Vol. 16(1), pp. 24-31, January-March 2022
DOI: 10.5897/AJBR2017.0927
Article Number: 8AD4B5168613
ISSN 1996-0778

http://www.academicjournals.org/AJBR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Biochemistry Research

Full Length Research Paper

Changes in lipid class and fatty acid composition during the development of African pear (*Dacryodes edulis*) fruit pulp

Uhunmwangho E. S.[1*] and Omoregie E. S.[2]

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, University of Medical Sciences, Ondo City, Ondo State, Nigeria.
[2]Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

Received 7 January, 2017; Accepted 19 April, 2017

The pulp of the African pear (*D.edulis*) fruits were investigated for its oil compositions, major lipid classes and constituents fatty acids from 4 weeks after anthesis (WAA) to fruit maturation. The oil was extracted with n-hexane using soxhlet extractor and characterised by gas chromatography. Fatty acid profile showed a saturated acid content of between 19.5 and 36.5% and unsaturated fatty acid content of 63.5 to 80.5%. Fractions of different fatty acids were synthesised at different stages of fruit development and the predominant fatty acids were palmitic acid (31.0%), stearic isomer (20.9%), oleic acid (7.1%) and linoleic acid (43.8%) at matured stage (20 WAA) of fruit development and 10.4, 12.9, 22.7 and 39.8% respectively at the immature stage (4 WAA) of fruit development. Polyunsaturated fatty acids were not detected in the African pear pulp oil throughout maturation. The major phospholipids were phosphatidylcholine (65.2%), phosphatidylinositol (25.8%) and phosphatidylethanolamine (8.9%), while phosphatidylserine (PS) and lysophosphatylcholine (LPC) remains as traces throughout fruits maturation. The pulp major sterol lipids were sitosterol accounting for about 71.3% and campesterol 12.4% of the total sterol lipids. The immature fruit pulp glyceride lipids were diacylglycerides (DAG) and triacylglyceride (TAG) which accounted for 70.2 and 18.6% respectively. In mature fruit pulp, TAG had a dramatic increase to 72.5% while DAG decreased to 22.1%. Major changes occurred in the TAG with fruit maturity with increased in concentration to 72.5% at 20 WAA. Based on these changing patterns of lipid fractions with fruit maturity, possible pathways of TAG synthesis have been proposed. In conclusion, the results at the 18 to 20 WAA showed that high quantities of essential fatty acids are present in the African pear pulp oil at mature stage of the fruits.

Key words: *Dacryodes edulis,* anthesis, n-hexane, gas chromatography, triacylglyceride.

INTRODUCTION

African pear (*D. edulis*) is well known plants in West Africa. The fruits are edible and the bark, leaves, stem and roots are used in ethno medicinal practice for treatment of diseases (Ajibesin et al., 2008). *D. edulis* is an indigenous fruit tree in the humid low lands and plateau regions of West, Central African and Gulf of Guinea countries. It belongs to the Burseraceae family, an evergreen tree indigenous to the Central Africa and Gulf of Guinea regions. The genus name is derived from the Greek word '*Dakruon*'(a tear) in reference to the resin

droplets that appears on the bark surface of its species (Burkill, 1985; Edem et al., 2009). The species-specific name *edulis* means edible (Anonymous, 2011a). The genus *Dacryodes* comprises about 40 species occurring in the American, Asian and African tropics. In Africa, about 20 species have been described (Anonymous, 2011b). In South-East Nigeria, the trees are grown around homesteads and flowering takes place from January to April. The major fruiting season is between May and October (Kengue and Nyagatchou, 1990).

D. edulis is a tree cultivated widely for its edible and nutritious fruits. Generally, the fruit may be soaked in hot water, or roasted/baked in an oven at about 50°C. The roasted fruit can be eaten with maize, plantain, cassava, cocoyam and bread (Sofowora, 2008).

The entire plant has pharmaceutical properties that are variously exploited by many African communities (Kengue, 2002). For instance, in the Western parts of Cameroon, the bark is crushed and used in concoctions against dysenteries while in Central Cameroon the bark is used to treat toothache. The leaves are boiled in combination with *Lantana camara, Cymbopogon citratus* and *Perseaamericana* to yield a steam bath taken to treat fever/headaches and malaria in Republic of Congo. The leaves and seed are used in Nigeria for animal feed (Ajibesin et al., 2008); the resin from the bark has long been reported to be effective against parasitic skin diseases and jiggers.

D. edulis is a versatile plant in African ethnomedicine as its various parts are employed to treat several diseases. The bark of the plant has long been used to cicatrize wound in Gabon (Adebayo-Tayo and Ajibesin, 2008). In Democratic Republic of Congo, the plant is employed for the treatment of leprosy, tonsillitis and dysentery, anaemia, spitting blood, pains and stiffness and skin diseases (Adebayo-Tayo and Ajibesin, 2008). In Congo Brazzaville, the leaves are boiled with those of *Lanata camara*, *Cymbopogon citretus* in water to form a decoction for treating malaria (Ikhuoria and Maliki, 2007). The bark resin is used in Nigeria to treat parasitic skin diseases and jiggers. When applied in lotions and creams, the resin smoothens and protects the skin. The leaves are often crushed and the juice used to treat generalized skin diseases such as scabies, ringworm, rashes and wounds, while the stems are employed as chewing sticks for oral hygiene (Ajibesin et al., 2008).

The essential oil of the plant has been shown to possess potent antibacterial activity against *Staphylococcus aureus, Bacillus aurens, Escherichia coli* and *Proteus minibillis* (Koudou et al., 2008; Okwu and Nnamdi, 2008). No part of *D. edulis* is known to be toxic (Ajibesin et al., 2008; Dike, 2010).

Fruit pulps form a major part of the diet of Nigerians, consumed as a meal as well as ingredients of local soups. Despite the increased popularity of this fruit pulp, *Dacryodes edulis* have not been used to produce oil on an industrial scale, or cultivated systematically because of lack of basic chemicals and biological knowledge of their values. The present work was carried out with a view to studying the oil potential and determining the suitability of *D. edulis* pulp oil for edible and/or industrial purposes.

MATERIALS AND METHODS

Plant materials

Matured fruits of African pear were collected from private farm land in Okada Town of Ovia North-East LGA of Edo State, Nigeria. The fruits were authenticated by the Department of Botany, Faculty of Sciences, University of Medical Science, Ondo City, Nigeria. A voucher specimen of each plant was there after deposited in the herbarium of the same Department.

Preparation of sample

Forty fruits were collected randomly from each of the studied trees at biweekly intervals starting from the fourth week after fruit set until senescence. The collected fruits were cleaned with a moist soft cotton wool and then the seeds were carefully separated from the fruits. Part of the separated pulp and nut were immediately used for the moisture content and oil extraction, while the remaining part was dried at 65°C for 4 h in an oven, crushed with a laboratory mortar and pestle and kept in a well labelled air tight polythene bags or screw-capped bottles at 4°C for subsequent biochemical analysis.

All reagents used were of analytical grade purchased from Sigma Chemicals Co, London, and BDH Chemicals Ltd., England.

Extraction of oil

The soxhlet extraction method was employed. The sample (5 g) was weighed into a weighed filter paper and folded neatly. This was placed inside the pre-weighed thimble (W_1). The thimble with the sample (W_2) was inserted into the soxhlet apparatus and extraction under reflux was carried out with the n-hexane (40-60°C boiling range) for 6 h. At the end of extraction, the thimble was dried in the oven for about 30 min at 100°C to evaporate off the solvent and was cooled in a desiccator and later weighed (W_3). The fat extracted from a given quantity of sample was then calculated.

Calculations

% Crude Fat (W/W) = [Loss in Weight Sample/Original Weight of Sample] × 100

*Corresponding author. E-mail: esosasam@yahoo.com. Tel: +2348128819787.

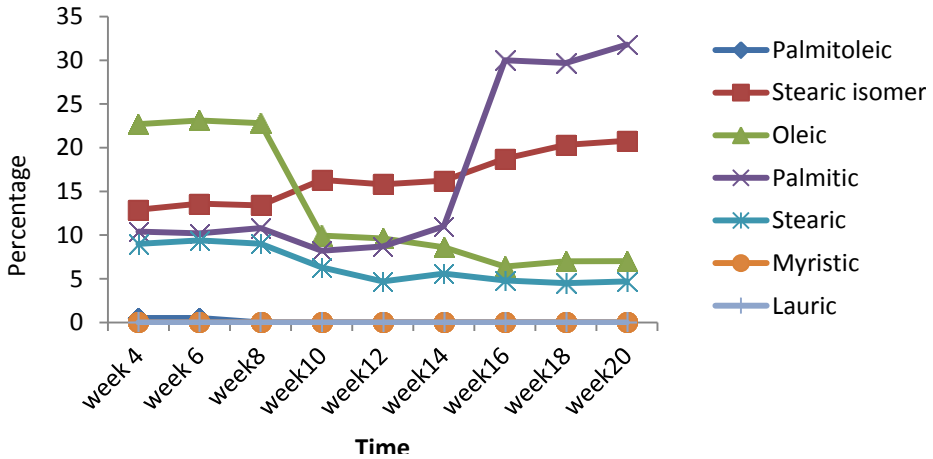

Figure 1. Saturated fatty acids composition in *D. edulis* pulp oil 4-20th WAA of fruits development. Values are mean ± SEM.

Determination of fatty acids

Fatty acids were determined according to the method of Manni and Caron (1995) as reported by Siedlecka et al. (2008). The lipids were converted to methyl esters by refluxing for 1 h with methanolic H_2SO_4 at 70°C. Fatty acid esters (FAME) were analysed on a 6820 gas chromatography (GC) system (Agilent Technologies). A FFAP capillary column (185^{o}C temperature) was used, 30 m × 250 µm × 0.25 µm (Quadrex Corporation). Carrier gas - nitrogen, flow 1.0 ml/per min, detector - FID, temperature programme used: 60 to 200°C (20°C/min, 10 min), injector, 250°C, detector, 300°C. The samples were dosed by a HT 300A automatic dosing device at an injection size of 1 µl of 5 to 10% heptane of methyl esters using the split method and a 30:1 splitting ratio. The needle was then withdrawn and noted the formation of a small peak on the chart paper due to solvent making start reference point.

The reference standard mixture of known composition was analysed in the operating conditions as those employed for the sample and the retention times for the common fatty esters were measured. The resulting peaks for the sample were identified from the graph. Fatty acids appeared on the chart in increasing number of carbon atoms and increasing unsaturation.

Phytosterol lipids analyses were determined according to the standard method of AOAC 994.10 (1996a), phospholipids were determined according to Raheja et al. (1973) and glycerides analysis followed the procedure of the ASTM (2012).

Statistical data analysis

All values are expressed as the means ±SEM of triplicate determinations. All statistical analyses are performed using Graph Pad Prism version 6. To test for differences between the group means, one way analysis of variance (ANOVA) is employed. Significant differences between the means are determined by Duncan's multiple range tests, and P values < 0.05 are regarded as significant (Kyari, 2008).

RESULTS

Nineteen FAs were determined in *D.edulis* fruits oil extracted biweekly intervals starting from the fourth week after fruit set until senescence is shown in (Figures 1 to 5). The notable FAs contained in extracted oil from *D. edulis* pulp fruits were palmitic, stearic, stearic isomer, oleic and linoleic acid. The pattern of oil accumulation in *D. edulis* fruits at different physiological maturity stages is presented in Figures 4 and 5.

The results (Figure 4) revealed the presence of monoglyceride, diglyceride and triglyceride.

DISCUSSION

Determination of oil content in plants is important because it predicts the profitability of given plants as potential source of oil. High oil content in plant seeds implies that processing them for oil would be economical (Ikhuoria et al., 2008). Fatty acids are consumed in a wide variety of end use industries as food, medicine, rubber, plastics, detergents and cosmetics (Gunstone, 1996). The fatty acid composition of *D. edulis* (Table 1) showed palmitic acids as the major saturated fatty acids. Stearic isomer and linoleic acid were the major unsaturated fatty acids in the matured fruits (20 WAA). This is in agreement with earlier reports by Umaru and Dere (1986) who reported high palmitic acid and linoleic acids in *D.edulis* pulp oil. The concentration of linoleic acid in *D. edulis* pulp oil fell within the range (38.2 to 43.8%) (Table 2) which is higher than what is found in common edible oil, such as cotton seed, grape seed, canola oil, soybean oil, corn oil and sunflower oil (Dubois et al., 2007). In the early stages of maturity, oleic acid was highly concentrated, but declined as the fruit matures. This was probably used up as energy for growth or for synthesis of other compounds (Ikhuoria et al., 2008). The most important edible oils are those containing palmitic, stearic, oleic and linoleic acids, of which oleic and linoleic acids are most valuable (Wang et al., 2012). Also, this study showed that *D. edulis* fruit oils

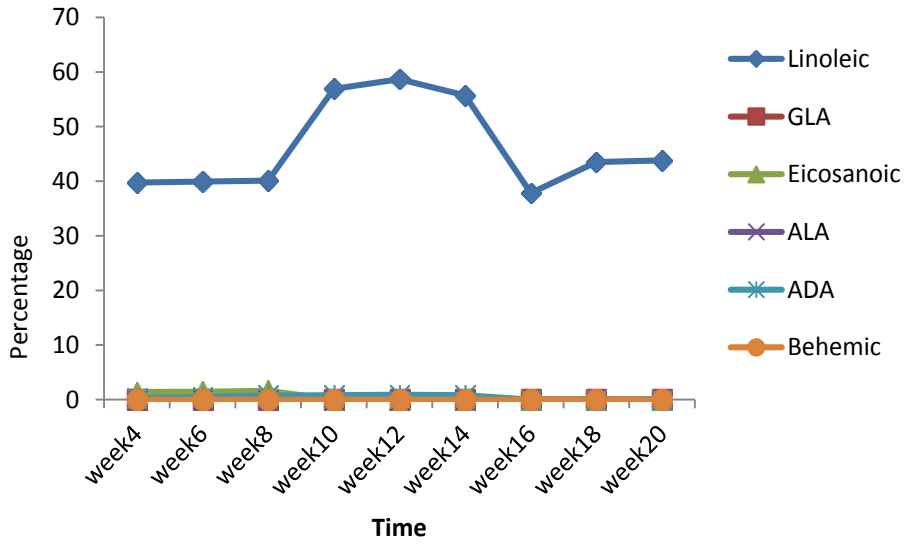

Figure 2. Composition of polyunsaturated fatty acids in *D. edulis* pulp oil 4-20th WAA of fruits development. Values are mean ± SEM.

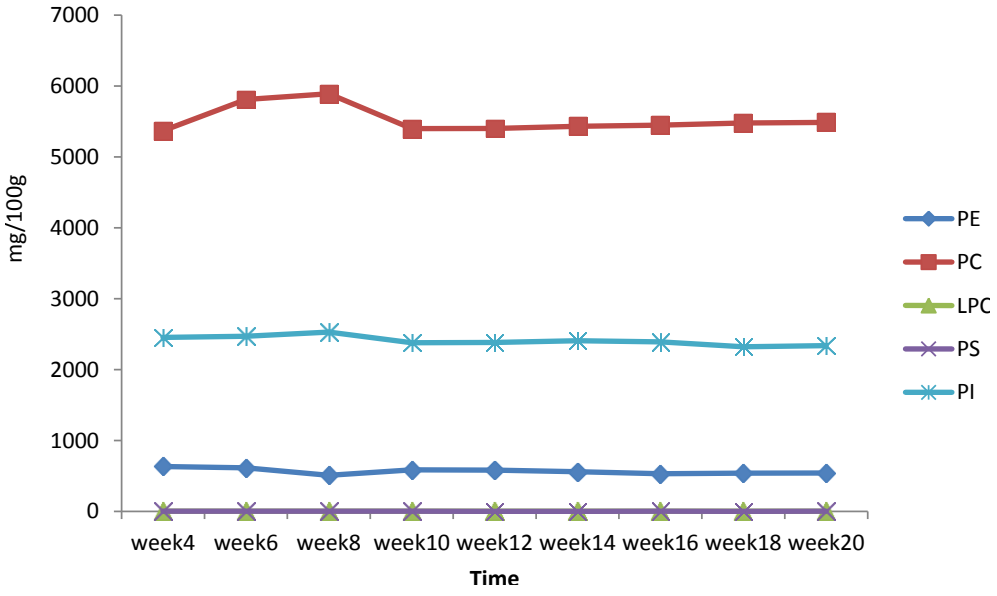

Figure 3. Phospholipids Composition in *D. edulis* pulp oil 4-20th WAA of fruits development. Values are mean ± SEM.

are rich in palmitic acid which is the principal composition of cooking oil (Wang et al., 2012).

The present study revealed that increase in the synthesis of triacylglycerols was accompanied by corresponding decrease in the level of diacylglycerides (Table 4) throughout the period of maturation (4 to 20 WAA) of the fruit (Table 3). The pattern of accumulation of triacyglycerides (TAG) indicate possible pathway that may be involved in their synthesis. In the plants, large amounts of free fatty acids (FFA) characterised the immature stage (4 to 12 WAA) with high levels of oleic acid, stearic acid and palmitoleic acid while the amount of TAGs formed was low. However, as the fruits mature, there were decreases in stearic, oleic and palmitoleic acids levels with a dramatic increase in TAG level. The reductions in the levels of FFAs with a corresponding dramatic increase in the TAG content suggest that most of the available FFAs were incorporated into TAG synthesis. This pattern of synthesis and lipid accumulation was observed in previous studies of some tropical oil

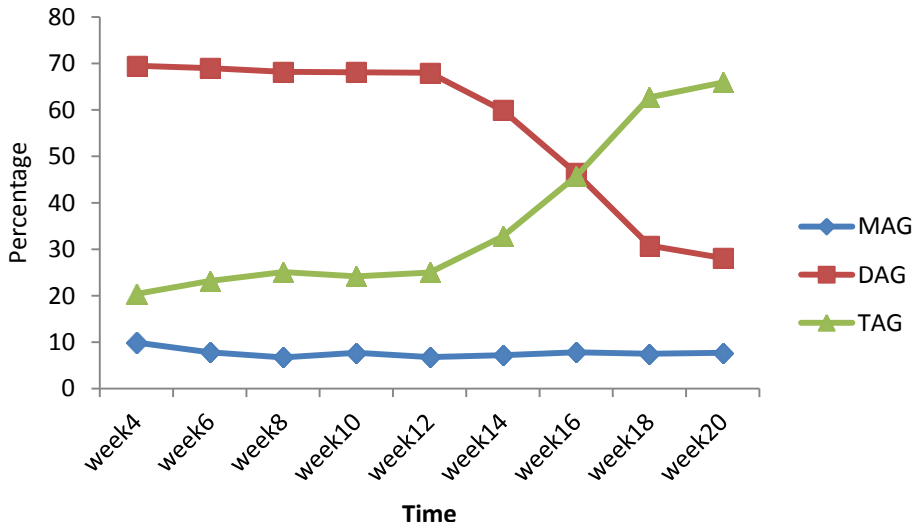

Figure 4. Composition of Neutral lipids in *D. edulis* pulp oil 4-20th WAA of fruits development. Values are mean ± SEM.

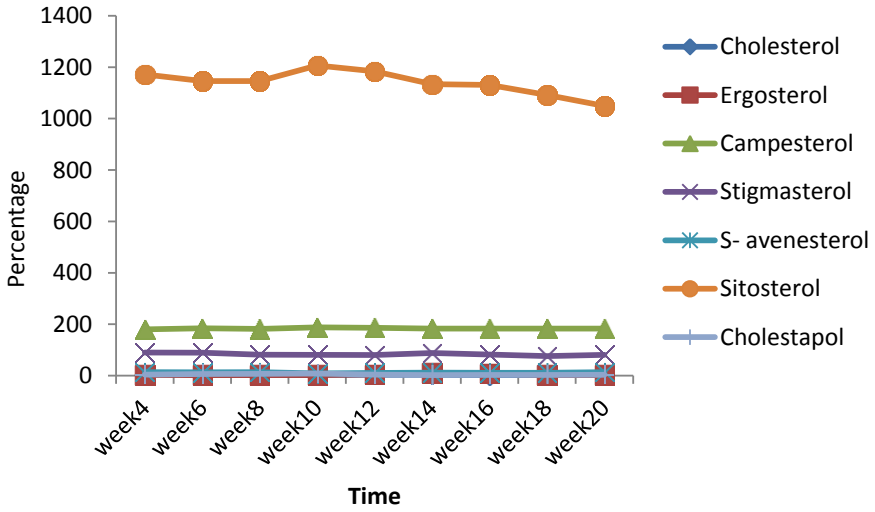

Figure 5. Percent sterol lipids in *D. edulis* pulp oil 4 to 20th WAA of fruits development. Values are mean ± SEM.

Table 1. Fatty acid composition of *D. edulis* at week 4 to 20 of fruit development.

Week	Lauric C12:0	Myristic C14:0)	Palmitic C16:0	Palmitoleic C16:1 Cis9)	Stearic C18:0	Stearic isomer (C18:1 (Cis 6)	Oleic C18:1 (Cis 9)
4	ND	ND	10.41±0.06	0.51±0.01	9.00±0.01	12.91±0.04	22.71±0.06
6	ND	ND	10.22±0.02	0.52±0.03	9.41±0.04	13.60±0.03	23.11±0.08
8	ND	ND	10.85±0.01	ND	9.02±0.02	13.38±0.08	22.81±0.03
10	ND	ND	8.23±0.31*	ND	6.31±0.03*	16.32±0.04*	9.95±0.10*
12	ND	ND	8.68±0.04*	ND	4.71±0.07*	15.81±0.05*	9.62±0.10*
14	ND	ND	11.00±0.03	ND	5.62±0.09*	16.22±0.03*	8.61±0.07*
16	ND	ND	30.90±0.04*	ND	4.80±0.07*	18.72±0.01*	6.40±0.06*
18	ND	ND	29.70±0.06*	ND	4.51±0.08*	20.32±0.08*	7.02±0.09*
20	ND	ND	31.81±0.08*	ND	4.70±0.08*	20.80±0.11*	7.02±0.01*

Values are mean ± SEM (* = P<0.05) compared with week 4. ND = Not detected.

Table 2. Fatty acid composition of *D. edulis* at weeks 4 to 20 of fruit development.

Week	Linoleic C18:2 (Cis9,12)	GLA C18:3 (Cis6,9,12)	Eicosenoic C20:1 (Cis11)	ALA C18:3 (Cis912,15)	EDA C20:2 (Cis 11,14)	Behenic C22:0	% Unsaturation	% Saturation
4	39.76±0.08	ND	1.41±0.01	ND	0.30±0.03	ND	80.50±0.81	19.51±0.031
6	39.92±0.06	ND	1.42±0.06	ND	0.50±0.03	ND	78.88±1.34*	20.10±0.56
8	40.11±0.10	ND	1.60±0.08	ND	0.73±0.04	ND	83.24±0.36*	15.30±0.81*
10	56.92±0.03*	ND	ND	ND	0.80±0.02	ND	84.20±0.611*	14.22±1.31*
12	58.70±0.08*	ND	ND	ND	0.90±0.08	ND	80.12±0.85	17.82±0.08*
14	55.71±0.13*	ND	ND	ND	0.81±0.04	ND	62.90±0.56*	35.29±0.73*
16	37.82±0.04*	ND	ND	ND	ND	ND	70.80±1.76*	28.53±0.61*
18	43.50±0.08*	ND	ND	ND	ND	ND	65.81±0.76*	34.22±1.33*
20	43.80±0.33*	ND	ND	ND	ND	ND	63.50±0.59*	36.52±0.61*

Data are the average of three replicates ± SEM (P>0.05) compared with week 4. ND = Not detected.

Table 3. Phospholipids composition of *D. edulis* at week 4-20 of fruit development.

Week	PE (mg/100 g)	PC (mg/100 g)	PS (mg/100 g)	LPC (mg/100 g)	PI (mg/100 g)
4	633.62±0.91	5370.50±0.10	2.54±0.08	3.61±0.13	2455.72±0.41
6	614.11±0.44*	5811.71±1.31*	2.57±0.04	3.08±0.36	2471.81±0.51*
8	509.86±0.71*	5891.77±0.93*	2.55±0.46	3.16±0.78	2530.04±1.06*
10	586.12±0.36*	5400.96±1.34*	2.31±0.31	3.29±0.07	2380.71±0.81*
12	582.17±0.04*	5402.34±0.64*	2.04±0.36	3.10±0.14	2384.38±0.98*
14	561.71±0.41*	5431.31±0.31*	2.14±0.05	3.44±0.36	2407.19±0.46*
16	531.81±0.49*	5449.16±0.89*	2.21±0.16	3.41±0.04	2391.12±0.76*
18	539.89±0.25*	5478..89±0.38*	2.19±0.35	3.27±0.81	2322.07±0.41*
20	541.12±0.36*	5487.10±0.43*	2.29±0.04	3.38±0.03	2338.23±0.49*

Data are the average of three replicates ± SEM (* = P<0.05) compared with week 4. PC = Phosphatidylcholine. PE = Phoshatidylethanolamine. PI = Phosphatidylinositol. PS = Phosphatidylserine. LPC = lysophosphatidylcholine.

Table 4. Composition of glyceride lipids in *D. edulis* at week 4 to 20 of fruit development.

Week	MAG (%)	DAG (%)	TAG (%)
4	9.91±0.03	69.51±0.20	20.43±0.12
6	7.81±0.438	69.04±0.54	23.17±0.24*
8	6.70±0.09*	68.19±1.04	25.11±0.72*
10	7.72±0.77*	68.14±0.99	24.16±1.01*
12	6.87±0.69*	68.11±1.44	25.02±1.44*
14	7.21±0.21*	59.89±1.00*	32.83±0.86*
16	7.86±0.51*	46.43±0.65*	45.79±0.73*
18	7.56±0.81*	30.80±0.95*	62.67±1.24*
20	7.77±0.31*	28.14±0.31*	65.87±0.60*

Values are mean ± SEM (* = P<0.05) compared with week 4.

seeds and fruits in which the disappearance of the partial glycerides and FFA which characterised the immature stage mesocarplipid coincided with the formation of TAG (Bafor and Osagie, (1988); Wang et al., 2012).

Another possible mechanism for TAG synthesis in the oil palm fruit mesocarp is the glycerol-3-phosphate pathway via phosphatidic acid (PA) acting as a transient intermediate. This is due to the fact that phosphatidic acid

Table 5. Sterol lipids in *D. edulis* pulp oil at week 4 to 20 of fruit development.

Week	Cholesterol (mg/100 g)	Cholesta-pol (mg/100 g)	Ergos-terol (mg/100 g)	Campesterol (mg/100 g)	Stigmasterol (mg/100 g)	Save-nesterol (mg/100 g)	Sitosterol (mg/100 g)
4	4.94±0.06	4.17±0.01	2.54±0.46	180.88±0.33	89.75±0.46	13.35±0.08	1177.63±0.07
6	3.86±0.03	6.17±0.06•	2.42±0.04	184.71±0.03•	89.16±0.16	12.81±0.07	1146.01±0.18•
8	3.01±0.76*	7.00±0.08•	2.11±0.05	182.01±0.38•	81.40±0.26•	13.41±0.03	1146.28±0.18•
10	3.20±0.65•	8.11±0.05•	1.84±0.81	188.06±0.22•	80.91±0.91•	8.94±0.91•	1207.01±0.23•
12	3.10±0.33•	4.53±0.41	5.10±0.06•	186.14±0.71•	80.14±0.02•	10.81±0.41•	1184.50±0.08•
14	3.12±0.09•	2.29±0.95•	9.95±1.86•	183.01±•0.86•	87.85±0.75•	11.52±0.66•	1134.04±0.15•
16	ND	2.46±0.44•	8.41±0.04•	183.23±1.24•	82.11±0.04•	11.01±0.06•	1131.14±0.18•
18	ND	3.47±0.41•	2.38±0.77	183.65±1.80•	75.87±1.14•	13.47±0.17	1092.06±0.41•
20	ND	2.01±0.31•	2.02±0.31	183.88±1.31•	80.71±0.41•	13.11±0.50	1049.30±0.97•

Data are the average of three replicates ± SEM (* = P<0.05) compared with week 4.

(PA), a major lipid fraction in some immature oil seed, was not detected in the matured oil palm fruit mesocarp during lipids accumulation. The PA is probably hydrolysed by a specific phosphotidate phosphydroxylase enzyme to give a 1, 2-diacylglycerol which is acylated to TAG in a reaction that is catalysed by a 1, 2-diacylglycerol acyltranferase enzyme (Bafor and Osagie, 1988). In support of this is the dramatic drop in the level of diacylglycerides at 20 WAA. This pattern of TAG synthesis has been supported as the most widely occurring pathway in most seed tissues (Bafor and Osagie, 1986; Wang et al., 2012).

The major phospholipids of the immature and matured stages (weeks 4 to 20) were phosphatidylinositol and phosphatidylethanolamine in both fruit oils (Table 3). This agrees with previous findings of Bafor and Osagie (1988) and Ekman et al. (2008) with oil palm. Lysophosphatidylcholine (LPC), phosphatidylserine (PS) and phosphatidylethanolamine (PE) were low throughout fruits maturation. The phospholipids content reported here are in concert with literatures (Bafor and Osagie, 1986, 1988) implicating PC and PE as an intermediate both in the synthesis of membrane phospholipids and that TAG and PA as a metabolites in the biosynthesis of other polar lipids.

In *D. edulis* pulp oil, cholesterol, cholestapol, ergosterol, s-avenasterol and sitosterol were detected at various levels (Table 5). Sitosterol was more abundant a constant level throughout. Bafor and Osagie (1988) reported the importance of free sterols in controlling membrane stability and permeability in beet and barley roots.

Conclusion

The results at the 18 to 20 WAA showed that high quantities of essential fatty acids are present in the African pear pulp in the mature stage and could as well be regarded as the physiological mature stage of the fruits that would present the optimum values of the properties which could be incorporated in food products. Hence, the African pear fruit which is a nutritious food could be harvested at this period for industrial and domestic uses.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors wish to thank the Management of the Nigerian Institute For Oil Palm Research (NIFFOR) for providing the space, extraction apparatus and valuable advice during the extraction of the oil.

REFERENCES

Adebayo–Tayo BC, Ajibesin KK (2008). Antimicrobial activity of *Coula edulis.* Research Journal of Medicinal Plant 2(2):86-91.

Ajibesin KK, Ekpo BJ, Bala DN, Essien EE, Adesanya SA (2008). Ethnobotanical survey of Akwa Ibom state of Nigeria. Journal of Ethnopharmacology 115(3):387-408.

Anonymous (2011a). *Dacryodes edulis*. Agroforestry Tree Database, World Agroforestry Centre P 159.

Anonymous (2011b). *Dacryodes edulis.* Portabase, Plant Resources of Tropical Africa (PROTA).

Association of analytical chemists (AOAC) (1996a). AOAC official method 994.10: Cholesterol in foods- Direct saponification – Gas chromatography method. Association of analytical chemists, Arlington, VA.

ASTM (2012). ASTM method D6584: The standard test method for the determination of free and total glycerine in B-100 Biodesiel methoyl esters by gas chromatography. Fisher Scientific, Runcorn, Chesire, UK.

Bafor ME, Osagie AU (1986). Changes in lipid class and fatty acid composition during maturation of Mesocarp oil palm (*Elaeis guineesis*) variety Dura. Journal of the Science of Food and Agriculture 37(9):825-832.

Bafor ME, Osagie AU (1988). Changes in some non-polar lipids composition of Deloping oil palm fruit (*Elaeis guineesis*) mesocarp, *Var. Duru.* Journal of the Science of Food and Agriculture 43(4):261-

264.
Burkill HM (1985). The useful plants of West Africa. Royal Botanic Gardens Kew 1:960.
Dike MC (2010). Proximate, phytochemical and nutrient compositions of some fruits, seeds and leaves of some species at Umudike, Nigerians ARPIN. Journal of Agricultural and Biological Science 5(1):7-16.
Dubois V, Breton S, Linder M, Fanni J, Parmentier M (2007). Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential. European Journal of Lipid Science and Technology 109(7):710-732.
Edem CA, Dosunmu MI, Bassey FI (2009). Determination of proximate composition, Ascorbic Acid and Heavy metal content of African walnut *Tetracarpidium conophorum*. Pakistan Journal of Nutrition 8(3):225-226.
Ekman Å, Hayden DM, Dehesh K, Bülow L, Stymne S (2008). Carbon partitioning between oil and carbohydrates in developing oat (Avena sativa L.) seeds. Journal of Experimental Botany 59(15):4247-4257.
Gunstone FD (1996). Fatty Acid and Lipid Chemistry. Springer 264 p.
Ikhuoria EU, Maliki M (2007). Characterization of *Avocado* pear (*Paesea 8 ignallin*) and African pear (*Dacryodes edulis*) extracts. African Journal of Biotechnology 6(7).
Ikhuoria EU, Aiwonegbe AE, Okoli P, Idu M (2008). Characteristics and composition of African oil bean seed (*Pentaclethra macrophylla* Benth). Journal of Applied sciences 8(7):1337-1339.
Kengue J (2002). Safou. *Dacryodes edulis*, International Centre for Underutilise Crops, Southampton, UK. RPM Reprographics, Chinchester, England P 147.
Kengue J, Nyagatchou J (1990). Problème de la conservation du pouvoir germinative chez les signal de safoutier (*Dacryodes edulis)*. Fruits 45(4):409-412.
Koudou J, Edou P, Obame LC, Bassole IH, Figueredo G (2008). Volatile components, antioxidant and antimicrobial properties of the essential oil of *Dacryodes edulis* from Gabon. Journal of Applied Sciences 8(19):3532-3535.
Manni G, Caron F (1995). Calibration and determination of volatile fatty acids in waste leachates by gas chromatography. Journal of Chromatography A 690(2):237-242.
Okwu DE, Nnamdi DA (2008). Evaluation of the chemical composition of *Dacryodes edulis and Raphia hookeri*. Maun and wendi exudates used in herbal medicine in south eastern Nigeria. African Journal of Traditional, Complementary and Alternative Medicines 5(2):194-200.
Raheja RK, Kaur C, Fingh A, Bhatia IS (1973). New coloromentary method of the quantitative estimation of phospholipids without acid digestion. Journal of Lipid Research 14(6):695-697.
Siedlecka EM, Kumirska J, Ossowski T, Glamowski P, Gołębiowski M, Gajdus J, Kaczyński Z, Stepnowski D (2008). Determination of Volatile Fatty Acids in Environmental Aqueous Samples. Polish Journal of Environmental Studies 17(3):351-356.
Sofowora A (2008). Medicinal plants and traditional medicine in Africa. 2nd Edn., Spectrum books Ltd. Ibadan, Nigeria.
Umaru O, Dere AO (1986). Characrerisation and Composition of the pulp oil and Cake of the African Pear, *Dacryodes edulis (G. Don).* Journal of the Science of Food and Agriculture 38(1):67-72.
Wang X, Ronghua XU, Ruling W, Aizhong L (2012). Transcriptome analysis of Sacha Inchi (*Plukenetia volubilis L*.) seeds at two developmental stages. BMC Genomics 13(716):1471-1483.