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Full Length Research Paper

Estimation of total free fatty acid and cholesterol content in some commercial edible oils in Ethiopia, Bahir DAR

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In the present investigation an attempt has been made to find out total free fatty acid and cholesterol content in some commercial edible oils in Ethiopia, Bahir Dar. Acid value, peroxide value, saponification value and cholesterol content were determined in a total of nine varieties of edible vegetable oils of which six are branded and three are non-branded. The analysis, performed using Liebermann-Burchard method, revealed varying levels of cholesterol content. Cholesterol was detected in seven of the vegetable oils while for two oil samples (Niger seed k-16 and Niger seed k-7) it was nil. Rapeseed branded vegetable oil has significantly highest cholesterol concentration (257.1 ± 0.42 mg/L) while branded palm oil has the least concentration (88.8 ± 0.85 mg/L) of cholesterol. Unlike the peroxide value, all the acid values and some saponification values show high values in comparison with the maximum permissibility level of codex standard for named vegetable oils (CODEX STAN210-1999). In conclusion, this study could be a guide line, to understand the quality of vegetable oils in Ethiopia, Bahir Dar. Consequently, these studies have been able to show that there is no cholesterol free oil in the market as opposed to what is claimed on the vegetable oil brand labels. Therefore, companies producing and marketing vegetable oils are enjoined to desist from misleading the public by labeling their products as "cholesterol free". They should indicate the amount of cholesterol present in the vegetable oil, no matter how small the quantity may be.

Key words: Edible oils, free fatty acid, cholesterol, peroxide value, saponification value and liebermann-Burchard test.

INTRODUCTION

Human beings from time immemorial have been using plants for a multitude of purposes. Oils have always been an integral part of human foods, being essential for health. Industrially, they play an important role in the development of different areas of chemical products, pharmaceutical, cosmetics, paints and most importantly, food (Atef, 2010).

Oils are naturally occurring esters of long straight-chain carboxylic acids. They belong to the saponifiable group (contain an ester groups) of lipids. Lipids are biologically produced materials that are relatively insoluble in water but soluble in polar and non-polar organic solvents. Edible oils are constituted of triacylglycerol molecules, mainly formed by unsaturated (oleic, linoleic, linolenic acids etc.) and saturated fatty acids (myristic, palmitic, stearic acids etc.) esterified to Glycerol units (Andersson et al., 2010). They can be formed from a single fatty acid that could be esterified up to three times into glycerol backbone, or at least by three different ones.

Almost every adult at present times develops some degrees of atherosclerosis, commonly known as "hardening of the arteries". Atherosclerosis leads to strokes, heart attacks and other serious health problems. High cholesterol, smoking and high blood pressure are the principal risk factors linked to heart disease.

The word "cholesterol" may quickly be associated with chronic heart disease and other heart problems. However,

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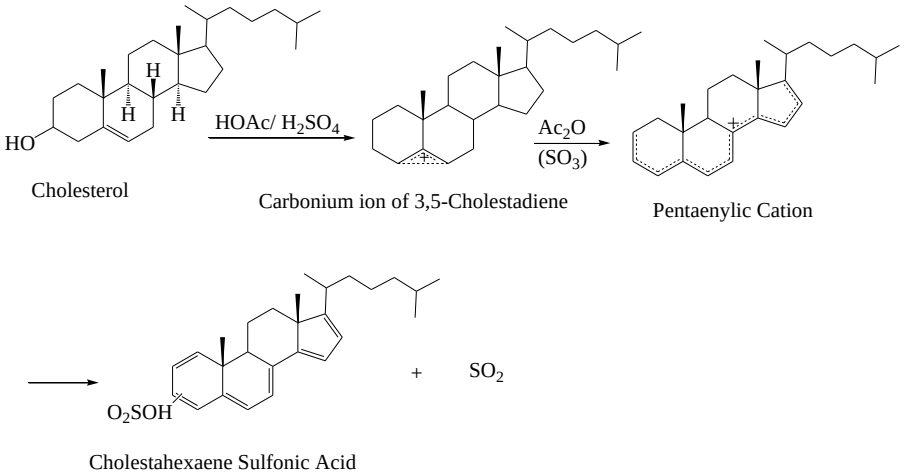

Figure 1. Mechanism for Liebermann-Burchard reaction.

cholesterol also has essential functions in the body such as providing essential components of membrane and serving as a precursor of bile acids, steroid hormones and vitamin D. Consuming cholesterol in our diet increases the level of low density lipoproteins (LDLs). There are so many different varieties of vegetable oil brands in our markets and all of them claim to be cholesterol free. Due to increasing awareness on the health implications of high cholesterol in the diets, most people now prefer to purchase cholesterol free vegetable oils (Attarde et al., 2010).

In general the chemistry of sterols includes a large amount of knowledge relating to the chemical properties, chemical synthesis, and analysis of sterols. The present investigations also include estimation of cholesterol in some commercial edible oil samples using UV-visible spectrophotometer by Liebermann-Burchard method (Attarde et al., 2010). Liebermann-Burchard test is used for colorimetric test to detect cholesterol, which gives a deep green color. The formation of a green or green-blue color after a few minutes mean is positive result. This color begins as a purplish, pink color and progresses through to a light green then very dark green color. The color is due to the hydroxyl group (-OH) of cholesterol reacting with the reagents and increasing the conjugation of the unsaturation in the adjacent fused ring (Figure 1). In this reaction the acetic acid in the Liebermann reagent reacts with cholesterol in the sample, which gives a green color whose absorbance, can be determined by UV-visible spectrophotometer at 640 nm (Burke et al., 1974).

MATERIALS AND METHODS

In this study, the term branded vegetable oil (BVO) is used to indicate edible vegetable oils that are produced from registered industries in Ethiopia and imported oils with brand name labeled on the product purchased from the market, while unbranded vegetable oil (UVO) refer to the locally produced edible vegetable oils without a brand, they were obtained specifically from their raw materials processed in small scale in Bahir Dar city.

Sources of edible vegetable oils (EVOs) sold in market of Ethiopia includes both imported and derived from local oil seeds. Samples of six brands of vegetable oils produced from a variety of oil seeds such as: (oil palm, rapeseed oil, cottonseed oil, mixture of 80% cottonseed and 20% rapeseed Oil, olive oil and sunflower oil) were purchased from Bahir Dar supermarket and Modjo Edible Oil Complex, Addis Ababa, under various brand names. Samples of three non-branded vegetable oils are: two Niger seed oils from different sites and rapeseed oil were purchased from Bahir Dar in which they are produced in small scale.

Each edible oil sample was grouped into three sets: The first were produced from Ethiopian oil factory; Modjo Edible Oil Complex (cottonseed, rapeseed and 80% cottonseed + 20% rapeseed), the second were imported oils (palm oil, sunflower and olive oil) and the third were oils produced in small scale, non-branded (niger seed oils and rapeseed oils). These were purchased as packaged by plastic, highland, and stored under room temperature in the laboratory until it was required for analysis. Selected analytical methods adopted by American Oil Chemist's Society (AOCS) for determination of the composition and stability of fats and oils include peroxide value (PV), anisidine value (AV), acid value (AV), iodine value (IV), saponification value (SV) and also gas chromatography measurements, spectroscopic measurements such as UV, NMR and IR (Barbara et al., 2005; Yookyung et al., 2007; Otunola et al., 2009).

Acid value

Each oil sample (1.0 g) was weighed and dissolved with 50 ml of ethanol in a conical flask. Two drops of phenolphthalein indicator were added and titrated to pink end point (which persisted for 15 minutes) with 0.1 N potassium hydroxide solution (KOH). Acid value was calculated (Equation 1) (Okpuzor et al., 2009):

$$\text{Acid value} = \frac{56.1xVxC}{m} \quad (1)$$

Where 56.1 is equivalent weight of KOH, V is the volume in ml of standard volumetric KOH solution used, C is the exact

Table 1. Liebermann-Burchard method for cholesterol estimation.

Reagents (ml)	S_1	S_2	S_3	S_4	S_5	S_6
Standard cholesterol solution	0.4	0.6	0.8	1.0	1.2	-
Liebermann-Burchard reagent	2	2	2	2	2	2
Chloroform	7.6	7.4	7.2	7.0	6.8	8

Table 2. Absorbance of standard cholesterol solutions for calibration curve at different concentrations at 640 nm.

Concentration (mg/L)	Absorbance
80	0.074±0.001
120	0.257±0.000
160	0.445±0.003
200	0.654±0.011
240	0.922±0.000

concentration in KOH solution used (0.1 N); m is the mass in grams of the test portion (1 g).

Saponification value

Saponification value was determined according to titremetric method discussed by Pearson (1981). Two grams of oil samples were weighed into a conical flask and 25 ml ethanolic potassium hydroxide was added. The solution was refluxed for 2 h with time to time shaking. One ml phenolphthalein was added and titrated with 0.5 N hydrochloric acid (HCl). The same process was conducted for blank determination. The value was calculated (Equation 2).

$$\text{Saponification value} = \frac{(V_o - V_1)\, x C x 56.1}{m} \quad (2)$$

Where 56.1 is equivalent weight of KOH, V_0 is the volume in ml of standard HCl solution used for the blank test, V_1 is the volume in ml of the standard HCl solution used for sample, C is the exact concentration of the standard HCl (0.5 N) solution and m is the mass in gram of the test portion (2 g).

Peroxide value

Peroxide value was evaluated according to AOCS Official Method Cd 8-53 (2003). Five grams oil samples were weighed into a conical flask and 30 ml of solvent mixture of glacial acetic acid-chloroform in the ratio of 3:2, respectively, were added to the oil samples. Half ml saturated potassium iodide (KI) solution was added to the solution and allowed to stand for 1 min thereafter, 30 ml of distilled water were added and titrated with 0.01 N sodium thiosulfate solution using starch indicator until the yellow color was discharged. A blank was prepared alongside the oil samples. Peroxide value was calculated (Equation 3).

$$\text{Peroxide value} = \frac{10x(V_1 - V_2)}{m} \quad (3)$$

Where: V_1 volume of $Na_2S_2O_3$ for determination of test sample in ml, V_2 volume of $Na_2S_2O_3$ for determination of blank solution in ml and m is mass of test portion in g (5 g).

Cholesterol estimation

Cholesterol content was estimated using Liebermann-Burchard reagent (Attarde et al., 2010). Standard cholesterol solution used was 2 mg/ml as stock solution. Liebermann-Burchard reagent was prepared with 7 ml concentrated sulfuric acid and 5 ml glacial acetic acid and was covered with black paper and kept in ice bucket in dark place.

Preparation of sample solutions and standard cholesterol solutions

Six volumetric flasks were marked as S_1, S_2, S_3, S_4, S_5 and S_6. Standard cholesterol solution was added as 0.4, 0.6, 0.8, 1.0 and 1.2 ml in five volumetric flasks whereas, flask six was kept blank. Two ml of the Liebermann-Burchard reagent were added to all six volumetric flasks and diluted to final volume of 10 ml with chloroform (Table 1). Flasks were covered with black carbon paper and kept in dark for 15 min. Then, set zero of spectrophotometer with blank (s_6) at 640 nm. The absorbance of all standards (six flasks) were determined on SP65 UV/Vis spectrophotometer (Table 2) and standard graph was plotted (Figure 2).

Three ml of sample solutions were taken and their absorbances were determined on SP65 UV/Vis Spectrophotometer after adding 1 ml oil sample, 2 ml Liebermann-Burchard reagent and 7 ml chloroform. Cholesterol concentration of sample solutions was determined (Table 3) using a standard curve constructed graphically plotting the absorbance against mg/l cholesterol.

RESULTS AND DISCUSSION

The research work was conducted to determine the extent of oxidative deterioration and cholesterol content

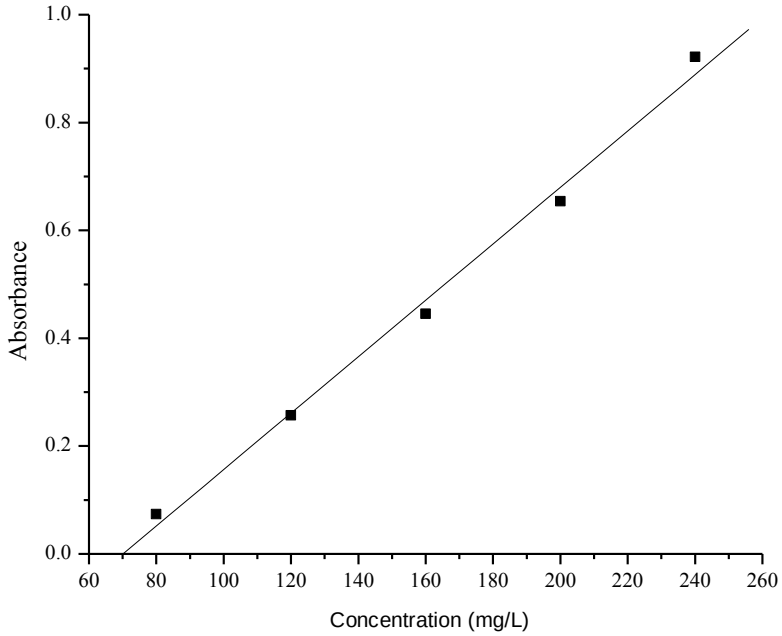

Figure 2. Calibration curve using cholesterol standard.

Table 3. Acid value, peroxide value, saponification value and cholesterol content for nine vegetable oil samples.

Sample	Acid value (mg KOH/g)	Peroxide value (meq.peroxide/kg)	Saponification value (mg KOH/g)	Cholesterol (mg/L)
Cottonseed oil	7.57 ± 0.39^{bcd}	0.60 ± 0.85^{b}	228.55 ± 0.00^{b}	131.90 ± 0.14^{f}
80% cottonseed + 20% rapeseed oil	8.98 ± 0.79^{bc}	3.00 ± 1.41^{ab}	228.22 ± 0.46^{b}	190.60 ± 0.28^{d}
Rapeseed	7.01 ± 1.19^{cd}	2.10 ± 0.14^{ab}	32.54 ± 0.16^{e}	257.10 ± 0.42^{a}
Rapeseed k-16	10.38 ± 1.19^{ab}	3.50 ± 0.14^{ab}	31.99 ± 0.92^{e}	139.20 ± 0.28^{e}
Niger seed k-16	8.69 ± 0.39^{bc}	3.80 ± 0.28^{a}	162.76 ± 0.69^{c}	ND
Niger seed k-7	12.06 ± 0.40^{a}	3.10 ± 0.42^{ab}	34.12 ± 2.08^{e}	ND
Olive oil	7.29 ± 0.79^{cd}	1.70 ± 0.42^{ab}	228.39 ± 0.23^{b}	234.80 ± 0.00^{b}
Palm oil	4.77 ± 0.39^{d}	2.20 ± 0.00^{ab}	260.22 ± 1.38^{a}	88.80 ± 0.85^{g}
Sunflower oil	5.05 ± 0.79^{d}	2.07 ± 0.11^{ab}	65.27 ± 0.04^{d}	204.60 ± 1.13^{c}

ND: not detected. Values with same letters in the same columns are not significantly different from each other according to Tukey HSD ($p < 0.05$).

from some edible oils under room temperature. Which attempts to make comparative quality assessment of industrial processed BVO against small scale processed UVOs found in Bahir Dar. This also provides supportive information for routine quality monitoring of edible vegetable oils that are used for food stuff.

The absorbance of each standard solution was measured three times at 640 nm following the procedure discussed earlier. The resulting absorbances are shown in Table 2. The equation of the calibration curve was obtained from the resulting absorbance versus concentration (mg/l) curve (Figure 2): $y = 0.005 x – 0.366$: $R^2 = 0.9967$. The analytical data for acid value, peroxide value, saponification value and cholesterol content of nine oil samples are shown in Table 3.

Acid value

Acid value is a measure of the free fatty acids in oil. Normally, fatty acids are found in the triglyceride form, however, during processing the fatty acids may get hydrolyzed into free fatty acid. The higher the acid value found, the higher the level of free fatty acids which translates into decreased oil quality. Acceptable levels for all oil samples should be below 0.6 mg KOH/g (measured in potassium hydroxide per gram) (AOCS Official Method Cd 8-53, 2003).

According to overall analysis of variance (ANOVA), acid value significantly varied among samples ($F = 18.7$, $P < 0.0001$, $R^2 = 0.94$). Niger seed k-7 and rapeseed k-16 have significantly higher acid value than the rest of oil samples (Table 3). Cottonseed, olive, rapeseed, sunflower, palm and olive oils had significantly lower acid values than the rest (Table 3). The minimum acid value was recorded for palm oil.

The acid values of UVOs were higher than BVOs, and the variations were statistically significant ($p < 0.05$). This hydrolysis is probably caused by a variety of agents presence of moisture in the oil, elevated temperature (above room temperature) and, most important of all, lipases (enzyme) coming from the source or contaminating microorganisms. This observation supports previous study that unrefined vegetable oils had higher acid value than refined oils (Rajko et al., 2010).

Among all oil samples the UVO, Niger seed oil k-7, has significantly ($p < 0.05$) highest (12.06 ± 0.40 mg KOH/g) acid value which indicate high free fatty acids and leads to a tendency to become rancid that is off-flavor (Tamzid et al., 2007). Probably, during processing the fatty acids may react with water and get hydrolyzed into the free fatty acids. In addition, niger seed oil k-7 is processed other than chemical refining methods (neutralization), which lower the free fatty acid content (Cmolik and Pokorny, 2000). Long storage of the oil seeds before or after processing may also have been responsible. Kalua et al. (2008) discussed that there were changes in oil quality during cold temperature storage of the fruit. This high content of free fatty acid levels has high probability for decreasing the ability of liver to store sugars (Gur and Harwood, 1991; Charlotte, 2004).

In contrast, palm oil has significantly ($p < 0.05$) lowest (4.77 ± 0.39 mg KOH/g) acid value. This is probably due to the higher content of saturated palmitic acid, which is less prone to oxidation than unsaturated fatty acids, linolenic acid, linoleic acid etc (Paul et al., 1992). The high degree of saturated fatty acids enables oils to be stable against oxidative rancidity and confer a longer shelf-life (Nwinuka and Barine, 2009). Interestingly, remaining seven oil samples have the acid values between these two oil samples.

All vegetable oil samples presented in this study have higher acid value than the Codex Standard for Named Vegetable Oils (CODEX-STAN210-1999) (0.6 mg KOH/g). Barring acid value, there is no significance difference ($p > 0.05$) estimated among branded local edible oils and imported edible oils. But in case of acid value branded local edible oils shows significantly high content, which implies that much free fatty acid may produced by factors.

Peroxide value

According to the overall ANOVA, peroxide value significantly varied among samples ($F = 4.2$, $P = 0.0226$, $R^2 = 0.79$). PV is a measure of oxidation during storage and the freshness of lipid matrix. In addition, it is a useful indicator of the early stages of rancidity occurring under mild condition and it is a measure of the primary lipid oxidation products. One of the most important parameters that influence lipid oxidation is the degree of unsaturation of its fatty acids. When double bonds of unsaturated fats are oxidized, peroxides are among the oxidation products formed. High peroxide value is an indicator of oxidation level and the greater the peroxide value, the more oxidized the oil is (http://www.bioriginal.com).

Peroxide values of all the oil samples were in agreement with the maximum Codex standard peroxide value (10 meq O_2/Kg) for vegetable oil deterioration. Niger seed k-16 has significantly high (3.80 ± 0.28 meq O_2/kg) peroxide value and hence high degree of unsaturation. This observation helps to suggest that niger seed oil has high content of unsaturated fatty acids, linoleic (C18:2) and oleic acid (C18:1), which are responsible for oxidative rancidity (Mohamed and Jörg-Thomas, 2002). A significantly low value was observed in cottonseed oil (0.60 ± 0.85 meq.O_2/kg). More significantly, peroxide values of all edible oil samples are in agreement to the Codex Standard for named Vegetable Oils (CODEX-STAN210-1999) maximum permissibility level (10 meq O_2/kg). PV of all the UVOs was higher than that of the BVOs, and the variations were statistically significant ($p < 0.05$). 80% cottonseed + 20% rapeseed oil shows high (3.00 ± 1.41 meq O_2/kg) PV, and is significantly higher than its branded counter parts. The present studies indicate that the UVO have higher PVs, which is very likely in products that do not contain preservatives, either natural or synthetic. Acid values are also in agreement with this observation. The industrial processing of EVO includes preservatives and hence reduces the high PV over time, and present results are in line with the reported literature (Chabiri et al., 2009).

Saponification value

Saponification value is an indication of the molecular weights of triglycerides in oil and significantly ($p < 0.05$) high proportion of saponification value in the palm oil (260.22 ± 1.38 mg KOH/g) suggests that the oil is a good raw material for soap industries. Higher Saponification value indicates high proportion of lower fatty acids since saponification value is inversely proportional to the average molecular weight or chain length of the fatty acids (Muhammad et al., 2011). Therefore, shorter the average chain length (C_4-C_{12}) the higher is the saponification number (Tamzid et al., 2007). The value obtained for palm oil during this study show that it contains high amounts of short chain fatty acids (< C_{12}), which is greater than the regulation of codex standard permissibility level (CODEX-STAN210-1999) (190-209 mg KOH/g).

Rapeseed k-16 has significantly ($p < 0.05$) lowest (31.99 ± 0.92 mg KOH/g) saponification value (except rapeseed and Niger seed k-7) that is also lower than codex standard permissibility level (168-181 mg KOH/g), which suggest that it contains high molecular weight long chain fatty acids hence are unsuitable for soap making and also unsuitable for human nutrition (Akinhanmi and Atasie, 2008).

Among nine oil samples studied, cottonseed oil (228.55 ± 0.00 mg KOH/g), olive oil (228.39 ± 0.23 mg KOH/g), 80% cottonseed + 20% rapeseed oil and palm oil (260.22 ± 1.38 mg KOH/g) have high saponification value than the regulation of codex standard. Typically, these oils have low molecular weight fatty acids. On the other hand, rapeseed oils (branded (32.54 ± 0.16 mg KOH/g) and unbranded (31.99 ± 0.92 mg KOH/g)), sunflower oil (65.27 ± 0.04 mg KOH/g) and niger seed oils both k-7 (34.12 ± 2.08 mg KOH/g) and k-16 (162.76 ± 0.69 mg KOH/g) have low saponification values compared to the guide line values. It indicates that they have high content of long chain fatty acids.

Cholesterol content

Representative analytical data for cholesterol content of said oil samples are presented in Table 3. Out of these, seven oil samples contained cholesterol include cottonseed oil, 80% cottonseed + 20% rapeseed oil, rapeseed both branded and non-branded oils, olive oil, palm oil and sunflower oil. Among these rapeseed oil has significantly ($p < 0.05$) maximum (257.10 ± 0.42 mg/L) cholesterol content and palm oil has significantly ($p < 0.05$) low (88.8 ± 0.85 mg/L) cholesterol content. Surprisingly, the two Niger seed oils (niger seed k-16 and niger seed k-7), contained nil cholesterol content. However, cholesterol content of all the BVOs was higher than that of the UVOs, and the variations were statistically significant ($p < 0.05$). The peroxide value of these samples supports the observation, that is, Niger seed may have high content of the essential fatty acid; linoleic (C18:2) acid, which has the ability to decrease cholesterol levels, stimulate cholesterol excretion into the intestine and inhibit biosynthesis of cholesterol in the liver. Oils containing high level of polyunsaturated fatty acid are found to inhibit the activity of hydroxymethylglutaryl-coenzymeA-reductase (HMG-CoA-reductase) which is the regulatory enzyme in cholesterol biosynthesis (Carl et al., 2009; Seddigheh et al., 2009; Ejikeme et al., 2010).

Finding from this study supports previous work that cholesterol is present in vegetable oils, although in small proportion (Okpuzor et al., 2009; Syed et al., 2003).

REFERENCES

Akinhanmi TF, VN Atasie (2008). Chemical Composition and Physicochemical Properties of Cashew nut (*Anacardium occidentale*) Oil and Cashew nut Shell Liquid. J. Agric. Food Environ., Sci. 2: 1-10.

Andersson B, WPD Caroline, RC Francinete, S Fabio, A Cesar, GF Antonio (2010). A simple methodology for the determination of fatty acid composition in edible oils through ^{1}H NMR spectroscopy. Magn. Reson.Chem., 48: 642-650.

AOCS (2003). Official Method Cd 8-53. American Oil Chemists Society, Champaign, IL. Accessed on www.bioriginal.com.

Atef MA (2010). Physiological effects of some plant oils supplementation on streptozotocin-induced diabetic rats. Res. J. Med. Sci., 5: 55-71.

Attarde D, J Pawar, B Chaudhari, S Pal (2010). Estimation of sterols content in edible oil and ghee samples. Int. J. Pharm. Sci. Rev. Res., 5: 135 -137.

Barbara M, L Bernhard, M Antonio, JA Maria (2005). Direct monitoring of lipid oxidation in edible oils by Fourier transform Raman spectroscopy. Chem. Phys. Lipids, 134: 173-182.

Burke RW, BI Diamondstone, RA Velapoldi, O Menis (1974). Mechanisms of the Liebermann-Burchard and Zak Color Reactions for Cholesterol. Clin. Chem., 20: 794-801.

Carl JL, VM Richard, RM Mandeep, OV Hector (2009). Omega-3 Polyunsaturated Fatty Acids and Cardiovascular Diseases. J. Am. Coll. Cardiol., 54: 585-594.

Chabiri SA, SS Hati, GA Dimari, VO Ogugbuaja (2009). Comparative Quality Assessment of Branded and Unbranded Edible Vegetable Oils in Nigeria. Pacific J. Sci. Technol., 10: 927-934.

Charlotte G (2004). Medicine Net. Com.

Cmolik J, J Pokorny (2000). Physical refining of edible oils. Eur. J. Lipid Sci. Technol., 102: 472-486.

Ejikeme PM, LN Obasi, AC Egbuonu (2010). Physico-chemical and toxicological studies on *Afzelia africana* seed and oil. Afr. J. Biotechnol., 9: 1959-1963.

Gur MI, JL Harwood 1991. Lipid Biochemistry: An introduction. London: Chapman and Hall, p. 406. http://www.bioriginal.com. Accessed on 16/2/2011.

Kalua CM, DR Bedwood, AG Bishop, PD Pienzler (2008). Changes in virgin oil quality during low temperature fruit storage. J. Agric. Food Chem., 56: 2415-2422.

Mohamed FR, M Jörg-Thomas, (2002). Proximate Neutral Lipid Composition of Niger (*Guizotia abyssinica Cass*.) Seed. Czech. J. Food Sci. 20: 98–104.

Muhammad N, E Bamishaiye, O Bamishaiye, L Usman, M Salawu, M Nafiu, O Oloyede, (2011). Physicochemical Properties and Fatty Acid Composition of *Cyperus esculentus* (Tiger Nut) Tuber Oil. Biores. Bull., 5: 51-54.

Nwinuka NM, IN Barine, (2009). Physico-chemical Properties and Fatty Acid Composition of *Dennettia tripetala* Fruit Oil (Pepper Fruit). Niger. J. Biochem. Mol. Biol., 24: 42 - 46.

Okpuzor J, VI Okochi, HA Ogbunugafor, S Ogbonnia, T Fagbayi, C Obidiegwu, (2009). Estimation of cholesterol level in different brands of vegetable oils. Pak. J. Nutr. 8: 57-62.

Otunola GA, GB Adebayo, OG Olufemi (2009). Evaluation of some physicochemical parameters of selected brands of vegetable oils sold in Ilorin metropolis. Int. J. Phys. Sci. 4: 327-329.

Paul JN, N Manny, BG Baryan, M Rosemary, MC Peter, A Mavis (1992). Plasma lipoprotein lipid and lipid changes with substitution of elaidic acid for oleic acid in the diet. Am. J. Clin. Nutr., 55: 46-50.

Pearson D (1981). The Chemical Analysis of Food. Churchill Publishing, London, pp. 580-581.

Rajko V, V Sergeja, A Helena (2010). Biochemical parameters and oxidative resistance to thermal treatment of refined and unrefined vegetable edible oils. Czech J. Food Sci., 28: 376–384.

Seddigheh A, N Bahar, S Nizal, P Sahar, S Salbali, E Ahmad, A Leila, (2009). Evaluation of fatty acid content of some Iranian fast foods with emphasis on *trans* fatty acids. Asia Pac. J. Clin. Nutr., 18: 187-192.

Syed MS, H Imran, DAG Syed, (2003). Estimation of sterols in edible fats and oils. Pak. J. Nutr., 2: 178-181.

Tamzid HM, MT Alam, MAU Islam (2007). Physico-chemical and nutritional studies of *Terminalia belerica* roxb. Seed oil and seed kernel. J. bio-sci., 15: 117-126.

Yookyung K, SH David, EK Sandra (2007). ATR-Fourier transform mid-infrared spectroscopy for determination of *trans* fatty acids in ground cereal products without oil extraction. J. Agric. Food Chem., 55: 4327-4333.