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Investigation of Cholesterol status of Selected Fat-based Drugs and Foods in Osogbo metropolis, Southwestern Nigeria

Oni Peter Gbenga[1], Agbaje Wasiu[1], Awolesi Oluwafemi[2]
[1](Department of Pure and Applied Chemistry, Osun State University, Osogbo, Nigeria)
[2](Department of Chemical Sciences, Olabisi Onabanjo University, Ago-Iwoye, Nigeria)

Abstract:
Background*: Cholesterol is subsumed a seminal lipid molecule in cell membranes and lipoprotein, vital for normal body function except at abnormal levels, as these have precipitated different human diseases including stroke, heart diseases and brain diseases. As a result, accurate measurement of cholesterol is pivotal particularly for people at high risk for the aforementioned diseases. This study investigates the cholesterol status in milk, egg, and cod liver oil samples obtained from different locations in Osogbo metropolis, Osun State, Nigeria. Fourier Transform Infrared (FTIR) spectrophotometer was used for identification of the functional groups in the samples while the cholesterol contents were determined using Ultraviolet (UV) spectrophotometer***.*
Materials and Methods*: Tin milk and cod liver oil samples of different brands were purchased from local retail stores while average-sized eggs were purchased from the local markets and washed properly. All were retrieved from within Osogbo metropolis, Osun state, Nigeria. The samples were transported to the laboratory and subsequently analyzed for cholesterol determination. These samples were coded on the basis of brand and/or point of sales. Cholesterol standards (0.6 mg/mL and 0.7 mg/mL) were prepared by dissolving 6 mg and 7 mg cholesterol each in different 10 mL of methanol; each solution was then shaken thoroughly to obtain a homogenized solution. Cholesterol was subsequently extracted from the various samples of milk, egg and cod liver oil. The absorbances of the standard and samples were read using an Ultraviolet (UV) spectrophotometer at 565nm.*
Results*: The results show that the cholesterol content in Popular Milk Sample 2 (PPMS2) was higher with the value of 24.63 ± 2.52 (in mg/ml) than other milk samples obtained within Osogbo metropolis. Oceanic cod liver oil sample had a cholesterol concentration of 1.99 ± 0.01 (in mg/ml), which was higher than the other cod liver oil samples obtained within Osogbo metropolis. The cholesterol level (26.19 ± 0.03 in mg/ml) in egg sample obtained from Oke-Baale area was higher than other egg samples obtained within Osogbo metropolis*.
Conclusion: *The egg samples contained the highest concentration of cholesterol while cod liver oil samples had the lowest. However, of all cod liver oil samples, Oceanic was above recommended threshold value, making its intake by those already having a heart disease possibly detrimental.*
Key Words*: Cholesterol; Cod liver oil; Milk; Egg; FTIR; UV spectrophotometer.*

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I. Introduction

Cholesterol is the major sterol in the human body, belonging to the class of molecules called steroids[1]. It is a vital structural component of animal cell membranes and is needed for maintaining proper membrane permeability and fluidity[2]. Most of cholesterol is produced in liver, adrenal glands, intestines, and in gonads, whereas 20 to 25% of cholesterol comes from the diet of animal origin in addition to egg and meat[3, 4]. Dairy products are particularly rich in cholesterol, because it is obtained from the animal source[3]. One of the major functions of cholesterol is to participate in the biosynthesis of bile acids in the liver which subsequently disintegrates dietary fats into smaller droplets and helps the subsequent digestion[5]. Cholesterol also participates in the absorption and the production of vitamin D[6]. Moreover, cholesterol is a precursor of steroids, such as testosterone and estrogen[7]. Besides, cholesterol is also a key component in lipoproteins, which are used to transport hydrophobic molecules (such as fats) in hydrophilic media, like blood[8]. Cholesterol is an important precursor for production of hormones[3]. Up to certain levels, cholesterol is beneficial for human body; meanwhile, higher levels of cholesterol are associated with many diseases[8].

It has been observed that high intake of fat-based foods and drugs in the bloodstream are an extremely important cause of atherosclerosis[9]. In this disorder, deposits of cholesterol and other fatty substances circulating in the blood accumulate in the interior walls of the blood vessels[2]. These fatty deposits build up, thicken, and become calcified, eventually converting the vessel walls to scar tissue while the deposits narrow the

channels of the blood vessels and thus can constrict the blood flow, causing heart attacks and strokes[2]. Indeed, fat-based products have been found to have a detrimental effect on health. A cholesterol molecule contains three major parts: 1) tetracyclic carbon ring (A–D) as the core of steroids, 2) polar hydroxyl group attached to ring A, and 3) short non-polar carbon chain attached to ring D (fig. 1)[7, 10].

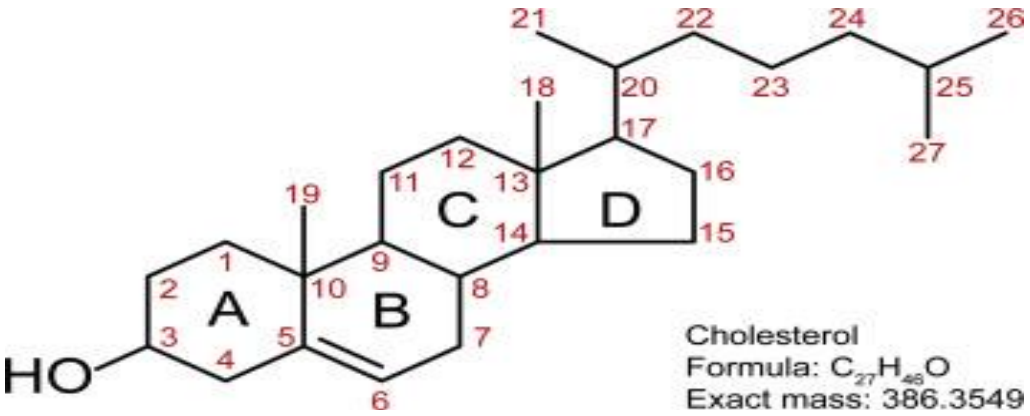

Fig. 1: Structure of cholesterol with the numbering of the carbon atoms (Adapted from Li et al., 2019)

Because it contains both hydrophobic and hydrophilic groups, cholesterol serves to help solubilize the nonpolar material that must be transported by the lower density lipoproteins[7, 10].

Low-density lipoproteins (LDL) are the major cholesterol carriers in the blood, transporting and supplying cholesterol to different parts of the body. They are also referred to as "bad" cholesterol because if too much LDL cholesterol circulates in the blood, it may slowly build-up in the arteries feeding the heart and brain[11, 12]. This can subsequently lead to formation of plaque, a thick, hard deposit that can clog those arteries, resulting in atherosclerosis[13]. The LDL cholesterol level should be less than 160 mg/dL (or 100 mg/dL for someone already having heart disease) [14].

High-density lipoproteins (HDL), on the other hand, also known as "good" cholesterol, are believed by medical experts to carry cholesterol away from the tissues and back to the liver, its main source of origin in the body, where it is then metabolized and passed out of the body[12, 15]). It is also believed that HDL removes excess cholesterol from plaques and thus slows their growth, helping to reduce the risk of heart attack or stroke[16]. So a high HDL level is a good sign of a healthy body; but a low HDL cholesterol level (less than 40 mg/dL for men and less than 50 mg/dL for women) also indicates a greater risk[17].

Research on the various biological functions of cholesterol and concerns over its pathophysiology are the driving force for the development and application of analytical methods for the determination of cholesterol concentration in various food products[4, 18]. Cholesterol levels in foods have been determined using gravimetric and spectrophotometric analysis[19]. Various kinds of methods to determine the concentration of cholesterol have also been reported, including colorimetric and spectrophotometric estimations, gas-liquid chromatography, high performance liquid chromatography (HPLC), and fluorometric and other methods[20, 21, 22]. Most of the reported protocols involve the use of complex reagents or complicated methods, and some of these are not applicable for samples containing complex matrices. However, the data from various chromatographic techniques are very dependent on the extraction procedures and intensity of the saponification steps[23, 24, 25, 26]. In recent years, however, among all of the quantitative techniques for determining cholesterol in food, gas phase and liquid phase chromatography stand out and are in widespread use, employing a range of detection methods[27]. It is important to know the cholesterol concentration in our dietary intake to inform individuals about healthy consumption and appropriate food choices to reduce nutrient-related diseases[28]. This study therefore aims to develop and validate a simple spectrophotometric method and to eliminate unnecessary steps to create a simple, precise, accurate, and productive method to quantify cholesterol in fat-based foods and drugs. The analytical procedure was characterized to ensure its selectivity, accuracy, and precision for the analysis.

II. Material And Methods

Sample Collection

Tin milk and cod liver oil samples of different manufacturers were purchased from local retail stores while average-sized eggs were purchased from the local markets and washed properly. All were retrieved from within Osogbo metropolis, Osun state, Nigeria.

Sample Preparation

The samples were transported to the laboratory and subsequently analyzed for cholesterol determination. These samples were coded on the basis of brand and/or point of sales (Table 1).

Table 1: Sample Information

Milk Samples	Brand	Cod Liver Oil Samples	Brand	Egg Samples	Location/Market
PKMS1	Peak Milk	EVS1	Everseas	ESOK	Oke-baale
TCMS1	Three Crown Milk	EVS2	Everseas	ESOS	Orisumbare
OLMS1	Olympic Milk	OCN1	Oceanic	ESAS	Asubiaro
PPMS1	Popular Milk	OCN2	Oceanic	ESDE	Dada Estate
PKMS2	Peak Milk	DPS 1	Dip Sea	ESOL	Olaiya
TCMS2	Three Crown Milk	EVS3	Everseas	ESAY	Ayepe
OLMS2	Olympic Milk	OCN3	Oceanic	ESIG	Igbona I
PPMS2	Popular Milk	DPS2	Dip Sea	ESSA	Sabo
PKMS3	Popular Milk	DPS3	Dip Sea	ESIB	Igbona II
TCMS3	Three Crown Milk	EVS4	Everseas	ESID	Idi-seke

Preparation of Cholesterol Standard Curve

Cholesterol standard (0.6 mg/mL) was prepared by dissolving 6 mg cholesterol in 10 mL of methanol; this solution was then shaken thoroughly to obtain a homogenized solution (Pompe, 1994). The absorbance of the standard was read using an Ultraviolet (UV) spectrophotometer at 565nm. Cholesterol standard of 0.7 mg/mL was also prepared by dissolving 7 mg cholesterol in 10 mL of methanol. The value of the absorbance was plotted against the concentration.

Extraction of Cholesterol from Milk Samples

Three ml of milk was accurately weighed using measuring cylinder and transferred into a beaker[23]. Direct saponification was achieved with 3ml of 10% (w/v) ethanolic potassium hydroxide (KOH). The mixture was placed in the water bath for 30 minutes at a temperature of 70^0C. 10ml of n-hexane and 2 ml of distilled water were added to the mixture, which was transferred into the separating funnel for extraction of the unsaponified fraction. The hexane extraction was repeated three times and the sample was rinsed thoroughly. The final extract was heated in the water bath at a temperature of 45^0C for 30 minutes to remove the hexane used. 3ml glacial acetic acid, 2ml of colouring reagent (a solution of ferric chloride/glacial acetic acid/sulphuric acid) was added to the extract, and was shaken vigorously. The absorbance of the standard and samples were read using an Ultraviolet (UV) spectrophotometer at 565nm. The absorption of the sample was compared against external cholesterol standard.

Extraction of Cholesterol from Egg Samples

Each egg was washed properly and then boiled in distilled water. The egg yolk was carefully separated from the boiled egg. For the preparation of saponified sample, 1 g of the egg yolk was dispensed in 2 mL of ethanol to which 18 mL of ethanolic KOH was added and mixed in a conical flask. The sample was then heated at 60^0C for 30 minutes in water bath for proper homogenization. The resultant mixture was then transferred into a separating funnel and extracted with 10 mL hexane with regular shaking. To ensure complete extraction of cholesterol, the extraction process was repeated twice with 5 mL of hexane. The hexane layer was then pooled and evaporated to dryness in water bath. 3ml glacial acetic acid, 2ml of colouring reagent was added to the extract, and was shaken vigorously. The absorbances of the standard and samples were read using an Ultraviolet (UV) spectrophotometer at 565nm (Pye Unicam UV1 Double Beam Scanning Spectrophotometer). The absorption of the sample was compared against external cholesterol standard.

Extraction of Cholesterol from Cod Liver Oil Samples

One milliliter of sample oil was dissolved in 10ml of chloroform (in the ratio 1:10). The mixture was evaporated to dryness in a water bath at 50^0C. 3ml glacial acetic acid, 3ml of colouring reagent was added to the extract, and was shaken vigorously. The absorbance of the standard and samples were read using an Ultraviolet (UV) spectrophotometer at 565nm. The absorption of the sample was compared against external cholesterol standard.

Structural Elucidation of Standard Cholesterol and the extracted cholesterols

In order to validate the presence of the functional groups that are characteristic of cholesterol, standard cholesterol as well as two differently categorized samples of extracted cholesterol would be subjected to FTIR spectrometric analysis.

III. Result

Two different concentrations of standard cholesterol solutions (6 mg/mL and 7mg/mL) were prepared and their absorbance was read using the ultraviolet spectrophotometer. The results are displayed in Table 2.

Table 2: Result of Cholesterol analysis obtained from Standard Cholesterol Sample

Absorbance (A^0)	Concentration (M)
0.462	0.60
0.468	0.70

The value of the absorbance was plotted against the concentration. The regression line was found to be Y = 0.06X + 0.426, with a correlation coefficient of 1 showing the linearity of the calibration (Fig. 1).

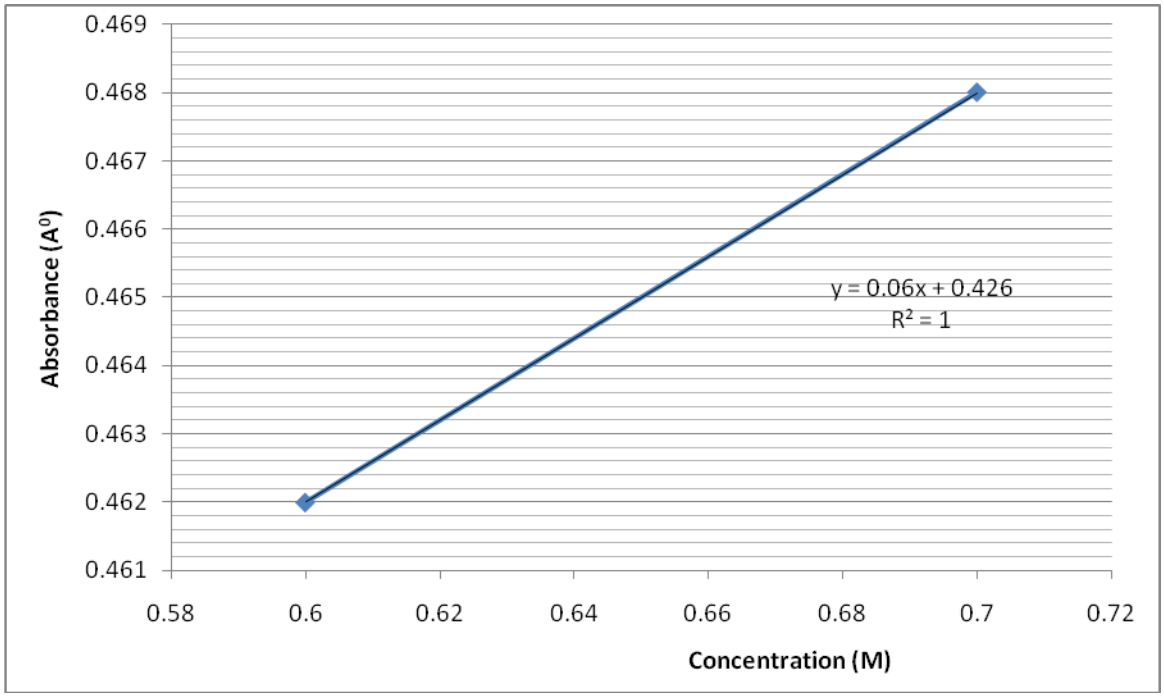

Fig 2: Cholesterol Standard Graph

The absorbances of milk, egg and cod liver oil samples were compared with the standard cholesterol graph, and the cholesterol concentrations were extrapolated from the graph. The results are presented in the Table 3. Each value is expressed as mean ± standard deviation.

Table 3: Concentrations of Cholesterol in milk, egg and cod liver oil samples

Milk Samples	Cholesterol Concentration (mg/ml)	Cod Liver Oil Samples	Cholesterol Concentration (mg/ml)	Egg Samples	Cholesterol Concentration (mg/ml)
PKMS1	12.29 ± 2.85	EVS1	0.69 ± 0.01	ESOK	26.19 ± 0.03
TCMS1	18.74 ± 0.46	EVS2	0.86 ± 0.02	ESOS	26.02 ± 0.03
OLMS1	16. 87 ± 2.77	OCN1	1.60 ± 0.03	ESAS	24.93 ± 0.09
PPMS1	23. 06 ± 3.94	OCN2	1.99 ± 0.01	ESDE	21.84 ± 0.09
PKMS2	10.06 ± 0.19	DPS 1	0.46 ± 0.02	ESOL	25.76 ± 0.11
TCMS2	16.20 ± 1.57	EVS3	0.83 ± 0.10	ESAY	26.07 ± 0.06
OLMS2	18.30 ± 2.96	OCN3	1.79 ± 0.17	ESIG	22.67 ± 0.18
PPMS2	24.63 ± 2.52	DPS2	0.48 ± 0.02	ESSA	25.92 ± 0.05
PKMS3	13.06 ± 3.24	DPS3	0.52 ± 0.05	ESIB	26.15 ± 0.08
TCMS3	18.23 ± 1.03	EVS4	0.85 ± 0.03	ESID	26.15 0.02

Structural Elucidation of Standard Cholesterol and the extracted cholesterol from Popular milk and Oceanic cod liver oil using FTIR

The FTIR spectra of the cholesterols are presented in Fig. 3 (standard cholesterol), Fig. 4 (cholesterol extracted from Popular milk) and Fig. 5 (cholesterol extracted from Oceanic cod liver oil).

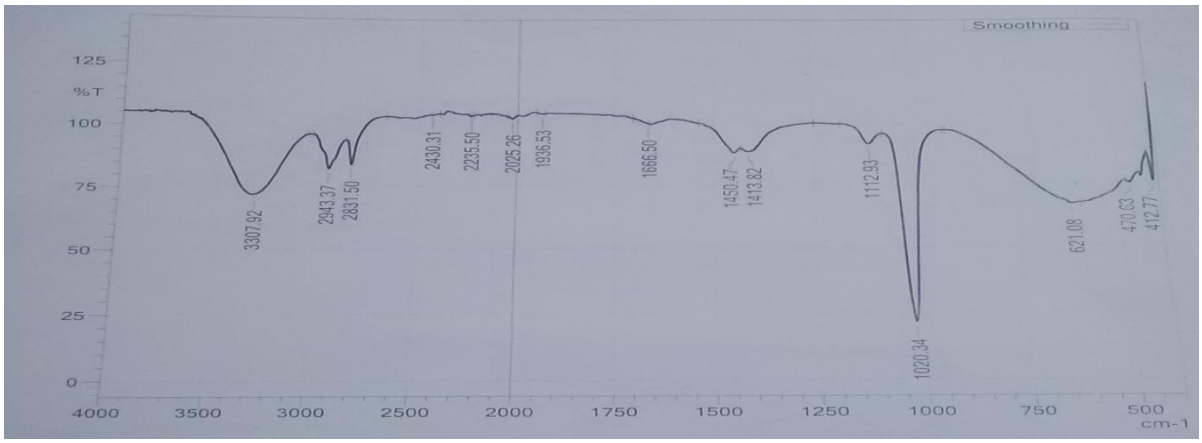

Fig. 3: FTIR Spectrum of Standard Cholesterol

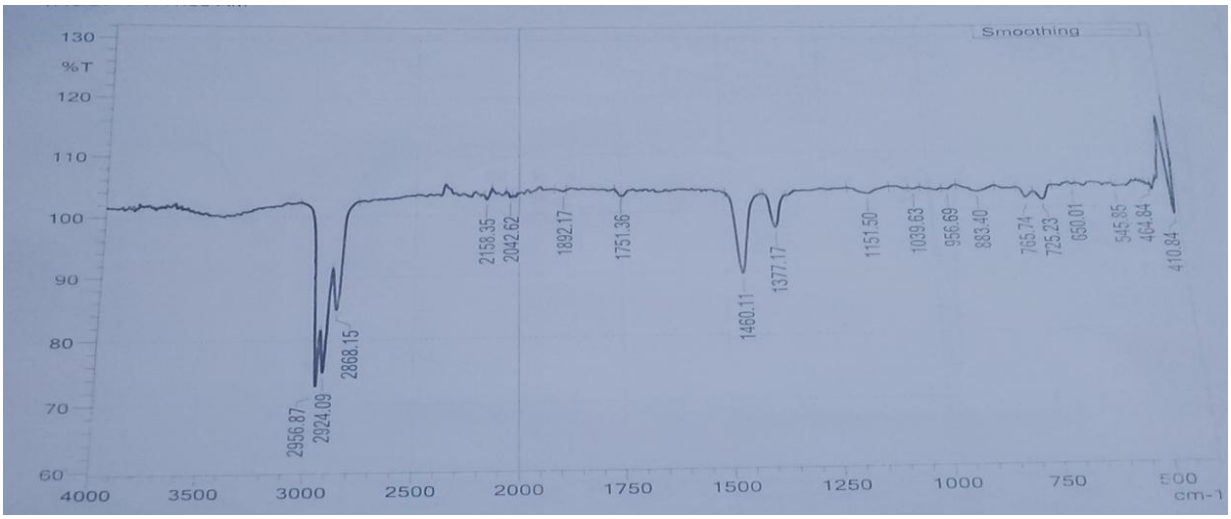

Fig. 4: FTIR Spectrum of Cholesterol extract from Popular milk sample

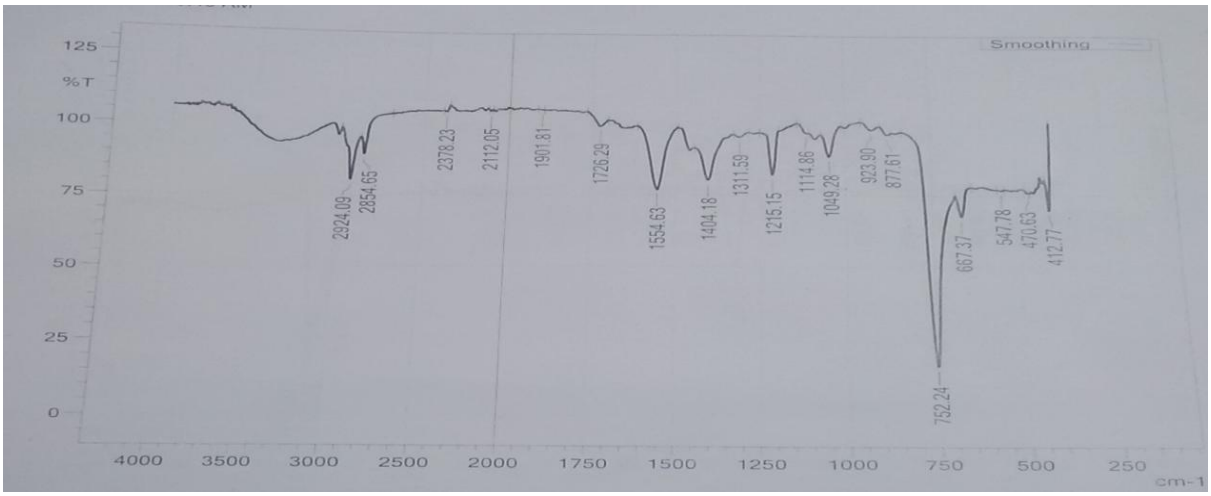

Fig. 5: FTIR Spectrum of Cholesterol extract of Oceanic cod liver oil sample

IV. Discussion

There was no significant difference at 95% confidence interval in the cholesterol concentration. From Table 3, it is established that the cholesterol level in milk samples ranged from 10.06mg/ml (Peak milk sample (PKMS2)) to 24.63mg/ml (in Popular milk sample (PPMS2)). Cholesterol level in cod liver oil samples ranged from 0.46mg/ml as in Dip Seas (DPS1) to 1.99mg/ml as in Oceanic (OCN2). Cholesterol level in egg samples ranged from 21.84mg/ml (ESDE) to 26.19mg/ml (ESOK). The threshold value of cholesterol concentration is

160 mg/dL (or 100 mg/dL for someone already having heart disease). If the cholesterol concentration exceeds 160mg/dL, it would result to detrimental effect on health[29]. After converting the cholesterol content obtained from the samples from mg/ml to mg/dL. It was established that the cholesterol content of all the samples used was above the threshold value, except Dip Seas and Everseas cod liver oil samples. This implies that, inasmuch as Oceanic cod liver oil is therapeutic, high doses by those already having a heart disease is possibly detrimental. Fig. 3 presents FTIR spectrum of standard cholesterol with the absorption bands at 412.77, 470.63, 621.08, 1020.34, 1112.93, 1413.82, 1450.47, 1666.50, 1936.53, 2025.26, 2235.50, 2430.31, 2831.50, 2943.37, and 3307.92 cm^{-1}. Fig. 4 presents FTIR spectrum of cholesterol extract from Popular milk sample, with absorption bands at 410.84, 464.84, 545.85, 650.01, 725.23, 765.74, 883.40, 956.69, 1039.63, 1151.50, 1377.17, 1460.11, 1751.36, 1892.17, 2041.62, 2158.35, 2868.15, 2924.09, and 2956.87 cm^{-1}. Fig. 5 presents FTIR spectrum of cholesterol extract of Oceanic cod liver oil sample, with absorption bands at 412.77, 470.63, 547.78, 667.37, 752.24, 877.61, 923.90, 1049.28, 1114.86, 1215.15, 1311.59, 1404.18, 1554.63, 1726.29, 1901.81, 2112.05, 2378.82, 2854.65, and 2924.09 cm^{-1}. The spectra can be characterized based on findings up to par with that in literature[30, 31]. The tables below show different peaks observed in the FTIR spectra represented in Fig. 3, 4 and 5, with the assignment of bonds.

Table 3: Functional Groups from the FTIR spectrum of Standard Cholesterol

Peak position on FTIR spectrum (cm^{-1})	*Assignment of bonds*
412.77, 470.63,	Unknown
621.08	O-H out-of-plane (bend)
1020.34, 1112.93	C-O vibration (stretch)
1413.82	O-H vibration (bend)
1450.47	C=C-C Aromatic ring (stretch)
1666.50	C=C (stretch)
1936.53, 2025.26	Aromatic combination bands
2235.50, 2430.31	Unknown vibration
2831.50, 2943.37	Asymmetric vibrations of C-H bonds of methyl groups of cholesterol structure (stretch)
3307.92	H-bonded O-H bond vibration (stretch)

Table 4: Functional Groups from the FTIR spectrum of Cholesterol Extract from Popular Milk Sample

Peak position on FTIR spectrum (cm^{-1})	*Assignment of bonds*
410.84, 464.84, 545.85	Unknown
650.01	O-H out-of-plane (bend)
725.23, 765.74	C-H out-of-plane vibrations (bend)
883.40, 956.69, 1039.63	C-C backbone weak vibration (stretch)
1151.50	C-O vibration (stretch)
1377.17	Methyl C-H symmetric vibrations (bend)
1460.11	C=C-C Aromatic ring (stretch)
1751.36, 1892.17	Aromatic combination bands
2041.62, 2158.35	Unknown vibration
2868.15, 2924.09, 2956.87	Asymmetric vibrations of C-H bonds of methyl groups of cholesterol structure (stretch)

Table 5: Functional Groups from the FTIR spectrum of Cholesterol Extract from Oceanic Cod liver Oil Sample

Peak position on FTIR spectrum (cm^{-1})	*Assignment of bonds (mode of vibration)*
412.77, 470.63, 547.78	Unknown
667.37, 752.24	O-H out-of-plane (bend)
877.61, 923.90,	C-C backbone weak vibration (stretch)
1049.28, 1114.86, 1215.15	C-O vibration (stretch)
1311.59	O-H in-plane (bend)

1404.18	O-H vibration (bend)
1554.63	C=C-C Aromatic ring (stretch)
1726.29, 1901.81	Aromatic combination bands
2112.05, 2378.82	Unknown vibration
2854.65, 2924.09	Asymmetric vibrations of C-H bonds of methyl groups of cholesterol structure (stretching)

From the structure of cholesterol (Fig. 1) 45 C-H bonds from five CH_3 groups; each comprising three C-H bonds, one C-O bond linking carbon and hydroxyl group, one C=C bond, and 29 C-C bonds can be observed. Asymmetric stretch of C-H bonds in the methyl group of aromatic and aliphatic compounds reveal vibrations in the regions 2800 – 2960 cm^{-1} (Tables 3, 4 and 5). Cholesterol comprises 5 methyl groups containing a sum of 15 C-H bonds. Asymmetric stretch of the C-H bonds can result in peaks at 2831.50, 2854.65, 2868.15, 2924.09, 2943.37, 2956.87 in the FTIR spectra of standard cholesterol and cholesterol extracts [30, 31]. H-bonded O-H stretch gives a broad band in the regions 3200 – 2570 cm^{-1}, which may have contributed to the band at 3307.92 cm^{-1}.

V. Conclusion

The cholesterol contents of milk, cod liver oil and egg samples obtained in Osogbo metropolis have been determined using the ultraviolet (UV) spectrophotometer. It is established that the egg samples contained the highest level of cholesterol, while the cod liver oil had the least. However, of the cod liver oil samples, Dip Seas and Everseas samples had concentrations below the threshold value of 160 mg/dL. This implies that, inasmuch as Oceanic cod liver oil is generally therapeutic, high doses by those already having a heart disease is possibly detrimental. The authors propose to take steps to determine the concentrations and further elucidate the structures of the extracted cholesterols using other spectroscopic techniques and recovery studies in order to validate the degree of accuracy and precision of results.

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