

Hypolipidemic Effect of Alpha-Linolenic Acid Rich Blended and Interesterified of Refined Palm Olein Oil With Flaxseed Oil as Compared to Native Oil Fed Rats

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Keywords Alpha linolenic acid; Omega-3 fatty acid; Blended oil; Interesterified oil; Flaxseed oil

Abbreviations FXO: Flaxseed Oil; RPOO: Refined Palm Olein Oil; PUFA: Polyunsaturated Fatty Acid; TAG: Tri Acyl Glycerol; LDL: Low Density Lipoproteins; HDL: High Density Lipoproteins; VLDL: Very Low-Density Lipoproteins; ALA: Alpha Linolenic Acid; DHA: Docosa Hexaenoic Acid; ARA: Arachidonic Acid

Abstract

In the present study, refined palm olein oil (SFO) and Flaxseed Oil (FXO) were chosen for blending and interesterification to obtain an ideal ratio and enriched with omega-3 fatty acid. FXO is a good source of α -linolenic acid (ALA, omega-3 FA) whereas an RPOO contains a higher amount of palmitic acid (SFA) and oleic acid (MUFA). But it is devoid of omega-3 fatty acids. To incorporate omega-3 FA into RPOO and to make up the S:M:P ratios 1:1:1, blends of RPOO with FXO was prepared in different ratios. The ideal combination was selected based on fatty acid composition. The mixture was subjected to enzymatic interesterification to improve the functional properties. Among the combinations, 40:60 ratios have shown ideal ratio fatty acid near to 1. The Triacyl Glycerol (TAG) molecular species revealed that the interesterification of oil alter the composition of major TAG molecular species even though the overall FA composition of blended and interesterified oils remains the same. In conclusion, the RPOO and FXO combination (40:60) is suitable to obtain the S: M: P ratio (1:1:1) in blends. This rearrangement of fatty acids enhance the functional properties and beneficial for cholesterol lowering properties. The influence of blending and interesterification with RPOO+FXO was studied on various lipid parameters in experimental animals. Rats fed blended oil and interesterified oil showed a decrease in cholesterol respectively as compared to RPOO. The serum and liver TAG level of rats fed mixed oil and interesterified oil were also significantly reduced as compared to natural oil of RPOO. Feeding of an omega-3 PUFA rich diet resulted in the accumulation of long-chain omega-3 PUFA and reduction in the long chain omega-6 PUFA. These studies clearly indicated that the incorporation of ALA and EPA+DHA into RPOO might beneficially hypo lipidemic and helpful for the heart, brain and retinal function.

Introduction

Omega-3 and omega-6 are two types of dietary polyunsaturated fatty acids (PUFA) and considered as essential fatty acids. These essential fatty acids cannot synthesise by mammals [1]. Hence it must be attained through the diet. An omega-6 fatty acid such as linoleic acid (LA, 18:2) which found in many vegetable oils such as groundnut oil, sunflower oil, soybean oil, corn oil and safflower oil [2]. Whereas the omega-3 fatty acid such as alpha-linolenic acid (ALA, 18:3) found in soybean, rapeseed and canola oil to the extent of 6 to 10% [1]. Omega-3 and omega-6 PUFA alters membrane fluidity and lipid composition of the membranes. Since different fatty acid has different physiological functions [3]. Omega-3 fatty acids have the ability to lower cholesterol in the blood. Omega-3 fatty acids have beneficial effects in controlling cardiovascular diseases, inflammation, immune disorders and cancer. Omega-3 fatty acids are helpful for normal functioning of brain and retina [4-6]. Most of the people in India are vegetarians. They would not eat fish to get preformed EPA and DHA. Alpha-linolenic acid (ALA) is the only source of omega-3 fatty acids for the vegetarian section of population. ALA can convert to EPA and DHA. ALA has many health benefits. ALA lowers blood cholesterol level, and it has an antithrombotic effect [7]. It acts as a nutraceutical and reduces stroke and stimulates neuronal protection and brain artery vasodilatation [8-11].

This higher ratio of omega-6/omega-3 fatty acid leads to atherosclerosis, oxidative stress, prone to inflammation and cause cancer. A lower ratio of omega-6 to omega-3 fatty acid is desirable for reducing risk factors for chronic diseases [1,12,13]. Hence, there is a need to prepare the proper balance of essential fatty acid which helps to lessen the development of cardiovascular disease and normal functioning of the brain. Therefore, alternative ways to increase consumption of omega-3 fatty acids from vegetarian sources have to be explored and assessed. The aim of the present study was to prepare a balanced ratio of fatty acids and incorporated omega-3 fatty acid through blending

and enzymatic interesterification of Refined Palm Olein Oil (RPOO) with Flaxseed Oil (FXO). RPOO is one of the major edible vegetable oils, but devoid of omega-3 fatty acids. Flaxseed Oil (FXO) is an excellent source of short chain omega-3 fatty acid. We evaluated whether there is a change in fatty acid composition and triglyceride molecular species of blended and interesterified oils.

Materials and Methods

Materials

Refined Palm Olein Oil (RPOO) was procured from a local supermarket. Cold pressed Flaxseed Oil (FXO) were obtained from Dhanayam organic, Chennai, India Immobilized 1, 3-specific lipase, Lipozyme® RM IM from Rhizomucor Miehei, from Novozymes, Bangalore, India. All the chemicals used in the experiment were procured from Sigma-Aldrich Co., St. Louis, MO, USA. All the solvents were obtained from Rank laboratories.

Preparation of blended and interesterified oil with RPOO and FXO

Blended oils were prepared by mixing of one oil of RPOO with another oil of FXO. A 200g mixture of two oils was placed in 500 ml beaker in duplicate for each blend and was mixed by using a mechanical stirrer at 180 rpm for 1hr in 40:60 ratios. The temperature was maintained at 40 °C during mixing. Interesterified oil helpful to move from one TAG molecule to another. Interesterified oils of RPOO with FXO were prepared using lipase enzyme (lipozyme IM RM) Rhizomucor Miehei at 5% level as described by [14]. The reaction was carried out in a shaking water bath (BS-31) at a speed of 160 rpm for 12 h at 37°C. After the interesterification reaction, the oil sample was decanted to separate enzyme; and washed with hexane and dried for reuse.

Animal experiments

In this study, we used Male Wistar weaning rats weighing 60±5g and were grouped (6 rats in each group) by random distribution and housed in cages under a 12 h light/dark cycle in a controlled temperature, in an approved animal house facility at K.M Pharmacy College, Madurai. The experimental protocol was approved by the institutional animal ethics committee (IAEC/288/2016/2014825101/TNAU/KMCP). The rats were fed AIN-93 diet [15] containing 10% RPOO+FXO blend, 10% RPOO+FXO interesterified and native oil 10%FXO for 60 days. Control rats received 10%RPOO with no omega-3. The AIN-93 diets (g/Kg diet) were prepared with the components of cornstarch 400g, casein 200g, alpha corn starch 132g, sucrose 100g, cellulose 50g, L-cystine 3g, choline chloride 2.5g, mineral mixture 40g, and vitamin mixture 10g. Diets used in this study were similar in all components except the fat sources. The rats had free access to food and water throughout the study. All efforts were made to minimize animal suffering. All protocols and procedures used were approved by the Committee of Institutional animal care and utilization. The rats were weighed at regular intervals. After 60 days of feeding, rats were fasted overnight and anaesthetized with ketamine (90mg/ml) and xylazine (10mg/ml). Blood was drawn by cardiac puncture into syringe pre-filled with ice cold heparin and plasma was separated by centrifugation at 1500g for 15min at 4°C. Liver, heart, and brain were harvested. Flash freeze all the samples and stored at -80°C.

Plasma and Liver lipid analysis

Plasma and Liver lipids were extracted according to [16]. The extracted chloroform layer was used for lipid analysis. Plasma lipid analysis such as LDL+VLDL cholesterol, HDL cholesterol, Triacylglycerols was analyzed by using Agappe diagnostic kits. Plasma clinical enzymes such as SGOT, SGPT, ALP, Creatine, Urea and Protein were analyzed by using Agappe diagnostic kits. Liver cholesterol was measured according to the method of [17] and triacylglycerols by the method of [18], using tripalmitin as the reference standard. Liver cholesterol levels were quantified using the method of Searcy and Bergquist (1960). Triglycerides (TG) were estimated by the method of Fletcher (1968), using tripalmitin as the reference standard (30–300ug). Phospholipids were estimated by a ferrous ammonium thiocyanate method [19], using dipalmitoyl phosphatidyl choline as the reference standard

Fatty acid composition

Fatty acid composition of dietary lipids of native, blended, interesterified oils, plasma lipids and liver lipids were analyzed by GC (Fisons GC fitted with a flame ionization detector) [20]. The oils were saponified with 0.5 M KOH and methylated with 40% BF₃ in methanol. The fatty acid methyl esters were separated using an Omega wax capillary column. The operating conditions were as follows: initial column temperature 120°C, raised by 15°C/min to 220°C, injection temperature 230°C, and detector temperature 240°C and Nitrogen used as the carrier gas as described by [2].

HMG-COA reductase activity

After sacrificing the rats, the liver was quickly removed and washed with saline. One gram of liver was homogenised in 3.5mL of ice cold buffer (pH 7.0) containing 3.1 mol/L of triethanol amine, 0.02 mmol of ethylenediamine tetra acetic acid and 2 mmol/L of dithiothreitol, pH 7.0. Liver microsomes were isolated according to the method by [21]. HMG-CoA reductase activity was measured by following the formation of CoA as described by [22].

Statistical analyses

Results are expressed as means±SD for each experimental group. The data were analyzed by one-way ANOVA followed by a post hoc Tukey test to compare blended and interesterified oil with native oil; p-values less than 0.05 were considered as statistically significant. All statistical analysis was performed using Graph pad prism and SPSS statistical software package version 17.0.

Results and Discussion

Fatty acid composition and nutraceuticals of dietary lipids of rats given blended or interesterified oil of RPOO with FXO

The dietary fatty acids of native, blended and interesterified oil are presented in Table 1. RPOO has a high amount of SFA about 50% but deficient in omega-3 PUFA. The major fatty acid present in RPOO was palmitic acid and oleic acid (Figure 1a). On the other hand, FXO has a higher amount of short chain omega-3 PUFA about 55% of alpha-linolenic acid, 14% linoleic acid, 17% oleic acid but deficient in SFA (Fig 1b). Blend of RPOO with FXO (40:60) has 26% palmitic

Table 1: Fatty acid composition of native blended and interesterified oils of refined palm olein oil with flaxseed oil.

Fatty acid	RPOO	FXO	RPOO+FXO (B) 60:40	RPOO+FXO (I) 60:40
C12:0	0.2±0.1 ^a	nd	0.1±0.03 ^a	0.1±0.03 ^a
C14:0	1±0.2 ^a	0.6±0.1 ^a	0.8±0.1 ^a	0.8±0.1 ^a
C16:0	41.1±2.6 ^c	7.7±0.9 ^a	27.7±2.0 ^b	26.5±2.5 ^b
C16:1	0.2±0.1 ^a	nd	0.1±0.02 ^a	0.1±0.03 ^a
C18:0	4.4±0.4 ^a	4.7±0.3 ^a	4.5±0.6 ^a	4.2±0.5 ^a
C18:1	42.3±2.8 ^c	17.2±2.5 ^a	32.3±3.1 ^b	31.6±2.6 ^b
C18:2	10.8±1.2 ^a	14.2±0.9 ^b	12.2±1.5 ^a	13.5±1.3 ^a
C 18:3	nd	55.6±3.2 ^b	22.2±2.1 ^a	23.2±2.2 ^a

Values are mean ± SD, n = 4; mean in a row with different superscript differ significantly at P<0.05, RPOO: Refined Palm olein oil, FXO: Flaxseed oil, B-blended, I-interesterified.

acid, 32%oleic acids, 12% linoleic acid and 23% alpha-linolenic acid (Figure 1c,1d & Figure 2). Among the different combination blends, RPOO+FXO blended oils (40:60) showed a balanced combination of SFA:MUFA:PUFA ratio near to 1:1:1 and 23% incorporation of omega-3 fatty acid (Table 2& Figure 3a,3b).There was no significant difference in the fatty acid composition of blended oil and interesterified oil (Figure 1c &d). RPOO showed a higher amount of total tocopherols of 425mg/Kg in which α-tocopherol was the major one (369mg/Kg oil), and FXO showed a higher amount of polyphenols and lignans. Total tocopherol content was found to be 351,378 and 370mg/kg respectively in FXO, blended oil and interesterified oil respectively. Blended and interesterified oil showed increased amount of tocopherols and phytosterols as compared to FXO native oil. Blended and interesterified oil showed incorporation of phenolics and lignans as compared to RPOO native oil which has devoid of phenolics and lignans.

Table 2: Distribution of saturated, monounsaturated, polyunsaturated fatty acid in native, blended and interesterified oils of RPOO with FXO.

Fatty acid	RPOO	FXO	RPOO+FXO (B)	RPOO+ FXO (I)
SFA%	46.7 ^c	13.0 ^a	33.2 ^b	31.6 ^b
MUFA%	42.5 ^c	17.2 ^a	32.4 ^b	31.7 ^b
PUFA%	10.8 ^a	69.8 ^c	34.4 ^b	36.7 ^b
S:M:P	1.4:1.3:0.3	0.4:0.5:2.1	1:01:01	1.0:0.9:1.1

Values are mean ± SD, n = 4; mean in a row with different superscript differ significantly at P<0.05, RPOO: Refined Palm olein oil, FXO: Flaxseed oil, B-blended, I-interesterified.

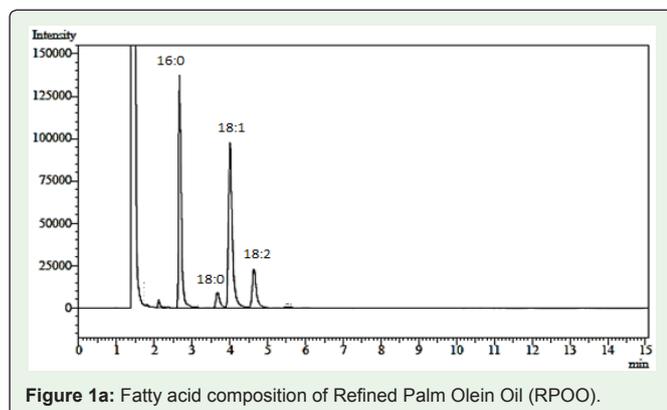


Figure 1a: Fatty acid composition of Refined Palm Olein Oil (RPOO).

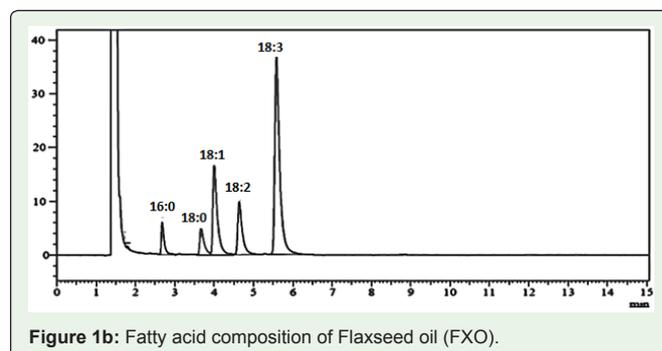


Figure 1b: Fatty acid composition of Flaxseed oil (FXO).

Effect of feeding dietary lipids on growth parameters, food intake, organ weights and plasma clinical enzymes of native, blended and interesterified oil of RPOO with FXO

Rats fed a diet containing native oil, blended oil and interesterified oil for 60 days. There was no significant change in Food intake, body weight gain and food efficiency ratio was observed in rats given different dietary lipids (Table 3). The percentage of fat absorption was 99% of the total lipids fed to rats and similar in all groups. This indicated that modified oils like blended and interesterified oil were absorbed well and on par with that of native oils. The weight of heart, brain, liver and kidney of rats given blended oils, interesterified oils were comparable to native oils (Table 4). There was no significant difference in clinical enzymes viz ALP, SGPT, SGOT, LDH and creatine kinase in blended oil and interesterified oil as compared to native oils (Table 5). There was no significant change in the total protein, creatinine and urea content of plasma of native, blended and interesterified oils (Table 5).

Table 3: Effect of feeding dietary lipids on growth parameters and food intake of native, blended and interesterified oil of RPOO with FXO.

	RPOO	FXO	RPOO+FXO(B)	RPOO+FXO(I)
Food Intake (g/day)	12.3±1.2 ^a	12.4±1.0 ^a	12.6±0.9 ^a	13.2±0.9 ^a
Body weight gain (g)	249±20 ^a	254±15 ^a	245±19 ^a	256±22 ^a
Food Efficiency ratio	0.35±0.03 ^a	0.33±0.04 ^a	0.34±0.02 ^a	0.35±0.03 ^a
Fat absorption (%)	99.8±0.2 ^a	99.5±0.04 ^a	99.6±0.02 ^a	99.4±0.04 ^a

Values are mean ± SD, n = 6rats/group; Mean in a row with different superscripts differ significantly at P<0.05. nd: not detected, RPOO: Refined Palm olein oil, FXO: Flaxseedoil, B: Blended, I: Interesterified.

Table 4: Effect of feeding dietary lipids on organ weights of native, blended and interesterified oil of RPOO with FXO.

Organ weight	RPOO	FXO	RPOO+FXO(B)	RPOO+FXO(I)
Liver(g/100g body wt)	3.48±0.2 ^a	3.53±0.3 ^a	3.28±0.2 ^a	3.38±0.3 ^a
Kidney(g/100g body wt)	0.78±0.1 ^a	0.81±0.2 ^a	0.73±0.1 ^a	0.66±0.1 ^a
Heart(g/100g body wt)	0.32±0.07 ^a	0.28±0.05 ^a	0.31±0.03 ^a	0.29±0.06 ^a
Brain(g/100g body wt)	0.65±0.1 ^a	0.59±0.06 ^a	0.68±0.03 ^a	0.56±0.04 ^a

Values are mean ± SD, n = 6rats/group; Mean in a row with different superscripts differ significantly at P<0.05. nd: not detected, RPOO: Refined Palm olein oil, FXO: Flaxseed oil, B:Blended, I: Interesterified.

Plasma lipid profile of rats given native, blended and interesterified oil of RPOO with FXO

Table 5: Effect of feeding dietary lipids on plasma clinical enzymes of native, blended and interesterfied oil of RPOO with FXO.

Plasma Clinical enzymes	RPOO	FXO	RPOO+FXO(B)	RPOO+FXO(I)
ALP (U/L)	289±58 ^a	308±49 ^a	275±56 ^a	316±62 ^a
SGPT(U/L)	44.5±5.3 ^a	48.2±4.1 ^a	46.9±3.8 ^a	47.3±5.6 ^a
SGOT(U/L)	200±32 ^a	187±28 ^a	192±33 ^a	185±25 ^a
CRT-Kinase(U/L)	2525±121 ^a	2628±201 ^a	2563±156 ^a	2612±187 ^a
LDH(U/L)	1349±57 ^a	1154±69 ^a	1170±61 ^a	1289±50 ^a
Creatine (mg/dl)	0.82±0.1 ^a	0.88±0.2 ^a	0.81±0.1 ^a	0.85±0.1 ^a
Urea(mg/dl)	21.5±3.6 ^a	20.8±2.9 ^a	22.6±3.2 ^a	23.9±3.0 ^a
Protein(mg/dl)	13.3±1.6 ^a	12.9±0.9 ^a	11.8±1.0 ^a	12.3±0.8 ^a

Values are mean ± SD, n = 6rats/group; Mean in a row with different superscripts differ significantly at P<0.05. nd: not detected, RPOO: Refined Palm olein oil, FXO: Flaxseed oil, B:Blended, I:Interesterfied.

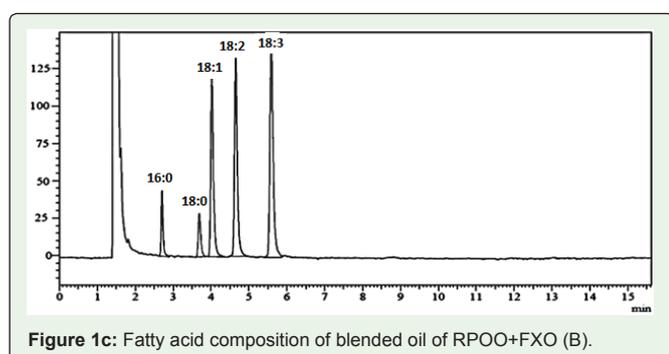


Figure 1c: Fatty acid composition of blended oil of RPOO+FXO (B).

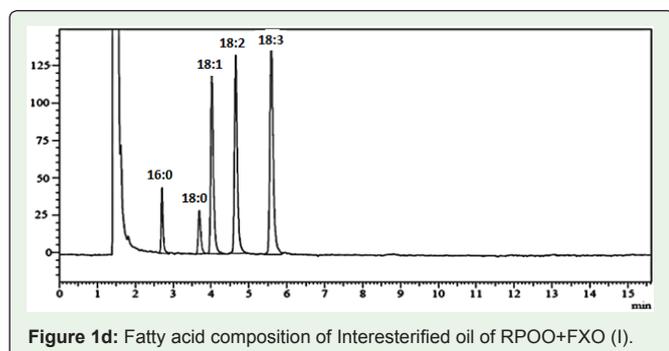


Figure 1d: Fatty acid composition of Interesterfied oil of RPOO+FXO (I).

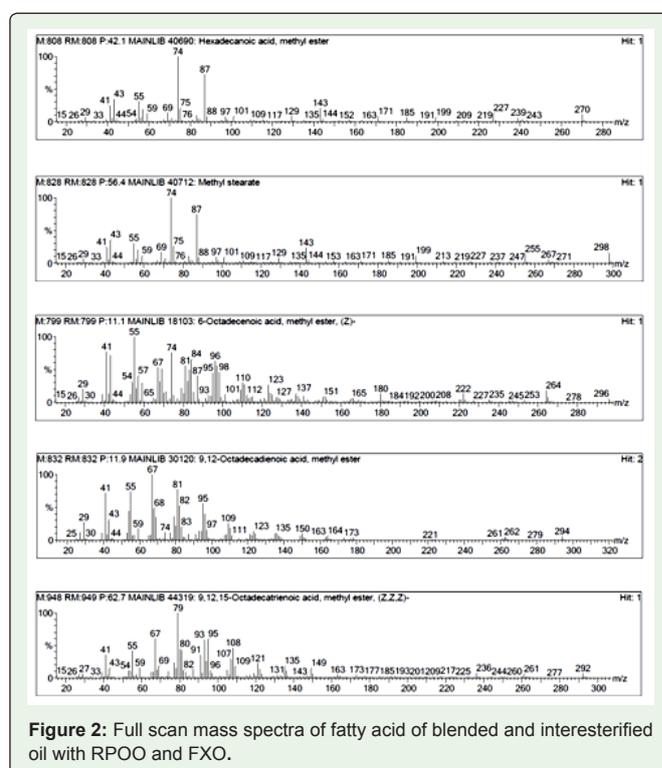


Figure 2: Full scan mass spectra of fatty acid of blended and interesterfied oil with RPOO and FXO.

Rats given flaxseed oil had total cholesterol of 70.1mg/dL in plasma which was 27.7% lower than that observed in the rats fed with RPOO. Total cholesterol levels in rats given blended oil and interesterfied oil showed a reduction of 22 and 37% as compared to rats given RPOO (Figure 4). In comparison with rats given blended oil, the total cholesterol level in rats given interesterfied oil showed a further reduction of 10% respectively. The HDL cholesterol content of rats given FXO was reduced marginally but significantly different from rats given RPOO. But the HDL cholesterol content was similar in rats given blended oils and a marginal increase in interesterfied oil as compared to rats given RPOO. In the case of rats given FXO, the LDL-C level was reduced by 36.9% as compared with rats given RPOO. LDL-C concentration of rats given blended and interesterfied of RPOO+FXO showed a 35.3% and 67.2% reduction respectively as compared to rats given RPOO. The phosphor lipid content of rats given FXO showed a reduction in 11.6% as compared to that given RPOO. Rats given blended and interesterfied oil showed a reduction

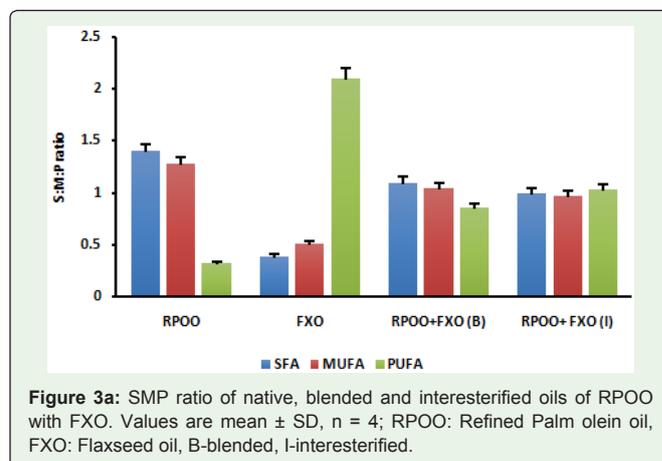
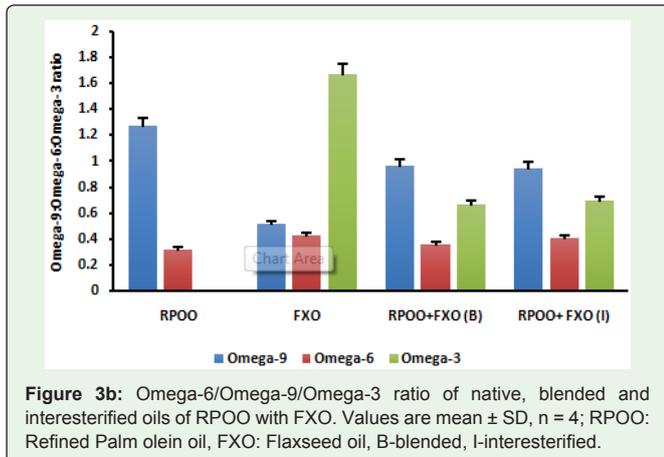


Figure 3a: SMP ratio of native, blended and interesterfied oils of RPOO with FXO. Values are mean ± SD, n = 4; RPOO: Refined Palm olein oil, FXO: Flaxseed oil, B-blended, I-interesterfied.

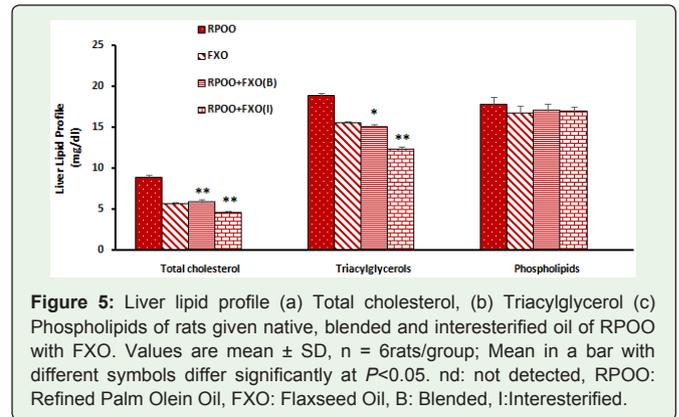
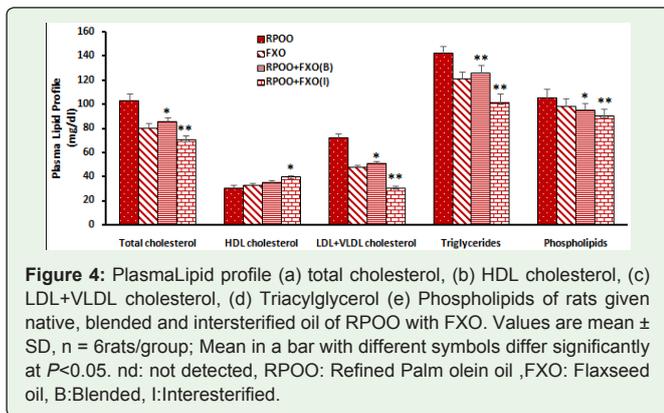


in the phosphor lipid content by 14.5% and 19.2% respectively as compared to that given RPOO. However, the phosphor lipid concentrations remain unaltered in blended and interesterified oil (Figure 4).

The TAG concentration in the plasma of rats given FXO was lowered by 14.7%, while those given with blended oil showed a reduction of 11.3% as compared to rats given RPOO. The TAG concentration in the plasma of rats given interesterified oil was decreased by 28.9% in comparison with RPOO. The plasma TAG content of rats given interesterified oil RPOO+FXO (I) was decreased by 19.8% in comparison with rats given blended oil RPOO+FXO (B). Hence, interesterified oil containing omega-6 and omega-3 PUFA showed a significantly higher hypolipidemic effect as compared to rats given blended oils and RPOO (Figure 4).

Liver lipid profile of native, blended and interesterified oil of RPOO with FXO

Liver plays an important role in lipid metabolism. Total cholesterol level in liver of rats given FXO was 5.6mg/ g tissue while those rats given RPOO had liver cholesterol concentration of 6.9mg/g tissue. Thus an 18.8% decrease in liver cholesterol was observed in rats given FXO as compared to those RPOO (Figure 5). The liver cholesterol concentration of rats given blended oil and interesterified oil were 5.8 and 4.5mg/g tissue respectively, which was lower by 15.9% and 34.7% lower than that observed in rats given RPOO. The rats given interesterified oil showed a reduction in total cholesterol



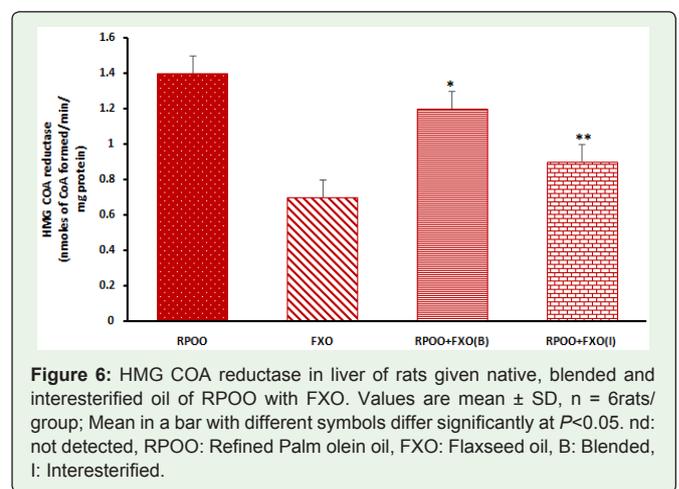
level by 22.4% as compared to rats given blended oil. The liver TAG concentration was also altered by the type of dietary lipid given to the rats. There was a significant decrease in liver TAG concentration by 17.5% and 19.6% in rats given FXO and blended oil respectively as compared to that given RPOO. Rats given interesterified oil showed a significant reduction in TAG concentration by 18.5 and 34.5% as compared to blended oil and RPOO. There was no significant difference in hepatic phospholipids of rats given dietary lipids in all the groups (Figure 5).

Effect of dietary lipids on HMG-CoA reductase activity in liver microsomes of rats given native, blended and interesterified oil of RPOO with FXO

Liver plays a vital role in cholesterol homoeostasis in the body and its biosynthetic pathway regulated by HMG-CoA reductase. Rats given blended oil and interesterified oil has shown marginally lowered the activity of HMG-CoA reductase activity by 14.2 and 35.7% respectively in liver microsomes (Figure 6). Moreover, rats fed FXO; the HMG-CoA reductase activity was decreased significantly by 50% as compared to rats given RPOO.

Fatty acid composition of plasma lipids of rats given native, blended and interesterified oil of RPOO with FXO

Plasma fatty acid composition showed a significant incorporation of omega-3 fatty acids in rats given FXO, blended oil and



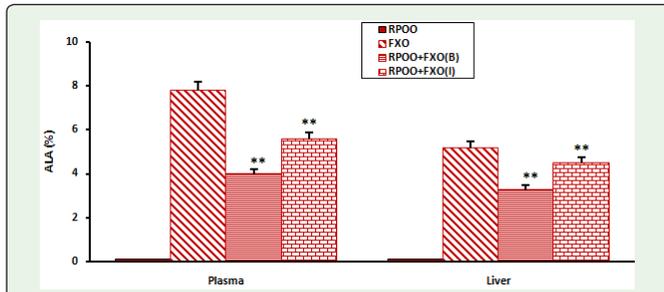


Figure 7a: Alpha linolenic acid content in plasma and liver of rats given native, blended and interesterified oil of RPOO with FXO. Values are mean ± SD, n = 6rats/group; Mean in a bar with different symbols differ significantly at P<0.05. nd: not detected, RPOO: Refined Palm olein oil, FXO: Flaxseed oil, B: Blended, I:Interesterified.

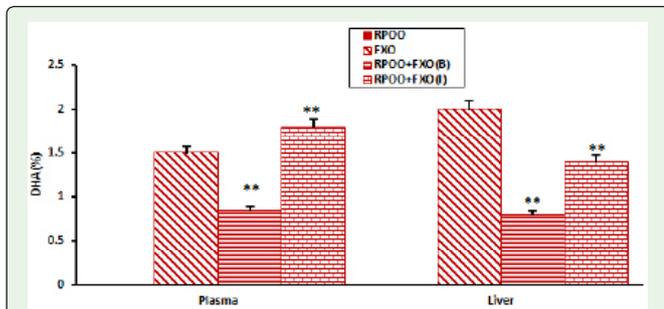


Figure 7b: DHA Content in plasma and liver lipids of rats given native, blended and interesterified oil of RPOO with FXO. Values are mean ± SD, n = 6rats/group; Mean in a bar with different symbols differ significantly at P<0.05. nd: not detected, RPOO: Refined Palm olein oil, FXO: Flaxseed oil, B: Blended, I:Interesterified.

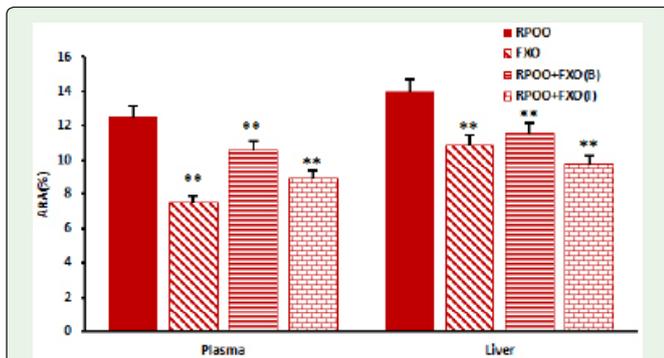


Figure 7c: Arachidonic acid Content in plasma and liver lipids of rats given native, blended and interesterified oil of RPOO with FXO. Values are mean ± SD, n = 6rats/group; Mean in a bar with different symbols differ significantly at P<0.05. nd: not detected, RPOO: Refined Palm olein oil, FXO: Flaxseed oil, B: Blended, I:Interesterified.

interesterified oil. Rats given FXO, blended and interesterified oil observed ALA to the extent of 7.8%, 4% and 5.6% of the total fatty acids (Figure 7a,b,c). The EPA+DHA content in plasma of rats given blended and interesterified oil are 3% and 5.2%, indicating that ALA from modified oils was converted to EPA and DHA. Rats given FXO alone also showed a conversion of EPA and DHA to the extent of 7.2% of total fatty acids. Results also observed that rats given FXO, blended and interesterified oils showed a significant decrease in the arachidonic acid (AA) levels by 28.8%, 20% and 32.6% as compared

to rats given RPOO. Rats given a diet containing RPOO had no omega-3 fatty acids in plasma lipids (Figure 7a- c).

Fatty acid composition of Liver lipids of rats given native, blended and interesterified oil of RPOO with FXO

Fatty acid analysis of liver lipids reflected on the type of dietary lipids fed to rats. The fatty acid composition of liver lipids of rats given different oils is given in (Figure 7a- c). Rats given FXO, blended and interesterified oil contained ALA to the extent of 5.2%, 3.3% and 4.5% of total fatty acid respectively. The EPA levels of rats given FXO, blended and interesterified oil was found to be 3%, 2.1% and 2.7% respectively. The DHA content in rats given FXO, blended and interesterified oil to the extent of 2.2%, 1.1% and 1.7% respectively (Figure 7a- c). There was a little conversion of ALA to EPA and DHA in blended and interesterified oil. Rats given RPOO had no omega-3 fatty acids in liver lipids.

Discussion

The primary findings of this study showed that dietary feeding of RPOO and FXO blended oil and interesterified oil significantly decreased Total cholesterol, TAG, LDL-C in serum and liver as compared to rats given RPOO. FXO, when fed as the sole source of fat in the diet showed a higher hypolipidemic effect compared to FXO blended oil and RPOO. Dietary intervention is one of the approaches for reducing the risk for cardiovascular disease. Many studies have suggested that omega-3 fatty acids can play a role in ameliorating cardiovascular disorders such as thrombosis. Diets rich in omega-3 PUFA (ALA, EPA and DHA) reduce platelet aggregation, plasma triglyceride levels and vascular contractility and alter tissue lipid composition. ALA functions as the precursor of DHA. ALA-rich diets when consumed increase the levels of ALA, EPA, DHA and total omega-3 fatty acid content of cell membrane phospholipids which ultimately increase the fluidity of membranes and alter their properties [9,23].

The direct source of DHA is available so far on marine foods like fish oils from sardine, salmon, mackerel, anchovy, halibut, sword fish, cod, rainbow trout and shark. DHA can be owed from other sources such as krill oil, seal and squid. Most of the people are vegetarians. Hence there is a need to identify the indirect sources. The indirect source of DHA is vegetables and plant seed oils. Plant seed such as flaxseed, canola, soybean, mustard contains short chain omega-3 fatty acid as Alpha-Linolenic Acid (ALA) [24,25]. Even though, the body has the ability to synthesize ALA to DHA. But the conversion of ALA to DHA is less in humans [26]. The metabolic engineering of plant seed oils contains alpha linolenic acid as short chain omega-3 fatty acid, and it converted to DHA. Various attempts have been approached to enhance the conversion of ALA to DHA by metabolic engineering of plant seed oils, algal biomass, structured lipid and dietary approaches with modified foods and through nanotechnology [27]. The biotechnological approaches are helpful to produce transgenic plants by using a new approach of gene tinkering and lipidomic approach providing better oil synthesis for accumulate ALA with DHA. Those plants are *Linum usitatissimum*, *Araphidosis ThalianaT2*, *Glycine Max* and *Camelina Sativa*. Algal biomass includes *Isochoric T-iso*, *Pavlova lutheri*, *Thalassiosira pseudonanfrom*, *Nano chloropsis osculate*, *gaditana*, *Rhodomonas Salina*, *Tetraselmis Suecica* and *Phaeodactylum tricornutum*. Eggs

from poultry fed with a feed containing linseed or fish meal or micro algae [24]. Nano-encapsulated ALA or DHA-rich oils enhance the DHA level to the extent of two-fold increased the bioavailability [28-30]. All these delivery systems are attempted for increasing the bioavailability.

Amongst the edible oils, flaxseed oil is very high in ALA [25]. If this might be made available and also find a method to upsurge the effective conversion of ALA to DHA, then vegetarian sections of the population can readily accept it as a part of a healthy diet which can help them to meet their DHA requirement. Dietary feeding of FXO, blended oil and interesterified oil significantly increased ALA, EPA and DHA content in plasma and liver lipids. The ration of omega-6 to omega-3 is importance for conversion of ALA to EPA and DHA through delta-6 desaturase acts on both n-3 and n-6 PUFAs. The conversion of ALA to EPA and DHA is affected by the relative amounts of LA and ALA in the diet. Hence, omega-6 to omega-3 ratio is important for metabolism of ALA to EPA and DHA. The n-6 to n-3 PUFA was maintained at 2.3-2.6 in blended oils and interesterified oils, and the conversion of ALA to EPA and DHA was observed in blended oil and interesterified oil-fed rats. This study clearly demonstrates that the conversion of ALA to long chain omega-3 PUFA taken place at 30% concentrations of ALA blended oil and interesterified oil-fed rats. Moreover, the LA content in blended and interesterified oil did not influence or affect $\Delta 6$ desaturase activities in the conversion of ALA to DHA. There was no significant difference in plasma and liver LA content in FXO, blended and interesterified oil. But, there was a significant reduction in AA levels was observed in rats given blended oil and interesterified oils as compared to RPOO native oils.

Conclusion

In summary, the RPOO and FXO combination (40:60) is suitable to obtain the omega-6/omega-3 ratio (1:1) in blends. The results indicated that blending and interesterification of oils resulted in changes in the TAG molecular species of oils even though overall fatty acid composition remains the same. This predisposition of fatty acids enhanced the functional properties and beneficial for cholesterol lowering properties. The interesterified oil and blended oil had no adverse effects on growth of the animals and efficiently absorbed. ALA rich FXO and blended oil significantly lowered the plasma lipids and liver lipids as compared to rats given RPOO. However, interesterified oil exhibited better hypo cholesterolemic and hypotriglyceridemic effects in rats as compared to RPOO and blended oil.

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References

1. Simopoulos AP. Evolutionary Aspects of the Dietary Omega-6/Omega-3 Fatty Acid Ratio: Medical Implications: In *Evolutionary Thinking in Medicine*. 2016; 119-134.
2. Sugasini D and Lokesh BR. Uptake of α -linolenic acid and its conversion to long chain omega-3 fatty acids in rats fed microemulsions of linseed oil: *Lipids*. 2012; 47: 1155-1167.
3. Hashimoto M, Hossain S, Al Mamun A, Matsuzaki K, and Arai H. Docosahexaenoic acid: one molecule diverse functions: *Critical Reviews in Biotechnology*. 2017; 37: 579-597.
4. Swanson D, Block R, and Mousa SA. Omega-3 fatty acids EPA and DHA: health benefits throughout life. *Advances in Nutrition: An International Review Journal*. 2012; 3: 1-7.
5. Calder PC. Very long chain omega-3 (n-3) fatty acids and human health: *European Journal of Lipid Science and Technology*. 2014; 116: 1280-1300.
6. Salem Jr N and Eggersdorfer M. Is the world supply of omega-3 fatty acids adequate for optimal human nutrition?: *Current Opinion in Clinical Nutrition and Metabolic Care*. 2016; 18: 147-154.
7. Lenihan-Geels G and Bishop KS. Alternative Origins for Omega-3 Fatty Acids in the Diet. In *Omega-3 Fatty Acids*: Springer International Publishing. 2016; 475-486.
8. Dhavamani S, Rao YPC and Lokesh BR. Total antioxidant activity of selected vegetable oils and their influence on total antioxidant values in vivo: A photochemiluminescence based analysis. *Food chemistry*. 2014; 164: 551-555.
9. Blackwood DP, Rodriguez-Leyva DP, and Pierce, GN. The potential for using alpha-linolenic acid as a therapy for cardiovascular disease: *Curr. Res. Cardiol*. 2014
10. Sugasini D and Lokesh BR. Enhanced incorporation of docosahexaenoic acid in serum, heart, and brain of rats given microemulsions of fish oil: *Molecular and cellular biochemistry*. 2013; 382: 203-216.
11. Stark AH, Reifen R and Crawford M A. Past and Present Insights on Alpha-linolenic Acid and the Omega-3 Fatty Acid Family: Critical reviews in food science and nutrition. 2016; 56: 2261-2267.
12. Kris-Etherton PM, Harris WS, and Appel LJ. Omega-3 fatty acids and cardiovascular disease: new recommendations from the American Heart Association: *Arterioscler Thromb Vasc Biol*. 2003; 23: 151-152.
13. Dyal SC. Long-chain omega-3 fatty acids and the brain: a review of the independent and shared effects of EPA, DPA and DHA: *Frontiers in aging neuroscience*. 2015; 7: 52.
14. Sharma M and Lokesh BR. Modification of serum and tissue lipids in rats fed with blended and interesterified oils containing groundnut oil with linseed oil: *Journal of Food Biochemistry*. 2012; 37: 220-230.
15. Reeves PG. Components of the AIN-93 diets as improvements in the AIN-76A diet: *The Journal of nutrition*. 1997; 127: 838-841.
16. Bligh EG and Dyer WJ. A rapid method of total lipid extraction and purification: *Canadian journal of biochemistry and physiology*. 1959; 37: 911-917.
17. Searcy RL and Bergquist LM. A new color reaction for the quantitation of serum cholesterol: *Clinica chimica acta*. 1960; 5: 192-199.
18. Fletcher MJ. A colorimetric method for estimating serum triglycerides: *Clinica Chimica Acta*. 1968; 22: 393-397.
19. Stewart JCM. Colorimetric determination of phospholipids with ammonium ferrioxalate: *Analytical biochemistry*. 1980; 104: 10-14.
20. Morrison WR and Smith LM. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol: *Journal of lipid research*. 1964; 5: 600-608.
21. Shapiro DJ and Rodwell VW. Regulation of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase and cholesterol synthesis: *Journal of Biological Chemistry*. 1971; 246: 3210-3216.
22. Hulcher FH and Oleson WH. Simplified spectrophotometric assay for microsomal 3-hydroxy-3-methylglutaryl CoA reductase by measurement of coenzyme A: *Journal of Lipid Research*. 1973; 14: 625-631.

23. Rodriguez-Leyva D, Bassett CM, McCullough R and Pierce GN. The cardiovascular effects of flaxseed and its omega-3 fatty acid, alpha-linolenic acid: Canadian Journal of Cardiology. 2010; 26: 489-496.
24. Lane K, Derbyshire E, Li W and Brennan C. Bioavailability and potential uses of vegetarian sources of omega-3 fatty acids: a review of the literature: Critical reviews in food science and nutrition. 2014; 54: 572-579.
25. Goyal A, Sharma V, Upadhyay N, Gill S and Sihag M. Flax and flaxseed oil: an ancient medicine and modern functional food: Journal of food science and technology. 2014; 51: 1633-1653.
26. Barceló-Coblijn G and Murphy EJ. Alpha-linolenic acid and its conversion to longer chain n-3 fatty acids: Benefits for human health and a role in maintaining tissue n-3 fatty acid levels : Progress in lipid research. 2009; 48: 355-374.
27. Kanjilal S, Kaki SS, Rao BV, Sugasini D, Rao YP, Prasad RB, et al. Hypocholesterolemic effects of low calorie structured lipids on rats and rabbits fed on normal and atherogenic diet: Food Chemistry. 2013; 136: 259-265.
28. Sugasini D, VC Devaraj, Mullangi Ramesh and BR Lokesh. "Lymphatic transport of α -linolenic acid and its conversion to long chain n-3 fatty acids in rats fed microemulsions of linseed oil." Lipids. 2014; 49: 225-233.
29. Sugasini D and Lokesh BR. Rats given linseed oil in microemulsion forms enriches the brain synaptic membrane with docosahexaenoic acid and enhances the neurotransmitter levels in the brain: Nutritional neuroscience. 2015; 18: 87-96.
30. Sugasini D and Lokesh BR. Curcumin and linseed oil co-delivered in phospholipid nanoemulsions enhance the levels of docosahexaenoic acid in serum and tissue lipids of rats: Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA). 2017; 119: 45-52.