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Fatty Acids Composition, Variation and Distribution in Different Accessions of the West African Pear (*Dacryodes edulis*) and Potential Health Benefits

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Authors' contributions

This work was carried out in collaboration among all authors. Author GOI conceptualized the research study, developed the methodology with GC-MS investigations, interpretation of GC-MS data, validation of data, preparation of final manuscript and visualization of general study outcome, Author HNEO developed study design, supervised the research work, reviewed and edited the draft manuscript. Author GEE carried out formal analysis and selection of statistical model, data curation; data validation and choice of software for preparation of manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The pulp of *Dacryodes edulis* is eaten raw, cooked or roasted by West and central Africans. The aim of this study was to determine the fatty acid composition, type, variation and distribution among different accessions of *D. edulis* purchased from different locations of Eastern Nigeria, using GC-MS data. The results showed that *D.edulis* is rich in ω-3,6,9 unsaturated fatty acids. Saturated fatty acids namely, pentadecanoic, tetradecanoic (myristic), tridecanoic, hexadecanoic (palmitic), undecanoic and octadecanoic (stearic) acids were common to all accessions. Unsaturated fatty acids identified in the four accessions included, Oleic, linoleic, linolenic, linoelaidic and several other polyunsaturated fatty acids including brassidic and vacennic acids, and 19,19-Dimethyl-Eicosa-8,11-dienoic acid (6.83±0.37%) a derivative of oleic acid which was found only in Nsukka samples. Two novel polyunsaturated fatty acids namely; Methyl,9,12-

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Heptadecadienoic acid and 2,6,10,14-Hexadecatetraenoic acid were identified in the present study. The ratio of saturation to unsaturation was calculated to be approximately 1:2. The concentration of TSFAs fell in the range of 27.07±1.75-39.87±1.72%, while TMUFAs was 3.14±0.22-10.77±0.55%, and TPUFAs was 46.81±1.22-58.71±2.33% respectively. We concluded that, the presence of polyunsaturated fatty acids in *D. edulis* may provide cardio-protective benefits in terms of human nutrition and could be substitutes for olive oil in healthy normo-cholesterolemic human diets. We further concluded that the differences in fatty acid composition, distribution, variation and concentration between the locations studied may be affected not only by their biogenetic origin but also by soil type and climatic conditions.

Keywords: Dacryodes edulis; fatty acids; unsaturation; saturation; cardio-protection.

1. INTRODUCTION

The West African Pear (*Dacryodes edulis*), is a perennial and herbaceous plant that fruits seasonally especially in the rainy season. The plant grows also in Central African countries including Cameroun, Angola, Congo, Gabon; and some parts of East Africa. People in West Africa consume the fruit raw, boiled or roasted. The pulp and the seed have been reported to contain fatty acids and mineral elements [1]. The fatty acid concentration, its distribution and variation among different accessions, and the classification of the oil has not been reported. The oil has been evaluated as surface coating driers in a recent study [2].

Fig. 1. Mature fruits of *D. edulis*

The variation in the nutrients and bioactive compounds of different accessions of the West African pear, and implications for dietary intake assessment and health has recently been reported [3].

Fatty acids occur generally in plant oils and animal fats. The commonly occurring and dominant fatty acids in foods of plant and animal origin include capric, caprylic, myristic, stearic, lauric, palmitic, arachidonic, oleic, linoleic and linolenic acids [4]. Fatty acids are grouped into saturated (without double bonds) and unsaturated fatty acids (with double bonds). Unsaturated fatty acids with double bonds form what is nutritionally referred to as Omega-3, Omega--6 and Omega-9 oils, which are known to be the best sources of cooking oils [5]. Omega fatty acids have been variously reported to be cardio-protective oils against all forms of heart disease [5,6]. Unsaturated fatty acids, generally referred to as Omega oils are considered the best source of oils for human nutrition and health [5,6].

The West African pear oil has been reported by [4] and [7] to contain palmitic acid (9.06%), stearic acid (15.46%), oleic acid (26.63%) and linoleic acid (30.85%). A previous report by [7] gave the fatty acid composition and ranges as palmitic acid 47.89% (35 – 65%), oleic acid 31.25% (16 – 35%) and linoleic acid 17.5% (14 – 27%) but did not classify the oil type and the studies did not indicate variation and distribution between locations. This study aim to address these gaps. Fatty acids are required for cellular processes, and for the correction of metabolic and endocrine dysfunction, and the production of other necessary and essential omega-3 and omega-6 fatty acids in the body [6,8,9,10,11].

It has been established that humans cannot synthesize linoleic acid (Omega-6 fatty acid) and α-linolenic acid (Omega-3 fatty acid). Thus these group of fatty acids are referred to as essential fatty acids because they must be taken into the body through the diet [9,11]. Omega-6 and omega-3 fatty acids derived from linoleic and α-linolenic acids are needed conditionally by many mammals including humans [9]. They are formed in the body from their parent fatty acids but not always at levels needed to maintain optimal health or development [9,11]. Infants for instance are thought to have a conditionally essential need for docosahexaenoic acid (DHA) which is derived from α-linolenic acid, and perhaps from arachidonic acid which is derived from linoleic

acid. This is because, the hydrocarbon molecules of unsaturated fatty acids have two carbons that share double or triple bonds and are therefore not completely saturated with hydrogen atoms. Unsaturated fatty acids molecules are not stable at room temperature and therefore easily disintegrate. The molecules do not stick to the walls of blood vessels or the body as is the case with saturated fatty acids [9,12].

However, saturated fatty acids which do not contain any double bond in their structure are considered to be unhealthy source of edible oils. These include Palm oil (Palmitic acid) and refined palm kernel oil (Lauric acid) as well as stearic acid [13,14]. A saturated fatty acid structure is fully hydrogenated as in palmitic, lauric and stearic fatty acids. The molecule is said to be very stable (usually solid at room temperature) and hard to break-up or disintegrate. This allows it to store and provide more energy than carbohydrates and makes it more likely to stick to the body as cholesterol resulting in high arterial and plasma cholesterol, also known as hypercholesterolemia [13,14,15]. High arterial and plasma cholesterol predisposes hypertension. Studies have shown clearly that hypercholesterolemia; hyperlipidemia, dyslipidemia and diabetes are some of the pre-disposing factors of cardiovascular disease (CVD), and indeed hypertension [13,14,16].

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Fresh mature fruits of *Dacryodes edulis* (*D. edulis*) were purchased from four local markets in the Eastern region of Nigeria namely: Akpabuyo and Ikom in Cross River State; Ikot Ekpeya in Akwa Ibom State and Nsukka market in Enugu State respectively. The fruit samples were authenticated by Dr. Michael Eko, a botanist in the Department of Botany, University of Calabar, Nigeria. A dried reference sample packaged in a polythene bag and labeled BCM/003/2019 was placed in the Samples Reference Room (SRR) of the Department of Biochemistry, University of Calabar, Nigeria.

2.2 Sample Preparation for GC-MS Analysis of Fatty Acids

Five hundred (500) grammes of the endocarp of each sample was cut into small pieces, washed and dried at room temperature (27±2.0°C) in a well ventilated room for 7 days. The dried endocarp was blended into powdered form using a manual blender and stored in air tight plastic containers until required. 200 g of each accession was placed in a thimble, and its oil was extracted with petroleum ether (40-60°C) for 3 h using a Soxhlet apparatus on an electrothermal mantle. The extract was concentrated *in vacuo* at 40°C, using a rotary evaporator to remove all alcohol and to afford 22 g, 21.4, 20.9 and 20.85 g of golden brown oil respectively. The oil samples were stored under refrigeration until required for GC-MS analysis.

2.3 Fatty Acids Methyl Esters (FAMEs) Derivatization of the Oil Sample

Twenty five (25) ng of oil sample was weighed into 10 mL micro-reaction vessel and 2 mL BCl3-MeOH 12% w/w was added. This was followed by the addition of 1 mL 2, 2-dimethoxyproprane. The mixture was mixed thoroughly and then heated for 5 minutes at 60°C. It was then cooled to below 30°C and 1 mL distil water and 1 mL n-hexane was added and mixed thoroughly again, then allowed to stand for 10 minutes. The upper (organic) layer was pipette into a sterile and clean vial and covered with a stopper and stored for GC-MS analysis. This was repeated for the 4 accessions.

2.4 GC-MS Analysis of FAMEs Samples for Fatty Acids Studies

The method reported by [16] was used to carry out the GC-MS analysis with a slight modification. Samples of FAMEs prepared from each sample oil extract, were injected manually through the injector pot of the GC. An Agilent 6890 GC coupled with a 5973i mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) was used, with a ChemStation Integrator to interprete data. The GC was equipped with a HP-5MS capillary column (30m × 250 µm i.d. × 0.25 µm, Agilent Technologies). Helium was used as the carrier gas with a constant flow rate of 1 mL/min to the column. The initial oven temperature was at 40°C, holding for 2 min, then raised to 150°C at 5°C/min; and finally raised to 280°C at 15°C/min, holding for 2 min. The injection pot was maintained at split less mode. The mass detector was operated at 150°C in electron impact (EI) mode at 70 eV. The ion source temperature was at 230°C and the transfer line temperature was maintained at 250°C. The chromatograms were recorded by monitoring the total ion currents in the 15–450 mass range. MS was detected with 2

min solvent delay. Analysis of each sample at each condition was repeated twice to ensure consistency. C6-C24 *n*-alkanes were ran under the same chromatographic conditions in order to calculate the retention indices (RI) of detected compounds. Identification of free fatty acids and other volatile constituents was based on retention indices relative to n-alkanes (C6-C24), and computer matching with the WILLEY 275.L library, and those contained in the NIST08 database; and confirmed by comparison of the retention times (RIs), as well as by comparison of their mass spectra and fragmentation patterns with those reported in literature [16].

2.5 Statistical Analysis

All determinations were done in duplicates and results were expressed as Mean ± SEM, for n=2, and subjected to analysis of variance (ANOVA) to check for statistical significance ($p<0.05$) of % abundance for individual fatty acids.

3. RESULTS AND DISCUSSION

Fatty acid compounds were identified by comparing their mass spectra with those contained in the NIST08 database, and confirmed by comparison of the retention times of the separated constituents with those of the authentic samples, and by comparison of retention indexes (RIs) of the separated constituents with the RIs reported in literature.

The most commonly found saturated fatty acids in the four locations studied (Akpabuyo, Ikot Ekpeya, Ikom and Nsukka), included Pentadecanoic acid, Octadecanoic (Stearic) acid, Undecanoic acid, Hexadecanoic (Palmitic) acid, Tetradecanoic (Myristic) acid and Tridecanoic acid; with Hexadecanoic acid (palmitic acid) being the most dominant saturated fatty acid (Tables 1-4). The total saturated fatty acids in the four locations, varied between 27.07±1.75% (Ikom) to 39.87±1.72% (Ikot Ekpeya) (Table 5).

The present study identified Trans-13-Octadecenoic (Cinnamic) acid, and its optical isomer Cis-13-Octadecenoic (Brassidic) acid. Other unsaturated fatty acids identified included, Methyl, 11, 14-Octadecadienoic acid, Cis-Vaccenic acid, 11-Octadecenoic (Vaccenic) acid. (9Z, 12E)-Octadecadienoic (Linoleic) acid; (Z,Z)-9,12-Octadecadienoic (Cis-Cis-Linoleic) acid; (E,E)-9,12- Octadecadienoic (Linolelaidic) acid, 2-(2-hydroxyethoxy) ethyl ester-Octadecanoic acid and Methyl,9,12-Heptadecadienoic acid. Methyl (9Z,15Z)-9,15-Octadecadienoic acid, which is a Methyl, trans-9,trans-15-Octadecadienoic acid, and is a methylated form of linoleic acid, and classified as an omega-3 fatty acid was also detected. Other forms of linoleic and linolenic trans-fatty acids and their isomers found in the four locations studied included, Methyl, 11, 14-Octadecadienoic acid, Methyl 9-cis,11-trans-Octadecadienoic acid and Methyl,10-trans, 12-cis-Octadecadienoic acid (Tables 1-4).

The total unsaturated fatty acids varied between 50.00 ± 0.78% (Ikot Ekpeya) to 69.48 ± 1.52% (Akpabuyo). Total unsaturated fatty acids in Ikom and Nsukka accessions gave 60.81± 0.95% and 59.44±1.17% respectively (Table 5). The most abundant unsaturated fatty acid in the 4 locations studied was linoleic acid and its isomers. The commonly occurring unsaturated fatty acids in the four accessions included, Oleic acid which occurred in its two isomeric forms as (Z)-9-Octadecenoic and (Z)-7-Octadecenoic acids. Other unsaturated fatty acids found in the 4 samples included linoleic acid, linolenic acid and linolelaidic acid. Samples from the four locations gave the following mean values of linoleic acid; Akpabuyo (47.64±1.35%), Ikot Ekpeya (38.72±2.17%), Ikom (34.85±0.74%), and Nsukka (38.89±2.21%) respectively. These results compared favourably with the linoleic acid content in Olive oil (19-22%); Sunflower oil ((40-46%) and peanut oil (28-31%) respectively. The concentration of linoleic acid did not vary significantly between the locations (Tables 1-4).

Linoelaidic acid, also known as (E,E)-9,12-Octadecadienoic acid ($C_{18}H_{32}O_2$) was shown in the present study to be common to all accessions of the West African Pear. Linoelaidic acid is an omega-6 trans fatty acid. It is a geometric isomer of linoleic acid, or a conjugated linoleic acid. Also, common to all the accessions is α-linolenic acid, also known as (Z,Z)-9,12-Octadecadienoic acid. α-linolenic acid (LNA) is an omega-3 fatty acid. Linoleic acid is undoubtedly the most important polyunsaturated fatty acid in abundance and distribution in nature. It is found distributed in all the accessions studied. It is one of the nutritionally essential fatty acids. It was reported that several research programmes were initiated some years ago to improve on the linoleic acid content of edible vegetable oils, and minimize the content of less desirable fatty acids in them. This was aimed at improving methods of agriculture, particularly in

the area of genetic engineering, so as to improve on strains and varieties [17]. The results in some instances were reported to be remarkable [17]. The present study suggests that such technology could be applied to further improve on the fatty acid composition in *D. edulis* in order to improve on its nutritional application. Linoleic and linolenic acids are similarly named omega fatty acids even though they have different roles in human health and nutrition. Despite their names, these omega-6 fatty acids are more similar to one another, and like linoleic acid, they generally co-occur in nuts, seeds and vegetable oils. This supports the findings in this study in which the isomeric forms of some of the fatty acids co-occurred in all the 4 accessions of the *D. edulis* studied.

Also identified as unsaturated fatty acids in the *D. edulis* samples included, Methyl,7,10-Hexadecadienoic acid and 2,6,10,14-Hexadecatetraenoic acid, 3,7,11,15-tetramethyl-9-(phenylsulphonyl)-ethyl ester (E,E,E) in Ikom; and 1,2-Benzenedicarboxylic acid (Phthalic acid), Bis (2-Ethyl, Hexyl)-Phthalic acid and Didodecyl, Phthalic acid in Nsukka samples. Phthalic acid and its derivatives including, Di-n-Octyl Phthalic acid, Phthalic (1,2-Benzene–dicarboxylic) acid and 2-Ethoxy-ethyl-isobutyl ester, as well as 4-Methoxy-Anthranilic acid, and β-Sitosterol acetic acid were found only in Akpabuyo accession. Phthalic acid occurs as three isomers including ortho-, meta- and para-isomers [18]. The present study did not identify phthalic acid and its derivatives in Ikom and Ikot Ekpeya samples (Tables 2 & 3); but found significant concentrations of the acid and its derivatives in Akpabuyo and Nsukka samples (Tables 1 & 4). Phthalic acid is an aromatic dicarboxylic acid used mainly in the form of the anhydride to produce perfumes, saccharin, phthalates and other useful products [18]. Phthalic acid has been reported to have low toxicity in mammals, with an LD_{50} in mice of 550 mg/kg body weight [18]. Phthalic acid is a derivative of naphthalene and this may be responsible for the characteristic aroma and flavour of *D. edulis.*

Two key fatty acids, 19,19-Dimethyl-Eicosa-8,11-Dienoic acid (a derivative of Oleic acid), and Carbamodithioic (dithiocarbamic) acid were identified in Nsukka accession. The two fatty acids were absent in Akpabuyo, Ikot Ekpeya and Ikom locations. Carbamodithioic acid has been reported to elicit anti-microbial effect *in vivo* and *in vitro* and this may be beneficial to human health. It is one of the nutritionally essential fatty acids with a wide application in clinical nutrition interventions.

The present study found the presence of Methyl,9,12-Heptadecadienoic acid as a novel odd number polyunsaturated fatty acid (Table 1) and a new highly polyunsaturated fatty acid identified as 2,6,10,14-Hexadecatetraenoic acid (Table 3). These fatty acids may be nutritionally very important, and have never been reported in literature, and are not part of the fatty acids data bank list. The only other forms of polyunsaturated hexadecatetraenoic acids are, 6(Z), 9(Z), 12(Z)-Hexadecatetraenoic acid, and 6(Z), 9(Z), 12(Z), 15-Hexadecatetraenoic acid. These forms of fatty acids have never been reported in literature. The concentrations of Methyl,7,10-Hexadecadienoic acid (also known as 2,3-Dinor-linoleic acid), and 2,6,10,14-Hexadecatetraenoic acid, 3,7,11,15-tetramethyl-9-(phenylsulphonyl)-methyl ester (E,E,E) (7.52% abundance), and Methyl,9,12-Heptadecadienoic acid may be significant in addressing some cardiovascular disease (CVD) and metabolic diseases. It is also significant to note that, Methyl,9,12-Heptadecadienoic acid was found only in Akpabuyo and Nsukka samples, but was absent in Ikom and Ikot Ekpeya samples. These fatty acids are unique and may be among the fatty acids that are scarce in nature [19].

The present study also detected a special fatty acid known as trans-11-Octadecenoic (Vaccenic) acid. Trans vaccenic acid (TVA trans-11, 18:1) is a positional and geometric isomer of Oleic acid. Trans vaccenic acid can be converted into cis-9 trans-11 conjugated linoleic acid (C9, T11-CLA), a CLA isomer that has many beneficial effects by stearoyl COA desaturase 1 (SCD1) in the mammary gland [20]. Vaccenoic acid is found mostly in mammals and ruminants convert it to rumenic acid, which is a conjugated linoleic acid. Its stereoisomer is cis-Vaccenoic acid also known as omega-7 fatty acid. Vaccenic acid and its stereoisomers are known to possess anti-carcinogenic properties in humans [20]. Both its cis- and trans-isomers are present at a total concentration of 1.5-3.5% in *D. edulis.* And this is significant for its role as an anti-carcinogenic agent. Vaccenic acid and palmitoleic acid have been reported to be the two most common omega-7 fatty acids in nature [20].

Vaccenic acid appeared in Ikom and Akpabuyo accessions, but was absent in Ikot Ekpeya and Nsukka samples. The presence of vaccenic acid in Ikom samples (northern) and Akpabuyo (Southern) shows that its presence or absence from any location was not dependent upon climatic variables or soil type, but may be due to specie biogenetic origin.

Table 1. Fatty acids distribution in accessions of *D. edulis* from Akpabuyo

Peak no.	RT (Min)	Name of fatty acid	Mol. Wt	Mol. Form.	% Abundance
1	12.60	14-Methyl-Methyl Ester, Pentadecanoic Acid	270	$C_{17}H_{34}O_2$	4.07±0.25
2	12.67	Pentadecanoic Acid	242	$C_{15}H_{30}O_2$	2.89±0.44
3	13.04	Octadecanoic Acid	284	$C_{18}H_{36}O_2$	1.69±0.12
4	13.17	Undecanoic Acid	186	$C_{11}H_{22}O_2$	0.22±0.05
5	13.40	Hexadecanoic Acid	256	$C_{16}H_{32}O_2$	20.82±1.29
6	13.55	Tridecanoic Acid	214	$C_{13}H_{26}O_2$	0.16±0.01
7	13.94	Methyl, 11, 14-Octadecadienoic Acid	294	$C_{19}H_{34}O_2$	1.67±0.04
8	13.95	Pentadecyl, Trifluoro Acetic Acid	324	$C_{17}H_{31}F_3O_2$	0.45±0.02
9	13.99	Trans-Cinnamic Acid	148	$C_9H_8O_2$	0.45±0.01
10	14.03	Cis-Vaccenic Acid	282	$C_{18}H_{34}O_2$	3.91±0.13
11	14.04	(Z,Z)-9,12-Octadecadienoic (linoleic) Acid	280	$C_{18}H_{32}O_2$	47.64±1.35
12	14.11	(E,E)-9,12- Octadecadienoic (linolelaidic) Acid	280	$C_{18}H_{32}O_2$	1.47±0.11
13	14.12	2-(2-hydroxyethoxy)ethyl esther-Octadecanoic Acid	460	$C_{26}H_{52}O_6$	3.66±0.07
14	14.30	Methyl,9,12-Heptadecadienoic Acid	280	$C_{18}H_{32}O_2$	0.80±0.03
15	14.31	Di-n-Octyl Phthalic Acid	390	$C_{24}H_{38}O_4$	0.80±0.02
16	15.75	4-Methoxy-Anthranilic Acid	168	$C_7H_7NO_2$	3.85±0.35
17	15.77	Oleic Acid	282	$C_{18}H_{34}O_2$	1.66±0.05
18	15.80	Phthalic Acid,2-Ethoxy-ethyl-isobutyl ester	280	$C_{14}H_{16}O_6$	0.54±0.03
19	15.90	1,2-Benzene –dicarboxylic Acid	170	$C_8H_6O_4$	0.05±0.01
14	16.73	β-Sitosterol Acetic Acid	456	$C_{31}H_{52}O_2$	7.58±0.24
				Total	**99.27±2.54**

Values are expressed as mean ± SEM, n = 2 Total Saturated Fatty Acids = 33.51± 1.04%, Total Unsaturated Fatty Acids = 60.81± 0.95%; Ratio of Saturation: Unsaturation approx. 1:2, Classification of Oil = Omega 3/6/9 rich oil

Table 2. Fatty acids distribution in accessions of *D. edulis* from Ikot Ekpeya

Peak no.	RT (Min)	Name of fatty acid	Mol. Wt	Mol. Form.	(%) Abundance
1	13.126	Pentadecanoic Acid	242	$C_{15}H_{30}O_2$	3.16±0.08
2	13.229	n-Hexadecanoic Acid	256	$C_{16}H_{32}O_2$	22.37±0.73
3	13.338	Tetradecanoic (Myristic) Acid	228	$C_{14}H_{28}O_2$	2.07±0.05
4	13.641	Octadecanoic (Stearic) Acid	284	$C_{18}H_{36}O_2$	6.73±0.41
5	13.922	(E,E)-9,12-Octadecadienoic (Linolelaidic) Acid	280	$C_{18}H_{32}O_2$	8.14±0.33
6	13.962	15-Hydroxypentadecanoic Acid	256	$C_{15}H_{30}O_3$	3.36±0.12
7	14.414	(Z,Z)-9,12-Octadecadienoic (cis-cis Linoleic) Acid	280	$C_{18}H_{32}O_2$	38.72±2.17

Peak no.	RT (Min)	Name of fatty acid	Mol. Wt	Mol. Form.	(%) Abundance
8	16.520	Hexadecanoic acid, 2,3-dihydroxy-propyl ester	330	$C_{19}H_{38}O_4$	1.08±0.07
9	16.600	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (2-palmitoyl, glycerol) Propylene Glycol Mono–Oleic Acid	330	$C_{19}H_{38}O_4$	1.10±0.14
10	17.830		340	$C_{21}H_{40}O_3$	3.14±0.22
				Total	**98.77±2.89**

Values are expressed as mean ± SEM, n = 2, Total Saturated Fatty Acids = 39.87±1.72%, Total Unsaturated Fatty Acids = 50.00 ± 0.78%, Ratio of Saturation: Unsaturation approx. 1:2, Classification of Oil = Omega 3/6 rich Oil

Table 3. Fatty acids distribution in accessions of *D. edulis* from Ikom

Peak no.	Ret Time (Min)	Name of fatty acid	Mol. Wt	Mol. Form.	(%) Abundance
1	12.67	Pentadecanoic Acid	242	$C_{15}H_{30}O_2$	2.66±0.22
2	12.89	Decanoic Acid (Silver (+1))	172	$C_{10}H_{20}O_2$	0.07±0.01
3	13.04	Hexadecanoic Acid	256	$C_{16}H_{32}O_2$	12.84±1.03
4	13.28	Tridecanoic Acid	214	$C_{13}H_{26}O_2$	0.49±0.02
5	13.31	Tetradecanoic (Mysistic) Acid	228	$C_{14}H_{28}O_2$	0.77±0.12
6	13.55	Undecanoic Acid	186	$C_{11}H_{22}O_2$	5.03±0.21
7	13.45	Octadecanoic (Stearic) Acid	284	$C_{18}H_{36}O_2$	4.02±0.32
8	13.53	Methyl 9-cis,11-trans-Octadecadienoic Acid	294	$C_{19}H_{34}O_2$	1.19±0.11
9	13.78	(E,E)-9,12-Octadecadienoic (Linolelaidic) Acid	280	$C_{18}H_{32}O_2$	8.62±1.31
10	13.82	Methyl,10-trans, 12-cis-Octadecadienoic Acid	294	$C_{19}H_{34}O_2$	0.69±0.05
11	13.83	Trans -13-Octadecenoic (Brassidic) Acid	338	$C_{22}H_{42}O_2$	0.65±0.05
12	13.86	11-Octadecenoic (Vaccenic) Acid	282	$C_{18}H_{34}O_2$	1.24±0.24
13	13.90	9,12-Octadecadienoic (Linoleic) Acid	280	$C_{18}H_{32}O_2$	2.97±0.13
14	13.92	Cis-13-Octadecenoic Acid	282	$C_{18}H_{34}O_2$	3.29±0.21
15	13.95	Oleic acid	282	$C_{18}H_{34}O_2$	5.59±0.08
16	14.11	(Z,Z)-9,12-Octadecadienoic (cis, cis Linoleic) Acid	280	$C_{18}H_{32}O_2$	34.85±0.74
17	14.35	(9Z, 12E)-Octadecadienoic (Linoleic) Acid	280	$C_{18}H_{32}O_2$	2.07±0.14
18	16.50	15-Hydroxypentadecanoic Acid	258	$C_{15}H_{30}O_3$	1.19±0.06
19	17.79	Methyl 7,10-Hexadecadienoic Acid	266	$C_{17}H_{30}O_2$	0.80±0.02
20	19.00	2,6,10,14-Hexadecatetraenoic Acid, 3,7,11,15-tetramethyl-9-(phenylsulphonyl)-ethyl ester (E,E,E)	458	$C_{27}H_{38}O_4$	7.52±0.49
				Total	**96.55±2.42**

Values are expressed as mean ± SEM, n = 2, Total Saturated Fatty Acids = 27.07±1.75%, Total Unsaturated Fatty Acids = 69.48 ± 1.52%, Ratio of Saturation: Unsaturation approx. 1:2, Classification of Oil = Omega 3/6 rich Oil

Table 4. Fatty acids distribution in accessions of *D. edulis* from Nsukka

Peak No.	Ret Time (Min)	Name of fatty acid	Mol. Wt	Mol. Form.	(%) Abundance
1	12.69	Pentadecanoic Acid	242	$C_{15}H_{30}O_2$	2.11±0.12
2	13.20	n-Hexadecanoic Acid	256	$C_{16}H_{32}O_2$	16.53±1.14
3	13.26	Tetradecanoic Acid	228	$C_{14}H_{28}O_2$	1.29±0.04
4	13.30	Octadecanoic Acid	284	$C_{18}H_{36}O_2$	0.66±0.01
5	13.46	Tridecanoic Acid	214	$C_{13}H_{26}O_2$	2.41±0.07
6	13.50	Carbamodithioic Acid	93	CH_3NS_2	3.36±0.05
7	13.55	(Z,Z)-9,12-Octadecadienoic (linoleic) Acid	280	$C_{18}H_{32}O_2$	38.89±2.21
8	13.59	Cis-13-Octadecenoic Acid	282	$C_{18}H_{34}O_2$	3.29±0.06
9	13.89	(E,E)-9,12-Octadecadienoic (linolelaidic) Acid	280	$C_{18}H_{32}O_2$	1.39±0.08
10	13.91	(Z,Z)-9,15-Octadecadienoic Acid	310	$C_{18}H_{30}O_4$	1.25±0.03
11	13.93	Trans -13-Octadecenoic (Brassidic) Acid	338	$C_{22}H_{42}O_2$	0.91±0.14
12	13.95	(Z)-9-Octadecenoic (Oleic) Acid	282	$C_{18}H_{34}O_2$	0.44±0.05
13	13.96	(Z)-7-Octadecenoic (Oleic) Acid	282	$C_{18}H_{34}O_2$	2.19±0.17
15	14.36	Methyl, 9,12-Heptadecadienoic Acid	280	$C_{18}H_{32}O_2$	4.25±0.21
15	14.42	2-Hydroxy-1-(Hydroxymethylethyl)-Hexadecanoic Acid 15-Hydroxypentadecanoic Acid	330	$C_{19}H_{38}O_4$	0.76±0.11
16	16.10	Methylene bis[dimethyl]-Hexanoic Acid	256	$C_{15}H_{30}O_3$	1.64±0.02
17	16.35	1,2-Benzenedicarboxylic Acid (Phthalic Acid)	130	$C_7H_{14}O_2$	4.01±0.12
18	16.38	Bis (2-Ethyl, Hexyl)-Phthalic Acid	166	$C_8H_6O_4$	2.21±0.02
19	16.65	Didodecyl, Phthalic Acid	390	$C_{24}H_{38}O_4$	2.27±0.01
20	16.73	19,19-Dimethyl-Eicosa-8,11-Dienoic Acid	502	$C_{32}H_{54}O_4$	0.53±0.04
21	27.76		336	$C_{22}H_{40}O_2$	6.83±0.37
				Total	**97.22±2.17**

Values are expressed as mean ± SEM, n = 2, Total Saturated Fatty Acids = 29.41±1.27%, Total Unsaturated Fatty Acids = 59.44±1.17%, Ratio of Saturation: Unsaturation approx 1:2, Classification of Oil = Omega 3/6 rich Oil

Table 5. Composition of saturated, monounsaturated, polyunsaturated fatty acids in the four accessions of Akpabuyo, Ikot Ekpeya, Ikom and Nsukka locations

Fatty Acid Type	Location and composition			
	Akpabuyo	Ikot Ekpeya	Ikom	Nsukka
Total SFAs (%)	33.51±1.04	39.87±1.72	27.07±1.75	29.41±1.27
Total MUFAs (%)	5.57±0.08	3.14±0.22	10.77±0.55	6.83±0.74
Total PUFAs (%)	55.24±1.15	46.86±1.22	58.71±2.33	52.61±1.58
Summary				
Total SFAs (%)	33.51±1.04	39.87±1.72	27.07±1.75	29.41±1.27
Total TUFAs (%)	60.81± 0.95	50.00 ± 0.78	69.48 ± 1.52	59.44±1.17
TSFAs : TUFAs	1:2	1:2	1:2	1:2
Classification of oil	Omega 3/6	Omega 3/6	Omega 3.6	Omega 3/6

SFAs = Saturated fatty acids; MFUs = Monounsaturated fatty acids; PUFAs = Polyunsaturated fatty acids; TSFAs = Total saturated fatty acids; TUFAs = Total unsaturated fatty acids

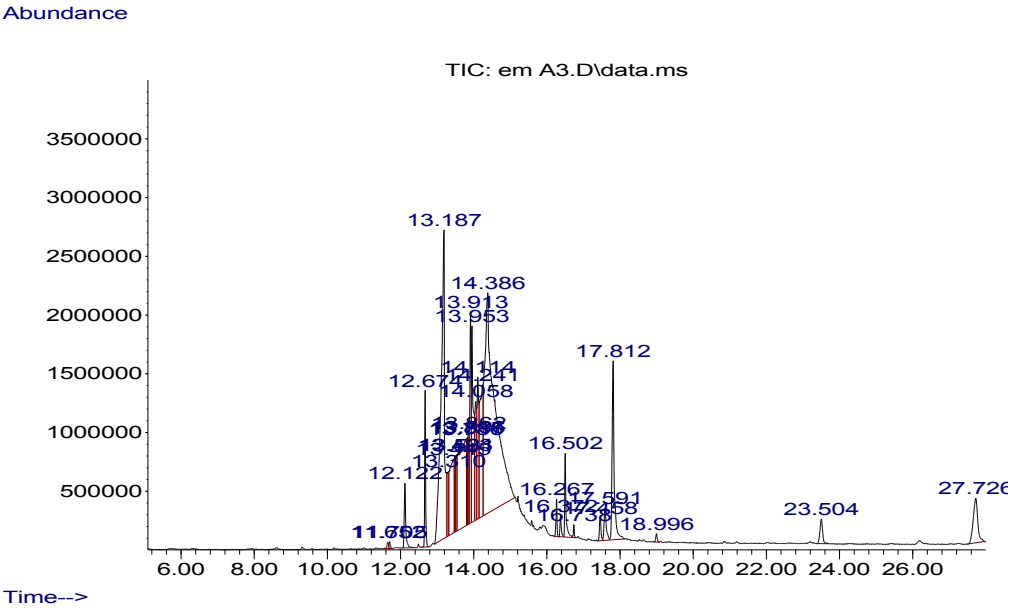

Fig. 2. GC-MS chromatogram of fatty acid distribution in *D. edulis* from Akpabuyo

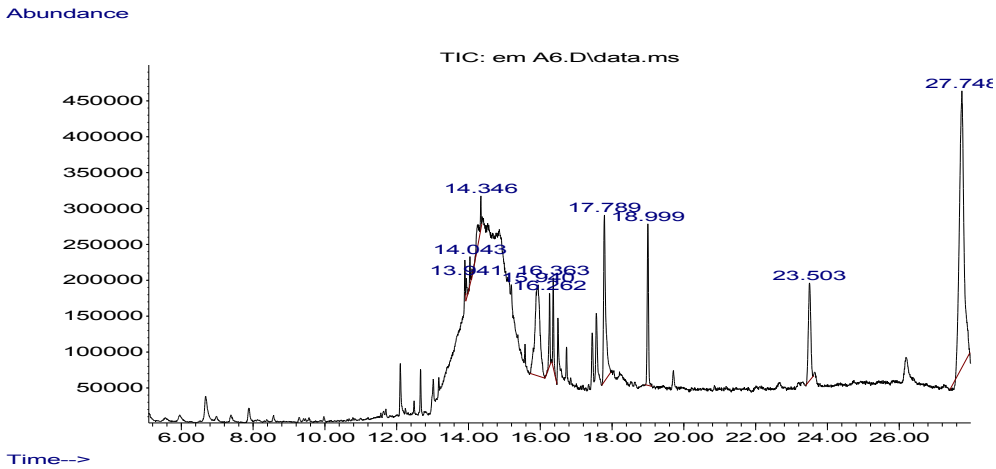

Fig. 3. GC-MS chromatogram of fatty acid distribution in *D. edulis* from Ikot Ekpeya

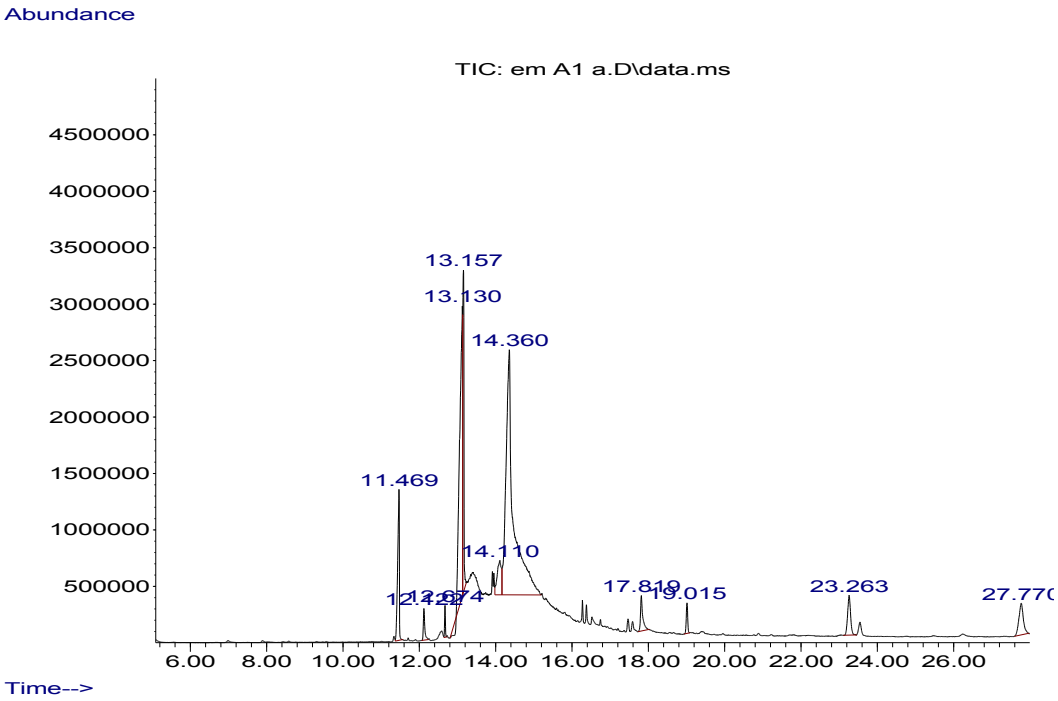

Fig. 4. GC-MS chromatogram of fatty acid distribution in *D. edulis* from Ikom

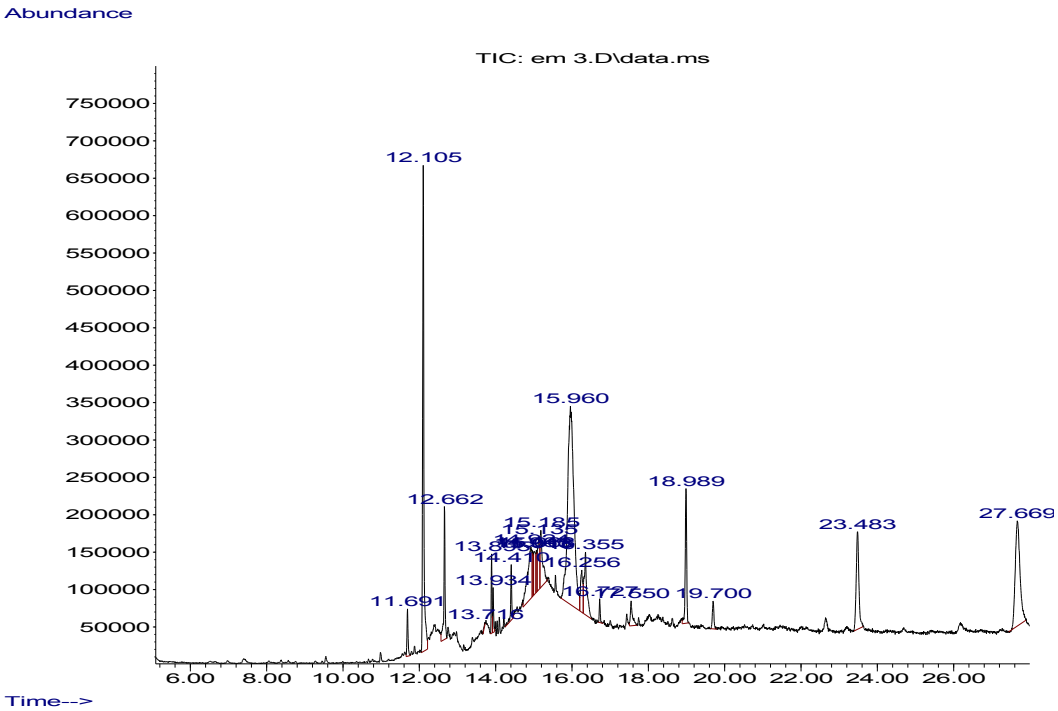

Fig. 5. GC-MS chromatogram of fatty acid distribution in *D. edulis* from Nsukka

Oleic acid (Z-9-Octadecenoic acid), and its isomer Z-7-Octadecenoic acid was found to be present at 0.80-2.6% in the four locations studied. It was found mainly in Nsukka samples (2.6% total abundance). Oleic acid is a member of the fatty acids group known as decenoic acids.

Oleic acid is a monounsaturated omega-9 fatty acid that occurs widely and naturally in animal and vegetable oils. It is the most commonly found fatty acid in human cells which is why it is not considered an "essential fatty acid" like omega-3 and omega-6 oils. Oleic acid was reported to have enormous benefits to the heart, brain, mood, skin cells and waistline. It is an antioxidant which prevents oxidative stress leading to various health benefits including anti-cancer and anti-ulcer effects [21,22,23].

We also reported special fatty acid in *D. edulis* (Trans-13-Octadecenoic or Brassidic acid). It could be described as a long chain monounsaturated Trans-(E) double-bond C22-carbon atom fatty acid denoted as 22:1ω9. It is the Trans-Docos-13-enoic acid which is the trans-isomer of Erucic acid. It is absent in *D. edulis* samples from the southern part of the Eastern region of Nigeria, but present at less toxic levels in the upland and more northern parts of the region including, Ikom (0.65%) and Nsukka (0.91%).

Cinnamic acid detected in this study was found only in Akpabuyo and Ikot Ekpeya samples, but absent in Ikom and Nsukka samples. Cinnamic acid is an organic compound with the molecular formula $C_9H_8O_2$. It is a white crystalline compound that is slightly soluble in water, and freely soluble in many organic solvents including glycerol. It is classified as an unsaturated carboxylic acid, and occurs naturally in many plants. Trans-Cinnamic acid is used in the manufacture of flavors, dyes, and pharmaceuticals; but its major use is for the production of its methyl, ethyl, and benzyl esters. These esters are important components of perfumes. Cinnamic acid is also a precursor to the sweetener aspartame, which is widely used in food and beverages as flavouring agent [24].

A significant finding was the presence of an Eicosadienoic fatty acid derivative (19,19-Dimethyl-Eicosa-8,11-Dienoic acid) in *D. edulis* samples from Nsukka. This fatty acid did not appear in the other three locations studied. Its molecular formula is $C_{22}H_{40}O_2$, and belongs to the family of the Eicosadienoic acids. Eicosadienoic acids are polyunsaturated omega-6 acids (20:3n6). It is the elongation product of γ-linoleic acid (GLA), and the direct precursor of DGLA. Levels of this fatty acid in the plasma reflect the levels of all other polyunsaturated omega-6 fatty acids in plasma [25].

The presence of oleic acid (omega-9), Linoleic acid (omega-6) and linolenic acid/linolelaidic acid (omega-3 and 6), shows that *D. edulis* is rich in omega oils. Both omega-3 and some omega-6 oils are essential polyunsaturated fatty acids with well established health benefits [21,22,23,25]. Omega oils are believed to have a wide range of benefits, including anti-oxidative properties, anti-cancer, anti-inlammatory, anti-osteoporotic, cardioprotective and neuroprotective effects [9,26]. Omega fatty acid oils are used for the prevention of heart attacks, lowering of high blood pressure, lowering of cholesterol, and for the reversal or "hardening of the blood vessels" a condition referred to as atherosclerosis [21,22,23,25]. There are several reports, suggesting that linoleic acid and its isomeric forms, and other polyunsaturated fatty acids, enhanced bone formation [27,28]. Thus, omega fatty acids are responsible for stronger bones formation by blocking of excess production of an inflammatory substance known as PGE2 [28]. Linoleic and linolenic acids are also known to possess body weight management properties including the reduction of body fat and increase in lean muscle mass. These are some of the potential benefits which *D. edulis* may confer on populations consuming the fruit.

Trans -13-Octadecenoic (Brassidic) acid was found at 0.65% in Ikom accessions, and 0.91% in Nsukka accessions. These two locations are the most northern in the Eastern region of Nigeria. The presence of brassidic acid in these two locations may be soil type-dependent, and dependent on climatic variables. Therefore, the presence and absence of some fatty acids as observed in this study may be affected by climatic variables and soil type. The presence of trans-fatty acid (TFA) in these two locations may be significant in that, the trans fatty acid occurred at low concentrations. A number of studies have shown an association of TFA consumption and increased risks of cardiovascular disease (CVD) [29,30]. This increased risk was said to be attributable to the association between trans fatty acids (TFA) and increase in the ratio of LDL cholesterol to HDL cholesterol [29,30].

In this study, the concentration of total unsaturated fatty acids (TUFAs) showed that the total monounsaturated fatty acids (TMUFAs) are present at a concentration of 3.14±0.22-10.77±0.55%, while the total polyunsaturated fatty acids (TPUFAs) content is 46.86±1.22-58.71±2.33% (Table 5). This discovery is consistent with the values of unsaturated fatty

acids previously found in different oil seeds including, sunflower, soybean, peanut and olive oil.

4. CONCLUSION

The West Africa Pear (*D. edulis*) containing oleic acid and linoleic acid which are key unsaturated fatty acids in nutrition interventions could be substitutes for olive oil in healthy normo-cholesterolemic human diets. The presence of oleic, linoleic, linolenic, linolelaidic acids and several forms of polyunsaturated fatty acids in *D. edulis* suggests that the oil is rich in MUFA and PUFA of omega-3, omega-6 and omega-9 origin. It was concluded that, the ratio of TSFAs to TUFS (saturation to unsaturation) in *D. edulis* is 1:2; and the concentration of MUFA and PUFA suggest that the oil should be classified as Omega oil.

Two novel polyunsaturated fatty acids namely; Methyl,9,12-Heptadecadienoic acid and 2,6,10,14-Hexadecatetraenoic acid were identified in the present study. The study showed that linoleic acid an ω-6 (C18:2) fatty acid and its isomeric forms (Z,Z and E,E) are undoubtedly the most widely distributed unsaturated fatty acids in the West African pear (*D. edulis*), and this is consistent with previous reports linoleic acid is the most important polyunsaturated fatty acid in abundance and in distribution in the plant kingdom. Its concentration in *D. edulis* compares favourably with the concentration found in several oil seeds including soybean, sunflower, peanut and olive oil. We concluded that oil from *D. edulis* may provide cardio-protective benefits to humans consuming the fruit. We further concluded that the composition, distribution and variation of fatty acids in *D. edulis* between the four locations studied may be attributable to differences in soil types, hydrology and climatic variables.

5. RECOMMENDATIONS

The researchers are open to collaboration with researchers from any part of the world that would be interested in carrying out further studies on isolation and characterization of secondary compounds, and studies on endocrinology and metabolism.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ajayi AI, Adesanwo O. Comparative study of the mineral element and fatty acid composition of *Dacryodes edulis* pulp and seed. World Journal of Agricultural Sciences. 2009;5(3):279-283.
2. Ibanga O. Isaac, Okon D. Ekpa, Udofot J. Ekpe. Extraction, characterization of African pear (*Dacryodes edulis*) oil and its application in synthesis and evaluation of surface coating driers. Inter. J. Adv Res. Cheml Sci. 2014;1(4):14-22.
3. Ene-Obong Henrietta, Igile Godwin, Ekpo Anthonia, Egbung G. Eneji, Agbo Michael. Variations in the nutrients and bioactive compounds of different accessions of the West African pear (*Dacryodes edulis*): Implications for dietary intake assessment and health. J. Food Comp. Anal. 2019;79:80-86.
4. Ikhuoria EU, Maliki M. Characterization of Avocado pear (*Persea americana*) and Africa pear (*Dacryodes edulis*) extracts. African J. Biotech. 2007;7:950-954.
5. Onntag NO. Structure and composition of fats and oils. In Bailey's Industrial Oil and Fat Products, Vol. 1, 4th Edition. Edited by Daniel Swern. 1979;1-83.
6. Lunn J, Theobald HE. The health effects of dietary unsaturated fatty acids. British Nutrition Foundation, Nutrition Bulletin. 2006;31:178-224.
7. Umoti U, Okyi DA. Characteristics and composition of oil and cake of African pear. J. Sci., Food Agr. 1987;38:67–72.
8. Shimura T, Miura T, Usami M, Ishihara E, Tanigara K, Ishida H, Sieno Y. Docohexanoic acid (DHA) improved glucose and lipid metabolism in KKay mice with genetic non-insulin dependent diabetes mellitus (NIDDM). Biol. Pharm. Bull. 1997;20:507-510.
9. Morgan NG, dhayal S. Unsaturated fatty acids as cytoprotective agents in the pancreatic β-cell. Prostaglandins Leukot. Essent. Fatty Acids. 2010;82:231-236.
10. Acosta-Montano P, Garcia-Gonzalez V. Effects of dietary fatty acids in pancreatic β-cell metabolism, implications in homoestasis. Nutrients. 2018;10(4):1-14.
11. McColl J. An introduction to essential fatty acids in health and nutrition. Bioriginal Reports; 2019.
12. Cimen I, Kocatark B, Koyuncu S, Tufanli O, Onat UI, Yildirin AD. Prevention of Atherosclerosis by bioactive palmitoleate

through suppression of organelle stress and inflammasome activation. Sci. Transl. Med. 2016;8:358,358ra126.
13. Carta G, Murru E, Lisai S, Sirigu A, Piras A, Banmi S. Dietary triacylglycerol with palmitic acid in sn-2 position modulate levels of N-acylethanolamides in rat tisues. PLoS ONE. 2015;10:1-12,e0120424.
14. Carta G, Murru E, Banmi S, Manca C. Palmitic acid: Physiological role, metabolism and nutritional implications. Frontiers in Physiology. 2017;8:1-14.
15. Igile GO, Iwara AI, Ekpe OO, Isika AI, Mgbeje BIA. Antihypertensive and electrolyte modulatory potential of Vernonioside E (Steroidal Saponin) from *Vernonia amygdalina* (*Asterecea*) in adult albino wistar rats. World Journal of Advance Healthcare Research. 2018;2(4): 2457-0400.
16. Igile GO, Okoi UL, Iwara IA, Eteng MU. Volatile organic constituents of two fractions of leaves of *Ficus vogelii* and their potential health implication. Journal of Chemical, Biological and Physical Sciences. 2018;8(4):811-819.
DOI: 10.24214/jcbps.B.8.4.81119
17. Bellaloui N, Bruns HA, Reddy KN. Agricultural practices altered soybean seed protein, oil, fatty acids, sugars and minerals in the Mid-South USA. Front. Plant Sci. 2015;6:31.
18. Lorz PM, Towae FK, Enke W, Jackh R, Bhargara N, Hillesheim W. Phthalic acid and derivatives. In: Ullmann's Encyclopaedia of Industrial Chemistry; 2007.
DOI: 10.1002/14356007.020_181pub2
19. Toyama Y, Tsuchiya T. The highly unsaturated acids in sardine oil X. The separation of highly unsaturated C28-acids. Bull. Chem. Soc. Japan. 1935;10: 543-547.
20. Lim J, Oh J, Lee H. Trans-11, 18:1 vaccenic acid has a direct anti-carcinogenic effect on MCF-1 human mammary adenocarcinoma cells. Nutrients. 2014;6(2):627-636.
21. Haug A, Hostmark T, Inadera H. Bovine milk in human nutrition – A review. Lipids in Health and Disease. 2007;6:25.
22. Sumich AL, Matsudaira T, Heasman B, Gow RV, Ibrahimovic A, Taylor E. Fatty acid correlates of temperament in adolescent boys with attention deficit hyperactivity disorder. Prostaglandins Leukot. Essent. Fatty Acids. 2013;88:431-436.
23. El-Fakharany EM, Abu-Serie MM, Litus EA, Permyakov SE, redwan EM. The use of human, bovine and carmel milk albumins in anticancer complexes with oleic acid. Protein Journal. 2018;37:203.
24. Sidhu JS, Zafar TA. Bioactive compounds in banana fruits and their health benefits. Food Quality and Safety. 2018;2(4):183-188.
25. Henderson RA, Jesen RG, Lammi-Keefe CJ, Ferris AM, David KR. Effect of fish oil on the fatty acid composition of human milk and maternal and infant erythrocytes. Lipids. 1992;27(11):863-869.
26. Wedland E, Farmer A, Glasziou P, Neil A. Effect of alpha linolenic acid on cardiovascular risk markers: A systematic review. Heart. 2006;92:166–169.
27. Sharif PS, Asalforoush M, Ameri F, Larijani B, Abdullahi M. The effect of fatty acids on bone biomarkers in Iranian postmenopausal osteoporotic women: A randomized clinical trial. Springer, Age. 2009;32:179-186.
28. Dansgaard CJ, Molgaard C, Matthiessen J, Laritzen L. The effects of n-3 long chain polyunsaturated fatty acids on bone formation and growth factors in adolescent boys. Pediatric Research. 2012;71:713-719.
29. Bhardwaj S, Passi SJ, Misra A. Overview of trans fatty acids: Biochemistry and health effects. Diabetes Metab. Syndr. 2011;5(3):161-164.
30. Igbal MP. Trans fatty acids – A risk factor for cardiovascular disease. Pakistan Journal of Medical Sciences. 2014;30(1): 194-197.

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