ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL SCIENCES

CENTER FOR FOOD SCIENCE AND NUTRITION

Physicochemical properties and Trace metal level of oils sold in Ethiopia and their effect on the serum of rats

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Getacher kebede

Advisors: Ashagrie Zewdu (Ph.D)

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Science and Nutrition.

June, 2015

Addis Ababa, Ethiopia

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DECLARATION

I, the undersigned, declare that this thesis is my original work and that all sources of materials used for the thesis have been dully acknowledged.

Signature: _____ Date:_____

The Thesis has been approved for Submission by:

Name of Supervisor(s) Signature(s) Date

Dr. Ashagrie Zewed (Advisor) _____ _____

This work is dedicated to:

My dear mother **Wosenyelesh Haile Silassie** who set the ground to my success, but not alive today to see the finals.

Acknowledgments

First and foremost, I would like to state my most sincere thankfulness to my advisory Dr. Ashagrie Zewdu for his valuable support and important contributions to my thesis.

I would like to thank Dr. Aweke kebede and Ato Binyam Tesfaye who helped me in every direction of my research from the beginning to the end. And Feyissa Challa for his valuable support to assist me in doing all biochemical parameters. I would like to extend my thanks to all the staff members of food science and nutrition department at the Ethiopian public health institute.

I would also like to thank Dr. Dawud Gashu for his support during my difficult times.

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List of Abbreviations

ALA	Alpha-Linolenic Acid
ApoB	Apolipoprotein B
CE	Cholesterol Esterase
CHD	Coronary Heart Diseases
CV	Cardio vascular
CVD	Cardiovascular Disease
DAG	Diacylglycerols
FAO	Food and Agriculture Organization
FC	Free Cholesterol
HDL-c	High Density Lipoprotein Cholesterol
HIV/AIDS	Human Immune deficiency Virus/ Acquired Immune Deficiency Syndrome
HMG	3-hydroxy-3-methyl-glutaryl-coenzyme A
IDL	Intermediate Density Lipoprotein
I-FABP	Intestinal Fatty Acid-Binding Protein
IL-1	Interleukin 1
LA	Linoleic Acid
LDL-c	Low Density Lipoprotein Cholesterol
MAG	Mono Acyl Glycerol

oLDL	Oxidized Low Density Lipoprotein Cholesterol
TC	Total Cholesterol
TG	Triglycerides
TNF	Tissue Necrosis Factor
VLDL	Very Low Density Lipoproteins
TFA	Trans Fatty Acid

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Abstract

Objective: Considering the fact that elevated serum concentration of cholesterol is a major risk factor for atherosclerotic disease, the effect of consumption palm oil, soya bean oil, Sheno lega and Niger seed (Nuge) oil-supplemented diet on serum lipid profile of Wistar albino rats was investigated. These oils were analyzed spectrometrically for their metal (Cu, Fe, Pb, Ni, and Zn) levels by Graphite Furnaces Atomic Absorption Spectrometer. The four samples of vegetable oils were also examined for peroxide value, acid value, iodine value and % free fatty acid (%FFA).

Methods: Growing male Wistar albino rats (*Rattus novergicus)* weighing (165-220g) were used for this study. The rats were housed individually in cages in groups of 4, and maintained at room temperature ($21^oC \pm 2^oC$) with 12 hrs light and dark cycles. Each group had 6 rats and fed with 10% palm oil, 10% Soya bean oil, 10% Sheno lega and 10% Niger seed (Nuge) oil - supplemented diets for 12 weeks. The blood samples were drawn by cardiac puncture after 4, 8 and 12 weeks. The serum levels of Total cholesterol (TC), Triglycerides (TG), Low-density lipoprotein (LDL), and High-density lipoprotein (HDL) were determined at 4-, 8-, and 12-week intervals. The trace metals levels of Copper, iron, zinc, Nickel and lead were determined by AOAC 1995 official method 990.05 by using GF AAS, after extracted from low quantities of oil (0.3290-0.3590 g) with a 65% nitric acid solution and Hydrogen per oxide30% using Microwave assisted digestion and quantization after preparing series of working standard solutions by diluting metal stock solution with deionized water to set calibration curve. Physicochemical analysis peroxide value (PV), iodine value (IV), were determined following the method of the AOAC official method 965.33 (2000) and AOAC official method(1995) respectively . The acid value (AV) was determined titrimetrically using the procedure of AOAC official method 940.20 (1995).

Results: In sheno lega group, 4 weeks of sheno lega supplemented fed significantly ($p<0.05$) increased serum total cholesterol than did Niger seed, palm oil or soya bean oil supplemented group . After 8 weeks of fed TC was highest in sheno lega supplemented group. After 12 weeks of fed TC decrease by all groups but no statistical difference was observed by palm oil and Niger seed group. High Density Lipoprotein Cholesterol (HDL-c),In sheno lega group, 4 weeks of Sheno lega supplemented fed significantly($p<0.05$) increased HDL-c than Niger seed oil, Palm oil or soya bean oil supplemented group. Niger seed oil supplemented group showed significant increase ($p<0.05$) after 8 weeks of fed. After 12 weeks neither group showed difference. Low

Density Lipoprotein Cholesterol (LDL-c), in palm oil, 4 weeks of Palm oil supplemented fed significantly ($p<0.05$)increased serum LDL-c than Soya bean oil, Sheno lega or Niger seed oil. After 8 weeks of fed, Niger seed oil supplemented group showed significant increased ($p<0.05$). After 12 weeks of fed Niger seed oil supplemented group showed significance higher value. Triglyceride (TG), in sheno lega group, 4 weeks of sheno lega supplemented fed showed increased serum TG. After 8 weeks of fed TG was significantly ($p<0.05$) increased by sheno lega supplemented group. After 12 weeks of fed all groups decreased TG but Sheno lega showed higher level. Trace metal level Cu (0.1715 µg/g) was found the highest metal concentrations in Niger seed oil, Sheno lega for Ni (1.7187 µg/g), Niger seed oil for Fe (4.082 µg/g), Soya bean oil contain lower amounts for Fe (2.453 µg/g), and Niger seed (Nuge) oil for Ni (0.8584 µg/g) compared to the other vegetable oil, Pb and Zn were not detected. Niger seed (nuge) oil with the highest acid value 10.84, Sheno lega, Palm oil and soya bean oil have 1.96, 1.54 and 1.47 mg KOH/g acid value respectively. Per oxide value was 6.40, 4.10, 3.99 and 3.10 meq.peroxide/kg in Niger seed oil, Soya bean oil, Sheno lega and Palm oil respectively. Iodine value 128.30, 112.56, 54.56 and 3.77 in Soyabean oil, Niger seed oil, Palm oil and Sheno lega respectively. Percent Free fatty acid (%FFA) 5.45, 0.99, 0.77, and 0.74 in Niger seed oil, Shenolega, palm oil, soyabean oil respectively.

Conclusions:

In short fed sheno lega acutely raises serum total cholesterol. After long fed fed palm oil and Niger seed oil rise serum total cholesterol but no difference showed between palm oil and Niger seed oil. In short fed sheno lega acutely increased HDL-c, Niger seed oil showed chronic effect. In short fed Palm oil acutely raises LDL-c. Chronic effect on LDL-c is the same by all oil types. Sheno lega affects triglycerides level in both acute and chronic use. Fe in all Oil groups were above the maximum values recommended for FAO/ WHO(2011). Cu level in Niger seed oil was above the maximum values recommended for FAO/WHO (2011), Nickel level in sheno lega and palm oil was above the margin approved requirement. The peroxide value and acid value in was highest for Niger seed oil . Acid value was above permissible value in all groups. Sheno lega existed with more saturated fatty acid with lowest iodine value.

CHAPTER ONE

INTRODUCTION

1. INTRODUCTION

1.1 Background

Diet is known to influence lipid metabolism, an important factor in the aetiology of cardiovascular disease, while hypercholesterolemia is considered a risk factor for atherosclerosis. Cardiovascular disease is one of the major health problems in the world. It is dramatically increasing in the last 10 years (Yamada *et al*., 1997).

Blood lipid profile determines the risk of cardiac disease. Lipid profile includes total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) often called good cholesterol, and Low density lipoprotein cholesterol (LDL-C) often called bad cholesterol and triglycerides (TG). The association between diet, plasma lipid concentrations and atherosclerosis has been well documented (Steinberg, 2006).

Atherosclerotic lesions in man and in animals appear to be related to elevated plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), decreased high-density lipoprotein cholesterol (HDL-C) and excess fat consumption. The mechanisms by which hypercholesterolemia (almost always associated with increased LDL-cholesterol) caused atherosclerosis is not clear. There is considerable evidence to suggest that oxidative damage to LDL significantly increased LDL atherogenicity (Jaarin *et al*., 2006).

Oxidised LDL (oLDL) is believed to have several mechanisms of promoting atherogenicity. Oxidised LDL may directly alter both the structure and function of the endothelial cells. Secondly it may chemotactically attract monocytes or macrophages to the endothelium which develop then into lipid laden foam cells of an atheromatous plaque. Oxidized LDL is also taken up by macrophages more rapidly than unoxidised LDL. Inflammatory and immune processes

have been shown to be implicated in the pathogenesis of atherosclerosis. The mediators of immune and inflammatory response, such as cyokines IL-1 and TNF may influence their development (Jaarin *et al*., 2006).

In recent years, the dominance of chronic diseases as major contributors to total global mortality has emerged and has been described in detail (Adeyi *et al*., 2007; WHO, 2008b). By 2005, the total number of cardiovascular disease (CVD) deaths (mainly coronary heart disease, stroke, and rheumatic heart disease) had increased globally to 17.5 million from 14.4 million in 1990. Of these, 7.6 million were attributed to coronary heart disease and 5.7 million to stroke. More than 80 percent of the deaths occurred in low and middle income countries (WHO, 2009e).

The World Health Organization (WHO) estimates there will be about 20 million CVD deaths in 2015, accounting for 30 percent of all deaths worldwide (WHO, 2005). By 2030, researchers project that non-communicable diseases will account for more than three-quarters of deaths worldwide; CVD alone will be responsible for more deaths in low income countries than infectious diseases (including HIV/AIDS, tuberculosis, and malaria), maternal and perinatal conditions, and nutritional disorders combined (Beaglehole & Bonita, 2008). Thus, CVD is today the largest single contributor to global mortality and will continue to dominate mortality trends in the future (WHO, 2009e).

The fats and oils most frequently are used for food preparation and as ingredients include soybean, canola, palm, cottonseed, olive, coconut, peanut, lard, beef tallow, butterfat, sunflower, corn, palm kernel, and safflower (Yeong, 2001). Palm oil has a saturated fatty acid content of 45 percent, and partially hydrogenated soybean oil, although much lower in saturated fat, contains transfatty acids introduced as a byproduct of hydrogenation (Khan & Mensah, 2009).

Despite widespread agricultural production and consumption of oil seeds, most refined edible oil in Ethiopia has been imported for many years, with import share of national consumption ranging from 65% to more than 90% over the past decade (Minstry of Health, 2011). Soya and palm oil are the leading source of the world supply of oils and fats (Yeong, 2001).The exponential growth in the use of tropical oils (specifically palm oil) and partially hydrogenated soybean oil in low and middle income countries is troubling because both these oils contain high levels of fatty acids that are atherogenic and linked to an increased risk of myocardial infarction (Yeong, 2001).

The edible oil consumption in Ethiopia is rising dramatically; available statistics indicate that more than double from 2000 to 2007 according to the FAO, and Ethiopia Food Balance Sheet (2007) projected to double thereafter. As both Imports and domestically produced source of supply, are rising steeply there is no sufficient study on the effect of those oils on lipid profile as well on cardio vascular disease in Ethiopia.

1.2 Statement of the problem

The exponential growth in the use of tropical oils and partially hydrogenated oil in low and middle income countries is troubling because both these oils contain high levels of fatty acids that are atherogenic and linked to an increased risk of myocardial infarction.

Ethiopia is one of the major centers of origin and/or diversity for several oil crops. Gomenzer (*Brassica carinata*), noug (*Guizotia abyssinica*), sesame (S*esamum indicum*) and linseed (*Lens culinaris*) are the major indigenous oil crops are primarily used as sources of oil for local consumption (CSA, 2009). But the country imports large amounts of edible oil, mainly palm oil and this value is about 40 to 50% of the export earnings of oil seeds. In 2008 the value of edible

oil imports more or less on the same level as the lue of oil seeds exports (Wijnands *et al*., 2009). However, complains on public health following the consumption of imported oil is increasing.

This project studied the effect of palm oil, soya bean oil, sheno lega and Niger seed oil in serum HDL-c, LDL-c and triglyceride level of rats fed with these oils, the physicochemical property and trace metal level.

1.3 significance of the study

The intake of oil/fat currently available in the market was measured on the effect of serum lipid profile in rats. The result gives a clue about the cardiovascular health safety of available oils/fats in market. The study answers the health complain following the consumption of imported oil and this study could also serve as a base line for future studies since similar study was not so far conducted in our context.

1.4. Research Question

This study answers the following research questions:

- ✓ Do Palm oil, soya bean oil, sheno lega and Niger seed oil have different effect in rats with respect to serum levels of TC, LDL-C, HDL-C and Triglyceride?
- ✓ Does the metal level namely Copper, Iron, Zinc, Nickel, and Lead in palm oil, soya bean oil, shenolega and Niger seed oil within acceptable range?
- ✓ Does the physico-chemical property of Palm oil, soya bean oil , shenolega and Niger seed oil are within acceptable limit or not?

1.5. Objective

1.5.1. General Objective

This project evaluated serum TOTAL CHOLESTEROL, LDL-C, HDL-C and TRIGLYCERIDE LEVELS in rats fed with palm oil, soybean oil, sheno lega and Niger Seed (Nuge) oil. The study also evaluated their physicochemical properties and trace metal levels.

1.5.2. Specific Objective

The specific objectives are to:

- ✓ Determine serum TC, LDL-C,HDL-C and Triglyceride level in rats fed with Palm oil, soya bean oil, sheno lega and Niger seed oil
- ✓ Determine the metal level, namely Copper, Iron, Zinc, Nickel, and Lead from palm oil, soya bean oil, shenolega and Niger seed oil
- ✓ Determine the physico-chemical property of Palm oil, soya bean oil , shenolega and Niger seed oil

CHAPTER TWO

LITERATURE REVIEW

2. LITERATURE REVIEW

2.1. LIPID AND LIPOPROTEINS

Lipids are fats that are either absorbed from food (Dietary fats) are important components of the human diet, providing energy, essential fatty acids (linoleic acid [LA] and alpha-linolenic acid [ALA]) and serve as a source of fat-soluble vitamins or synthesized by the liver. Triglycerides (TGs) and cholesterol contribute most to disease, although all lipids are physiologically important. Cholesterol is a fat-like substance (lipid) that is present in cell membranes and is a precursor of bile acids and steroid hormones. Cholesterol travels in the blood in distinct particles containing both lipid and proteins (lipoproteins). Three major classes of lipoproteins are found in the serum of a fasting individual. These are low density lipoproteins (LDL), high density lipoproteins (HDL), and very low density lipoproteins (VLDL) (GHAFOORUNISS, 2009).

LDL cholesterol typically makes up 60–70 percent of the total serum cholesterol. It contains a single apolipoprotein, namely apo B-100 (apo B). LDL is the major atherogenic lipoprotein and has long been identified by National Cholesterol education program (NCEP) as the primary target of cholesterol- lowering therapy. This focus on LDL has been strongly validated by recent clinical trials, which show the efficacy of LDL-lowering therapy for reducing risk for CHD (Sacks & Campos, 2003).

HDL cholesterol normally makes up 20–30 percent of the total serum cholesterol. The major apolipoproteins of HDL are apo A-I and apo A-II. HDL-cholesterol levels are inversely correlated with risk for CHD. Some evidence indicates that HDL protects against the development of atherosclerosis, although a low HDL level often reflects the presence of other atherogenic factors (Bruckert & Hansel, 2007).

The VLDL is triglyceride-rich lipoproteins, but contains 10–15 percent of the total serum cholesterol. The major apolipoproteins of VLDL are apo B-100, apo Cs (C-I, C-II, and C-III), and apo E. VLDL are produced by the liver and are precursors of LDL; some forms of VLDL, particularly VLDL remnants, appear to promote atherosclerosis, similar to LDL. VLDL remnants consist of partially degraded VLDL and are relatively enriched in cholesterol ester. Strictly speaking, IDL belongs to remnant lipoproteins although, in clinical practice, IDL is included in the LDL fraction (Sacks & Campos, 2003).

A fourth class of lipoproteins, chylomicrons, is also triglyceride-rich lipoproteins; they are formed in the intestine from dietary fat and appear in the blood after a fat-containing meal. The apolipoproteins of chylomicrons are the same as for VLDL except that apo B-48 is present instead of apo B-100. Partially degraded chylomicrons, called chylomicron remnants, probably carry some atherogenic potential (Law, 1999).

Although LDL receives primary attention for clinical management; growing evidence indicates that both VLDL and HDL play important roles in atherogenesis. VLDL and HDL receive consideration after LDL in the overall management of persons at risk for CHD. The positive relationship between serum cholesterol levels and the development of first or subsequent attacks of CHD is observed over a broad range of LDL-cholesterol levels; the higher the level, the greater the risk. Early prospective data suggested that the risk of CHD plateaued at lower cholesterol levels, but this apparent plateau has disappeared in larger studies. Only in populations that maintain very low levels of serum cholesterol, e.g., total cholesterol <150 mg/dL (or LDL cholesterol <100 mg/dL) throughout life do we find a near-absence of clinical CHD (Law, 1999).

A low HDL cholesterol (<40 mg/dL) also counts as a major risk factor for setting lower LDL goals, whereas a higher HDL cholesterol (>60 mg/dL) takes away one other risk factor. Many

prospective epidemiological studies have reported a positive relationship between serum triglyceride levels and incidence of CHD. When triglyceride levels are >200 mg/dL, the presence of increased quantitites of atherogenic remnant lipoproteins can heighten CHD risk substantially beyond that predicted by LDL cholesterol alone (Austin, Hokanson & Edwards, 1998).

Table 2.1. ATP III Classification of Total Cholesterol, LDL Cholesterol and Triglyceride Levels

Totalcholesterol(mg/dL)		LDLcholesterol(mg/dL)		HDL-c(mg/dL)		Triglycerides(mg/dL)	
<200	Desireable	<100	Optimal	<40	Low	<150	Normal
200-239	Border line high	100-129	Above optimal	≥60	High	150-199	Border
≥240	High	130-159	Border line high			200-499	High
		160-189	High				
		≥ 190	Very high			≥500	Very high

National Cholesterol Education Program National Heart, Lung, and Blood Institute National Institutes of Health NIH Publication No. 02-5215, September 2002

2.1.1 Lipid Metabolism

The major lipids in the body are triglycerides (Tg), cholesterol (free cholesterol, FC, and cholesterol esters, CE), and phospholipids (Pl). Triacylglycerols (fats, triglycerides) constitute 90% of dietary lipid, and are the major form of energy storage in humans. Cholesterol serves as a component of cell membranes and as a precursor for steroid hormones and bile acids. Phospholipids are major components of cellular membranes and lipid-transporting lipoproteins. Fats are insoluble in water. Digestive enzymes, however, are water soluble. Digestion of fats must therefore take place at the interface where fat meets water. Obviously more digestion can occur if more surface area is exposed. Two things act in this regard to aid fat digestion - 1) motion of the intestine and 2) bile acids (secreted by the liver), which act as "digestive detergents" to emulsify fats. Triacylglycerols in the body are derived primarily from: The Diet,

Biosynthesis, and Storage in adipocytes. In humans, the first step in dietary fat digestion starts in the stomach with mechanical emulsification and partial TG hydrolysis by gastric lipase, resulting in the lipolytic products DAG and free fatty acids. Gastric lipase does not hydrolyze PL or cholesterol ester, but its activity in the stomach accounts for 10 to 30% of TAG-lipolysis (Shashikala & Naidu, 2014).

The remaining part of TG digestion is brought about in the duodenal lumen by pancreatic lipase, which acts mainly on the sn-1 and sn-3 position of TAG molecules, releasing 2-MAG and free fatty acids. Pancreatic lipase is abundantly present in pancreatic juice, in accordance with the clinical observation that only severe pancreatic insufficiency results in lipid malabsorption. In the presence of bile salts, pancreatic lipase requires the cofactor pancreatic co-lipase[1] for adequate TG hydrolysis, since TG droplets covered with bile salts are not accessible to pancreatic lipase. Binding of pancreatic co-lipase to the TG/water interface facilitates binding of pancreatic lipase (Borgstrom, 2014).

Digestion of phospholipids occurs entirely in the duodenal lumen, predominantly by pancreatic phospholipase A2. Phospholipase A2, requiring calcium and the presence of bile salts for activation, hydrolyzes phospholipids at the sn-2 position, resulting in free fatty acids and lyso-phosphatidylcholine (lyso-PC). Dietary cholesterol is mainly present as free cholesterol and only 10–15% as cholesterol ester. Cholesterol esters must be hydrolyzed in the duodenum by pancreatic cholesterol esterase (CE) before absorption can take place. Human cholesterol esterase (also known as carboxyl ester lipase, bile salt-stimulated lipase, monoglyceride lipase, pancreatic non-specific lipase, or human milk lipase) does not only hydrolyze cholesterol esters but also acts on TG (sn-1, sn-2, sn-3), PL (sn-1, sn-2), and lipidic vitamin esters and its activity is greatly enhanced by the presence of bile salts (Lombardo & Guy, 1980).

A micelle is formed when soaps surround a non-polar substance in water. Lipid digestion by pancreatic lipases generates mono and diacylglycerols that are absorbed in the small intestine. Bile acids aid in this process too, forming micelles. Blocked bile ducts inhibit absorption of fats considerably. Remember also that vitamins A, D, E, and K are fat soluble, and their absorption too is dependent on bile acids. Once inside the intestinal cells, fatty acids complex with a protein in the cytoplasm called intestinal fatty acid-binding protein (I-FABP) that increases their effective solubility and protects the cell from their detergent effects (Vassileva *et al*., 2000).

The mono and diacylglycerols in the digestive cells are converted back to triglycerides, and packaged into lipoproteins in the bloodstream called chylomicrons .They travel into the blood stream via the lymph system. Triacylglycerols are also synthesized by the liver where they are packaged as very low density lipoproteins (VLDLs) and released into the blood. Upon arrival in adipose tissue and muscle cells, lipoprotein lipase cleaves them to free fatty acids and glycerol. Fatty acids are taken up by these tissues and glycerol is transported to liver or kidneys where it is converted to dihydroxyacetone phosphate (glycolysis intermediate) by glycerol kinase (puts phosphate on) and glycerol-3-phosphate dehydrogenase (oxidizes to DHAP) (Buhman, Accad & Farese, 2000).

2.1.2 Lipid Transport

Lipids by definition are insoluble in water. In order to transport lipids such as fatty acids, triacylglycerols, steroids and fat soluble vitamins in the blood plasma, a carrier protein is required. Fatty acids are carried from the adipose tissue to the muscle, heart and liver tissues by serum albumin. Vitamin A is carried by the retinol binding protein. There are steroid carrier proteins that carry steroids to the target cells. The bulk of the body's lipids (cholesterol,

phospholipids and triacylglycerols), are transported in the plasma by large complexes called lipoproteins (Miles, 2003).

These lipoproteins consist of a core of hydrophobic lipids surrounded by a shell of phosphotidyl glycerols and proteins. The protein components of lipoproteins solubilize the hydrophobic lipids and contain the cell targeting signals. Lipoproteins are classified according to their density. The lowest density lipoproteins are the chylomicrons followed by the chylomicron remnants, very low density lipoproteins VLDLs, intermediate density lipoproteins, IDLs, low density lipoproteins, LDLs, and high density lipoproteins, HDLs. The densities of these lipoproteins are related to the relative amounts of lipids to proteins in the complex. The higher the protein content the higher the density of the lipoprotein (Miles, 2003).

During lipid absorption, the intestine releases large amounts of TG-rich chylomicrons into the circulation. During fasting, however, the liver is the major source of TG-rich lipoproteins by secreting VLDL. Both liver and intestine are capable of synthesizing HDL, which are secreted as nascent particles containing predominantly phospholipids and unesterified cholesterol. Another major lipoprotein, LDL, is formed in the plasma compartment as a product of VLDL catabolism. Additionally, the liver synthesizes apoproteins that are essential structural and enzymatic components of lipoproteins. Apoproteins act as cofactors for enzymes crucial for cholesterol esterification or triglyceride lipolysis. Apo A-I activates the cholesterol-esterifying enzyme LCAT, which is also synthesized in the liver. Apo C-II is required for lipoprotein lipase activation, which hydrolyzes lipoprotein triglycerides, thus converting chylomicrons into chylomicron remnants and VLDL into IDL and ultimately LDL. Apo E and apo B are crucial for receptor-mediated uptake of lipoproteins by peripheral cells, as well as for hepatic uptake of end products of lipoprotein catabolism. Lipoprotein remnant uptake by the liver, mediated by SR-B1

and hepatic lipase, provides a feedback inhibition mechanism for cholesterol homeostasis by regulating activity of HMG-CoA reductase, the key enzyme in hepatic cholesterol neosynthesis (Miles, 2003).

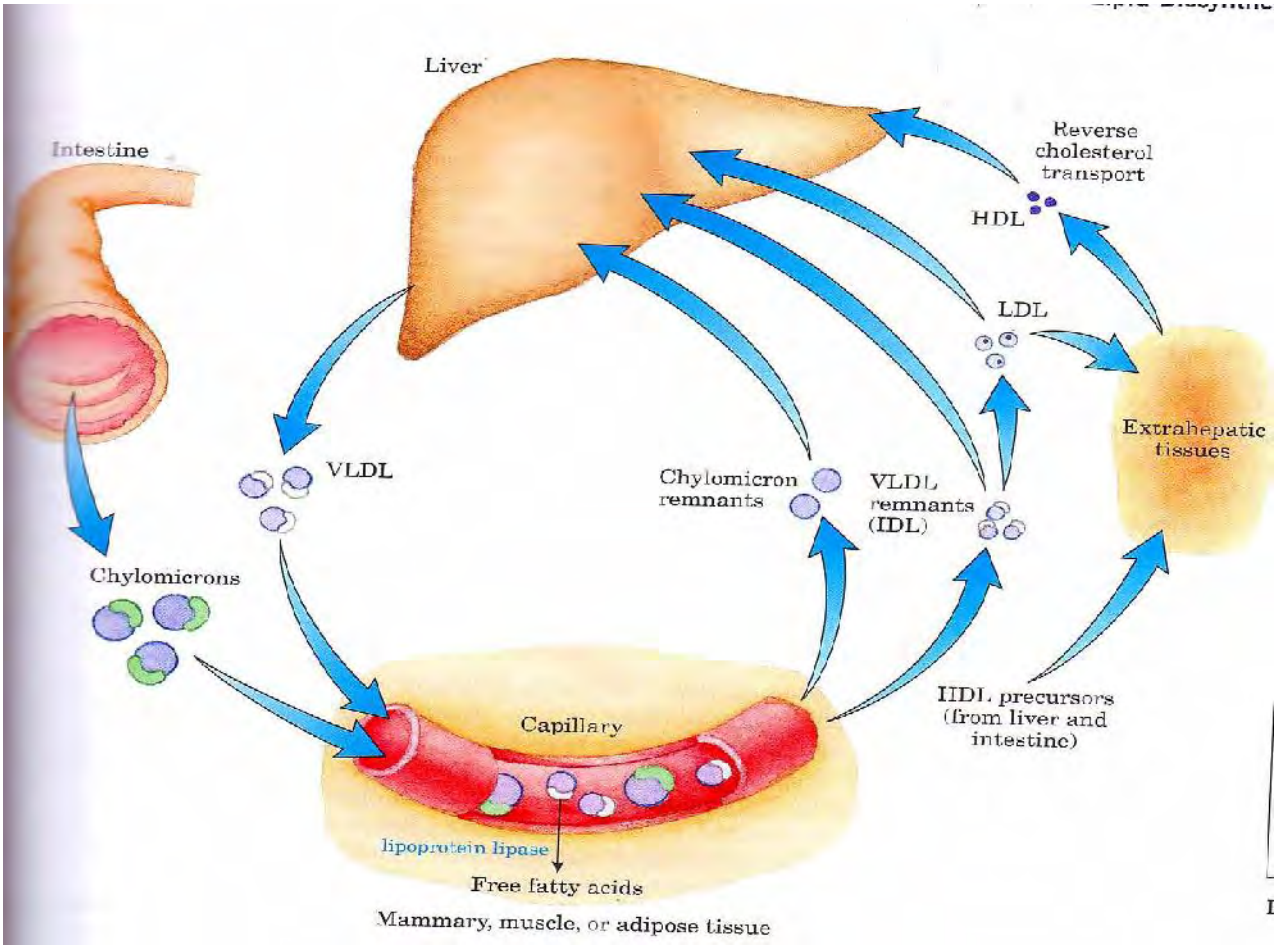


Fig 2.1.Lipoprotein transport (Miles, 2003*)*

2.1.3. Lipoprotein Metabolism

Lipoproteins are complex particles, composed of various lipids and proteins that transport endogenous and dietary fats in the blood stream. There are five types of lipoprotein particles: from smallest to largest, HDL, LDL, IDL, VLDL (high, low, intermediate, and very low density lipoproteins), and chylomicrons. Abnormal lipoprotein levels are associated with several human

diseases, most commonly atherosclerosis. Individuals with atherosclerotic coronary heart disease almost invariably have one or more of four lipoprotein abnormalities: increased LDL-C; decreased HDL-C, usually associated with increased levels of triglyceride-rich lipoproteins (VLDL); increased levels of IDL-C and chylomicron remnants; and high levels of an abnormal lipoprotein, called lipoprotein(a) [Lp(a)], which is a complex of LDL and a large glycoprotein called apolipoprotein(a) [apo(a)l. Lipoproteins synthesized by the liver transport endogenous TGs and cholesterol. Lipoproteins circulate through the blood continuously until the TGs they contain are taken up by peripheral tissues or the lipoproteins themselves are cleared by the liver. Factors that stimulate hepatic lipoprotein synthesis generally lead to elevated plasma cholesterol and TG levels (Breslow, 1993)

Lipid and lipoprotein metabolism can be divided into two pathways, the exogenous pathway involved in the transport of dietary lipids, and the endogenous pathway. In the intestine, absorbed and re-esterified TG, CE and Pl are packed into apoB-48-containing CM, and secreted via the lymph to the circulation. Thereafter, CM TG are rapidly hydrolyzed by lipoprotein lipase (LPL) and to some extent also by hepatic lipase (HL), which process, together with other changes in the lipid and apolipoprotein content of the particles, results in the formation of smaller, cholesterol-enriched CM remnants. CM remnants are removed from the plasma mainly by the liver through the LDLR and to a smaller extent through the LDL receptor-related protein (LRP-1), or cell surface proteoglycans (PG). Under normal conditions, most of the absorbed TG carried by the CM is used in the extrahepatic tissues whereas nearly all cholesterol is delivered to the liver. A small portion of CM remnants seems to be cleared by peripheral tissues as well (Tall, 1998).

The endogenous lipid transport system can be divided into two subsystems: the apoB-100 lipoprotein system (VLDL, IDL and LDL) and the apoA-I lipoprotein system (HDL). The apoB-100 system begins with the hepatic assembly and secretion of apoB-100-containing VLDL particles .VLDL contain apoprotein B-100 (apo B), are synthesized in the liver, and transport TGs and cholesterol to peripheral tissues (Breslow, 1993).

VLDL is the way the liver exports excess TGs derived from plasma FFA and chylomicron remnants; VLDL synthesis increases with increases in intrahepatic FFA, such as occur with high-fat diets and when excess adipose tissue releases FFAs directly into the circulation (eg, in obesity, uncontrolled diabetes mellitus). Apo C-II on the VLDL surface activates endothelial LPL to break down TGs into FFAs and glycerol, which are taken up by cells. Thereafter, VLDL TG are hydrolyzed in peripheral tissues by LPL, and the particles converted to smaller TG-depleted remnant particles (Breslow, 1993).

A part of VLDL remnants are directly cleared from the plasma by hepatic and possibly also peripheral receptor-mediated mechanisms. The remaining particles enter into the VLDL-IDL-LDL cascade, where most of the core TG in the particles is hydrolyzed by LPL and HL, leading to the formation of IDL and LDL particles. Some of the IDL particles are conceivably removed by the liver via LDLR or LRP-1. The rest are converted to LDL particles, the products of VLDL and IDL metabolism, are the most cholesterol-rich of all lipoproteins. About 40 to 60% of all LDL are cleared by the liver in a process mediated by apo B and hepatic LDL receptors. The rest are taken up by either hepatic LDL or nonhepatic non-LDL (scavenger) receptors (Breslow, 1993).

Hepatic LDL receptors are down-regulated by delivery of cholesterol to the liver by chylomicrons and by increased dietary saturated fat; they can be up-regulated by decreased

dietary fat and cholesterol. Nonhepatic scavenger receptors, most notably on macrophages, take up excess oxidized circulating LDL not processed by hepatic receptors. Monocytes rich in oxidized LDL migrate into the subendothelial space and become macrophages; these macrophages then take up more oxidized LDL and form foam cells within atherosclerotic plaques . The size of LDL particles varies from large and buoyant to small and dense. Small, dense LDL is especially rich in cholesterol esters, is associated with metabolic disturbances such as hypertriglyceridemia and insulin resistance, and is especially atherogenic. The increased atherogenicity of small, dense LDL derives from less efficient hepatic LDL receptor binding, leading to prolonged circulation and exposure to endothelium and increased oxidation (Breslow, 1993).

The metabolism of the apoAI-containing HDL-particles is intimately connected with both the exogenous and endogenous lipid transport pathways. HDL particles are derived from precursor complexes secreted by the liver and intestine. They are the main mediators of the reverse cholesterol transport system whereby cholesterol synthesized or deposited in peripheral cells is returned to the liver (Tall, 1998).

This process begins with the removal of FC from cell membranes to nascent HDL particles (Tall, AR., 1998) and esterification of FC by lecithin cholesterol acyl transferase (LCAT), after which the CE is transferred to the hydrophobic core of the HDL particle. In this process, nascent HDLs are converted to spherical lipid-rich HDL. Part of the HDL core CE is then transferred to apoB-48 or apoB-100-containing lipoproteins in exhange for TG by the cholesteryl ester transfer protein (CETP), whereafter these transferred CEs can either be removed from the circulation by the liver or redistributed to peripheral cells. The CEs remaining in the HDL particles are taken up by hepatocytes either via receptor-mediated endocytosis by of apoE-containing HDL particles by

the LDLR, LRP-1, or the putative HDL holoparticle receptor, or through selective removal of HDL CE by the hepatic scavenger receptor BI . At the same time, TG transferred from other lipoproteins to HDL are hydrolyzed by HL, leading to the conversion of TG-rich HDL_2 to TG-poor HDL_3 particles, and release of free apo-AI and lipid-poor HDL to be reused in the reverse cholesterol transport cycle . Besides the exchange of CE for TG, the complex interplay of HDL with other lipoproteins during reverse cholesterol transport involves exchange of other components as well, such as apolipoproteins and Pl. Thus, HDL particles can be considered to serve in plasma as a reservoir of lipids and apolipoproteins for apoB-100 and apoB-48-containing lipoproteins (Tall, 1998).

2.2 ATHEROSCLEROSIS

Atherosclerosis is the leading cause of death and disability in the world (Glass & Witztum, 2001). High blood concentrations of low-density lipoproteins (LDL) and low blood concentrations of high-density lipoproteins (HDL) are primary factors for the development of atherosclerotic disease risk for CAD, if HDL-c is low, is accompanied by other lipid alterations, such as hypertriglyceridemia (especially if the TC/HDL-C is high), an increased concentration of remnant lipoproteins, and small and dense LDL particles. These alterations may be associated with the metabolic syndrome (Expert Panel on Detection, 2002).

Atherosclerosis affects various regions of the circulation preferentially and yields distinct clinical manifestations depending on the particular circulatory bed affected. Atherosclerosis of the coronary arteries commonly causes myocardial infarction and angina pectoris. Atherosclerosis of the arteries supplying the central nervous system frequently provokes strokes and transient cerebral ischemia. In the peripheral circulation, atherosclerosis causes intermittent claudication and gangrene and can jeopardize limb viability. Involvement of the splanchnic

circulation can cause mesenteric ischemia. Atherosclerosis can affect the kidneys either directly (e.g., renal artery stenosis) or as a frequent site of atheroembolic disease (Glass & Witztum, 2001).

Although the development of atherosclerosis has been linked to hypercholesterolemia, the formation of an atherosclerotic plaque is not simply the accumulation of cholesterol and cholesterol esters within arterial walls; instead, it is a complex dynamic process involving mechanisms that include release of chemotaxis factors and cytokines from endothelial cells, chemoattraction and migration of monocytes into the sub-endothelial space, migration and proliferation of smooth muscle cells and apoptosis (Berliner *et al*., 1995).

2.2.1 Initiation of Atherosclerosis

2.2.2 Fatty Streak Formation

An integrated view of experimental results in animals and study of human atherosclerosis suggests that the “fattystreak” represents the initial lesion of atherosclerosis. The formation of these early lesions of atherosclerosis most often seems to arise from focal increases in the content of lipoproteins within regions of the intima. This accumulation of lipoprotein particles may not result simply from an increased permeability, or “leakiness,” of the overlying endothelium. Rather, these lipoproteins may collect in the intima of arteries because they bind to constituents of the extracellular matrix, increasing the residence time of the lipid-rich particles within the arterial wall. Lipoproteins that accumulate in the extracellular space of the intima of arteries often associate with proteoglycan molecules of the arterial extracellular matrix, an interaction that may promote the retention of lipoprotein particles by binding them and slowing their egress from the intima (Glass & Witztum, 2001).

Lipoprotein particles in the extracellular space of the intima, particularly those bound to matrix macromolecules, may undergo chemical modifications. Accumulating evidence supports a pathogenic role for such modifications of lipoproteins in atherogenesis. Two types of such alterations in lipoproteins bear particular interest in the context of understanding how risk factors actually promote atherogenesis: oxidation and nonenzymatic glycation (Ross, 1999).

2.2.3 Lipoprotein Oxidation

Lipoproteins sequestered from plasma antioxidants in the extracellular space of the intima become susceptible to oxidative modification. Oxidatively modified low-density lipoprotein (LDL), rather than being a defined homogenous entity, actually comprises a variable and incompletely defined mixture. Both the lipid and protein moieties of these particles can participate in oxidative modification. Modifications of the lipids may include formation of hydroperoxides, lysophospholipids, oxysterols, and aldehydic breakdown products of fatty acids. Modifications of the apoprotein moieties may include breaks in the peptide backbone as well as derivatization of certain amino acid residues. A more recently recognized modification may result from local hypochlorous acid production by inflammatory cells within the plaque, giving rise to chlorinated species such as chlorotyrosyl moieties. Considerable evidence supports the presence of such oxidation products in atherosclerotic lesions (Jaarin *et al*., 2006).

2.2.4 Nonenzymatic Glycation

In diabetic patients with sustained hyperglycemia, nonenzymatic glycation of apolipoproteins and other arterial proteins likely occurs that may alter their function and propensity to accelerate atherogenesis. A good deal of experimental work suggests that both oxidatively modified and glycated lipoproteins or their constituents can contribute to many of the subsequent cellular events of lesion development (Jaarin *et al*., 2006).

2.2.5 Leukocyte Recruitment

After the accumulation of extracellular lipid, recruitment of leukocytes occurs as a second step in the formation of the fatty streak. The white blood cell types typically found in the evolving atheroma include primarily cells of the mononuclear lineage: monocytes and lymphocytes. A number of adhesion molecules or receptors for leukocytes expressed on the surface of the arterial endothelial cell likely participate in the recruitment of leukocytes to the nascent fatty streak. Oxidised LDL (oLDL) is believed to have several mechanisms of promoting atherogenicity (Krauss, 1998). Oxidised LDL may directly alter both the structure and function of the endothelial cells. Secondly it may chemotactically attract monocytes or macrophages to the endothelium which develop then into lipid laden foam cells of an atheromatous plaque. Oxidised LDL is also taken up by macrophages more rapidly than unoxidised LDL. Inflammatory and immune processes have been shown to be implicated in the pathogenesis of atherosclerosis. The mediators of immune and inflammatory response, such as cyokines IL-1 and TNF may influence their development (Jaarin *et al*., 2006).

2.2.6 Foam Cell Formation

Once resident within the intima, the mononuclear phagocytes differentiate into macrophages and transform into lipid-laden foam cells. The conversion of mononuclear phagocytes into foam cells requires the uptake of lipoprotein particles by receptor mediated endocytosis (Chait & Heinecke, 1994).

Patients or animals lacking effective LDL receptors due to genetic alterations (e.g., familial hypercholesterolemia), however, have abundant arterial lesions and extraarterial xanthomata rich in macrophage-derived foam cells. Also, the exogenous cholesterol suppresses expression of the LDL receptor, such that under hypercholesterolemic conditions the level of this cell-surface

receptor for LDL decreases. Candidates for alternative receptors that can mediate lipid-loading of foam cells include a growing number of macrophage “scavenger” receptors, which preferentially endocytose modified lipoproteins, and other receptors for oxidized LDL or VLDL (very low density lipoprotein), a type of lipoprotein commonly encountered in certain hypercholesterolemic states (Chait & Heinecke, 1994). By ingesting lipids from the extracellular space, the mononuclear phagocytes bearing such scavenger receptors may remove lipoproteins from the developing lesion.

Some lipid-loaded macrophages may leave the artery wall, functioning to clear lipid from the artery. Lipid accumulation, and hence propensity to form atheroma, ensues if the amount of lipid entering the artery wall exceeds that exported by mononuclear phagocytes or other pathways. Macrophages may thus play a vital role in the dynamic economy of lipid accumulation in the arterial wall during atherogenesis. Some lipid-laden foam cells within the expanding intimal lesion perish. Some foam cells may die as a result of programmed cell death known as *apoptosis*. This death of mononuclear phagocytes results in formation of the lipid-rich center, often called the *necrotic core*, of more complicated atherosclerotic plaques (Lusis, 2000).

Macrophages taking up modified lipoproteins, much like intrinsic vascular wall cells, may elaborate cytokines and growth factors that can further signal some of the cellular events in lesion complication. A number of growth factors or cytokines elaborated by mononuclear phagocytes can stimulate smooth-muscle cell proliferation and production of extracellular matrix, which accumulates in atherosclerotic plaques. Cytokines found in the plaque, including IL-1 or TNF-_, can induce local production of growth factors such as forms of platelet derived growth factor (PDGF), fibroblast growth factor, and others that may contribute to plaque evolution and complication. Other cytokines, notably interferon _ (IFN-_) derived from activated

T cells within lesions, can inhibit smooth-muscle proliferation and synthesis of interstitial forms of collagen. These examples illustrate how atherogenesis likely depends on a complex balance between mediators that can promote lesion formation and other pathways that can mitigate the atherogenic process (Libby, 2001).

2.3. FATS AND OILS

Dietary oils remain the major source of lipid in diets. They have lower densities than water, and may have consistencies at ambient temperature of solid, semisolid, or clear liquid. When they are solid-appearing at a normal room temperature, they are referred to as “fats,” and when they are liquid at that temperature, they are called “oils.” For simplification purposes, the terms "fat" and "oils" are used interchangeably (Strayer *et al*., 2006).

The fats and oils most frequently are used for food preparation and as ingredients include soybean, canola, palm, cottonseed, olive, coconut, peanut, lard, beef tallow, butterfat, sunflower, corn, palm kernel, and safflower (Yeong, 2001). Edible oils form an essential part of the modern diet. These oils play a role as an energy source, and provide the diet with many beneficial micronutrients. Although a popular conception may be that fat should be avoided, certain edible oils as a dietary supplement may play an important role in the improvement of cardiovascular health. Cardio vascular disease (CVD) has become one of the leading causes of death worldwide. CVD includes heart attack and stroke, is the leading cause of death, and the most predominant is coronary heart disease. The second strokes, are generally the blockage or hemorrhage of blood vessels leading to the brain causing inadequate oxygen supply and often long term impairment of sensation or functioning part of the body. Atherosclerosis, the third, is the gradual blocking of the arteries with deposit of lipids, smooth muscle cells and connective tissue (Laaksonen, 2000).

In the last decade or two, research on the consumption of dietary fats and oils has become an important topic. High fat content, together with the type of fat in the diet, has been blamed for causing such conditions. Recent research has indicated that the quality or type of fat may be more important than the quantity of fat reducing CVD risk (Puskas *et al*., 2004).

The levels of total cholesterol and the LDL and HDL fractions in the blood are influenced by several factors, including age, sex, genetics, diet and Physical inactivity. Since diet and physical exercise may be controlled by man, they are the basis for recommendations to reduce risk factor for coronary artery disease (Schrauwen & Westerterp, 2000).

Dietary supplementation with different oils may have beneficial effects on cardiovascular health. While olive oil and sunflower-seed oil are known to reduce serum cholesterol, fish oil has become well known for reducing potentially fatal cardiac arrhythmias. Recently, red palm oil research has shown beneficial effects on cardiac recovery from ischaemia– reperfusion injury. It is clear that dietary supplementation with edible oils may play a vital role in reducing the mortality rate due to heart disease. Despite widespread agricultural production and consumption of oil seeds, most refined edible oil in Ethiopia has been imported for many years, with import share of national consumption ranging from 65% to more than 90% over the past decade. Available statistics indicate that edible oil consumption in Ethiopia is rising dramatically, more than doubling from 2000 to 2007 according to the FAO, and according to several sources possibly doubling again since 2007(Ethiopia Food Balance Sheet, Food and Agricultural Organization (FAO, 2007). As both Imports and domestically produced source of supply, are rising steeply there is no sufficient study on the effect of those oils on lipid profile as well on cardio vascular disease in Ethiopia, Even though there have been a lot of studies in other countries.

A study by Klurfeld *et al.* (2001) compared the effects of a *trans* fat rich diet and a saturated fat diet on serum lipids. The trans fat diet, made from partially hydrogenated soybean oil, contained 9.3% energy as trans fat, whereas the saturated fat diet contained lauric acid at 6.8% energy. The LDL/HDL ratio was higher after the *trans* fat diet than after the lauric acid diet.

According to the study done by Judd *et al*. (2002), where subjects were fed diets containing high trans fat(8.3%), moderate trans fat (4.2%), stearic acid (10.9%), saturated fat (lauric, myristic, palmitic) (sum = 18%), and carbohydrates (54.5%). The results showed the high *trans* fat diet raised LDL cholesterol levels the most, followed by moderate trans fat and saturates, then stearic acid, carbohydrates and oleic acid. HDL levels were lowest with high *trans* fat, moderate trans fat and stearic acid diets, the highest value was with saturated fat diet, and Oleic acid and carbohydrate diets were intermediate.

A study by Seneviratne *et al*. (2011) shown the effect of the selected oil blends on serum lipid parameters of wistar rats was examined. The serum level of total cholesterol, LDL and triglycerides decreased and serum levels of HDL increased significantly in rats fed with the oil blend containing the seed of BO (40%) and Corn oil (60%) were comparable with the those of the rats fed with Soybean oil.

A study done by Oluba *et al.* (2011) investigated the effect of palm oil (PO) and melon oil (EMO) on serum and tissue lipid profile in rats. Serum TC concentrations was about 30 and 20% significantly lower in the groups fed PO and EMO respectively than in the Corn oil(CO) group. Although rats fed PO had higher reduction than rats fed EMO diet in serum TC, the difference was not significant. Serum TG concentrations were about 30% significantly lower in both PO and EMO fed rats compared to CO group. No significant difference ($p > 0.1$) was observed in

serum TG levels between PO and EMO groups. No significant differences in serum HDL-C were detected among the three groups at the end of the study. However, serum nHDL-C was significantly lower in both PO and EMO than in the CO group. A non significant ($p > 0.1$) lower concentration in serum nHDL-C was also observed in PO compared to EMO group

A study done by Onyeali *et al*. (2010) on effect of palm oil-supplemented diet on plasma lipid profile of Wistar albino rats was investigate by feeding the experimental animals with 20% palm oil-supplemented diets for 12 weeks. The plasma levels of Total cholesterol (TC), Triglycerides (TG), Low-density lipoprotein (LDL), and High-density lipoprotein (HDL) were determined at 4-, 8-, and 12-week intervals. The results showed significant ($p<0.05$) increases in TC, TG and LDL at the 4th week. However, the level of HDL did not show significant difference from control value. The TC/HDL ratio increased non-significantly. At the 12th week, significant decreases ($p<0.05$) were observed for TC, LDL-c, while no significant difference was noted for TG and HDL. The TC/HDL ratio decreased significantly ($p<0.05$).

A study done by Ibegbulem and Chikezie (2012), measured the effects of palm oil and palm kernel oil containing diet on serum Total Cholesterol, HDL-c, LDL-c, triglycerides VLDL-c in weaning male albino rats. The ratios of TC: HDL-C and LDL-C: HDL-C in the three groups of rats were in the increasing order of PO>PKO and TC:HDL-C>LDL-c:HDL-c.

In an animal study which was conducted in Iran, it has been observed that ghee oil consumption significantly increased HDL-C level, but did not have any significant effect on other serum lipids (Ahmadi-Asl *et al*., 2008).

There is a great amount of evidence confirming that the type of dietary fat is more determinant in CVD development than its amount (Ghafoorunissa , 2009). Saturated fatty acids (SFAs) cause an

increase in serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels (Guo *et al*., 2010).

In the past, the effects of fats on increasing plasma TC levels were estimated by their saturation degree (Volk, 2007). However, the evidence obtained during this past decade indicates that the trans fatty acid (TFA) existing in hydrogenated oils not only increases LDL-C, TC, and apolipoprotein (Apo) B levels, but also decreases high-density lipoprotein cholesterol (HDL-C) and ApoA levels. Therefore, TFAs are more harmful than SFAs (Lichtenstein *et al*., 2003).

Ethiopia as an importer of large volume of fats and oils no study was done in this regards. Considering the fact that elevated serum concentration of cholesterol is a major risk factor for coronary artery disease, it became necessary to ascertain the effect of consumption of these oils on biochemical parameters. This project studied the effect of palm oil, soya bean oil , Niger seed oil and Sheno lega on serum level of HDL-c, LDL-c, TG and total cholesterol level in rats.

2.3.1 Palm Oil

Palm Oil (PO) is a form of edible vegetable oil that is extracted from the fruit of the tropical palm tree *Elaeis guineensis* (Mukherjee & Mitra, 2009). In the unprocessed form palm oil is reddish brown in color, and it has a semisolid consistency at ambient temperature. Many countries plant oil palm to produce the oil to fulfill their local consumption. In contrast, Malaysia and to a certain extent Indonesia are unique in that the production of palm oil is meant for export. Ethiopia is importing palm oil from this region to full fill the majority of its consumption.

Palm oil, like all oils and fats, is made up mostly of glyceridic materials with some nonglyceridic materials in small or trace quantities (Sundram, Sambanthamurthi & Tan, 2003). Triglyceride and Fatty Acid Composition Triglycerides form the major component and bulk of the glyceridic

material present in palm oil with small amounts of monoglycerides and diglycerides, which are artifacts of the extraction process. The fatty acid chains present in the palm oil triglycerides could vary in the number of carbons present in the chain (chain length) and in structure (presence of double bonds, i.e., unsaturation). It is the variations in the structure and number of carbons in these fatty acid chains that largely define the chemical and physical properties of palm oil, about 50% of the fatty acids present in palm oil are saturated (palmitic acid, stearic acid) and about 50% are unsaturated (oleic acid, linoleic acid). The use of palm oil in food dates back 5000 years. For edible and nonedible uses, palm oil is normally refined (Wilson *et al*., 2005). However, even today, unrefined palm oil is still used for cooking in certain African villages much the same way as it used to be. Examples demonstrating the range of palm oil applications in food is shortening, margarine, vanaspati, deep frying fat, and specialty fats.

Regarding Cardiovascular studies with palm oil, most of the studies done on palm oil focused on its effect on the serum lipid profile. Being arbitrarily esterified as a saturated fat, palm oil has been postulated to increase serum cholesterol levels and hence enhance the risk of coronary heart disease (CHD) (Kritchevsky, 2000).

Recent findings, besides earlier published literature, however, indicate that this hypothesis is not uniformly true and suggested possible mechanisms by which palm oil may in fact reduce serum cholesterol. First, palmitic acid does not have an impact negatively on the serum lipid profile (Khosla & Sundram, 1997; Ong & Goh, 2002).

Second, the TAG conformation in palm oil has SFA on the sn-1 and sn-3 positions of the glycerol backbone, and unsaturated fatty acids on the sn-2 position in 75–87% of these molecules. This TAG conformation leads to absorption of more unsaturated fats than saturated fats, as the fatty acid on the sn-2 position is absorbed preferentially to those in the sn-1 and sn-3

positions (Renaud, Ruf & Petithory, 1995; Kritchevsky, Tepper & Kuksis, 1999). Furthermore, tocotrienols contained within palm oil have been shown to inhibit HMG-CoA reductase, a rate-limiting enzyme in cholesterol biosynthesis (Khor, Chieng & Ong, 1995).

RPO has a similar fatty acid composition to refined palm oil, and thus it also has a neutral effect on the serum lipid profile. It also has similar tocotrienol content to refined palm oil. It may therefore be accepted that RPO has similar effects on the serum lipid profile to palm oil. For instance, Kris-Etherton and co-workers (2002) demonstrated that feeding a palm oil diet to rats did not raise plasma cholesterol in comparison to a highly polyunsaturated corn oil diet. Similarly, Sugano and co-workers were unable to establish significant differences in plasma cholesterol in rats fed a palm olein diet compared to other polyunsaturated oils. Sundram and co-workers (2003) compared the effect of palm oil and its fractions with two commonly used polyunsaturated oils, namely soybean and corn oil. It was demonstrated that palm oil feeding did not elevate plasma cholesterol whereas high-density lipoprotein cholesterol (HDL-C) tended to be raised on the palm oil diet relative to the corn oil diet.

In a study done on rabbits by Hornstra (1988), with respect to dietary lipids and cardiovascular disease; effects of palm oil was found to be significantly less atherogenic than fish oil, linseed oil and olive oil. The result was obtained after rabbits supplemented diets with the different oils for 18 months. Study done by Wilson *et al*. (2005) shown, different palm oil preparations reduce plasma cholesterol concentrations and aortic cholesterol accumulation compared to coconut oil in hypercholesterolaemic hamsters. They found that supplementation of 10% palm oil to a standard hamster diet for 10 weeks had reduced total cholesterol, non-HDL-cholesterol and TG when compared with a coconut-supplemented group. In addition, they demonstrated that palm oil supplementation can be associated with significantly lower lipid hydroperoxide formation than

coconut oil supplementation. The authors suggest that this may be due to different antioxidant pathways associated with the oils, or an intrinsic effect of dietary fats. In the same study, RPO also reduced total cholesterol and non-HDL-cholesterol when compared with a coconut oil-supplemented group. In addition, the RPO led to a significant increase in HDL-cholesterol in this study. Results showed that RPO had the highest non-esterified cholesterol:cholesteryl ester ratio, indicating that it was the least atherogenic of all the oil preparations used.

In a study by Salinas *et al.* (2008), it was found that a 35 days supplementation of a deodorised and bleached form of palm oil could significantly reduce total cholesterol and increase HDL-cholesterol in rats with induced hyperlipidaemia by the addition of 5% egg yolk powder to the diet. It was concluded that the high concentrations of micronutrients and MUFA in palm oil altered the serum lipid profile favorably in hyperlipidaemic rats.

In a study by Girardet *et al*. (2001) rats were fed a diet containing 12% palm oil for 1 year and found that palm oil caused increased total serum cholesterol, but that it was accompanied with a lower aortic accumulation of cholesteryl esters when compared with sun flower oil; rapeseed oil, soyabean oil and butter.

2.3.2 Soybean Oil

Soybean oil is normally produced by extraction with hexane. The production consists of the following steps. The soybeans are first cleaned, dried and de-hulled prior to extraction. The soybean hulls need to be removed because they absorb oil and give a lower yield. This dehulling is done by cracking the soybeans and a mechanical separation of the hulls and cracked soybeans. Magnets are used to separate any iron from the soybeans. The soybeans are also heated to about 75 ^{o}C to coagulate the soy proteins to make the oil extraction easier. To extract the oil, first the soybeans are cut into flakes, which are put in percolation extractors and emerged in hexane.

Counter flow is used as extraction system because it gives the highest yield. After removing the hexane, the extracted flakes only contain about 1% of soybean oil and are used as livestock feed, or to produce food products such as soy protein. The hexane is recovered and returned to the extraction process. The hexane free crude soybean oil is then further purified (Kummerow, 2005).

Soybean oil is the dominant oilseed produced in the world in 2010-2011 of 41.874 million metric tons. The U.S. accounts for 20.6% of world soybean oil production, while Brazil produces 15.8% and the European Union accounts for 5.8%. The consumption of soybean oil rose 9.2% worldwide in 2010-2011, with the U.S. accounting for 18.6%, Brazil accounting for 12.4%, India accounting for 6.9%, and the European Union accounting for 6.4% of demand (Kummerow, 2009). Soybean oil has a high content of linoleic acid, and lower level of linolenic acid. These are both essential fatty acids for humans and therefore of dietary importance. Triacylglycerol (TG) is the primarily neutral lipid in soybean oil, nearly all the TG molecules contain at least two unsaturated fatty acids, and di- and trisaturates are essentially absent (Kummerow *et al*., 2007).

Soybean oil contains 52.5% linoleic (18:2 Δ9,12) acid, which is also known as 18:2n6 or omega–6. It also contains 7.5% linolenic (18:3 $\Delta^{9,12,15}$) acid also known as 18:3n3 or omega-3. During hydrogenation, the double bond at any of these 9,12 or 9, 12, 15 positions can be shifted to form new cis and trans unsaturated fatty acid isomers not present in soybean oil (Kummerow, 2009).

Soybean oil is classified as polyunsaturated oil which includes about 15 % saturates, 24 % monounsaturates and 61 % polyunsaturates of which 53.2 % is linoleic acid while the linolenic acid content is about 7.8 %. The nutritional advantages of this composition and its effects in regulating the plasma lipid and eicosanoids bio - synthesis are reviewed on the basis of results from several human clinical trials and studies. These studies have shown that soybean oil

effective in lowering the serum cholesterol and LDL levels, and likely can be used as potential hypocholesterolemic agent if used as a dietary fat and ultimately help prevent atherosclerosis and heart diseases (Kummerow *et al*., 2007).

Attention also drawn to the ability of soybean oil to regulate the eicosanoids balance in such a manner that reduce the atherosclerotic and thrombotic tendencies which are the main causes of heart attacks and strokes. These positive findings on the effect of soybean oil on the cardiovascular system are attributed to its unique fatty acid composition. Soybean oil provides the optimum linoleic to linolenic acid ratio which is the key to achieve the desired balance between the various categories of eicosanoids that help in prevention of heart diseases. Natural soybean oil contains several essential fatty acids that our body needs to work properly,including linoleic and linolenic acids. However, much of the soybean oil consumed in many parts of the world has been partially hydrogenated; that is, it's chemical composition has been changed. This hydrogenation removes the necessary essential fatty acids contained within the original oil. Some of the partially hydrogenated soybean oil has been converted to *trans* fatty acids. This study will evaluate its effect on serum HDL-c, LDL-C and and triglycerides of rats feed with soyabean oil sold in Addis Ababa market.

Study done by Uhegbu (2013) revealed that soybean oil supplemented diet caused a significant ($p<0.05$) decrease in TC, LDL, VLDL, TG and phospholipid and a significant ($p<0.05$) increase in the level of HDL when compared with the control.

2.3.3. Vegetable Ghee (Sheno lega)

Initially, vegetable ghee which is also known as vanaspati was produced using a single hydrogenated oil, for example cottonseed or groundnut oil. As the industry grew, products based on blends of oils or animal fats were produced. Presently, soybean, rapeseed, cottonseed, and

palm oil (PO) are the most commonly used oils in the formulation of vegetable ghee. These oils usually have to be hydrogenated in order to achieve the required characteristics for vegetable ghee. Hydrogenation is a fairly costly process and produces undesirable *trans* fatty acids (TFA). Most of the TFA content in the human diet derived from the partial hydrogenation of fats. Several published reports have indicated that TFA have a negative impact on plasma lipoprotein profile by lowering high-density lipoprotein (HDL) cholesterol and raising the low-density lipoprotein (LDL) cholesterol. Margarine, shortenings and vegetable ghee are manufactured by hydrogenation of vegetable oils, during which a reduction in the unsaturation of oils and an increase in the isomerization at the double bonds take place. Hence, there is a worldwide concern about the consumption of these hydrogenated fats (Nor-Aini *et al*., 2010).

In certain countries, vegetable ghee may be formulated for general purpose applications, cooking, frying, and baking (Mehmood, 2012). Vegetable ghee such as sheno lega, shagi lega are some of palm oil based products imported available in the market. The health benefit or the health detrimental effects of these oils are not studied. This project was evaluating their effect on serum HDL-c, LDL-c and Triglyceride levels in rats.

Cardiovascular studies in animal study, which was conducted in Iran, have shown ghee oil consumption significantly increased HDL-C level, but did not have any significant effect on other serum lipids (Ahmadi Asl N, 2008). Ghee oil is an important dietary fat used in India and other South Asian countries, which contains high amounts of SFAs (about 59% of its whole fatty cids). SFAs, except for stearic acid, increase serum TC (Sartika, 2011). Therefore, ghee oil, that is high in cholesterol and SFAs, is considered as harmful. On the other hand, ghee is a good source of oleic acid which is capable of protecting LDL-C particles from oxidation and prevents atherosclerosis (Gupta & Prakash, 1997).

2.3.4. Niger Seed Oil

Niger *(Guizotia abyssinica* Cass) is an oilseed crop that is cultivated mainly in Ethiopia and India (Getinet & Sharma, 1996). Niger seed provides 50 to 60% of Ethiopia's indigenous edible oil but only 2% of India's total oilseed production. It represents also a minor oilseed crop in some other African countries (Riley & Belayneh, 1989).

Niger seeds contain around 40 % oil and about 20 % protein. Getinet and Sharma (1996), reported that Niger oil has a fatty acid composition typical for other Compositae family oils, such as safflower and sunflower. Niger seed oil contains linoleic acid as the primary fatty acid (75-80%), followed by palmitic and stearic acids (7-8%) and oleic acid (5-8%), although Indian Niger oil is reportedly higher in oleic acid (25%) and lower in linoleic acid (55%).

Niger seed oil has a "nutty taste and a pleasant odour," this edible oil is the mainly produced in Ethiopia and India. Various methods and equipment are used to press the oil, including small cottage expellers and large oil mills. Besides cooking purposes, Niger oil is also used for lighting, anointing, painting and cleaning of machinery. It can also substitute for sesame oil for pharmaceutical purposes and can be used for soap-making (Getinet & Sharma 1996).

2.4. TRACE METALS IN OILS

2.4.1. Role of Metals in Lipid Oxidation

The quality of edible oils regarding their freshness, storability and toxicity can be evaluated by the determination of several trace metals. Levels of trace metals like Cu, Zn, Fe, Mn and Ni are known to increase the rate of oil oxidation while other elements such as As, Cd and Pb are very important on account of their toxicity and metabolic role (Anthemidis *et al*., 2005). The presence of metals in vegetable oils depends on several factors. They might come from the soil,

environment, genotype of the plant, fertilisers and/or metal- containing pesticides, introduced during the production process or by contamination from the metal processing equipment (Zeiner *et al*., 2005).

Heavy metals can be classified as potentially toxic (arsenic, cadmium, lead, etc.), probably essential (vanadium, cobalt) and essential (copper, zinc, iron, manganese, etc.). Toxic elements can be very harmful even at low concentration when ingested over a long time period (Unak *et al.*, 2007). The essential metals can also produce toxic effects when the metal intake is excessively elevated (Gopalani *et al*., 2007).

It is necessary to assess the levels of heavy metals in edible vegetable oils and to report possible contamination that would represent a health hazard. The most common contaminant in Vegetable oil is iron. Iron is as a result of wear and tear of machinery in the mills and transportation tank (Norhayati *et al.,* 2005). It is known that zinc, copper and iron are prooxidant metals which can catalyze the oxidation process and contributing to the oxidative deterioration. It was reported that copper accelerates the hydroperoxides destruction rate thereby increasing the production of secondary oxidation products while iron increase the rate of peroxide formation. Concentration of trace metals gives a great affect of oil qualities with regards to freshness, storage stability and their influence on human nutrition and health. The determination of trace levels of some metals is important because it could catalyze oxidation of fatty acid chains exerting deleterious effect on shelf life and nutritional value (Joao *et al.*, 2008).

According to the trace metal level of edible oils done by Leila and Mohammad (2014), determined lead (Pb), cadmium (Cd), nickel (Ni), Manganese (Mn), zinc (Zn), copper (Cu), iron (Fe), calcium (Ca) and magnesium (Mg) levels in olive oil, Canola oil, Sunflower oil and Soyabean oil produced in Iran by atomic absorption spectrometry (AAS).The concentration of

nickel, manganese, zinc, copper, iron, calcium and magnesium were observed in the range of 0.91–2.17, 0.14–1.76, 3.58–9.54, 0.18–0.68, 7.78- 28.93, 21.42–78.52, 5.34–36.49 µg/g, respectively. Lead and cadmium were found to be 4.56–15.82 and 1.87–8.58 µg/kg. Another study in china on Health risk assessment of eight heavy metals in nine varieties of edible vegetable oils consumed in China namely Soya bean, corn, peanut, sesame, rapeseed, cotton seed, olive, blend and sunflower oil (Zhu *et al*., 2011). Eight heavy metals, namely Cu, Zn, Fe, Mn, Cd, Ni, Pb and As, in nine varieties of edible vegetable oils collected from China were determined by inductively coupled plasma atomic emission spectrometry (ICPAES)and graphite furnace atomic absorption spectrometry (GF-AAS) after microwave digestion. The concentrations for copper, zinc, iron, manganese, nickel, lead and arsenic were observed in the range of 0.214–0.875, 0.742–2.56, 16.2–45.3, 0.113–0.556, 0.026–0.075, 0.009–0.018 and 0.009–0.019 µg/g respectively.

A study done by Acar (2012) and Ansari (2009), Pakistani edible vegetable oil Turky Olive oil, Sunflower oil and soya bean oil was analyzed for Ni, cu and Fe the result was 0,0,0, 0.06-0.11, 0.04-0.10,0.05-0.13,1.53-1.99,1.30-1.71,1.33-1.68, µg/g respectively.

2.4.2. Analysis of Metal in oils by GF AAS

GF AAS also known as Electrothermal Atomic Absorption Spectrometry (ETAAS)) is a type of spectrometry that uses a graphite-coated furnace to vaporize the sample. Briefly, the technique is based on the fact that free atoms will absorb light at frequencies or wavelengths characteristic of the element of interest (hence the name atomic absorption spectrometry). Within certain limits, the amount of light absorbed can be linearly correlated to the concentration of analyte present. Free atoms of most elements can be produced from samples by the application of high temperatures (Zhu *et al.*, 2011).

In GFAAS, samples are deposited in a small graphite or pyrolytic carboncoated graphite tube, which can then be heated to vaporize and atomize the analyte. The atoms absorb ultraviolet or visible light and make transitions to higher electronic energy levels. Applying the Beer-Lambert law directly in AA spectroscopy is difficult due to variations in the atomization efficiency from the sample matrix, and nonuniformity of concentration and path length of analyte atoms (in graphite furnace AA). Concentration measurements are usually determined from a working curve after calibrating the instrument with standards of known concentration. The main advantages of the graphite furnace comparing to aspiration atomic absorption are the following the detection limits for the graphite furnace fall in the ppb range for most elements, Interference problems are minimized with the development of improved instrumentation,the graphite furnace can determine most elements measurable by aspiration atomic absorption in a wide variety of matrices(Zhu *et al.*, 2011).

Most currently available GFAAs are fully controlled from a personal computer that has Windows-compatible software. The software easily optimizes run parameters, such as ramping cycles or calibration dilutions. Aqueous samples should be acidified (typically with nitric acid, HNO_3) to a pH of 2.0 or less. Discoloration in a sample may indicate that metals are present in the sample. For example, a greenish color may indicate a high nickel content, or a bluish color may indicate a high copper content. A good rule to follow is to analyze clear (relatively dilute) samples first, and then analyze colored (relatively concentrated) samples. It may be necessary to dilute highly colored samples before they are analyzed (Calapaj *et al*., 1988).

GF AAs are more sensitive than flame atomic absorption spectrometers, and have a smaller dynamic range. This makes it necessary to dilute aqueous samples into the dynamic range of the specific analyte. GFAAS with automatic software can also pre-dilute samples before analysis.

After the instrument has warmed up and been calibrated, a small aliquot (usually less than 100 microliters (µL) and typically 20 µL) is placed, either manually or through an automated sampler, into the opening in the graphite tube. The sample is vaporized in the heated graphite tube; the amount of light energy absorbed in the vapor is proportional to atomic concentrations. Analysis of each sample takes from 1 to 5 minutes, and the results for a sample is the average of triplicate analysis (Sun, H.J. 1989).

The presence of metals in edible oils occurs through natural contamination and by introduction during the refining process. Specific to refining is the introduction of nickel, which is used as a hardening agent. Lead and copper are potentially present in oils because of environmental contamination. The presence of metals in the final, refined oil is undesirable because the metals can facilitate oxidative degradation of the oil and decrease shelf life (Zhu *et al.*, 2011).

2.5 PHYSICOCHEMICAL PROPERTIES IN OILS

Fats and oils have been used for food and a variety of other applications since prehistoric times, as they were easily isolated from their source and fats and oils found utility because of their unique properties. These ingredients were found to add flavor, lubricity, texture, and satiety to foods. They have also been found to have a major role in human nutrition. Fats and oils are the highest energy source of the three basic foods (carbohydrates, proteins, and fats), carriers for oil soluble vitamins, and many contain fatty acids essential for health that are not manufactured by the human body (O'Brien, 2009).

The chemical and physical properties of edible oils depend primarly on composition and temperature. Pure fats and oils are generally white or yellow solids and liquids. Pure fats and oils are also odorless and tasteless (Otunola, Adebayo & Olufem, 2009). However, over a period of time fats become rancid they develop an unpleasant odor and tasted.

It has been found that oxidatively abused fats can complicate nutritional and biochemical studies because they can affect food consumption under *ad libitum feeding* conditions and also reduce the vitamin content of the food. If the diet has become unpalatable due to excessive oxidation of the fat component and is not accepted by the animal, a lack of growth by the animal could be due to its unwillingness to consume the diet. Thus, the experimental results might be attributed unwittingly to the type of fat or the nutrient being studied rather than to the condition of the ration. Knowing the oxidative condition of fats is extremely important in biochemical and nutritional studies in animals. This study determined properties of oil samples through the chemical tests like peroxide value, iodine value, acid value and percentage of free fatty acid as indicated by Strayer *et al*. (2006).

2.5.1. Peroxide Value

Lipid oxidation involves the continuous formation of hydroperoxides as primary oxidation products that may break down to a variety of nonvolatile and volatile secondary products (M. C. Dobarganes and J. Velasco .2002). The formation rate of hydroperoxides outweighs their rate of decomposition during the initial stage of oxidation, and this becomes reversed at later stages. Therefore, the peroxide value (PV) is an indicator of the initial stages of oxidative change. However, one can assess whether a lipid is in the growth or decay portion of the hydroperoxide concentration by monitoring the amount of hydroperoxides as a function of time (Shahidi & Wanasundara, 2002).

2.5.2. Iodine Value

The iodine value (*IV*) gives a measure of the average *degree of unsaturation* of a lipid: the higher the iodine value, the greater the number of C=C double bonds. By definition the iodine value is expressed as the grams of iodine absorbed per 100g of lipid**.** Iodine value (I.V.) is directly

proportional to the degree of unsaturation (No of double bonds) and inversely proportional to the melting point (M.P.) of lipid. An increase in I.V. indicates that high susceptibility of lipid to oxidative rancidity due to high degree of unsaturation.

$$\text{Iodine Value} = \frac{(B - S) \times N \text{ of } Na_2S_2O_3 \times 0.127\text{g/meq} \times 100}{\text{Weight of Sample (g)}} \quad \text{Eq.1}$$

B: V ml of $Na_2S_2O_3$ volume for blank

S: V ml of $Na_2S_2O_3$ volume for sample

N: normality of $Na_2S_2O_3$

2.5.3 Acid Value

The acid value (*AV) is* the number that expresses, in milligrams the quantity of potassium hydroxide required to neutralize the free acids present in 1 g of the substance. The acid value may be overestimated if other acid components are present in the system, *e.g.* amino acids or acid phosphates. The acid value is often a good measure of the breakdown of the triacylglycrols into free fatty acids, which has an adverse effect on the quality of many lipids.

$$RCOOH + KOH \longrightarrow KOH + KOH$$

Acid value is the measure of hydrolytic rancidity. In general, it gives an indication about edibility of the lipid. Edible oil contain > 1%.

$$AV = \frac{\text{ml of KOH x N x56}}{\text{Weight of sample}} \quad \text{Eq. 2}$$

Where: N= normality of KOH

$$\% \text{ Free Fatty Acid} = AV \times 0.503 \quad \text{Eq. 3}$$

CHAPTER THREE

MATERIALS & METHODS

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted in the Ethiopian Public Health Institute (EPHI) research laboratory and partly in Addis Ababa University center for food science and nutrition, Addis Ababa.

3.2. Oil Samples Collection

Edible oils Samples used for this study were Palm oil (GPMO), Soya bean oil (GSBO), Sheno Lega (GSHO) and Niger seed (Nuge) oil (GNSO). Two samples of edible vegetable oils produced by domestic producers and two imported from abroad a total of four samples were collected. Three kilo gram Sheno Lega and three liter Soya bean oil were purchased from Shoa super market found in Addis Ababa and three liter Palm oil was purchased from co-operative (*Shemachoch*) Shop and three liter Niger seed (Nuge) oil from Addis Modjo Vegetable oil complex Sh.co. The collected oil samples (Palm oil, Soyabean oil and Niger seed (Nuge) oil were packed in polyethylene bottle and Sheno lega in a metal container were stored in a refrigerator until analyzed.

Table 3.1. Sample identification and coding in this study

Name of Product	Oil Source	Group	Code	Origin	Date of Manf	Date of Exp
Palm oil	palm	G1	GPMO	Malaysia	02 Apr 13	04 Apr 16
Soya bean oil	Soya bean	G2	GSBO	Ethiopia	09 May 14	08Apr 2015
Sheno lega	Palm	G3	GSHO	Yemen	16 Jul 13	01 Jan 15
Niger seed (Nuge) oil	Niger seed	G4	GNSO	Ethiopia	--	--

The coding is based on taking the first two letter from name of the sample. Group Palm oil (GPMO), Group Soya bean oil(GSBO), Group Sheno lega (GSHO), Group Niger Seed oil (GNSO).

3.3. Animals, Diets and Study Design

3.3.1. Experimental Animals Handling and Ethical Consideration

Growing male Wistar albino rats (*Rattus novergicus)* weighing (165-220g) from the animal house of the Ethiopian public health institute the Former ENHRI, were used for this study. Prior to initiation of the study, they were fed *ad libitum* with premixed diet and water for 7 days as an adaptation period. The premix diet is the base line diet prepared from Wheat flour and chickpea powder at a proportion of 1:5 before addition of any oil/fat. Then rats were randomly assigned and housed individually in cages in groups of 4(fig.3.1), and maintained at room temperature ($21^oC \pm 2^oC$) with 12 hrs light and dark cycles. Each group had 6 rats. The study protocol and animals handling was conducted in compliance with the National Research Council of National Academies Guide for the care and use of laboratory animals (2011). The food intake was monitored every other day and the weight gain was monitored weekly. Rats were fasted before

blood sampling. Rats were placed in a Desiccator and anesthetized using diethyl ether (fig.3.3). The blood samples were drawn by cardiac puncture after 4, 8 and 12 weeks.

Fig.3.1. Rats assigned individually in Plastic cages

The study was started after Ethical approval granted by the Ethical Committee of the College of Natural Sciences Addis Ababa University Minutes No. 12 Ref. CNSDO/215/07 (see Anex I)

3.3.2 Diets

All experimental diets were prepared by having the same base line (premix) diet supplemented with 10% of respected tested oil samples. Base line (premix) contained Wheat flour 80%, chickpea flour 20% and sodium chloride (salt) 0.05%. Wheat flour was purchased from co-operatives (*Shemachoch*) shop from sholla area market. Chickpea flour was prepared at EPHI laboratory. Raw chickpea was purchased and it was soaked overnight. It was then allowed to dry to separate the seed from shell. The seed was milled in roater mill to make flour. The flour was sieved in a sieve size to have the same particle size with the wheat flour. The test oils were 10% Palm oil, 10% Soya bean oil, 10% Sheno lega and 10 % Nigere seed oil. All diets had the same

composition as Carbohydrates, lipids and proteins and sodium. The caloric values of the diets were the same. The diets were prepared twice a week and store at a temperature of 4^0c.

Table 3.2. Diet formulation in four study groups

	GPMO	GSBO	GSHO	GNSO
Wheat & chickpea flour mix (gm)	900	900	900	900
Palm oil(gm)	100	-	-	-
Soya bean oil (gm)	-	100	-	-
Sheno lega (gm)	-	-	100	-
Niger seed(nuge) oil(gm)	-	-	-	100
Salt (NaCl)	0.05%	0.05%	0.05%	0.05%

Palm oil group (GPMO), Soya bean oil group (GSBO), Sheno lega group (GSHO), and Niger seed oil group (GNSO).

This study used 10% oil from different oil sources which is similar to AIN 93 M diet mixes recommended for rats (Philip, 1997). Group's level was chosen with a view to study the biochemical implications that may arise under 10% oil/fat diet conditions.

Group 1 Palm oil group (Code: GPMO)

This group was fed with supplemented diet for the period of 4, 8and 12 weeks. The supplemented diet was prepared by mixing Palm oil and the premix diet in 1:9 (Palm oil: Premix diet) proportions.

Group 2 Soya bean oil group (Code: GSYO)

This group was fed with supplemented diet for the period of 4, 8 and 12 weeks. Which was prepared by mixing Soybean oil and the premix diet in 1:9 (Soybean oil: premix diet) proportions.

Group 3 Sheno lega group (Code: GSHO)

This group was fed with supplemented diet for the period of 4, 8 and 12 weeks. Supplemented diet was prepared by mixing Shenolega and premixed diet in the proportions 1: 9 (Shenolega: Pre mixed diet).

Group 4 Niger seed (Nuge) oil group (Code: GNSO)

This group was fed with Niger seed (Nuge) oil and the diet for the period of 4, 8 and 12 weeks. This supplemented diet was prepared in the proportions of 1:9 (Niger seed oil: Premix diet)

The test diet was prepared in pelletized form manually using manual extruder. Pellets are formulations that are formed through agglomerating powdered diet in to convenient way of providing diet to rats (Figure 3. 2). Diet powders prepared above, up on addition of tape water (with water to diet ratio, 350ml to 1000gm diet) made in to past with right consistency manually in stainless steel bowl and the formed clumps forced to pass through a hollow stainless steel tube to have a uniform cylindrical shape (diameter of 9mm); the extruded strip might have rough surface which could be removed by rolling the strips of diet on a smooth wooden slab on bench top and then the strip cut in to shorter length (about 6cm) with stainless steel cutting knife and finally placed in an oven to dry at 50^{o}C overnight, then removed from an oven and allowed to cool, then placed within air tight polyethylene zipped bag at refrigerator until provided to rats as per the method elucidated by Green and Turner (1974).

Fig.3.2. Diet in pelletized form

3.3.3. Experimental Design

Completely randomized controlled design was employed. Before the commencement of the experiment, animals were deprived food over night but allowed access to water *ad libitum*. Six rats from each group were anaesthetized and blood samples were taken to establish the baseline levels of the studied parameters. At the end of the experimental diet treatment, Rats from each group were fasted overnight. In early morning rats were anaesthetized and blood samples were taken. Rats were allowed free access to food and water for duration of the study. Diet consumption was measured every other day and body weight was measured weekly, correcting for food spillage.

3.4. EXPERIMENTAL FEED COMPOSITIONAL ANALYSIS

Dry matter content; proximate composition determination (crude protein, fat, ash content) were conducted as per the AOAC (2000) using official methods for Wheat and Chickpea flour. Determination of trace metal content of Palm oil, Soya bean oil, Sheno lega and Niger seed oil was done using atomic absorption spectrophotometer method after Microwave Assisted digestion and preparing series of working standard solutions by diluting metal stock solution with deionized water to set calibration curve. Physicochemical analysis peroxide value (PV), the weight (mg) of active oxygen contained in one gram of the oil or fat, and the iodine value (IV), the number of grams of iodine absorbed by 100 parts by weight of the oil or fat, were determined following the method of the AOAC official method 965.33 (2000) and AOAC official method (1995) respectively . The acid value (AV) was determined titrimetrically using the procedure of official method 940.20(1995).

3.4.1. Dry Matter (DM) Content

Moisture content was determined according to AOAC (2000) using the official method 925.09. A crucible was dried in an oven at 105^{o}C for 1 hour and placed in desiccators to cool. The weight of crucible (W1) was determined. 5gm samples was weighed in dry crucible (W2) and dried at 105^{o}C for 3 hour and after cooling to room temperature in desiccators it was again weighed (W3). The moisture content was determined by using Equation 4.

$$\text{Moisture Content in \%} = \frac{W2 - W3}{W2 - W1} * 100 \qquad \text{Eq. 4}$$

3.4.2. Proximate Composition Determination

Proximate analysis of two samples: Wheat flour and Chick pea flour was determined according to AOAC (2000).

3.4.2.1. Determination of Crude Protein

Protein content was determined according to AOAC (2000) using the official method 979.09. *Digestion:* About 0.5 gm of fresh samples in triplicate was added to a Tecator tube and 6 ml of acid mixture (5 parts of concentrated ortho-phosphoric acid and 100 parts of concentrated sulfuric acid) was added and mixed again on to this mixture 3.5 ml of 30% hydrogen peroxide was added step by step. As soon as the violet reaction had ceased, the tubes were shaken and placed back to the rack. Three grams of catalyst mixture (ground 0.5 gm of selenium metal with 100 gm potassium sulfate) was added in to each tube and allowed to stand for about 10 minutes before digestion. When the temperature of the digester attained 370^{o}C, the tubes lowered in to the digester. The digestion was continued until appearance of clear solution (at about 4 hours). The tubes in the rack were cooled in a fume hood: 25 ml of de-ionized water was added, and shaken to avoid precipitation of sulfate in the solution.

Distillation and titration: the digested and diluted sample solution was distilled using boric acid and the distillate was titrated using 0.1N hydrochloric acid until reddish color appeared (Persson, 2008).

The crude protein was determined as follow:

$$\text{Nitrogen \%} = \frac{(VHCl\ (1) * NHCl)}{Wo} * 14 * 100 \qquad \text{Eq. 5}$$

$$\%\ \text{Protein} = 6.25 * \%\ \text{Nitrogen} \qquad \text{Eq. 6}$$

Where: V is volume of HCl in Litter consumed to the end point of titration, N is normality of HCl (0.1N is usually used), Wo is sample weight on dry matter basis and 14 is molecular weight of nitrogen. The % of nitrogen is converted to % of protein by using the following conversion factor based on the assumption that proteins contain 16% nitrogen.

(% Protein = 6.25 * % Nitrogen for Chickpea flour and 5.7 for wheat flour)

3.4.2.2. Determination of Crude Fat

The crude fat was extracted according to AOAC (2000) Official method 4.5.01. About 2 gm of Wheat flour and 2gm of Chickpea flour was extracted with 50ml diethyl ether at least for 4 hours in a soxhlet extractor (SZC-D Fat Determinator, China). The solvent was then evaporated and the extracted fat was dried in an oven and cooled in a desiccators. The crude fat was determined as follow:

$$\text{Crude Fat \% Wt} = \frac{W2 - W1}{W} * 100 \qquad \text{Eq. 7}$$

Where: W1 = Weight of extraction flask (gm)

W2 = Weight of extraction flask plus dried crude fat (gm)

W = Weight of sample (gm)

3.4.2.3. Determination of Ash Content

The ash content was determined by AOAC (2000) using official method 923.03. Washed porcelain dishes with distilled and deionized water were placed in a muffle furnace for 30 minutes at 550^{o}C. The dishes were cooled in desiccators (with granular silica gel) for about 30 minutes at room temperature and were weighed to the nearest milligrams (M1). About 2.5 gm of fresh sample was weighed in a dish (M2). The dishes were placed on a hot plate under a fume-

hood and the temperature was slowly increased until smoking ceases and samples become thoroughly charred. The dishes with the samples were placed inside a muffle furnace (CARBOLITE, England) at 550^{o}C for 5 hours and then cooled in desiccators for 1 hour. The ash was clean and white in appearance. When cooled to room temperature, each dish with ash was reweighed to the nearest milligrams (M3).

$$\text{Total Ash \%} = \frac{M3 - M1}{M2 - M1} * 100 \qquad \text{Eq. 8}$$

Where: (M2 – M1) is sample mass in grams on dry base and (M3 – M1) mass of ash in grams.

3.5 SERUM PREPARATION

At pre- and post- diet treatment periods week 0, 4, 8 and week 12, rats were fasted over night. Rats were placed in a desiccators to be anaesthetized with diethyl ether (SIGEMA-ALDICH)(fig.3.4) in early morning, about 2 ml blood samples were drawn from each animal by cardiac puncture using 10 cc syringe and 21 ½ needle. Blood was collected in 10 ml non heparinzed vacutainer (BD) tubes. The blood was allowed to clot at room temperature for 1 h, and then centrifuged at 3000 g for 10 min. Top layer of serum was separated into plain tubes and frozen at -82°C until analysis.

Fig. 3.3. Anesthsizing rats using diethyl ether

3.6. SERUM LIPID PROFILE ESTIMATION

3.6.1 Serum Lipid Analysis

Total cholesterol (TC), HDL-cholesterol (HDL-c), LDL-cholesterol (LDL-c) and triacylglycerol (TAG) were quantified using enzymatic, colorimetric method using COBAS INTEGRA 400 plus Analyzer (fig. 3.6) (Roche Diagnostics GmbH, sandhofer strasse), fully automated closed system. Method was according to the manufacturer's instructions.

Test sample was prepared by mixing serum (10 µl) with assay reagent (1.0 ml). Standard sample was prepared by mixing a (10 µl) with assay reagent (1.0 ml). Blank sample was prepared by mixing distilled water (20 µl) with assay reagent (1.0 ml). The mixtures were incubated at 37 ^{0} C for 5 min and the absorbance was read at the respective wave using COBAS INTEGRA 400 plus Analyzer (Roche Diagnostics GmbH, sandhofer strasse)(fig. 3.4).

Materials: COBAS INTEGRA 400 plus Analyzer (Roche Diagnostics GmbH, sandhofer strasse), fully automated closed system .

Fig.3.4. COBAS INTEGRA400 Plus Fully automated cholesterol analyzer

3.6.2 Total Cholesterol (TC)

Reagents: Piperazine-,4-bis(2-ethanesulfonic acid) buffer: 225mmol/L, pH 6.8; Mg^{2+}: 10 mmol/L; sodium cholate: 0.6 mmol/L; 4-aminoantipyrine: ≥0.45 mmol/L; phenol: ≥ 12.6 mmol/L; fatty alcohol polyglycol ether: 3%; cholesterol esterase (Pseudomonas spec.):(≥ 1.5u/ml); cholesterol oxidase (E.coli): ≥0.45u/ml; peroxidase(horseradish): ≥0.75u/ml; stabilizers; preservative

Method: the method was based on Roeschaluand *et al* (1974) Testing. TC was carried out through Enzymatic; Colorimetric method where Cholesterol ester was cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol oxidase then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminoantipyrine to form a red quinine-imine dye. Absorbance was measured at 512/659 nm. COBAS INTEGR analyzers automatically calculated the analyte concentration of each sample.

$$\text{Cholesteryl ester} + H_2O \xrightarrow{\text{cholesterol ester hydolase}} \text{cholesterol} + \text{fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{4-(p-benzoquinone- monoimino)-phenazone} + 4\,H_2O$$

3.6.3 HDL-Cholesterol

Reagents: R1 HEPES buffer: 10.07 mmol/L; CHES;96.95mmol/L, pH 7.4; dextran Sulphate:1.5 g/L; magnesium nitrate hexahydrate:>11.7 mmol/L; HSDA:0.96mmol/L; ascorbate oxidase(Eupenicillium sp., recombinant):>50µkat/L; peroxidase(horseradish):>16.7µkat/L;

preservative SR HEPES buffer: 10.07 mmol/L,pH 7.0; PEG-cholesterol esterase (Pseudomonas spec.): > 3.33µkat/L; PEG-cholesterol oxidase(Streptomyces sp., recombinant:>127 µkat/L; peroxidase(horseradish):>333µkat/L; 4-amino-antipyrine: 2.46 mmol/L;preservative.

Method: HDL-Cholesterol was directly determined in the presence of VLDL, LDL AND CHYLOMICRONS BY The COBAS INTEGRA HDL-cholesterol plus. No sample pretreatment step was required. The method used PEG- modified enzymes and dextran sulphate (Sugluchi *et al* 1995) for direct determination of HDL cholesterol in serum and plasma. When cholesterol esterase and cholesterol oxidase enzymes are modified by PEG, they showed selective activities to ward lipoprotein fractions, with the reactivity in increasing order: LDL < VLDL = Chylomicrons < HDL.

Homogenous enzymatic colorimetric assay: In the presence of magnesium ions and dextran sulphate, water soluble complexed with LDL, VLDL and chylomicrons were formed which were resitsnt to PEG-modified enzymes. The cholesterol concentration of HDL-cholesterol was determined by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups (approximately 40%). Cholesterol esters were broken down quantatively into cholesterol and fatty acids by cholesterol esterase. In the presence of oxygen, cholesterol was oxidized by cholesterol oxidase to 4-cholestenone and hydrogen peroxide.

$$\text{HDL-Cholesterol esters} + H_2O \xrightarrow{\text{PEG-cholesterol esterase}} \text{HDL-cholesterol} + \text{fatty acid}$$

$$\text{HDL- Cholesterol} + O_2 \xrightarrow{\text{PEG-Cholesterol oxidase}} \text{cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{HSDA} + H^+ + H_2O \xrightarrow{\text{Peroxidase}} \text{purple blue pigment} + 5\ H_2O$$

HSDA = Sodium N-2-hydrxy-3-sulfopropyl)-3,5-dimethoxyaniline

The color intensity of the blue quinoneimine dye formed was directly proportional to the HDL-cholesterol concentration. It was determined by measuring the increase in absorbance at 583 nm.

3.6.4 LDL-Cholesterol

This automated method for the direct determination of LDL-C took advantage of the selective micellary solublization of LDL-C by a nonionic detergent and the interaction of sugar compound and lipoproteins (VLVL and chylomicrons). When a detergent was included in the enzymatic method for cholesterol determinations (cholesterol esterase cholesterol oxidase coupling reaction), the relative reactivities of cholesterol in the lipoprotein fractions increase in the order: HDL<chylomicrons<VLDL<LDL. In the presence of Mg^{++}, a sugar compound markedly reduces the enzymatic reaction of the cholesterol measurement in VLDL and chylomicrons. The combination of sugar compounds with detergent enables the selective determination of LDL-cholesterol in serum (Bachorik & Ross, 1995).

Reagents: MOPS (3-morphlio-propanesulfonic acid), HSDA, AOD (RECOMBINANT), POD (horseradish), Magnesium sulphate.$7H_2O$, 4-aminoantipyrine, cholesterol esterase (microbial), cholesterol oxidase (microbial).

Method: Homogenous enzymatic colorimetric assay was used;

$$\text{LDL-Cholesterol esters} + H_2O \xrightarrow{\text{deteregent/cholesterol esterase}} \text{cholesterol} + \text{free fatty acid (selective micellary solublization)}$$

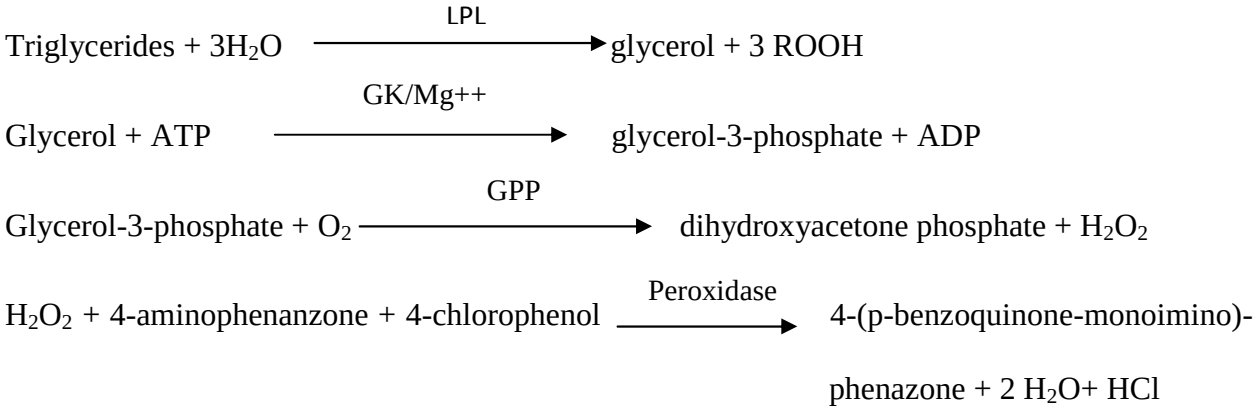
$$\text{LDL- Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{HSDA} + H^+ + H_2O \xrightarrow{\text{peroxidase}} \text{purple blue pigment} + 5\ H_2O$$

HSDA= sodium N-2-hydrxy-3-sulfopropyl)-3,5-dimethoxyaniline

3.6.5 Triglycerides

Method: This method was based on Wahelfeld (1974) using a lipoprotein lipase from microorganism for the rapid and complete hydrolysis of triglycerides for glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide reduced then reacted with a-aminophenanzone and 4-cholorophenol under the catalytic action of peroxidase to form a red dyestuff (Trinder endpoint reaction). The color intensity of the red dyestuff formed was directly proportional to the triglyceride concentration and measured photometricaly.



3.7 METAL DETERMINATION

3.7.1 Sample preparation

Reagents, standards and digestion of samples

All reagents and standard stock solutions used were from SIGMA_ALDRICH. The nitric acid and hydrogen peroxide was ultra high purity grade. Pipettes, digital weighing machine. All laboratory ware including pipette tips and auto sampler cups were cleaned thoroughly with deionized water, cleaned soaked in dilute nitric acid then rinsed thoroughly with deionized

distilled water. To avoid contamination of the specimens, all steps in the sample preparation procedure were carried out in a laboratory equipped for trace element analysis.

ETHOS one HPMDS, milestone Sri, helping chemist's microwave digester (fig. 3.5) was used.

3.7.2 Sample Digestion

Duplicate sample of oils (0.3290-0.3590 g portion) were weighed into 8 separate Teflon digestion vessels. A Teflon vessel was placed on the balance plate and Duplicate sample of oils (0.3290-0.3590 g portion) was weighed. The Teflon vessel was introduced into the safety shield. 7ml of HNO_3 65%, 1ml of 30%H2O2 were added into each vessel. The solution was gently swirled to homogenize the sample with the acids. The vessel was closed and it was introduced into the rotor segment, and then tightened with the torque wrench. The segment was inserted into the microwave cavity then the temperature sensor was connected. Digestion conditions for microwave system were applied as 3 min for 500 W, 5 min for 800 W, 8 min for 1000 W, 10 min for 1300 W, vent: 8 min, respectively. Blank solutions were also prepared by using the digestion procedure given above to check the possible analytic contaminations in the reagents used for the sample preparation.

The resulting solution was transferred into a 50 mL volumetric flask after washing the interior surface of the digestion vessel with nitric acid (1% v/v) three times and diluting the solution with ultra – pure water containing nitric acid (1% v/v) and then kept as a stock sample solution.

3.7.3 Standard Solutions

Four series of working standard metal solution (Table 4) was prepared by appropriate dilution of the metal stock solution (nitrate of the metal) with deionized water containing 2.4ml 3N HCl in

10ml volumetric flask. Calibration curve (concentration versus absorbance) for each element using the prepared standard solution was prepared.

The sample concentration was analyzed using GF AAS by aspirating deionized water. Sample blank solution was run with the sample solution.

Table 3.3. Series of working standard solution for copper, iron, Nickel, Zinc and Lead determination

Series No.	Standard Concentration (ppm)
1	0.0
2	0.5
3	1
4	3
5	4

Fig.3.5 ETHOS One Micro wave Digester (EPHI laboratory)

3.7.4. Determination of metals by GF AAS

Copper, iron, zinc and Nickel were determined by AOAC 1995 official method 990.05 by using GF-AAS, microwave assisted acid digestion and quantization. The method applicable to copper, iron, and nickel in oil and copper and iron in fats at ≥5µg/kg for copper and >10µg/kg for iron and nickel. Nickel, and iron were determined by the graphite furnace atomic absorption spectrometry (GFAAS) (SpectrAA 220, GTA 110, Varian, Australia), equipped with pyrolytically coated graphite tubes and deuterium background correction.

Flame atomic absorption spectrometry (F-AAS) (SpectrAA 220, Varian, Australia), equipped with deuterium background correction was used to determining zinc, Copper and Lead. The NH4H2PO4/Mg (NO3)2 was used as chemical modifier for determination

Test portion was vaporized in GF connected to AAS previously calibrated using standard solutions of organo compounds of Cu, Fe, and Ni. Metal content was determined from measured absorbances at selected wavelengths. Elements were determined sequentially.

Calculations: Peak height was measured and calibration curve was drawn by plotting absorbance of 3 working standard solutions, corrected for the blank, against their respective metal contents. Metal content of the sample was read from relevant calibration curve. Mean of the results of 2 determinations were reported as final result. Result was reported in mg/kg oil/fat corrected for any required dilutions.

$$\text{Metal Content (mg/100gm)} = \frac{[(a - b)*V]}{10*W} \qquad \text{Eq. 9}$$

Where: W = Weight (gm) of sample

V = 50ml = Volume of extract

a = Concentration (µg/ml) of sample solution

b = Concentration (µg/ml) of blank solution

3.8 PHYSICOCHEMICAL ANALYSIS

Reagents: Analytical grade hydrochloric acid, Diethyl ether, potassium iodide, sodium

thiosulphate, Iodine, ethanol, sodium hydroxide, potassium dichromate, potassium hydroxide, chloroform and glacial acetic acid, Starch, Phenolphthalein were used.

Equipment: Adventurer digital balance,250ml Erelnmeyer,250ml conical flasks, Pasture pipette Burette, Beaker were used in the determination measurements.

Method: The peroxide value (PV), the weight (mg) of active oxygen contained in one gram of the oil or fat, and the iodine value (IV), the number of grams of iodine absorbed by 100 parts by weight of the oil or fat, were determined following the method of the AOAC official method 965.33 (2000)and AOAC official method(1995) respectively . The acid value (AV) was determined titrimetrically using the procedure of official method 940.20.

3.8.1 Peroxide Value: 5.00±0.05 g sample was weighed into a 250 ml Erlenmeyer flask and 30ml acetic acid- chloroform (3:2) solution was added. 0.5ml saturated potassium iodide solution was added and the flask was swirled for one minute until the sample was dissolved and 30 ml distilled water was added. It was slowly titrated with 0.01N Sodium thiosulphate with constant and vigorous shaking. The titration was continued until the the colour changed to light yellow. 0.5 ml 1% soluble starch indicator was added which gave blue color, titrating was continue shaking the flask vigorously near endpoint which was faint blue color to librated all the iodine from chloroform layer. The sodium thiosulphate was added drop wise until the blue color disappeared. Blank determination was also conducted.

$$\text{Peroxide value} = \frac{(S-B) \times N}{W} \times 1000 \qquad \text{Eq.10}$$

Where: Peroxide value= mEq peroxide per Kg sample

S= Volume of sodium thiosulphate solution (titrant) in milliliter for sample

B= Volume of sodium thiosulphate solution (titrant) in milliliter for blank

N= Normality of sodium thiosulphate solution

1000= Conversion unit (g/Kg)

W= Sample mass (g)

3.8.2 Iodine value: 0.2543g oil was weighed in 500ml conical flask and 25 ml carbon tetrachloride was added. 25ml Hannus solution was added. The flasks were kept in dark for 30 minutes. After standing for 30 minutes 15ml potassium iodide solution and 100ml boiled and cooled water was added. The librated iodine was titrated with 0.1N sodoum thiosulphate solution using starch as an indicator and blue color disappeared. Blank determination was also conducted and Iodine value was calculated as follow:

$$\text{Iodine value} = \frac{12.69(B-S)N}{W} \qquad \text{Eq.11}$$

S= Volume of sodium thiosulphate solution (titrant) in milliliter for sample

B= Volume of sodium thiosulphate solution (titrant) in milliliter for blank

N= Normality of sodium thiosulphate solution

W= Sample mass (g)

3.8.3 Acid value: A 50 ml (1:1) Solvent mixture of diethyl ether and ethanol 95% was measured into an Erelnmeyer flask and 2g of oil was added and shaked. To the solution 1% 1 ml phenolphthalein was added and titrated with o.1N potassium hydroxide solution.

$$\text{Acid value} = \frac{56.1\ VN}{W} \qquad \text{Eq.12}$$

V= volume in ml of standard KOH

N= normality of KOH

W= weight in g of the sample

The acidity is frequently expressed as free fatty acid for which calculation be;

Free fatty acids as oleic acid= 28.2 VN/W percent by weight

A cid value = percent fatty acids(as Oleic) x1.99

Fig.3.6. Chemical analysis at EPHI laboratory

3.9. STATISTICAL ANALYSIS

Descriptive statistics were calculated and results shown in terms of mean and standard deviation. Four groups (GPMO, GSBO, GSHO & GNSO) were compared using one way analysis of variance (ANOVA) and statistical differences between means was tested by the Duncan's post hoc test where significant differences occurred at significance level of 5% ($P < 0.05$). Data was analyzed using the software Statistical Package for the Social Sciences (SPSS), version 20.0, IBM SPSS Inc. Chicago, USA.

CHAPTER FOUR

RESULTS & DISCUSSION

4. Results and Discussion

4.1 Experimental Feed percentage composition

Diet is the most important factor in experimental animal nutrition. In our study we have used cereal-based, unrefined or nonpurified diets which was the one among the three categories of diets for experimental animals. The results of proximate analysis in Table 4.1 shows the protein, fat, carbohydrate, moisture and ash content and the premixed diet composition in 100gm of diet. The result was found to be 15.01gm protein, 1.07gm fat and 68.98gm carbohydrate. The fats/oil under study was added in the amount based on the AIN-93M maintenance diet and the total energy per kg of diet in each group was 4,012.83 kilocalorie.

Table 4.1. Experimental Diet Proximate (g/100g) and Energy composition in Kcal/Kg

	% composition		**Premix diet composition**	**Experimental diet (10% test Oil &Premix Diet 90%)**	**Energy(kcal/Kg)**			
	Wheat	**Chickpea**	**80% wheat 20% chickpea**		**GPMO**	**GSBO**	**GSHO**	**GNSO**
Protein	13.08±0.46	23.1±1.03	15.08±0.23	135.72±0.21	542.88	542.88	542.88	542.88
Fat	0.47±0.01	3.44±0.14	1.07±0.07	9.63±0.06	86.67	86.67	86.67	86.67
Carbohydrate	70.92±2.37	61.2±1.62	68.98±1.99	620.82±1.79	2483.28	2483.28	2483.28	483.28
Moisture	13.28±0.27	9.8±0.17	12.58±0.22					
Ash	2.25±0.00	2.46±0.00	2.29±0.00					
Palm oil					900			
Soyabean oil						900		
Sheno lega							900	
Niger seed oil								900
Total (Kcal/Kg Experimental Diet)					4012.83	4012.83	4012.83	4012.83

Palm oil group (GPMO), Soya bean oil group (GSBO), Sheno lega group (GSHO), and Niger seed oil group (GNSO) Means ar
for values of triplicate determinations. Experimental diet(1: 9) 10% fat/oil with90% base line diet,
Energy is calculated as Protein(4kcal/g), Carbohydrate(4kcal/g) and Fat(9kcal/g)

4.2. Wight Gain

The weight gain of rats fed different fat/oils is presented in Table 4.2. In this study there was no significant difference ($p<0.05$) in the weight gain pattern among the groups of rats fed with diets containing different oils by week 4 and 8 and 12 the lipid profile of dietary fat has no effect on body weight gains which is in line with previous studies (Lopez-Ferrer *et al*., 1999; Nguyen *et al*., 2003; Kavouridou *et al*., 2008).

Table 4.2 Weight gain in rats fed diets based on 10 % Palm oil, Soya bean oil, Sheno lega and Niger seed oil.

Group	Week 0	week 4		week 8		Week12	
	Initial weight (g)	Weight after 4 week (g)	Weigh difference	Weight after week 8(g)	Weight difference	Weight after week 12(g)	Weight difference
GPMO	194.33±5.90	243.42±3.19	49±3.19[a]	320.00±3.00	126±3.00[a]	401.54±2.88	207±2.88[a]
GSBO	195.33±3.07	248.92±3.35	53±3.35[a]	312.00±2.55	117±2.55[a]	404.13±4.05	209±4.05[a]
GSHO	208.00±3.26	262.00±2.49	54±2.49[a]	334.42±2.32	126±2.32[a]	415.29±2.30	207±2.30[a]
GNSO	196.67±3.73	243.92±3.04	47±3.04[a]	323.25±2.88	127±2.88[a]	404.13±2.95	208±2.95[a]

Values are presented as mean ± SD for three determinations, and n=6 rats. Palm oil group (GPMO), Soya bean oil group (GSBO), Sheno lega group (GSHO), and Niger seed oil group (GNSO). Significant at $P<0.05$.

4.3 Serum lipid Analysis

Most studies on the relationship of diet to heart disease have focused on the theory that high levels of serum lipids are related to intake of dietary fats and oils and the reduction in the quality or change in the kind of dietary fat will lead to a lowering of lipid levels in the blood which in turn may help to stop or slow the progress of these disease. The amount and type of fat contained in a diet has long been linked with the risk of Coronary Heart Disease (CHD), with saturated fats being adverse while polyunsaturated fats being protective factors.

Palm oil, Sheno lega, soy bean oil and Niger seed oils are among the oils and fats imported and domestically produced fats and oils in Ethiopia (Sertse *et al*., 2011). These fats/oils are the leading to satisfy the highest need of the nation in terms of oil and fats demand. The

experimental diets differed with regard to their fatty acid profiles. Palm oil is mono unsaturated oil about 50% of the fatty acids present in palm oil is saturated (palmitic acid, stearic acid) and about 50% are unsaturated (oleic acid, linoleic acid). Sheno lega was produced using single hydrogenated oil, palm oil (PO). Hydrogenation is a fairly costly process and produces undesirable *trans* fatty acids (TFA). Most of the TFA content in the human diet derived from the partial hydrogenation of oil/ fats.

Several published reports have indicated that TFA have a negative impact on plasma lipoprotein profile by lowering high-density lipoprotein (HDL) cholesterol and raising the low-density lipoprotein (LDL) cholesterol. Soybean oil is classified as polyunsaturated oil which includes about 15% saturates, 24% monounsaturates and 61 % polyunsaturates of which 53.2 % is linoleic acid while the linolenic acid content is about 7.8 %. Natural soybean oil contains several essential fatty acids that our body needs to work properly, including linoleic and linolenic acids. However, much of the soybean oil consumed in many parts of the world has been partially hydrogenated; that is, its chemical composition has been changed. This hydrogenation removes the necessary essential fatty acids contained within the original oil. Some of the partially hydrogenated soybean oil has been converted to trans fatty acids. Ethiopian Niger seed oil contains linoleic acid as the primary fatty acid (75-80%), followed by palmitic and stearic acids (7-8%) and oleic acid (5-8%).

4.3.1 Total Cholesterol (TC)

In sheno lega group , 4 weeks of sheno lega supplemented fed(Table 4.3) significantly increased serum total cholesterol than did Niger seed, palm oil or soyabean oil supplemented group . After 8 weeks of fed TC was highest in sheno lega supplemented group. After 12 weeks of fed palm oil and Niger seed group showed no difference.

Sheno lega shows a negative health effect by acutely rising total cholesterol after short feeding period. The type of fat contained in diet has long being linked with risk of CHD with saturated fats being adverse. The acute total cholesterol rising effect following consumption of sheno lega is because of the saturated fat contained in it. Sheno lega with the lowest iodine value shows lowest degree of unsaturated fatty acid in it Table 4.5. The acute TC rising effect by Sheno lega may lead to cause an early exacerbation of CHD in prelipid disorder condition.

After 12 weeks of fed palm oil and Niger seed group showed no difference. The result of this study show similar effect with previous study done by Wilson *et al*. (2005) showed supplementation of 10% palm oil to a standard hamster diet for 10 weeks had reduced total cholesterol.

The chronic total cholesterol level is significantly highest by Palm oil and Niger seed oil. The TC rising effect of these two oils are not linearly proportional to their constituent nature. Palm oil is about 50% of the fatty acids present are saturated (palmitic acid, stearic acid) and about 50% are unsaturated (oleic acid, linoleic acid) (Wilson *et al*., 2005). Whereas Niger seed oil contains linoleic acid as the primary fatty acid (75-80%), followed by palmitic and stearic acids (7-8%) and oleic acid (5-8%) (Getinet & Sharma, 1996) but the cholesterol rising effect of the two oils are proportional. After long feed with palm oil and Niger seed oil TC is higher in the group. This finding suggests that prolonged feed with palm oil and Niger seed oil showed no significance different ($p>0.05$).

Long term feeding with palm oil didn't raise TC in comparison to highly polyunsaturated Niger seed oil. The CHD risk increases with palm oil and Niger seed oil up on chronic consumption. The present study is in line to previous study done by(Denke and Grundy, 1992) increased plasma total cholesterol with intake of palmitic acid, a major content of palm oil. Niger seed oil

with minor constituent of palmitic acid has comparable TC rising effect with palm oil. Palm oil found non significant (P>0.05) difference with Niger seed oil. This study was in line with previous studies by (Kritchvsky *et al 2001*) palm oil did't rise plasma cholesterol in comparison to highly poly unsaturated corn oil.

4.3.2 HDL-c

In sheno lega group, 4 weeks of Sheno lega supplemented fed (Table 4.3) significantly increased HDL-c than Niger seed oil, Palm oil or soya bean oil supplemented group. After 8 weeks of fed, Niger seed oil supplemented group showed significant increased level. After 12 weeks neither group showed difference.

Sheno lega has a protective effect in acute period. The study is in line with an animal study which was conducted in Iran, it has been observed that ghee oil consumption significantly increased HDL-C level (Ahmadi Asl N, 2008), which is also observed in this study during the short feeding period.

The protective effect of HDL-C is most widely attributed to its Key role in mediating the reverse cholesterol transport system whereby cholesterol synthesized or deposited in peripheral cells is returned to the liver for reutilization (Tall, 1998). Oxidation of LDL-c is a risk factor for atherosclerosis and coronary heart disease. Since HDL-c enhances the inhibition of LDL-c oxidation (Nofer, Kehrel & Foker, 2002).Epidemiological and controlled clinical trials suggest that each 1 mg/dl (0.026 mmol/L) increment in LDL cholesterol causes an increase in coronary risk of 1%. Epidemiological observations also show an increase of 2-3% in risk for each 1 mg/dl (0.026 mmol/L) decrease in HDL-c cholesterol (Mensink & Katan, 1992). Soya bean does not showed protection effect.

Palm oil Shows non significant ($p>0.05$) difference from the base line value 45.42± 3.95 mg/dl as compared to after 4 week fed 42.89±4.58, 8 week 45.58± 8.35 mg/dl and 12 week 45.42± 3.95 mg/dl. The Effect of Palm oil on Serum HDL-c in this study both the acute and chronic effect was not significantly ($p>0.05$) affected Table 4.3.

Soya bean oil Shows Significant decreased value from the base line 45.42± 3.95 mg/dl after 4 week fed to 37.48±4.68 mg/dl, 8 week 32.3±6.35 mg/dl and 12 week 36.12±4.91 mg/dl. The decreasing effect was not significant ($P>0.05$) between week 4, 8 and week12.

Partially hydrogenated soybean oil, although much lower in saturated fat, contains trans fatty acids introduced as a byproduct of hydrogenation (Khan & Mensah, 2009) ,trans faty acid in soyabean oil as a by product of processing are atherogenic and linked to an increased risk of myocardial infarction (Yeong, 2001) by decreasing HDL-c. The finding in this study showed soya bean oil significantly($p<0.05$) decrease serum HDL-C.

Sheno lega decrease HDL-c with increasing in the feeding period. It shows 56.9±2.21 mg/dl by HDL-c after 4 week fed, by week 8 decreases to 47.13±6.09 mg/dl and after 12 week o 41.3±4.83 mg/dl. The HDL-c value at week 4 was significantly ($p<0.05$) higher as compared to the value by week 8 and week 12. The difference between week 8 and 12 was not significant ($P>0.05$). In this study HDL-c was increased in short feeding period whereas the level showed non significant changes in long feeding period. Sheno lega has a protective effect in acute and but no changes in chronic period. The study is in line with an animal study which was conducted in Iran, it has been observed that ghee oil consumption significantly increased HDL-C level (Ahmadi Asl N, 2008), which is also observed in this study during the short feeding period. But it doesn't show significant ($p>0.05$) different on HDL-c during the study period.

Niger seed oil showed no difference from the base line value 45.42± 3.95 mg/dl to 48.24±3.65 mg/dl by week 4, after week 8 HDL-c increases from base line value 45.42± 3.95 mg/dl to 57.45±1.61 mg/dl. By week 12 the value showed non significant ($P<0.05$) increase from the base line 45.42± 3.95 mg/dl to 46.3±4.83 mg/dl. The result from the whole study period in this study Niger seed oil showed no difference by week 4 and week 12. But the result showed at week 8^{th} is significant ($p<0.05$) different from week 4 and week 12.

An initial significant ($p<0.05$) difference was observed among the groups at the 4^{th} week. On prolonged feeding to 12 weeks difference was not observed by all groups. Diet with increasing intake of linoleic and linolenic acids increases HDL-C and decreases LDL-c, while higher intake of oleic acid decreases LDL-c but doesn't affect HDL-C (LAWTON et al., 2000). The degree of saturation might not be the problem but the presence and level of trans fats existed in the oil during processing contributes to the decreasing effect of HDL-c in this study by soya bean oil is in line with (Lichtenstein *et al*., 2003). The overall effect on HDL-c is not significantly ($p<0.05$) different by palm oil, sheno lega and Niger seed oil whereas soya bean oil significantly ($p<0.05$) affects negatively.

4.3.3. LDL-c

In palm oil, 4 weeks of Palm oil supplemented fed (Table 4.3) significantly increased serum LDL-c than Soya bean oil, Sheno lega or Niger seed oil. After 8 weeks of fed, Niger seed oil supplemented group showed significant increased level. After 12 weeks Niger seed oil supplemented group showed significance higher value. But Palm oil, Soya bean oil, Sheno lega group showed no difference.

The result in this study is in line with previous study done by (Onyeali *et al*., 2010) results showed significant ($p<0.05$) increases at the 4th week There is a great amount of evidence

confirming that the type of dietary fat is more determinant in CVD development than its amount (Ghafoorunissa , 2009). The fatty acid composition found in palm oil 50% of the fatty acids is saturated (palmitic acid, stearic acid) and about 50% is unsaturated (oleic acid, linoleic acid).

Saturated fatty acids (SFAs) cause an increase in serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels (Guo *et al*., 2010). Another important aspect of the analysis of the influence of dietary oil on the lipid metabolism of rats is the polyunsaturated/saturated fatty acid ratio (PFA/SFA). An elevated PFA/SFA ratio is believed to reduce serum lipid levels. Monounsaturated fatty acids (MFA) have also been reported to reduce serum lipid levels as effectively as does PFA.

Niger seed oil the poly unsaturated oil showed significant ($p<0.05$) increase at 8^{th} week. It is because in the present study the trace metal level (Table 4.4) and the chemical analysis result (Table 4.5) were showed higher copper level with higher peroxide value (PV). Peroxide value is a measure the hydroperoxides formed in fats and oils. Hydroperoxides value increases with % free fatty acid level (% FFA) and the presence of trace metals. The Peroxide Value in Niger seed (Nuge) oil was higher than the other groups and the trace metal level was also higher for Iron and Copper. The hydroperoxides are highly correlated with the LDL-cholesterol and HDLcholesterol (Mehmood, 2012). The increase of LDL-cholesterol was positively correlated with hydroperoxides. The LDL-c values in Niger seed (Nuge) oil fed rats after 8 weeks fed was higher of the other groups. The increase LDL-c and thus consider harmful. This finding suggests Niger seed oil fed with high level of hydroproxides. This result is in line with the study done by (Mehmood, 2012) thermally oxidized ghee changes in the lipids profile of the animals Triglycerides, cholesterol and LDL-cholesterol.

LDL-c has several characteristics that are linked to atherogenesis: long residence time in plasma, and enhanced oxidizability, arterial proteoglycan binding, and permeability through the endothelial barrier (Krauss, 1998). This study was in argument with many other studies provided support for positive contribution of palmitic acid, a major component of palm oil, to hypercholesterolemic effect (Cuesta *et al*., 1998) with short feeding period.

Palm oil showed acute significant ($p<0.05$) LDL-C increase effect. Niger seed oil showed significant LDL rising effect upon chronic consumption among the group at $p<0.05$.

4.3.4 Triglyceride level (TG)

In sheno lega group , 4 weeks of sheno lega supplemented fed(Table 4.3) showed increased serum TG than did palm oil, Niger seed, or soyabean oil supplemented group. After 8 weeks of fed TG was significantly increased by sheno lega supplemented group. After 12 weeks of fed Sheno lega showed higher level.

The TGs in this study (Table 4.3) is significantly ($p< 0.05$) increased by Sheno lega. This increased TG level may arise from its hydrogenation process results sheno lega with more saturated fatty acid content. The physico chemical analysis result in Table 4.5 sheno lega showed the least iodine value 3.77±0.03. The less the iodine value the less the number of C=C.

Elevated triglycerides (TG) had been implicated in the development of CVD and such elevation is associated with obesity, pro-inflammatory and pro-thrombic biomarkers and type II diabetes which predispose to CVD (Hodis *et al*., 1999).

Sheno lega showed significant ($p<0.05$) increase from the base line value 58.98 ± 2.09 mg/dl to 98.28±5.06 mg/dl at the 4^{th} week. By the 8^{th} week the value showed significant ($p<0.05$) increase from the base line value 58.98 ± 2.09 mg/dl to 119.72±8.56 mg/dl. At the 12^{th} week the value

showed significant ($p<0.05$) increase from the base line value 58.98 ± 2.09 mg/dl to75.10±5.15 mg/dl. The whole study period showed increased value with feeding time from the baseline value. The observed result at the 4th week was significantly different ($p<0.05$) from the 8th week 12th week. The 8th week was significantly different ($p<0.05$) different from 12th week.

Niger seed oil increases triglyceride level from the base line value 58.98 ± 2.09 mg/dl to 78.72±5.14 mg/dl at the 4th week. By the 8th week, increase from the base line value 58.98 ± 2.09 mg/dl to100.26± 4.34 mg/dl. At the 12th week TG increase from the base line value 58.98 ± 2.09 mg/dl to 65.50± 4.07 mg/dl. The increase of TG was positively correlated with hydroperoxides in Niger seed oil. Niger seed oil is consumed with more oxidized form PV value 6.40±0.01. This finding suggests Niger seed oil fed with high level of hydroproxides. This result is in line with the study done by (Mehmood, 2012) thermally oxidized ghee changes in the lipids profile of the animals Triglycerides. The TGs in this study (Table 4.3) was significantly ($p< 0.05$) increased by Sheno lega because it is more saturated and Niger seed is more oxidized it affects lipid profile by increasing TG level.

Palm oil showed difference from the base line value 58.98 ± 2.09 to 90.43± 4.87 at the 4th week. By the 8th week, increase from the base line value 58.98 ± 2.09 to74.18±6.46. At the 12th week decrease from the base line value 58.98 ± 2.09 to 55.41±5.03. The effect was decreasing as the feeding time increased. The rising effect showed at the 4th week is significantly ($p<0.05$) decreased by the 8th and 12th week. The effect of each oil/fat on serum TG level showed on Table 4.3. Palm oil diet an effect considered beneficial to man in relation to risk factor for coronary heart disease (Yarnell *et al*., 2001). Similar decreases had been reported by other workers (Kritchevsky *et al*., 2001; Kamisah *et al*., 2005). The TGs level is consistently higher for Sheno lega even at 12th week

Soya bean oil showed no difference from base line value 58.98 ± 2.09 to 55.83± 3.94 mg/dl at 4^{th} week. At the 8^{th} week, increase from 58.98 ± 2.09 mg/dl to 75.58±4.09 mg/dl. At the 12^{th} week, increase from 58.98 ± 2.09 mg/dl to 68.12±2.39 mg/dl. The decreased effect observed at 4^{th} week is increased by 8^{th} week and 12^{th} week. The result showed at the 12^{th} week is not significant ($p>0.05$) different from the result at the 8^{th} week.

Table 4.3 Serum lipid concentrations of rats fed palm oil, Soya bean oil, Sheno lega and Niger seed (Nuge) oil-supplemented diets for 4, 8 and 12 weeks.

Type of oil	4 weeks	8 weeks	12 weeks
	Total cholesterol (mg/dl)		
Palm oil	78.06±2.81^{aq}	83.78 ±3.99^{aq}	65.09±3.03^{aqrs}
soya bean oil	64.51±2.32^{bq}	66.13 ±3.15^{bq}	50.14±2.33^{bqrs}
Sheno lega	91.56±3.29^{cq}	92.60± 4.41^{cq}	55.62±2.58^{crs}
Niger seed oil	84.06±3.02^{dq}	86.73± 4.13^{aq}	63.80±2.97^{ars}
	HDL-c level (mg/dl)		
Palm oil	42.89 ±4.58^{a}	45.58± 8.35^{a}	41.23±4.59^{ab}
soya bean oil	37.48±4.68^{bq}	32.3± 6.35^{bq}	36.12±4.91^{aq}
Sheno lega	56.90±2.21^{cq}	47.13±6.09^{ar}	41.30±4.83^{abr}
Niger seed oil	48.24±3.65^{d}	57.45±1.61^{cqr}	46.36±3.95^{bs}
	LDL-c level (mg/dl)		
Palm oil	47.71±3.09^{aq}	28.56 ± 1.85^{aqr}	18.31±1.19^{aqrs}
soya bean oil	30.88± 2.00^{bq}	19.51±1.26^{bqr}	18.10± 1.17^{aqr}
Sheno lega	34.08±2.21^{cq}	30.67±1.99^{adq}	19.00±1.22^{aqrs}
Niger seed oil	28.07±1.82^{cq}	32.84±2.13^{cqr}	23.16±1.50^{bqrs}
	Triglycerides level (mg/dl)		
Palm oil	90.43±4.87^{aq}	74.18±6.46^{aqr}	55.41±5.03^{ar}
soya bean oil	55.83± 3.94^{b}	75.58±4.09^{aqr}	68.12±2.39^{bqr}
Sheno lega	98.28±5.06^{cq}	119.72±8.56^{cqr}	75.10±5.15^{cqrs}
Niger seed oil	78.72±5.14^{dq}	100.26± 4.34^{dqr}	65.50± 4.07^{bqrs}
	TC	**HDL**	**LDL**	**TG**
Base line	56.88 ± 2.63	45.42± 3.95	2.43 ± 1.83	58.98 ± 2.09

Each data point represents the mean of six rats ± standard deviation; Letters a, b, c, d and e were used to compare statistical significance in columns ($p < 0.05$). Letters q, r, and s were used to compare statistical significance in rows ($p < 0.05$).q Statistically significant compared to base line value.r statistically significant compared to 4 weeks reading. s statistically significant compared to 8 weeks reading.

4.4. Trace metal analysis

The concentration of Cu, Ni, and Fe were detected and Pb and Zn were not detected because they are below the detection limit. Cu(1.715 µg/g) was found the highest metal concentrations in Niger seed oil, Sheno lega for Ni (1.718 µg/g), Niger seed oil for Fe (4.08 µg/g). Soya bean oil contain lower amounts for Fe (2.45 µg/g), and Niger seed (Nuge) oil for Ni (0.858 µg/g) compared to the other vegetable oil. The results of trace metal levels were presented in Table 4.4.

Some trace elements in food, which so far generally have been disregarded as contaminants in food, such as nickel, may provoke allergies in particularly sensitive consumers. Small amounts of heavy metals in edible oils are well known to have serious deteriorative effects on the stability of these oils. The difference in the concentrations of these elements can be used for adulteration determination. Trace element contamination of food grade fats and oils is regulated by Codex Alimentarius and national standards (FAO/WHO, 2011).

Fe- the obtained mean values in this work for all Oil groups Niger seed oil, Sheno lega, palm oil, soya bean oil 4.08, 3.82, 3.04 and 2.45 µg/g respectively are higher than the maximum values recommended for FAO/ WHO (Codex.,2011) 1-1.5 µg/g for Fe). Similar study was done in Pakistan Turky Olive oil, Sunflower oil and soya bean oil. Fe was determined 1.53-1.99, 1.30-1.71, 1.33-1.68 µg/g respectively by Acar (2012) and Ansari (2009). Fe was determined 16.2–45.3 µg/g by Zhu *et al*.,(2011) from nine varieties of edible vegetable oils collected from China. When these results are compared to our findings, Fe was less than from china edible oil and larger than from Pakistan vegetable oil.

Cu in Niger seed oil the mean concentration (0.1715 µg/g) was obtained which is above the maximum values recommended for FAO/WHO (2011) (0.1 µg/g for Cu) to be found in edible vegetable oils. In palm oil 0.0907, Sheno lega 0.05726, and Soya bean oil 0.0390 µg/g mean

concentration was found. This findings are comparable with the mean concentration in Pakistani vegetable oil, Olive oil, Sun flower oil and Soya bean oil 0.06-0.11, 0.04-0.10, 0.05-0.13 µg/g respectively (Ansari 2009 & Acar 2012).

Ni in this study was found in sheno lega 1.7187µg/g and from palm oil 1.623 µg/g, in soya bean oil 0.7839µg/g and 0.8584 µg/g in Niger seed oil which are not within the international requirements, the approved content of Nickel in oils is 0.2 µg/g (Ni). The obtained result in soya bean oil and Niger seed oil showed less and similar for sheno lega and palm oil in concentration found from Iran 0.91–2.17 µg/g by Leila and Mohammad (2014) and larger than the concentration 0.026–0.075 µg/g found in china by Zhu *et al*., (2011). Catalytic hydrogenation of fats and oils by use of nickel catalysts, sheno lega is the catalytic hydrogenation of product of palm oil use nickel as a catalytic metal. The nickel content of Sheno lega and palm oil is significantly different ($p<0.05$) than in niger seed oil and soya bean oil.

Lead in vegetable oils is 0.1µg/g FAO/WHO (Codex, 2011) as maximum permissible concentration. Lead can trigger both acute and chronic symptoms of poisoning. Acute intoxications only occur through the consumption of relatively large single doses of soluble lead salts. Chronic intoxications can arise through the regular consumption of foodstuffs only slightly contaminated with lead. The danger of chronic intoxications is the greater problem. Lead is a typical cumulative poison; fortunately Lead was not detected in all groups' oil in this study.

Levels of trace metals like Cu, Zn, Fe, Mn and Ni are known to increase the rate of oil oxidation (Anthemidis *et al*., 2005). The assessed levels of trace metals copper and iron are prooxidant metals which can catalyze the oxidation process and contributing to the oxidative deterioration since these were higher for Niger seed oil. It was reported that copper accelerates the hydroperoxides destruction rate thereby increasing the production of secondary oxidation

products while iron increase the rate of peroxide formation. Concentration of trace metals gives a great affect of oil qualities with regards to freshness, storage stability and their influence on human nutrition and health.

Table4.4. Results for the determination of trace element levels in edible vegetable oil by GFAAS(µg/g**)**

				GFAAS	
Sample ID	Cu	Ni	Zn	Fe	Pb
GPMO	0.0907±0.003[a]	1.623 ±0.050[a]	ND	3.045±0.003[a]	ND
GSBO	0.0390±0.029[b]	0.7839±0.196[b]	ND	2.453±0.012[b]	ND
GSHO	0.0572±0.013[c]	1.7187±0.133[a]	ND	3.829±0.000[c]	ND
GNSO	0.1715±0.048[d]	0.8584±0.026[b]	ND	4.082±0.014[c]	ND

Palm oil group (GPMO), Soya bean oil group (GSBO), Sheno lega group (GSHO), and Niger seed oil group (GNSO).Values are presented as mean ± SD for two determinations, ND(Not detected), P<0.05., Letters a, b, c, d and e were used to compare statistical significance in columns

4.5 Physicochemical analysis

The results of physicochemical analysis for peroxide value, iodine and acid values were presented in Table 4.5.

4.5.1 Peroxide value

The results in Table 4.5 showed that peroxide value of Niger seed (nuge) oil 6.40, soya bean oil 4.07, Sheno lega 3.99 and palm oil 3.10 Meq Oxygen/kg peroxide values. The peroxide value determination showed in decreasing order Niger seed oil>Soya bean oil>sheno lega>Palm oil. Niger seed oil showed significant difference ($p<0.05$) from Soya bean oil and from the whole group. Niger seed oil contains linoleic acid as the primary fatty acid (75-80%), followed by palmitic and stearic acids (7-8%) and oleic acid (5-8%). Soybean oil is classified as polyunsaturated oil which includes about 15% saturates, 24% monounsaturates and 61 % polyunsaturates of which 53.2 % is linoleic acid while the linolenic acid content is about 7.8 %.

Sheno lega was produced using single hydrogenated oil about 59% saturated fatty acid. Palm oil is mono unsaturated oil about 50% of the fatty acids present in palm oil is saturated (palmitic acid, stearic acid) and about 50% are unsaturated (oleic acid, linoleic acid). But there is no significant difference ($P<0.05$) between soya bean oil with more unsaturated fatty acid and Sheno lega the more saturated fat in the PV.

Peroxide value is used as a measured of the extent to which rancidity reaction have occurred during storage. The value obtained in the study is below the standards sated by FAO/WHO (1994) value which is ≤10.

The fatty acids existed in Niger seed oil is significantly ($P<0.05$) prone to rancid reaction than from soya bean oil with the more unsaturated fatty acid.

4.5.2 Iodine value

The result in Table 4.5 showed the iodine value. Soya bean oil is with the highest mean iodine number 128 followed by Niger seed (nuge) oil 113 and palm oil 55 and the saturated oil (vegetable fat) Sheno lega with iodine number 4.

The tendency of an oil to combine with oxygen of the air and become gummy (known as drying) is measured with the iodine number, which in fact is merely a measure of the level of unsaturation of the oil in question (a higher iodine number will indicate higher unsaturation seeing that iodine is absorbed primarily by the mechanism of addition to the double bonds characteristic of unsaturation) (Gertz, 2000). The iodine value in sheno lega is 4 means the fatty acids in this fat is existed as saturated. The physical state of Sheno lega is sold at room temperature because the majority of the fatty acids in the triglyceride bond are in saturated form.

The level of unsaturation is in the order Soya bean oil > Niger seed (nuge) oil> palm oil > Sheno lega.

4.5.3 Acid value

The result in Table 4.5 showed that acid value of Niger seed oil 10.84±0.57 KOH/g, Sheno lega 1.96 KOH/g, Palm oil 1.54 KOH/g and soya bean oil 1.47 KOH/g acid value respectively.

Niger seed oil showed significant ($p<0.05$) difference from the group but no significant ($p<0.05$) difference was observed between Sheno lega, Palm oil and soya bean oil.

Acid value is an important index of physicochemical properties of oil which is used to indicate the quality age, edibility, and suitability of oil for use in industries such as paint. Acid value is used to measure the extent to which glycerides in the oil has been decomposed by lipase and other physical factors such as light and heat. The presence of FFAs in an oil is an indication of insufficient processing, lipase activity, or other hydrolytic actions.

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).

Niger seed (nuge) oil with the highest acid vale 10.84 KOH/g, Sheno lega, Palm oil and soya bean oil have 1.96 KOH/g, 1.54 KOH/g and 1.47 KOH/g acid value respectively. The lower the acid value of oil, the few fatty acid it contains which makes it less exposed to the phenomenon of rancidity. In the view of the results shown unacceptable AV and %FFA from the accepted acid value0.6 mg KOH/g. GNSO significant($p<0.05$) 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. This result was comparable with Atinafu and Bedemo (2011) Highest Acid value (AV) and

peroxide level (PV) was found in Niger seed oil. The triglycerides in Niger seed oil is hydrolyzed in to free fatty acids.

Peroxide value was highest for Niger seed oil followed by soya bean oil and sheno lega and least PV value was showed by Palm oil. The PV, IV of all the oils under this study was in agreement with the recommended (standard) physico chemical characteristics sated by FAO/WHO (1994). The acid values are much higher than the value Sated ≤0.6 mg KOH per g sample of edible oil. Niger seed oil processing was not sufficient to met stated standards for refined vegetable oils.

Table 4.5 Peroxide value, Iodine value and Acid value for four vegetable oil samples

Sample	**PEROXIDE VALUE(meq.peroxide/kg)**	**IODINE VALUE**	**Acid Value(mg KOH/g)**	**% FFA**
GPMO	3.10 ± 0.01^{a}	54.56 ± 0.00^{a}	1.54 ± 0.39^{a}	0.77^{a}
GSBO	4.10 ± 0.28^{b}	128.30 ± 0.49^{b}	1.47 ± 0.07^{a}	0.74^{a}
GSHO	3.99 ± 0.02^{b}	3.77 ± 0.03^{c}	1.96 ± 0.03^{b}	0.99^{a}
GNSO	6.40 ± 0.01^{c}	112.56 ± 1.32^{d}	10.84 ± 0.57^{c}	5.45^{a}

Palm oil group (GPMO), Soya bean oil group (GSBO), Sheno lega group (GSHO), and Niger seed oil group (GNSO). Values are presented as mean ± SD for three determinations, $P<0.05$. Letters a, b, c, d and e were used to compare statistical significance in columns

CHAPTER FIVE

CONCLUSION & RECOMMENDATIONS

5. CONCLUSION AND RECOMMENDATIONS

5.1. CONCLUSION

In short fed period TC in rat serum is altered by sheno lega. In long fed period TC decrease by all groups. LDL-c affected higher by Palm oil in short fed. In longer fed Niger seed oil significantly increased but no difference showed between soya bean oil, palm oil and sheno lega. The HDL-c increased by Sheno lega in short period. In long period no difference was showed by soya bean oil, palm oil, sheno lega and Niger seed oil. The TG in rat serum was altered higher by sheno lega with short feeding period. In longer fed Sheno lega altered higher than Niger seed oil and soya bean oil but palm oil affects less than all groups.

Fe in all Oil groups were above the maximum values recommended for FAO/ WHO(2011). Cu level in Niger seed oil was above the maximum values recommended for FAO/WHO (2011), Nickel level in sheno lega and palm oil was above the margin approved requirement.

The peroxide value and acid value in Niger seed oil was different from soya bean oil, sheno lega and palm oil. Acid value was above permissible value in all groups.

The trace metal level and the physicochemical properties are also important factor for serum lipid parameters. When the trace metal level with in liquid oil increases, the level of oxidation also increases in the same condition. In this study the poly unsaturated Niger seed oil with high level of Fe and Cu showed high PV and AV. As the same time the serum level of TC and LDL-c is significantly different from its counterpart poly unsaturated soya bean oil but showed non significant difference from partially saturated palm oil. Ni level is high in saturated sheno lega

and palm oil than in Niger seed oil and soya bean oil, this might come from catalytic process of hydrogenation such process in fats and oils use nickel catalysts.

Generally the health effect of palm oil on lipid profile was not as the public view but Niger seed oil presented with more affected quality and lipid profile.

Limitations of the study

The oils under this study were not directly subjected to temperature and time by which most Ethiopian diets are prepared.

5.2. RECOMENDATIONS

This study was done on limited oil types available on the market and a broader future study to include all available oils/fats brands in the market

The trace metal level in poly unsaturated fatty acid oils is crucial to their effect to lipid profile so further analysis should be done to other groups of oil available in the market

Trace level of toxic metals in saturated oil should also be done in available products in the market

This study was done after drying the experimental diet at 50^0C in dry oven. So I recommend to extend this study to different cooking temperature

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ANNEX

Annex A: Ethical Approval from College of Natural Sciences Ethical Review Committee.

COLLEGE OF NATURAL SCIENCES
Addis Ababa University

OFFICE OF THE DEAN

Ref: CNSDO/215/07/15
Date: January 13, 2015

To Whom It May Concern

The Ethical Committee of the College of Natural Sciences in its meeting held on 22/12/2014 (Minutes No.12) has examined the project entitled **"The Effect of Consumption of Palm Oil and Shenolega on Serum HDL-C, IDL-C and Triglyceride Level in Rats"** by Getacher Kebede (Center for Food Science and Nutrition) for ethical approval.

The Proposal is approved for implementation. The decision will remain valid until December 21, 2015.

With regards,

Negussie Retta, (Professor)
Dean, College of Natural Sciences

Encl:

- RERC Minutes