, 2.821644% and refined, 2.017714% in the oil (Kirk and Sawyer, 1991).

The saponification value of the groundnut oil decreased from 192 to 188 mg/KOH after refining. This decrease generally could be due to the neutralization of fatty acids which may have resulted from the hydrolysis of the oil sample. According to Kirk and Sawyer (1991), the number of milligram of potassium hydroxide (KOH) used to neutralize the fatty acid determine the degree of hydrolysis of the oil sample.

The iodine value for groundnut oil decreases from 910 mg/100 g to 860 mg/100 g after refining. The decrease was not quite significant. Hence, refining did not affect iodine value significantly. The decrease in iodine value denotes decrease in the degree of unsaturation of the oil caused by the extent of oxidation and degree of heat treatment given to the oil during refining process (Kirk and Sawyer, 1991).

The acid value for groundnut oil decreased after refining from 2.890 to 0.420 mgKOH/g. The decrease was significant for the groundnut oil. Acid value had the highest decrease after refining (85.47%). According to Demian (1990), the acid value is used to measure the extent to which glyceride in oil has been decomposed by lipase and other actions such as light and heat and that it determination is often used as general indication of the condition and edibility of oils. This decrease is an improvement in the quality of the oil. It is an expected result, since a reduction in acid value is targeted in the refining process.

The Fatty acid composition in crude and refined groundnut oil is as shown in Table 2. The oil shows a moderate decrease in fatty acid composition from its crude form to refined form. According to Achinewhu and Akpapunam (1985), refining does not have much effect on fatty acid composition except for some slight inconsistent decrease in saturated and unsaturated fatty acid. This inconsistency was observed in this work with palmitic and palmitoleic acid. However, Caprylic acid shows the highest decrease after refining (75.96%). Oleic acid was the major unsaturated fatty acid and it recorded 58.7% in crude and 57.7% in refined followed by palmitic acid, 8.23% in crude and 11.74% in refined and linoleic acid 21.8% in crude and 21.5% in refined. The percentage of saturated fatty acid was recorded to be16.82% in

Table 2. Fatty acid composition of crude and refined groundnut oil.

Fatty acid	Crude composition (%)	Refined composition (%)
Caprylic acid (C8:0)	0.013346	0.003208
Capric acid (C10:0)	0.008544	0.005542
Lauric acid (C12:0)	0.275816	0.116076
Myristic acid (C14:0)	0.115270	0.1061
Palmitic acid (C16:0)	8.2280	11.7378
Palmitoleic acid (C16:0)	0.1073	0.1296
Stearic acid (C18:0)	2.4581	2.0606
Oleic acid (C18:1)	58.6871	57.6784
Linoleic acid (C18:2)	21.7656	21.5413
Linolenic acid (C18:3)	0.3446	0.2810
Arachidic acid (C20:0)	1.8313	1.4804
Behenic acid (C22:0)	3.8852	2.36610

Table 3. Composition of crude and refined free fatty acids and phospholipids of groundnut oil after refining.

Composition	Crude	Refined
Free fatty acid composition in groundnut oil (%)	2.82	2.01
Phospholipids Composition in groundnut oil (%)	5.53	23.12

crude and 20.37% in refined with palmitic acid recording the highest both in crude (8.23%) and refined (11.74%) respectively followed by stearic acid. Capric acid recorded the least reduction in saturated fatty acid in its crude form with 0.0085%.

The free fatty acid for groundnut oil is given in Table 3. The free fatty acid for crude groundnut oil (2.82%) is slightly greater than that in the refined oil (2.01%). From Table 3, it is vividly seen that groundnut oil shows higher percentage decrease after refining (28.49%). Crude oils with high free fatty acid content result in higher refined losses (International Conference on Palms and Palm Products, 1989). According to Esuoso and Odetokun (1995) free fatty acid of oil suitable for edibility purpose should not exceed 5%. In view of this, groundnut oil both crude and refined is edible. The amount of free fatty acid present is a measure of the quality of the unrefined as well as the refined oil (International Conference on Palms and Palm Products, 1899). Therefore, in view of the low free fatty acid in groundnut oil, both crude and refined, it makes higher quality oil. The decrease in free fatty acid could be as a result of removal of some fatty materials and free fatty acids during refining process. According to Kirk and Sawyer (1991), the presence of free fatty acid and other fatty materials in oil brings about the offensive odour and taste in the oil on long storage.

The phospholipids composition of the groundnut oil is also shown in Table 3. The amount of phospholipids on crude groundnut oil (23.12%) is higher than that recorded for refined groundnut oil. Phospholipids contribute to the stability of edible oil fats and fatty food through their antioxidation activity (Singleton, 1993; Carelli et al., 1997).

This shows that the natural antioxidative activity of refined groundnut oil may be lower than crude groundnut oil. Phospholipids are responsible for oil discoloration during deodorization so that their determination is necessary to evaluate the efficiency of degumming (International Conference on Palms and Palm products, 1989; Mounts and Mash, 1990, Mounts et al., 1992; Nzai and Proctor, 1998). On heating crude groundnut oil to deodorization temperature, the brown coloration that will be observed will be more than observed for refined groundnut oil.

The copper concentration of groundnut oil decreased from 0.37 to 0.036 mg/Kg after refining and the iron concentration of same oil decreases from 3.98 to0.31 mg/Kg after refining as shown in Table 4.

The percentage decrease of iron in groundnut oil after refining is the most significant (92.19%). Metals such as iron and copper catalyze oxidation and therefore are responsible for both an increase rate of oxidation (International Conference on Palm and Palm materials products, 1989). Therefore, in view of the higher concentration of metals in crude groundnut oil will be higher than that in refined groundnut oil.

Table 4. Copper and iron concentration in the groundnut oil samples.

Concentration of metals in the oils	Crude	Refined	% decrease in metals after refining
Concentration of copper in groundnut oil (mg/kg)	0.3738	0.0361	91.07
Concentration of iron in groundnut oil (mg/kg)	3.9832	0.3109	92.19

Conclusion

Refining slightly affects the physical and chemical characteristics of groundnut oil. There was decrease from the crude form to the refined form and there was a significant decrease in acid value. This significant decrease confirms the presence of oleic acid which is significant in groundnut oil. As for the major and minor constituents, refining did not have much effect on the fatty acid except for some slight inconsistent decrease in saturated and unsaturated fatty acids.

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