Estimation of Cholesterol Content and Free Fatty Acids in Edible Oils in Iraq

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Abstract

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 Iraq, city Babylon. Acid value, peroxide value, and cholesterol content were determined in a total of twenty varieties of edible vegetable oils. The analysis performed using methods Liebermann-Burchard and Ojiako and Akubugwo revealed varying levels of cholesterol content. Cholesterol was detected in twenty of the vegetable oils. Zakera oil branded vegetable oil has significantly highest cholesterol concentration (331±0.234mg/L) while Alkhair oil has the least concentration (99.6±0.404 mg/L) of cholesterol. Unlike the peroxide value, all the acid values and show high values in comparison with the maximum permissibility level of codex standard for named vegetable oils (CODEX STAN210-1999). 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.

Keywords: Free fatty acid, Edible oils, Leibermann-burchard, Cholesterol.

Introduction

The heart diseases are increasing day by day and the heart attack is a major cause of death world over. There are several reasons for heart diseases but one of the most important reasons is hypercholesterlemia. The blood stream carries cholesterol in particles called lipoproteins.

But too much circulating cholesterol injures arteries especially coronary arteries. This leads to accumulation of cholesterol-laden "plaques" in vessel linings, a condition called as Atherosclerosis. The word "cholesterol" may quickly be associated with chronic heart disease & other heart problems. However 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. Public, Patients and doctors are better informed about the risk associated with the elevated level of cholesterol, benefits of life style changes and medical measures aimed at lowering cholesterol [1].

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 [2]. Although

many plant parts yield oil, in actual commercial practices, oil is extracted primarily from seeds of oilseed plants and according to the USDA [3] , the oilseed plants commonly used worldwide include; soybean, cotton, palm, rape and groundnut. Cholesterol, contrary to popular belief, is present in plants [4]. 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) [5]. It has also been found to be a major constituent of the chloroplasts, shoots, pollens, seeds and leaf surfaces [6]. 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, 57 because its high level in the body has been associated with coronary heart diseases [7]. 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 [8]. However, what are becoming clearer and clearer is that it is not the amount of fat in the diet that matters but the type of fat [9]. 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 [10]. Industrial processing especially catalytic hydrogenation of vegetable oils affects their fatty acid composition [11]. Processing increases saturated fatty acids component of oils. Saturated fatty acid rich diets have been found to increase the level of cholesterol [12]. Thus, we are concerned by the fact that Iraqi markets are flooded with assorted processed vegetable oils from different parts of the world all labeled to be cholesterol free

Materials and Methods

Samples of 20 brands of edible oils produced from a variety of Vegetable Oils were purchased from various markets in Babylon Iraq. The label on each sample container was (NO CHOLESTEROL).

Chemicals:

Cholesterol (Babylon Research Lab, Iraq), Liberman- Burchard reagent (Acetic anhydride & Sulphuric acid (SIGMA-ALDRICH), Chloroform, potassium hydroxide, potassium iodide, ethanol, potassium iodide (SIGMA-ALDRICH).

Instrument:

UV – Visible Apel Spectrophotometer 2450 (Shimadzu/JAPAN)

Standard Cholesterol Solution

10 mg of standard cholesterol dissolved in 10 ml chloroform, shacked well.

Liberman – Burchaed Reagent:

0.5 ml of sulfuric acid dissolved in 10 ml of acetic anhydride. Covered and kept in ice bucket.

Sample Preparation

Samples were weighed to 1 gm, dissolved in chloroform to 10 ml and further diluted to 10 times (10,000 ppm). 3ml of diluted sample solution were mixed with 2ml of Liberman-Burchard reagent and 2 ml of chloroform. The tubes were covered with black carbon paper and kept in ice bucket in dark place for 15 min. Liberman-Burchard reagent react with the cholesterol to produced characteristic green color their absorbance were determined on spectrophotometer at 560 nm.

Procedure

Six volumetric flasks were marked as P1, P2, P3, P4, P5 and P6. 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. Flasks were covered with black carbon paper and kept in dark for 20 min. Then, set zero of spectrophotometer with blank (P6) at 560 nm. The absorbance of all standards (six flasks) was determined on SP65 Apel UV/Vis spectrophotometer (Table1) and standard graph was plotted (Figure 2). Three ml of sample solutions were taken and their absorbance were determined on SP65 Apel 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 2) using a standard curve constructed graphically plotting the absorbance against mg/l cholesterol.

Determination of Cholesterol Content

Method 1

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 C0. Glacial acetic acid (3.0 ml) and 3.0 ml of color 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 color reagent. After cooling for 30 minuts at room temperature, absorbance of standard and samples were read at 560 nm. Cholesterol content was estimated with the formula: Ojiako and Akubugwo (1997).

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

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 color which is measured spectrophotometrically.

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) [13]:

C17H33COOH + Naoh → C17h33coona + H2O …… (1)

Acid value = $\frac{56.1xVXc}{m}$ ……(2)

Where 56.1 is equivalent weight of KOH, V is the volume in ml of standard volumetric KOH solution used, C is the exact concentration in KOH solution used (0.1 N), m is the mass in grams of the test portion (1 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 2) (Official Method 965.33 Peroxide Value in Oils and Fats, 2000).

Peroxide Value = 10X(V1-V2) /M

I2+ 2Na2S2O3 →Na2S4O6+ 2nai……..(3)

Where: V1 volume of Na2S2O3 for determination of test sample in ml, V2 volume of Na2S2O3 for determination of blank solution in ml and m is mass of test portion in g (5 g)

Result and Discussion

Almost every adult living in an industrialized nation develops some degree of atherosclerosis, commonly known as “hardening of the arteries”. Atherosclerosis leads to strokes, heart attacks and other serious health problems. The heart disease is linked to risk factors. The principal risk factors are high cholesterol, smoking and high blood pressure. Other risk factors include: diabetes, obesity, family history of heart disease and stress. The present investigation is an overview of how food choices can affect one’s cholesterol level. Each animal cell, both human and non-human, contains cholesterol. Cholesterol is important in some of the functions of the liver cell and produces all the cholesterol the body needs. There

is no need to consume cholesterol in diet. Cholesterol and fats move thorough the body protein packages called lipoproteins.low density lipoproteins (LDLs) carry cholesterol to the organs through the arteries. The LDLs deposit their load through the inner walls of the arteries and promote atherosclerosis. they are known as bad cholesterol. The higher the LDL level greater the risk of heart problems."Good cholesterol" found in high density lipoproteins (HDLs) moves back to the liver where it is disposed of. People who exercise, do not smoke and stay their ideal weight tend to have higher level of HDLs. Blood cholesterol levels are affected by many factors. Population groups with an average cholesterol level of 150 mg/dL or less are largely free of atherosclerosis. For cholesterol level above 150 mg, the risk of heart disease increases [14].

Cholesterol is the most common steroid in animals and the precursor for all other animal steroids. Cholesterol numbering system applies to all of these molecules. Many of steroids containing methyl groups in positions 10 and 13 O8- to 10 carbon alkyl side chain at position 17. polyprenyl nature of this compound is particularly evident in the side chain. Many of steroids containing oxygen in the C-3, either in rigid hydroxyl group or carbonyl group in other steroids. To a large extent, and oriented towards the carbon in positions 10 and 13 and the alkyl group in position 17 is always on the same side of the steroid nucleus, orientation β-. Alkyl, which extends from the other side of the backbone of the steroid is α in orientation [15].

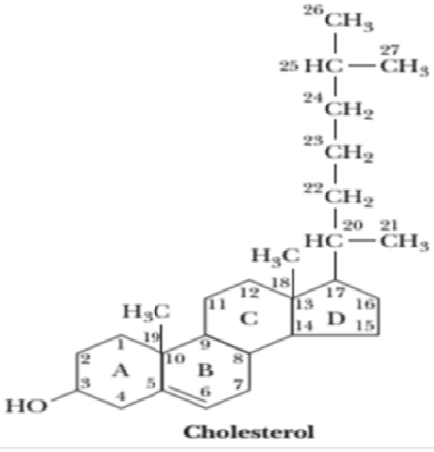

Figure 1: The Structure Of Cholesterol And Its Steroid Ring Designations And Carbon Numbering.

Cholesterol content can be estimated using Liebermann-Burchard reaction method [16]. 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 color which is measured spectrophotometrically. The Liebermann–Burchard or acetic anhydride test is used for the detection of cholesterol. The formation of a green or green blue color after a few minutes is positive. Lieberman–

Burchard is a reagent used in a colorimetric test to detect cholesterol, which gives a deep green color. 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. Since this test uses acetic anhydride and sulfuric acid as reagents, caution must be exercised so as not to receive severe burns .oil samples that are contained cholesterol include. Zakera oil has significantly maximum (331±0.234mg/L) cholesterol content and Alkhair oil has significantly low (99.6±0.404mg/L) cholesterol content. [17]

Table 1.Absorbance Of Standard Cholesterol Solutions For Calibration Curve At Different Concentrations At 560 Nm.

CONCENTRATION Mg / L	Absorbance ±Se
80	0.065±0.001
120	0.215±0.000
160	0.421±0.003
200	0.628±0.011
240	0.855±0.007
260	0.959±0.008

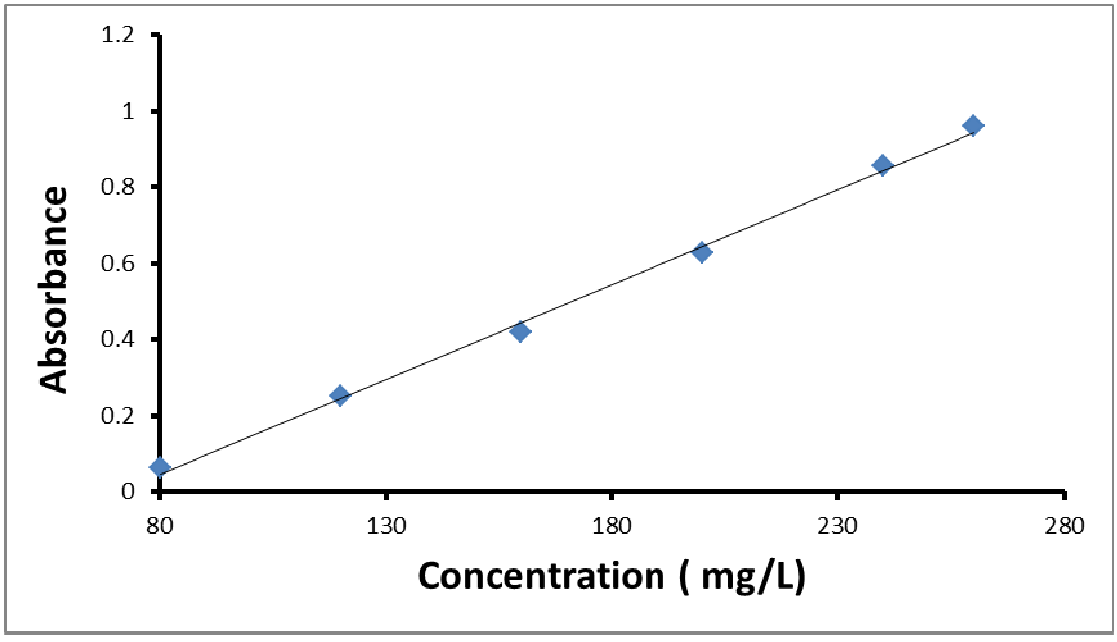

Figure 2.Calibration curve using cholesterol standard

Table 2: Amount Of Cholesterol In Oil Samples

Sample (Brand)	Ojiako And Akubugwo (Mg/Ml)	Liebermann-Burchard (Mg/Ml)
1. Alkhair	120±0.001	99.6±0.404
2. Orkide	254±0.146	274±0.561
3. Bizce	144±0.035	130±0.323
4. Sandy	143±0.277	145±0.186
5. Sunflower Oil	159±0.093	155±0.058
6. Noor Oil	130±0.069	137±0.095
7.Golden Cup	150±0.416	156±0.307
8.Altunsa Oil	100.7±0.263	108±0.141
9. Palm Oil	161±0.095	167±0.266
10.Olive Oil	175±0.029	177±0.097
11. Norrse Oil	123±0.035	145±0.020
12.Ottaman Oil	177±0.035	189±0.828
13.Jena Oil	151±0.236	155±0.097
14.Bimoli	221±0.541	230±0.028
15. Canola Oil	209±0.043	266±0.032
16. Castor Oil	299±0.055	310±0.048
17. Coconut Oil	399±0.134	321±0.121
18.Family Oil	177±0.043	187±0.032
19.Zakera Oil	320±0.277	331±0.234
20.Jamela Oil	288 ±0.197	254±0.198

Table 3: Acid And Peroxide Value For Vegetable Oil Samples

Sample (Brand)	Acid Value (Mg KOH/G)	Peroxide value (meq.peroxide/kg)
1. Alkhair	3.46±0.10	12.0±0.23
2. Orkide	0.46±0.02	5.45±0.14
3. Bizce	0.30±0.12	4.47±0.03
4. Sandy	0.43±0.01	4.39±0.27
5. Sunflower Oil	0.55±0.08	5.92±0.09
6. Noor Oil	0.66±0.06	3.01±0.06
7.Golden Cup	2.3±0.55	5.06±0.41

8.Turkey	1.50±0.01	9.79±0.26
9. Palm Oil	1.11±0.02	6.15±0.09
10.Olive Oil	0.44±0.03	7.56±0.02
11. Norrse Oil	3.54±0.05	2.35±0.03
12.Ottaman Oil	0.99±0.10	7.77±0.03
13.Jena Oil	2.44±0.01	5.19±0.23
14.Bimoli	0.44±0.03	2.16±0.54
15. Canola Oil	1.66±0.02	2.89±0.04
16. Castor Oil	0.90±0.01	9.99±0.05
17. Coconut Oil	2.34±0.02	3.99±0.13
18.Family Oil	0.77±0.03	7.71±0.04
19.Zakera Oil	2.51±0.23	3.20±0.27
20.Jamela Oil	1.61±0.09	8.8 ±0.19

Acid Value:

Acid value (AV) is an important indicator of vegetable oil quality. AV is expressed as the amount of KOH (in milligrams) necessary to neutralize free fatty acids contained in 1.0 g of oil. But the free fatty acid (%FFA) content is a conventional expression of the percentage mass-fraction of total fat. According to the nature of the fat it is expressed as lauric acid for coconut, palm kernel, and similar oils, as palmitic acid for palm oil and as oleic acid for all other oils .In the view of the results shown in Table 3, all the samples have acceptable AV and %FFA values except samples Alkhair oil and zakera oil have unacceptable higher values and this is may be due to the presence of higher free fatty acids as rancid oil which is hazardous for human uses [18].

Acid value determination the value as a general indication of the situation and edibility of oil. This is because is accompanied by an increase in the acid value by the development of objectionable flavors and odors [19] .

Peroxide Value

Peroxide value is an indication of the extent of oxidation suffered by oil. High peroxide value indicates high degree of unsaturation, which in turn responsible for oxidative rancidity [20]. The test sample is first dissolved in mixture of chloroform and acetic acid (2:3). By flowing nitrogen gas through the sample to dispel residual oxygen, add potassium iodide, and then titrate free iodine with 0.01mol/ sodium thiosulfate. The endpoint is determined by the maximum inflexion point on titration curve

I2+ 2Na2S2O3 →Na2S4O6+ 2nai……(4)

Initial peroxide values for the edible oils and fat ranged between 2.1 and 12.0 meq/kg-oil. The highest value was found in Alkhair oil (12.0 meq/kg-oil) and the lowest value from Bimoli (2.1 meq/kg-oil). The values for all the edible oils were within the FAO/WHO and TBS standards for edible vegetable oils. In general the peroxide value increased with storage time. Oils exposed to both atmospheric oxygen and light showed a much larger increase in peroxide value during storage.

The peroxide value of these samples supports the observation, that is, Alkhair oil, . Castor oil 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 [21].

Finding from this study supports previous work that cholesterol is present in vegetable oils, although in small proportion.

PV is a measure of oxidation during storage and freshness of the matrix fat. In addition, it is a useful indicator of the early stages of rancidity that occurs under mild conditions and it is the primary measure of lipid oxidation products. One of the most important criteria that affect fat oxidation is the degree of saturation of fatty acids. When oxidized double bonds of unsaturated fat, and peroxides are among the oxidation products formed. An indication of the high levels of peroxide oxidation and the greater the peroxide value, the more oxidized oil **.**

Cholesterol Content:

Representative analytical data for cholesterol content of said oil samples are presented in Table 2.We have used two different methods in our quest to find out if there is any Cholesterol in vegetable oils processed in or imported into Iraq. 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 finding from this study supports previous work by [22] , 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 . Our results may substantiate this claim as all the samples analyzed 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.

Following recommendations may be useful in reducing the level of cholesterol in the blood:

1. Cholesterol level can be decreased by cholesterol lowering drugs, but these are expensive and can be avoided in many cases. Lifelong dependence on drugs is not desirable, and there may be side effects for individuals..
2. Have your cholesterol level tested before drugs are necessary. Monitored regularly.
3. Use plant foods i.e. grains, beans, vegetables, fruits and berries. It should use vegetable oils for cooking instead of vanaspati ghee.
4. Mental stress causes an increased release of adrenaline which may elevate cholesterol levels. Relaxation techniques such as stretching, deep breathing or meditation help in lowering blood cholesterol.
5. Stop smoking and exercise more. Exercise raises HDL cholesterol level can also reduce the level of LDL cholesterol.
6. The use of dietary fiber may reduce cholesterol in the blood.

Conclusion

Cholesterol is a soft, fat-like, waxy substance found in the bloodstream and in all your body cells. As a lipid, or fat-like substance, it's an important part of a healthy body because it's used for building cells. But a high blood cholesterol level is a major risk for coronary heart disease, which can lead to heart attack. It's also a risk factor for stroke. One method of reducing high cholesterol levels is following a healthy diet by limiting foods high in cholesterol and saturated fats and eating more whole grains, fruits, vegetables and lean meats. Besides, It is better to not use oil edible Iraq among edible oils commonly sold in Iraq since it has low content of the essential fatty acid, which has the ability to increase cholesterol levels and no hence its content.

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References

[1] Hu, F.B., J.E. Menson and W.C. Willet, Types of dietary fats and risk of coronary heart diseases: A critical review. J. Am. College of Nutr. 2001., 20: 5-49.

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

[3] Behrman, E.J. and G. Venkat, Cholesterol and plants. J. Chemical Edu., 2005, 82: 1790-1793.

[4] Noda, M., M. Tunaka, Y. Seto, T. Aiba and C. Oku, Occurrence of cholesterol as major sterol component in leaf surface lipids. Lipids, 1988, 23: 439-444.

[5] Shukla, V.K.S., P.C. Dutta and W.E. Artz, Camelina oil and its unusual cholesterol content. J. Am. Oil Chem. Soc., 2002,79: 965-969.

[6] Laker, M., Understanding cholesterol. Family doctor publications, Dorset, United Kingdom, 2003, 116.

[7] Mensink, R.P., P.L. Zock, A.D. Kester and M.B. Katan, Effects of dietary fatty acids and carbohydrates on the ratio of serum to HDL cholesterol and serum lipids and apoproteins: a meta-analysis of 60 controlled trials. Am. J. Clin. Nutr., 2003, 77: 1146-1155.

[8] Gur, M.I. and J.L. Harwood, Lipid Biochemistry: An Introduction. London: Chapman and Hall, 1991, 406.

[9] Keys, A., J.T. Anderson and F. Grande, Serum cholesterol response to change in diet: Particularly saturated fatty acids in the diet. Metabolism, 1965.14: 776-787.

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

[1] Official Method 965.33 Peroxide Value in Oils and Fats, A.O.A.C. 17th edn, 2000.

[2] Anonymous, Relationship of Blood pressure, Serum Cholesterol, Smoking habits, Relative weight and ECG abnormalities to incidence of major coronary events. J. Chronic Dis., 1978, 31: 201-206.

[3] Garrett, R.H.; Grisham, C.M. Biochemistry y, 5th Edition, University of Virginia, Brooks/Cole, 20 Davis Drive, Belmont, CA, 2012, 94002-3098, USA.

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

[5] Smith, L. L. Cholesterol autoxidation; Plenum Press: New York, 1981.

[6] Hoenselaar, R., Saturated fat and cardiovascular disease: The discrepancy between the scientific literature and dietary advice, *Nutrition*, 2012, 28:118– 123.

[7] Nelson, D.L.; Cox, M.M.;Lehninger Principles of Biochemistry, Fourth Edition, Worth Publishers, Inc. New York, 2005,344.

[8] Dimberu, G. A.; Belete, B. ,Estimation of total free fatty acid and cholesterol content in some commercial edible oils in Ethiopia, Bahir Dar,

[9] Journal of Cereals and Oil seeds, 2011, 2(6):71-76.

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

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

[12] Noda, M., M. Tunaka, Y. Seto, T. Aiba and C. Oku, Occurrence of cholesterol as major sterol component in leaf surface lipids. Lipids, 1988, 23: 439- 444.

[13] Raven, H.P. and B.G. Johnson, Biology, 5th Edn. Mc Graw-Hill, New York, 1999, 1231.

[14] Official Method 965.33 Peroxide Value in Oils and Fats, A.O.A.C. 17th edn,2000.

[15] Anonymous, Relationship of Blood pressure, Serum Cholesterol, Smoking habits, Relative weight and ECG abnormalities to incidence of major coronary events. J. Chronic Dis., 1978, 31: 201-206.

[16] Garrett, R.H.; Grisham, C.M. Biochemistry y, 5th Edition, University of Virginia, Brooks/Cole, 20 Davis Drive, Belmont, CA, 2012, 94002-3098, USA.

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

[18] Smith, L. L. Cholesterol autoxidation; Plenum Press: New York,1981.

[19] Hoenselaar, R., Saturated fat and cardiovascular disease: The discrepancy between the scientific literature and dietary advice, Nutrition, 2012,28:118– 123.

[20] Nelson, D.L.; Cox, M.M.; ,Lehninger Principles of Biochemistry, Fourth Edition, Worth Publishers, Inc. New York, 2005,344.

[21] Dimberu, G. A.; Belete, B. ,Estimation of total free fatty acid and cholesterol content in some commercial edible oils in Ethiopia, Bahir Dar,

[22] Journal of Cereals and Oil seeds, 2011, 2(6):71-76.

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

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

[25] Noda, M., M. Tunaka, Y. Seto, T. Aiba and C. Oku, Occurrence of cholesterol as major sterol component in leaf surface lipids. Lipids, 1988, 23: 439- 444.

[26] Raven, H.P. and B.G. Johnson, Biology, 5th Edn. Mc Graw-Hill, New York, 1999, 1231.