BIOCHEMICAL EFFECT OF PALM OIL FRACTIONS ON RATS

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Abstract

This study aimed to discuss the effect of six different sources of fats (100% corn, 100% butter and 100% margarine) and three blends from margarine and butter with different percentages on plasma lipid profile, lipid risk ratios and rats body weight when add in rat diet through a six weeks experimental period. The free fatty acid (FFA)%, peroxide value (PV) and the fatty acid composition of the six types of fats were determined, 36 male Wistar strain rats were categorized to six groups, 6 rats of each. The food intake and body weight of rats were recorded once a week. At the end of the experiment, total food intake, body weight gain and food efficiency ratio were calculated. Total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglyceride (TG) were determined in plasma. It found that diet with the 100% margarine showed the highest FFA% and PV. Rat group fed on the 100% margarine diet showed the highest levels of TC, TG, LDL-C and the risk ratio (TC/HDL-C). The least HDL-C and risk ratio LDL-C/HDL-C values were also noticed in 100% margarine fed group, our article concluded that as the margarine percentage in diet increase this was accompanied by elevation in plasma lipid profile and body weight that is well known to have series of deleterious effects on body health.

Key words: Palm oil, Margarine, Butter.

Introduction

Hyperlipidemia is one of the most fatal disorder leading to sever diseases and accounts for about one third of total deaths around the world especially atherosclerosis of the most valuable arterial network of the main vital organs (Ayata et al., 2013, Jorgensen et al., 2013). Not all blood lipids are included in risk factors but blood cholesterol is the main milestone of high stroke incidence (Menet et al., 2018). High intake of SFAs has been related to hypercholesteremic condition, fatal cardiovascular diseases (CVDs) (Page et al., 1957). The later are fatal diseases of the heart and blood streams that include coronary heart disease (CHD) and cerebrovascular disease. CVDs responsible for 31% of global deaths (Holub, 2002). Chen et al., (2011) reported that in developing countries, the rate of CVDs mortality increase with the increase of Palm oil (PO) consumption. Palm oil is a vegetable oil obtained from the palm tree’s fruit (Elaeisguineensis). PO is considered to be the second biggest vegetable oil produced and traded all over the world. It is composed of 50% palmitic acid (PA) (16:0), 40% of hypocholesterolaemic monounsaturated oleic acid (18:1, omega-9), and 10% linoleic (18:2, omega-6) polyunsaturated fatty acid (Ayorinde et al., 2000), in addition to negligible values of the hypercholesterolemic saturated fatty acids (SFAs) lauric (12:0) and myristic (14:0) acids. The ratio of SFA to unsaturated fatty acid (UFA) of PO is thus near unity. PO contains minor amount of vitamin E, which are powerful antioxidants, represent a natural inhibitors for cholesterol synthesis and also permits a longer shelf-life for palm-based food products. Abundance of indigenous source of carotenoids, tocopherols and tocotrienols in PO provided it with high oxidative stability (Chong and Ng, 1991; Muller et al., 2010). The previously mentioned phytonutrients are associated with several health promoting properties including the anti-cancer, anti-angiogenesis, cardio-protection, cholesterol inhibition, neuro-protective properties, antioxidative defence mechanisms, and anti-diabetes (Loganathan et al., 2010). PO is ordinarily used in shortening, frying fats and margarines (Imoisi et al., 2015). Although many previous studies reported that PO-based diets induce a state of hypercholesteremia than do...
other vegetable oils like corn, soybean, and sunflower oils, PO consumption causes reduction in endogenous cholesterol level. Researchers render this phenomenon to its tococtrienols content and its peculiar FAs isomeric position.

The stereospecific distribution of SFA in the triacylglycerol (TAG) molecule of PO, decrease their metabolic effects and absorption rate. International guidelines, stated that SFAs intake should not exceed 10% of total energy, within a balanced diet; to avoid harmful effects that may occur on human health (mainly CVDs or cancer risk) (Marangoni et al., 2017).

The processed food industry is highly dependent on palm production that makes the PO industry critical. United States Food and Drug Administration’s ban on trans-fatty acids (TFAs) because of their potential adverse health impacts. Elevated consumption of PO as a potential replacement for TFA in ultra-processed foods could be anticipated. As trans-fats are considered unhealthy fractions, this directed all worldwide for PO as the healthy natural subrogation (Kadandale et al., 2019).

TFAs are certain class of dietary fatty acids that are produced by biohydrogenation process to fat of ruminant animals or by commercial hydrogenation of vegetable oils (Emken, 1984). Relative to UFAs, TFAs/hydrogenated fats consumption results in increase the risk ratio that represented by (higher LDL-C in relative to lower HDL-C). (Lichtenstein et al., 1999).

Hu et al., (2001) stated that the replacement of SFA and TFA with mono and polynomials saturated fats is beneficial and highly effective in preventing CHD than reducing the net fat consumption. TFA from fried foods elevated TG and the (LDL/HDL) cholesterol ratio more than did margarine/hydrogenated vegetable oil products (Sartika, 2011).

The relation of income and food source directed many scientist to discover margarine in 1869 to be cheap source substituted butter but it is not expensive as butter (Chrysam, 1996). Margarine is a water-in-oil emulsion. The aqueous phase composed of water, salt and some preservatives while the fatty phase, is a blend of oils and fats, emulsifiers and antioxidants. The solid fat content of margarine is a result of hydrogennation process to liquid oils. Hydrogennation results in the formation of TFAs where some cis double bonds are rearranged to Trans bonds (Fomuso and Akoh, 2001).

The main object of our study was to evaluate the extent of PO hazardous effect when formulated as margarine using different percentages of butter/margarine blends. This was followed by evaluation of plasma lipid profile and nutritional parameters in rats.

### Materials and Methods

#### Animals:

36 Male Wister albino rats weighing 50-70g. The rats were kept in stainless steel cages under standard conditions. Food and water were given ad-libitum. The animal experiment was carried out adopting the Ethics Committee of Cairo University, Giza, Egypt, on ethical approval Number: CU II F 30 19.

#### Diet:

The basal diet was formulated according to AOAC (2005). The composition of mineral and vitamin mixtures were also according to AOAC (2005).

#### Diet Preparation:

Refined, bleached and deodorized corn oil, Commercial Unsalted Creamery Butter product of New Zealand, it consists of (82.9% milk fat, 15.7% moisture, 1.4% solids Non-fat) and commercial margarine that is composed of (50% hydrogenated PO, 50% PO) was obtained from IFFCO Egypt Company for Edible Oils and Fats: Bldg. 28, Road 261, New Maadi, and Cairo, Egypt.

#### Chemical reagents:

Total cholesterol (TC), High density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglyceride (TG) were obtained from (BIODIAGNOSTICS®): 29 Tahreer St., Dokki, and Giza, Egypt.

#### Methods

Free Fatty acid and Peroxide Value:

Free Fatty acid and Peroxide value of oil and fat are the determined according to AOCS official methods (2009).

Fatty acid composition:

The fatty acid composition was determined by the conversion of oil to fatty acid methyl esters prepared by adding 1.0mL of n-hexane to 15 mg of oil followed by 1.0mL of sodium Wister albinomethoxide (0.4 mol), according to the modified method of (Zahran and Tawfeuk, 2019).

Experimental design:

36 male Wister albino rats were categorized to six groups, 6 rats each. Rats were fed on same basal diets varying only in its type of fat and classified as follows: Group I: Normal control received basal diet plus fat (100%
The FFAs percent of all six fat samples namely corn oil, butter, margarine and the three fat blends of butter/margarine were ranged between 0.12 and 0.25% (Table 1). The result showed that the corn oil possess the lowest FFAs content whereas the margarine possess the highest. It is clearly shown that there is a direct relation between FFAs content and the margarine % in fat blends that is to say, as the margarine % increase, the FFAs % also increase and vice versa. FFAs were reported to catalyze the oxidative dissolution of oils by enzymatic and or chemical hydrolysis to form off volatile components (Aniɔ'owska et al., 2016). Moreover, FFA value is an indication of lipase efficacy (Elabd et al., 2017). Dunford (2012) reported that high-quality PO possess high % of the neutral (TAGs, or TGs) and decreased FFAs % and this results coincide with the current study. The inevitable sequels of elevated levels of FFAs are the greater risks of cardiovascular events (Jakobsen et al., 2009).

Table 1 also presents the PV of the previously mentioned fat samples. The PV was ranged between 0.19 mEq./kg and 1.60 mEq./kg for butter and corn oil respectively. The PV is considered a strong indicator of peroxidation, and thus high PV of the oil is a sign of low oil resistance to wards oxidation process during storage, and a detector to deterioration level (Zahran and Tawfeuk, 2019). This wide variation in PV may render to the high UFAs percent content for corn oil compared to butter. Similarly margarine showed high PV 1.19 mEq./kg and thus may share corn oil in its low storage stability.

Fatty acid composition:
Table 2 showed FA composition of the fat samples under test. Corn oil possesses the highest UFA (67.55%) while butter showed the least (26.82). This is obviously shown in linoleic acid which represents the most abundant UFA in corn oil (35.61%), while butter has the least linoleic acid percentage (1.48%). Corn oil also has high percent of oleic acid (31.94%). Dermaux et al., (1999) reported that the most important FAs naturally occurring in the TGs of vegetable oils and margarine are PA, stearic acid (18:0), oleic acid, linoleic acid and linolenic acid (18:3) and such results coincides with our results seen in Table 2.

Six important fatty acids were shown in margarine sample and its blends, which are C14:0, C16:0 and C18:0 as SFAs, and C18:1, C18:2, and C18:3 as UFAs. PA was present in margarine as the highest SFA percent (45.32%), however, the short chain FAs C4:0 to C14:1 were absent in both corn oil and margarine, except myristic acid (C14:0) was reported in margarine in minor amount (1.03%). In addition, the highest SFA level (73.19%) was present in margarine as the highest SFA percent. The PV was ranged between 0.19 mEq./kg and 1.60 mEq./kg for butter and corn oil respectively. The PV is considered a strong indicator of peroxidation, and thus high PV of the oil is a sign of low oil resistance to wards oxidation process during storage, and a detector to deterioration level (Zahran and Tawfeuk, 2019). This wide variation in PV may render to the high UFAs percent content for corn oil compared to butter. Similarly margarine showed high PV 1.19 mEq./kg and thus may share corn oil in its low storage stability.
values increased by increasing the percentage of butter to be 1.73 (mixture II) in case of butter mixed with margarine at a ratio of 50:50, and to be 2.31 (mixture I) on mixing butter with margarine at a ratio of 75:25, respectively. It’s obvious that the fatty acid profile change by changing the margarine percentage in three blends under investigation.

The short chain fatty acids: butyric, caproic, caprylic, capric, and lauric acids decreased upon increasing the % of margarine to butter in the three butter/margarine blends. These results are in agreement with those reported by El-Hadad and Tikhomirova (2018). Fats and oils are made up of a mixture of TGs, which consists of a glycerol backbone to which three FAs are esterified. The positions of fatty acids attached to the glycerol backbone are referred by stereospecific numbering Sn-1, -2 and -3 Fig. 1. The stereospecific position of fatty acids in TG has a great impact on the properties of oils and fats.

Renaud et al., (1995) investigated that both native PO and interesterified PO has similar FA composition but different saturation levels at the Sn-1, -2 and -3 positions. In vivo studies confirmed that FAs at the Sn-2 position in dietary fats significantly influenced biological effects such as lipemia and platelet aggregation rather than the total FA composition of the dietary fats. The positional distribution of FAs in the TG molecule does give thus an influence of the properties of fats and oils, and should not be classified based on the overall fatty acid composition (Teh et al., 2018).

Effects of feed ingrates different fat types for 6 weeks on body weight, food intake and food efficiency ratio:

The results shown in Table 3 represent that increasing the percent of margarine in diet is accompanied by significant elevation in body weight much more clearly seen in 100% margarine diet compared to rat fed diet containing 100 % butter. Such elevation in body weight may be attributed to its dietary SFA content mainly PA. Fig. 2 demonstrated that the rat body weight increase with time with non-significant difference among the experimental groups.

According to (Padley et al., 1994) the stereospecific position of FAs in the TG molecule determines the physical characteristic of fats, affects the absorption of fatty acids, lipid metabolism and fat distribution in tissues in addition it plays a major role in serum cholesterol level. PA, the most abundant circulating SFA, The mechanism engaged by SFAs appears to be through TLR4, triggering acute and chronic inflammation in-vivo and in-vitro experimental models (Tian et al., 2015; Wang et al., 2017). TLR4 is an essential modulator of innate immunity, and links it with metabolic disorders like obesity. Ubhayasekera et al., (2013) reported that palmitate is one of the most abundant SFA recorded in obese children.

Mice fed with the chemically interesterified

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fat type</th>
<th>Free fatty acid</th>
<th>Peroxide Value (mEq./Kg oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Corn oil</td>
<td>0.12%</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>100% Butter</td>
<td>0.14%</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>75% Butter+25% Margarine (mixture I)</td>
<td>0.16%</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>50% Butter+50% Margarine (mixture II)</td>
<td>0.18%</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>25% Butter+75% Margarine (mixture II)</td>
<td>0.20%</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>100% Margarine</td>
<td>0.25%</td>
<td>1.19</td>
</tr>
</tbody>
</table>

Table 1: Free Fatty Acid % and Peroxide Value of Different Fat Types.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Corn oil</th>
<th>Butter (mixture I)</th>
<th>(mixture II)</th>
<th>(mixture III)</th>
<th>Margarine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyric acid (C4:0)</td>
<td>ND</td>
<td>0.58</td>
<td>0.47</td>
<td>0.12</td>
<td>ND</td>
</tr>
<tr>
<td>Caproic acid (C6:0)</td>
<td>ND</td>
<td>1.22</td>
<td>0.99</td>
<td>0.46</td>
<td>ND</td>
</tr>
<tr>
<td>Caprylic acid (C8:0)</td>
<td>ND</td>
<td>1.18</td>
<td>0.89</td>
<td>0.58</td>
<td>ND</td>
</tr>
<tr>
<td>Capric acid (C10:0)</td>
<td>ND</td>
<td>3.58</td>
<td>2.59</td>
<td>1.44</td>
<td>1.18</td>
</tr>
<tr>
<td>Lauric acid (C12:0)</td>
<td>ND</td>
<td>5.92</td>
<td>4.41</td>
<td>2.91</td>
<td>1.97</td>
</tr>
<tr>
<td>Myristic acid (C14:0)</td>
<td>ND</td>
<td>14.03</td>
<td>10.74</td>
<td>7.15</td>
<td>4.9</td>
</tr>
<tr>
<td>Myristoleic acid (C14:1)</td>
<td>ND</td>
<td>0.87</td>
<td>0.64</td>
<td>0.4</td>
<td>ND</td>
</tr>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>24.83</td>
<td>32.37</td>
<td>35.67</td>
<td>38.54</td>
<td>40.03</td>
</tr>
<tr>
<td>Stearic acid (C18:0)</td>
<td>7.62</td>
<td>12.39</td>
<td>11.51</td>
<td>11.21</td>
<td>11.16</td>
</tr>
<tr>
<td>Oleic acid (C18:1)</td>
<td>31.94</td>
<td>20.26</td>
<td>22.43</td>
<td>25.83</td>
<td>29.69</td>
</tr>
<tr>
<td>Linoleic acid (C18:2)</td>
<td>35.61</td>
<td>1.48</td>
<td>2.8</td>
<td>3.89</td>
<td>5.16</td>
</tr>
<tr>
<td>Linolenic acid (C18:3n3)</td>
<td>ND</td>
<td>0.81</td>
<td>1.45</td>
<td>2.77</td>
<td>4.07</td>
</tr>
<tr>
<td>α-Linolenic acid (C18:3n6)</td>
<td>ND</td>
<td>3.4</td>
<td>2.89</td>
<td>3.56</td>
<td>ND</td>
</tr>
<tr>
<td>Arachidic acid (C20:0)</td>
<td>ND</td>
<td>1.92</td>
<td>2.51</td>
<td>0.8</td>
<td>1.83</td>
</tr>
<tr>
<td>SFA</td>
<td>32.45</td>
<td>73.19</td>
<td>69.78</td>
<td>63.21</td>
<td>61.07</td>
</tr>
<tr>
<td>UFA</td>
<td>67.55</td>
<td>26.82</td>
<td>30.21</td>
<td>36.45</td>
<td>38.92</td>
</tr>
<tr>
<td>SFA/UFA ratio</td>
<td>0.48</td>
<td>2.73</td>
<td>2.31</td>
<td>1.73</td>
<td>1.57</td>
</tr>
</tbody>
</table>

ND= not detected
Table 3: Effects of Feeding Rats Different Fat Types for 6 Weeks on Body Weight, Total Food Intake and Food Efficiency Ratio.

<table>
<thead>
<tr>
<th>Diets containing 10% fats</th>
<th>Initial Body Weight (gm)</th>
<th>Body Weight (gm) after Week 1</th>
<th>Body Weight (gm) after Week 2</th>
<th>Body Weight (gm) after Week 3</th>
<th>Body Weight (gm) after Week 4</th>
<th>Body Weight (gm) after Week 5</th>
<th>Final Body Weight (gm) after Week 6</th>
<th>BWG (gm)</th>
<th>Total Food Intake (gm)</th>
<th>Food Efficiency Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil (basal diet)</td>
<td>61.83±1.25</td>
<td>94.03±2.35</td>
<td>113.80±2.60</td>
<td>133.65±1.16</td>
<td>150.47±1.39</td>
<td>165.23±1.84</td>
<td>182.72±1.08</td>
<td>120.88</td>
<td>500.10</td>
<td>0.26</td>
</tr>
<tr>
<td>100% Butter</td>
<td>62.83±0.91</td>
<td>99.38±1.20</td>
<td>116.32±2.63</td>
<td>135.87±2.77</td>
<td>155.07±1.44</td>
<td>172.52±1.06</td>
<td>190.95±0.90</td>
<td>128.12</td>
<td>526.50</td>
<td>0.25</td>
</tr>
<tr>
<td>75% Butter+25% Margarine</td>
<td>64.33±0.95</td>
<td>101.36±1.04</td>
<td>121.55±0.78</td>
<td>141.58±0.61</td>
<td>157.75±0.71</td>
<td>175.65±0.69</td>
<td>197.77±0.53</td>
<td>133.43</td>
<td>517.10</td>
<td>0.26</td>
</tr>
<tr>
<td>Margarine (mixture I)</td>
<td>59.83±1.87</td>
<td>103.14±1.50</td>
<td>128.55±1.38</td>
<td>146.50±1.58</td>
<td>161.75±1.22</td>
<td>183.38±1.14</td>
<td>201.60±0.90</td>
<td>141.77</td>
<td>515.90</td>
<td>0.28</td>
</tr>
<tr>
<td>50% Butter+50% Margarine</td>
<td>61.50±1.82</td>
<td>106.75±1.19</td>
<td>134.68±1.80</td>
<td>148.78±1.74</td>
<td>166.90±0.75</td>
<td>189.98±1.67</td>
<td>206.77±0.99</td>
<td>145.27</td>
<td>500.20</td>
<td>0.29</td>
</tr>
<tr>
<td>25% Butter+75% Margarine</td>
<td>59.83±1.60</td>
<td>109.13±1.99</td>
<td>135.33±1.47</td>
<td>151.45±0.52</td>
<td>171.40±0.52</td>
<td>191.65±0.78</td>
<td>210.43±2.91</td>
<td>150.60</td>
<td>485.75</td>
<td>0.31</td>
</tr>
<tr>
<td>100% Margarine</td>
<td>61.83±1.25</td>
<td>94.03±2.35</td>
<td>113.80±2.60</td>
<td>133.65±1.16</td>
<td>150.47±1.39</td>
<td>165.23±1.84</td>
<td>182.72±1.08</td>
<td>120.88</td>
<td>500.10</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SE (n=6). In each row different superscript letters mean significant differences at 0.05 probabilities.

References:
- Fernandez et al., 2012
- De Wit et al., 2015
- Musso et al., 2011
- Moulle et al., 2015
- Hernández-Cáceres et al., 2014
- Hrnéček et al., 2014
- Exley et al., 2012
- Karupaiah and Sundram, 2007
- Gouk et al., 2010
- Laugerette et al., 2014

The authors attributed this fat deposition in the long chain FA position of glycerol backbone. The biological effects of SFAs may depend on the length of carbon atoms in the FA chain, the degree of saturation and the stereospecific positioning of FAs in TAG and the stearate of the FA chain. The authors also explained that high-doses of PA induce pro-inflammatory markers in plasma (IL-6) and in white adipose tissue (WAT) IL-1β and TNFα (Langere et al., 2014).
inflammatory responses and leptin resistance, similarly to obesogenic-diets. Several studies carried out on animal models indicated that, during lactation, the consumption of normo-lipidemic diets, rich in SFAs derived from PO and/or in partially hydrogenated fats (PHF) induces fat retention in the young offspring (Silva et al., 2006). The role of PO consumption in T2DM is controversial, also other findings mentioned that PO-enriched diet impairs glucose tolerance may be due to the reduction in insulin-sensitivity (Kochikuzhyil et al., 2010) this also in conjunction with increase of serum TAGs which associated with insulin-resistance (Storlien et al., 2000). PA was found to markedly impair phosphorylation process and thus, activation of insulin receptor, insulin receptor substrate-1, and Act in several cell types, contributing to the onset of insulin-resistance (Mordier and Iynedjian, 2007).

Table 3 also indicated that there were no significant differences among the rat groups in total food intake.

### Table 4: Plasma Total Cholesterol and Triglyceride Levels (mg/dl) in Rats Fed Different fat types for 6 weeks.

<table>
<thead>
<tr>
<th>Diets containing 10% fats</th>
<th>TC (mg/dl) Feeding period (weeks)</th>
<th>TG (mg/dl) Feeding period (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero 2 4 6</td>
<td>Zero 2 4 6</td>
</tr>
<tr>
<td>Corn oil (basal diet)</td>
<td>119.21 ±3.11Bb 126.86 ±0.95ad 127.17 ±0.97ac 131.14 ±0.59ad</td>
<td>106.54 ±6.14Bb 109.20 ±2.82Bb 115.50 ±3.47Ab 126.34 ±7.43Ab</td>
</tr>
<tr>
<td>100% Butter</td>
<td>117.29 ±1.38Ba 129.37 ±0.80cd 140.76 ±0.64Bb 146.82 ±0.74Ac</td>
<td>104.54 ±8.79Bb 116.41 ±0.63Ab 120.44 ±0.79Ab 133.52 ±8.91Ab</td>
</tr>
<tr>
<td>75% Butter+25% Margarine (mixture I)</td>
<td>119.13 ±1.58Bda 131.14 ±0.59cd 140.98 ±2.56Ac 150.49 ±4.72Ac</td>
<td>105.34 ±1.32Da 118.01 ±1.28Ca 127.38 ±1.23Bb 134.19 ±1.51Ab</td>
</tr>
<tr>
<td>50% Butter+50% Margarine (mixture II)</td>
<td>117.83 ±4.01Ca 133.54 ±0.92Ac 143.20 ±2.80Ac 160.43 ±11.72Ac</td>
<td>107.34 ±1.08Da 122.26 ±1.65Ca 133.24 ±0.82Ab 138.70 ±2.62Ab</td>
</tr>
<tr>
<td>25% Butter+75% Margarine (mixture III)</td>
<td>121.73 ±3.40Ba 140.41 ±2.21Bb 157.08 ±1.85Bb 173.46 ±4.53Ab</td>
<td>109.20 ±2.82Ca 124.94 ±10.80Ab 130.06 ±1.30Ab 146.70 ±4.15Ab</td>
</tr>
<tr>
<td>100% Margarine</td>
<td>118.53 ±1.31Ca 148.22 ±2.09Ba 158.37 ±0.68Ba 189.21 ±11.10Ba</td>
<td>108.82 ±1.35Ba 128.08 ±10.70Ab 135.37 ±8.54Ab 149.22 ±1.18Ba</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SE (n=6).

a, b, c, d In each column the same superscript letter means non-significant difference while different superscript letters mean significant differences at 0.05 probability.

A, B, C, D In each row different superscript letters mean significant differences at 0.05 probabilities.

### Table 5: Plasma HDL-cholesterol and LDL-cholesterol levels (mg/dl) in Rats Fed Different fat types for 6 weeks.

<table>
<thead>
<tr>
<th>Diets containing 10% fats</th>
<th>HDL-cholesterol (mg/dl) Feeding period (weeks)</th>
<th>LDL-cholesterol (mg/dl) Feeding period (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero 2 4 6</td>
<td>Zero 2 4 6</td>
</tr>
<tr>
<td>Corn oil (basal diet)</td>
<td>33.31 ±1.09Ca 34.26 ±0.71Ca 38.80 ±0.26Ba 43.77 ±1.73Ba</td>
<td>60.20 ±2.68Ba 64.73 ±4.40Ab 70.97 ±4.78Ab 75.84 ±1.14Ac</td>
</tr>
<tr>
<td>100% Butter</td>
<td>35.34 ±1.19Ba 33.63 ±1.17Ac 32.60 ±0.70Ab 31.33 ±1.46Bb</td>
<td>67.51 ±4.25Bb 71.18 ±4.46Bb 73.02 ±6.47Bb 90.57 ±1.93Ab</td>
</tr>
<tr>
<td>75% Butter+25% Margarine (mixture I)</td>
<td>34.05 ±1.16Ba 31.13 ±0.88Ab 30.39 ±1.01Ab 29.39 ±1.54Ab</td>
<td>66.42 ±1.08Ca 73.42 ±2.20Bc 83.21 ±5.18Bc 96.23 ±5.97Ab</td>
</tr>
<tr>
<td>50% Butter+50% Margarine (mixture II)</td>
<td>33.63 ±1.26Ba 30.19 ±0.70Ab 29.59 ±1.49Ab 27.90 ±2.37Bb</td>
<td>62.11 ±2.34Da 76.21 ±3.16Bc 89.09 ±4.85Ab 109.61 ±3.62Ab</td>
</tr>
<tr>
<td>25% Butter+75% Margarine (mixture III)</td>
<td>32.91 ±0.36Ad 29.39 ±1.51Bc 27.50 ±1.28Bc 26.40 ±1.16Bc</td>
<td>64.91 ±4.52Ca 80.41 ±0.90Bb 90.68 ±0.98Bb 115.50 ±5.29Ab</td>
</tr>
<tr>
<td>100% Margarine</td>
<td>35.22 ±0.91Bb 29.99 ±1.78Ab 27.90 ±1.71Bc 25.16 ±1.42Ca</td>
<td>60.75 ±2.60Da 85.21 ±3.42Ca 99.14 ±4.65Bb 117.90 ±4.87Ab</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SE (n=6).

a, b, c, d In each column the same superscript letter means non-significant difference while different superscript letters mean significant differences at 0.05 probability.

A, B, C, D In each row different superscript letters mean significant differences at 0.05 probabilities.
The results shown in Tables 4 and 5 represented that increasing the margarine percentage in diet caused significant elevation in plasma TC, TG and LDL-C levels. The elevation in such lipid parameters represent a direct relation with the margarine % in diet blends compared to butter fed rat groups. Grundy et al., (1988) reported that consumption of diets containing 8 to 16 carbon-atom SFAs increases the serum concentration of LDL-C. Such elevation in plasma TC, LDL-C and TG was also approved by Denke and Grundy (1992) that render this to the high concentration of PA. Also Table 5 showed that significant reduction in plasma HDL-C levels as margarine % in diet increase. Certain eating pattern that is described by a high intake of total fat, SFA and cholesterol, but low in fiber and PUFAs content may play a crucial role in the CHD risk (Kratz, 2005). Table 4 and 5 and Figs. 3-8 reported the effect of the six different fat types under test on the plasma lipid profile and the risk ratios 1 and 2 with time. The results shown in Table 6 represented that increasing margarine percentage in diet caused significant elevation in risk ratios 1 and 2. This Table 6 also showed that the highest risk ratios 1 and 2 were found in rat group that was fed on margarine as 100% fat. These results are in the same boat with those reported by Asadi et al., (2010) but these results showed disagreement with those reported by Edem (2009). Several studies have demonstrated that PO was similar to unsaturated oils in its effects on blood lipids. PO provides a healthy alternative to TFA containing hydrogenated fats that have been demonstrated to have serious deleterious effects on health. The similar effects of PO on blood lipids, comparable to other vegetable oils could be due to the structure of the major TGs in PO, which has an UFA in the stereospecific numbers Sn-2 position of the glycerol backbone. Many nutritional studies have reported that PO with high monounsaturation at Sn-2 position is comparable to monounsaturated oils (like olive and canola oils) in its effect on lipid profile. In addition, PO is endowed with a bouquet of phytonutrients beneficial to health, such as tococtrienols, carotenoids, and phytosterols (Choo and Kalanithi, 2014). Qureshi et al.,
(1991) reported that tocotrienols in PO exerted a hypocholesterolemic effect in humans aged 30 to 60 years probably through the inhibition of cholesterol synthesis.

Hayes et al., (1991) have reported that PA appeared normocholesterolemic when the supply of linoleic acid was above 6 energy %. It is noticeably that diet contain the 25 and 50% margarine showed non-significant difference in TG level compared to butter, this may related to their comparable percentage of either the short chain FA namely C4, C6 and C8 or γ-Linolenic acid.

Substitution of saturated fats with non-hydrogenated vegetable oils was associated with a rapid decline in CHD mortality (Zatonski and Willett, 2005). This supports our results, which indicate that as hydrogenated oil % increase this is accompanied by a series of deleterious effects on body heath.

PO contains negligible amounts (less than 1.5%) of the hypercholesterolemic SFAs, namely lauric acid (12:0) and myristic acid (14:0). It possesses moderately rich amounts of the hypocholesterolemic, monounsaturated oleic acid (18:1, omega-9) and adequate amounts of linoleic acid (18:2, omega-6) (Chong and Ng, 1991).

Trans-fat increases LDL-C and decreases HDL-C (Mozaffarian et al., 2006). PO significantly increased TC, LDL-, and HDL-C concentrations compared to vegetable oils low in saturated fat. PO was also found to significantly increase HDL-C compared with trans-fat containing partially hydrogenated vegetable oils (Sun et al., 2015).

Tinahones et al., (2004) reported that diet rich in PA do not raise plasma TC, but reduce LDL-C or even keep it normal, and in addition it elevate plasma HDL. The researcher may render this to the increase in LDL-receptor activity, such results coincide with those of Lindsey et al., (1990). The mechanism for the LDL-C elevating effect of hydrogenated fat appears to be similar to that of saturated fat, primarily determined by decreased catabolism. These findings support current recommendations to reduce saturated and hydrogenated fat consumption. Rats fed with hydrogenated oils containing palm, this was accompanied by exacerbation to the atherosclerotic risk through elevation of both serum TC level and oxLDL and on the other hand reduction in paraoxanase-1 activity (Amini et al., 2017).

Harris et al., (1983) demonstrated that SFAs with carbon atoms 12, 14 and 16 are considered to have hypercholesterolemic effect. This result is consistent with ours that show elevation in total plasma cholesterol with values 150, 160, 173 and 189 mg/dl at P<0.05in the sixth week of the experiment in diet containing 25, 50, 75 and 100% of margarine respectively. This indicate a direct relation between the margarine percentage and plasma cholesterol level which may be related to the PA % in diet which was shown to be elevated as the concentration of margarine % in diet increase. Tinahones et al., (2004) also reported low LDL-C/HDL-C risk ratio in plasma of rat fed on PA whereas our results showed high levels of this risk ratio with its highest value seen in diet with 100% margarine when compared with butter rat fed group. In our study, plasma TG level increase as the % of margarine in rat diet increase these results come with that of (Lindsey et al., 1990; Tinahones et al., 2004).

Studies have also established that SFAs activate inflammatory and innate immune responses (Huang et al., 2012). PA increased serum levels of pro-inflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 (Wang et al., 2017). The levels of these cytokines were also elevated in the cardiac tissues. PA is the most common SFA found in the human body and

![Fig. 2: Effect of feeding rats several fat types (100% corn oil, 100% butter, (75% butter + 25% margarine), (50% butter + 50% margarine), (25% butter + 75% margarine) and 100% margarine) as 10% of total basal diet for 6 weeks on rat body weight.](image-url)
can be provided in the diet or synthesized endogenously from other FAs, carbohydrates and amino acids. PA represents 20–30% of total FAs in membrane phospholipids, and adipose TAG (Carta et al., 2015). In order to maintain membrane phospholipids balance an optimal intake of PA in a certain ratio with UFAs, especially PUFAs of both n-6 and n-3 families are important (Carta et al., 2017). PA is a major component of palm oil (44% of total fats), but significant amounts of PA can also be found in meat and dairy products (50–60% of total fats), as well as olive oil (8–20%). Furthermore, PA is also present in breast milk with 20–30% of total fats (Innis, 2016). The average intake of PA is around 20–30 g/d (Sette et al., 2011). Changes in PA intake do not influence significantly its tissue concentration (Song et al., 2017), as the intake is counterbalanced by PA endogenous biosynthesis via de novo lipogenesis (DNL). Specific nutritional factors and physiopathological conditions may induce DNL, caused elevated tissue content of PA that may lead to disrupted homeostatic control of its tissue concentration (Wilke et al., 2009). Whereas in normal physiological conditions, PA accumulation is prevented by enhanced delta desaturation to palmitoleic acid and/or elongation to stearic acid and followed by delta desaturation to oleic acid (Strable and Ntambi, 2010). PA homeostatic balance disruption is implicated in many pathological conditions such as atherosclerosis, neurodegenerative diseases and cancer.

**Conclusion**

The current study revealed that the use of margarine substitute of butter in foods should be avoided or partially replaced to prevent its hazardous effects on human health. In addition our study could not establish strong evidence against margarine/palm oil blends consumption relating to non-communicable diseases mortality. Further studies are needed to establish such a relation. Greater awareness should be taken on dealing with unhealthy dietary habits.
Fig. 5: Effect of feeding rats several fat types (100% corn oil, 100% butter, (75% butter+25% margarine), (50% butter+50% margarine), (25% butter+75% margarine), and 100% margarine) as 10% of total basal diet for 6 weeks on plasma HDL-C level. Plasma HDL-C level decrease with time in all groups except the corn oil fed group showed sharp elevation started from week 2 to the end of the experiment.

Fig. 6: Effect of feeding rats several fat types (100% corn oil, 100% butter, (75% butter+25% margarine), (50% butter+50% margarine), (25% butter+75% margarine), and 100% margarine) as 10% of total basal diet for 6 weeks on plasma LDL-C level. All rat groups showed significant LDL-C elevation with time except that for both the corn and the 100% butter groups as the former showed weak elevation and the latter showed some fluctuation.

Fig. 7: Effects of feeding rats several fat types (100% corn oil, 100% butter, (75% butter+25% margarine), (50% butter+50% margarine), (25% butter+75% margarine), and 100% margarine) as 10% of total basal diet for 6 weeks on plasma Risk Ratio 1. All rat groups showed elevation in plasma Risk Ratio 1 with time except for the corn rat fed group that showed sharp reduction from week 2 to week 6.
A healthy overall diet should still be prioritized for good health.

References


Fig. 8: Effects of feeding rats several fat types (100% corn oil, 100% butter, (75% butter+25% margarine), (50% butter+50% margarine), (25% butter+75% margarine), and 100% margarine) as 10% of total basal diet for 6 weeks on plasma Risk Ratio 2. All rat groups showed elevation in plasma Risk Ratio 2 with time whereas the corn rat fed group showed constancy with slight decrease at the end of the experiment.


