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Estimation of Cholesterol Level in Different Brands of Vegetable Oils

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Abstract: An analysis of twenty one assorted brands of vegetable oils in Lagos Metropolis Nigeria, reveals varying levels of cholesterol content. Cholesterol was found to be present in most of the oil brands sampled using three standard methods. Cholesterol was detected in seventeen of the vegetable oil brands with concentration of less than 1 mg/ml while seven of the oil brands had cholesterol concentrations ranging between 1-4 mg/ml. Low iodine values were obtained in four of the vegetable oil brands and three of them had high acid values. High performance liquid chromatography (HPLC) confirmed the presence of cholesterol at varying concentrations in all the oil brands and gave the lowest detectable cholesterol values in all the oil brands. The Laser brand made from rapeseed had the highest cholesterol concentration of 3.2 mg/ml while Grand brand made from groundnuts had the least concentration (0.12 mg/ml) of cholesterol using HPLC analysis. Leibermann-Burchard method showed that Gino brand from palm kernel had the least concentration of cholesterol (3.86 mg/ml ±0.032) and the highest concentration of 3.996 mg/ml ±0.0404 was obtained in Sesame seed oil brand. This report is important in view of health implications of cholesterol in our diets. Consequently, we have been able to show that there is no cholesterol free oil in the market as shown 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: Vegetable oils, heart diseases, leibermann-burchard, cholesterol

Introduction

Many vegetable oils are consumed directly or used as ingredients in food. Reports show that approximately 75% of the World's production of oils and fats come from plant sources (Raven and Johnson, 1999). Although many plant parts yield oil, in actual commercial practices, oil is extracted primarily from seeds of oilseed plants and according to the USDA (WASDE-320, 1996), the oilseed plants commonly used worldwide include; soybean, cotton, palm, rape and groundnut. Cholesterol, contrary to popular belief, is present in plants (Behrman and Venkat, 2005). Cholesterol has been detected in vegetable oils, where it could make up to 5% of the total sterols and a relatively high amount of cholesterol was described in camelina oil (about 200 mg/kg) (Shukla *et al*., 2002). It has also been found to be a major constituent of the chloroplasts, shoots, pollens, seeds and leaf surfaces (Behrman and Venkat, 2005, Noda *et al*., 1988).

Cholesterol, a lipid, plays a vital role in the physiological regulation of membrane fluidity and proper functioning of cells. It is also a major precursor in the production of bile acids, steroid hormones as well as vitamin D. Cholesterol found in the cell membrane of all cells, has been of great medical importance in recent years, because its high level in the body has been associated with coronary heart diseases (CHDs) (Laker, 2003). Coronary heart disease (CHD) is the leading cause of death in most industrialized countries and its importance as a major public health problem is increasing in developing countries (Murray and Lopez, 1996).

However, what is becoming clearer and clearer is that it is not the amount of fat in the diet that matters but the type of fat (Hu *et al*., 2001). Metabolic studies have shown that Trans fats have adverse effects on blood lipid levels, increasing LDL ("bad") cholesterol while decreasing HDL ("Good") cholesterol. This combined effect on the ratio of LDL to HDL cholesterol is double that of saturated fatty acids (Mensink *et al*., 2003).

Industrial processing especially catalytic hydrogenation of vegetable oils affects their fatty acid composition (Gur and Harwood, 1991). Processing increases saturated fatty acids component of oils. Saturated fatty acid rich diets have been found to increase the level of cholesterol (Keys *et al*., 1965).

Thus, we are concerned by the fact that Nigerian markets are flooded with assorted processed vegetable oils from different parts of the world all labeled to be cholesterol free. In this study, cholesterol content was

determined by three different methods, in 21 brands of vegetable oils sold in Lagos metropolis in order to ascertain this claim. The acid and iodine values of some of the samples were also determined.

Materials and Methods

Samples of 21 brands of vegetable oil produced from a variety of oil seeds (oil palm. Soya bean, rapeseed, sesame seed, cottonseed and peanut) were purchased from various markets in Lagos Metropolis. The label on each sample container was "NO CHOLESTEROL".

Chemicals, reagent and equipment: All chemicals were supplied by BDH chemicals Ltd, Pool. England. Spectrophotometer is Spectronic Genesys Tim_8, HPLC analysis carried out using Agilent 1100 series, C18 column (250*4.0 mm, 5 µm).

Determination of cholesterol content:

Method 1: As described by Ojiako and Akubugwo (1997).

Total 0.1 mL of sample oil each and standard cholesterol dissolved in chloroform in ratio 1:10 was evaporated to dryness in a water bath at 50°C. Glacial acetic acid (3.0 mL) and 3.0 mL of colour reagent (a solution of ferric chloride/glacial acetic acid/sulphuric acid), was added to each sample and the standard, then shaken vigorously to dissolve the oil. Blank contained 2.0 mL of chloroform, 3.0 mL glacial acetic acid and 3.0 mL of colour reagent. After cooling for 30 mins at room temperature, absorbance of standard and samples were read at 560 nm. Cholesterol content was estimated with the formula:

$$\text{Cholesterol mg /00 mL} = AB/AS \times CS$$

Where,
AB = Absorbance of oil sample.
AS = Absorbance of Standard cholesterol.
CS = Concentration of Standard cholesterol.

Method 2: Liebermann- Burchard method as described by Bloor, 1916.

The Liebermann-Burchard reaction method is a colorimetric method in which cholesterol is treated with chloroform, acetic anhydride and concentrated sulfuric acid to produce a green colour which is measured spectrophotometrically.

Method 3: High Performance Liquid Chromatography.
The oil samples were first saponified with 3% ethanolic KOH and the resulting nonsaponifiable lipids were then dissolved in chloroform and the analysis was carried out immediately. The HPLC analysis was done using an Agilent 1100 series, C18 column (250*4.0mm, 5 µm), acetonitrile/water (1:1) mobile phase and a UV detector at 239 nm at a flow rate of 0.4 ml/min.

Determination of iodine and acid values: The methods of British Pharmacpoeia (2000) were used to determine the Acid and Iodine value of the samples.

Iodine value: Chloroform (2%, 2.0 mL) and 5.0 mL of WIJ's solution (8.5g iodine/7.8 g iodide trichloride/450 mL glacial acetic acid in 1 liter acetic acid) were added to sample from a burette and mixed thoroughly. Blank contained 2 mL of chloroform and 5 mL of WIJ's solution. The test samples and the blank were left in the dark for 5 min and 3.0 mL of 7.5% w/v potassium iodide was added to all test samples and blank. Starch indicator (0.1 mL) was added to each sample and blank and titrated to a colorless end point using 0.1N sodium thiosulfate solution. Iodine value was calculated using the formula:

$$\text{Iodine value} = \frac{(\text{Titer value of blank} - \text{titer value of oil samples}) \text{ mL} \times 0.01269 \times 100}{\text{Weight of oil sample (g)}}$$

Acid value: Each oil sample (1.0 g) was weighed and neutralized with 50 mL of fat solvent. 2 drops phenolphthalein indicator were added and titrated to pink end point (which persisted for 15 mins) with 0.1 N potassium hydroxide solution. Acid value was calculated using the formula:

$$\text{Acid value} = \frac{V \times 0.00561 \times 1000}{\text{Weight of oil sample (g)}}$$

V = Volume of 0.1N KOH used.

Results

The analytical data for cholesterol content of twenty-one oil samples from the retail markets are shown Table 1. Seventeen of the samples had cholesterol levels lower than 1 mg/ml while cholesterol levels of seven of them ranged between 1-4 mg/ml. Paradoxically, HPLC method did not detect cholesterol in Lesieur oil brand and Coconut oil. Ojiaku and Akubugwo and Liebermann-Burchard, methods detected cholesterol (0.907±0.095mg/ml and 3.116±0.266mg/ml) respectively in Lesieur oil while Liebermann-Burchard method detected cholesterol (1.642 ± 1.198) in Coconut oil. Sesame seed oil brand which had the highest cholesterol content according to the methods of Ojiako & Akubugwo and Liebermann-Burchard however showed a moderate amount of cholesterol from our HPLC observations.

Table 1: Concentrations of cholesterol of oil samples

	Sample (Brand)	Ojiako and Akubugwo (mg/ml)	Liebermann-Burchard (mg/ml)	HPLC (mg/ml)
1	Sesame	1.20±0	3.996±0.404	0.57±0.200
2	Century	0.872±0.146	2.749±0.561	0.55±0.050
3	Sunola (grdnut)	0.368±0.035	1.711±0.323	0.44±0.010
4	Kings	0.662±0.277	0.622±0.186	0.92±0.030
5	Grand (soya)	1.054±0.093	1.508±0.058	0.839±0.04
6	Laser	0.846±0.069	-	3.20±0.010
7	Golden cup	0.400±0.416	0.928±0.307	0.22±0.010
8	Turkey	0.562±0.263	0.626±0.141	1.9±0.0400
9	Lesieur	0.907±0.095	3.116±0.266	Nil
10	Olive	0.421±0.029	0.702±0.097	0.58±0.02
11	Oki	1.095±0.035	0.569±0.020	0.4±
12	Grand (grdnut)	0.152±0.035	1.393±0.828	0.209±0.00
13	Harvop	0.258±0.236	0.460±0.097	0.42±0.010
14	Bimoli	0.224±0.541	0.456±0.028	0.54±0.020
15	Sania	Nd	0.638±0.032	1.2±0.5400
16	Savoil	Nd	0.460±0.048	0.2±0.0100
17	Famili	Nd	0.864±0.121	0.52±0.010
18	Gino	Nd	0.386±0.032	0.44±0.030
19	Zok	Nd	1.122±0.234	0.92±0.040
20	Coconut	Nd	1.642±0.198	Nil
21	Palm oil	Nd	0.561±0.012	0.149±0.01

*nd- not determined

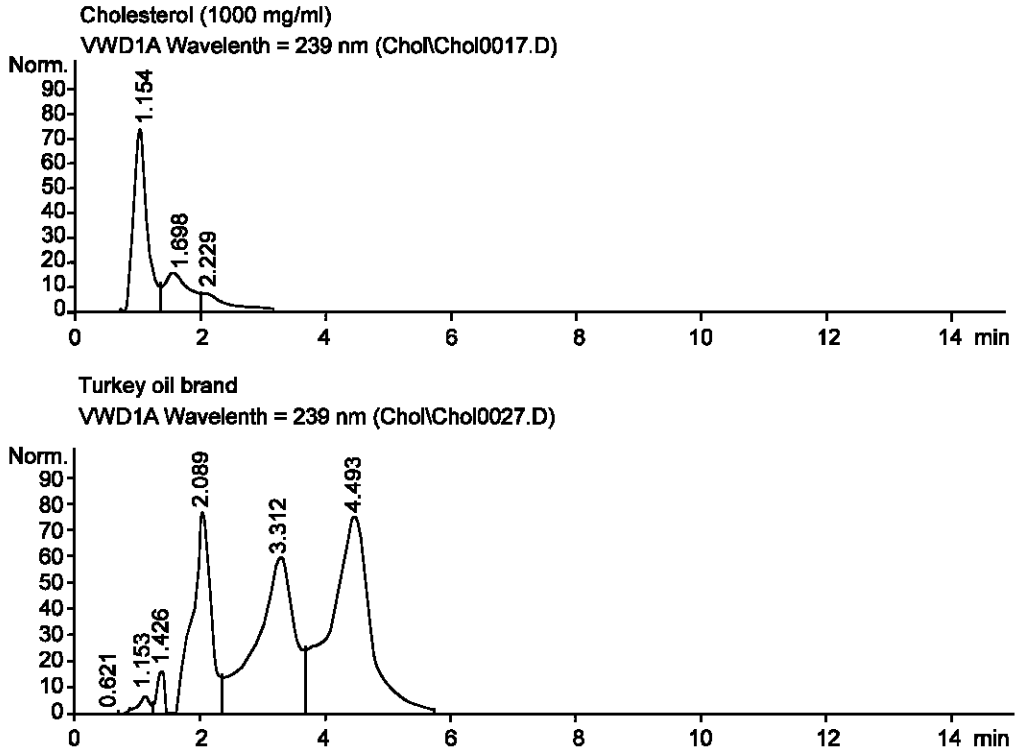

Fig. 1: Elution profile of standard cholesterol and Turkey brand of vegetable oil

Table 2 shows that the iodine values of seventeen of the oil samples including century, laser, sesame and Lesieur brands of vegetable oils were relatively high but lower than the values obtained for Oki, Havop and Envoy. The elution profiles of Turkey brand of vegetable oil and standard cholesterol are shown in Fig. 1. The profile shows that there is cholesterol (1.9 mg/ml) in Turkey oil brand. This is also the case in all other brands except Lesieur and coconut oil brand (profile not shown).

Table 2: Iodine and acid values from 18 brands of vegetable oil samples

Sample	Iodine value (g/100 g oil)	acid value (mg/100 g sample)
Sesame	104.9±1.01	17.46±0.10
Century	124.6±2.51	0.46±0.02
Sunola (groundnut)	86.7±2.08	2.70±0.10
Kings	73.7±4.04	3.86±0.24
Grand (soya)	93.3±4.66	0.17±0.00
Envoy	26.0±3.61	2.97±0.02
Sunola (cotton)	92.0±0.00	1.04±0.08
Laser	116.5±1.29	0.22±0.01
Golden cup	78.3±1.16	0.23±0.02
Turkey	65.3±2.25	0.76±0.01
Flora	64.0±0.00	1.53±0.03
Lesieur	101.5±0.50	0.34±0.02
Olive	96.7±2.29	3.42±0.01
Grand (groundnut)	93.2±0.76	0.28±0.00
Havop	42.7±1.42	2.16±0.03
Bimoli	57.9±1.00	0.19±0.01
Sania	79.1±0.17	0.19±0.02
Oki	42.2±0.25	0.98±0.01

Discussion

We have used three different methods in our quest to find out if there is any Cholesterol in vegetable oils processed in or imported into Nigeria. 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 our diets, most people now prefer to purchase cholesterol free vegetable oils.

Our findings from this study supports previous work by Shukla *et al.* (2002), which showed that cholesterol is present in vegetable oils, although in small proportion, (up to 5% of the total sterol). Indeed, an unusually high amount of cholesterol was detected in Camelina oil (about 200 mg/kg). Furthermore, cholesterol has been detected as one of the major sterols in the surface lipids of higher plants especially in the leaves of rape (Noda *et al.*, 1988). Our results may substantiate this claim as all the samples analysed by the three methods led to the detection of cholesterol in varying proportions. This contradicts the label claim by most of the producers of these vegetable oils.

As earlier stated, what is important in oil consumption is not the amount of fat but the type of fat. Lichtenstein *et al.* (1999) showed that consumption of products low in trans fatty acids has beneficial effects on serum lipoproteins. In his review Wilson *et al.* (2001) opined that trans fat is moderately hypercholesterolemic.

Cholesterol has been known as the 'oily killer' since the early-mid 60s, especially since several works then showed that it is the main cause of atherosclerotic lesions which are the major causes of coronary heart disease (Anthony, 2000; Hayden and Tyagi, 2005; Nicolosi *et al.*, 2004; Jaquish, 2007). Works done by Brown and Goldstein (1986) report on individuals with familial hypercholesterolemia (a rare genetic disorder characterized by a high cholesterol level), showed that the rate of LDL uptake and degradation affects the level of cholesterol in the body.

Some of the oils (Century, Sesame, Lesieur and Laser) had high iodine values suggesting that these oil brands have a high content of unsaturated fatty acids. The lipid profile of oils is considered contributory to the risk of Cardiovascular diseases and some oil seeds possess a higher ability to lower the level of low density lipoproteins than others as shown in Corn oil or olive/sunflower oil mixture (Wagner *et al.*, 2001); in soybean oil (Lichtenstein *et al.*, 2006); in sunflower oil (Binkoski *et al.*, 2005) and in Corn oil (Cuchel *et al.*, 1996). Almendingen *et al.* (1995) showed that serum level of total and low density lipoprotein cholesterol was elevated in hydrogenated fish oil diet compared to soybean oil diet.

High acid values indicative of high free fatty acids obtained from Sesame and Kings oil brands showed rancidity. Long storage of the oil seeds before or after processing may have been responsible. Kalua *et al.* (2008) showed that there were changes in oil quality during cold temperature storage of the fruit.

Moreover, the nutritional value of processed oils is lowered by processing, as nature designed foods so that both unsaturated fatty acids and vitamin E complex occur together in the same foods (Gur and Harwood, 1991). Processing destroys this vitamin. There was a modification of the volatile compounds in virgin olive oil after treatment with hot water (Perez *et al.*, 2003). Vega-Lopez *et al.* (2006) reported that plasma fatty acid profiles are altered when palm and partially hydrogenated soy oils are compared to soy and canola oils. Trans fatty acids are formed during dehydrogenation of oils and this is done to improve oxidative stability and functionality of oils (King and White, 1999). Several workers have associated changes in lipid profile of oils, to processing (Wilson *et al.*, 2005; Pedersen *et al.*, 2005). Ortiz *et al.* (2004) also showed that mode of extraction of oil has an effect on the microstructure of Avocado pulp.

Apart from processing technique, variability may reflect the differences in the growing season of the oilseed plant source. Some plant characteristics are affected by season of harvest. All the oil sample brands used for the study showed that the concentration of cholesterol depends on the sensitivity of the method. While the Libermann-Burchard method gave the highest cholesterol values, followed by Ojiako and Akubugwo, the HPLC method however, shows that only Lesieur and coconut oil brands have no cholesterol. The HPLC, due to its sensitivity confirms that there is really no cholesterol free oil in our markets as advertised. It is pertinent that oil producers and marketers should label their products correctly with the quantity of cholesterol in the oil brand no matter how minute the quantity therein.

It is then left to the consumers to make up their minds which oil brand satisfies their culinary needs.

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