FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 7 No. 3, June (Special Issue), 2023, pp 198 - 206
DOI: https://doi.org/10.33003/fjs-2023-0703-1843

EXTRACTION, PURIFICATION AND CHARACTERIZATION OF LIPOXYGENASE FROM CONOPHOR NUT (*Tetracarpidium conophorum*)

[1]Oladotun O. Oguntoyinbo, [2]Abayomi M. Abiodun, [3]Oludare D. Adebayo, *[3]Victor N. Enujiugha

[1]Department of Food Science and Technology, Lagos State University of Science and Technology, Ikorodu, Nigeria.
[2]Department of Nutrition and Dietetics, The Federal Polytechnic, Bida, Nigeria.
[3]Department of Food Science and Technology, Federal University of Technology, Akure, Nigeria.

*Corresponding authors' email: vnenujiugha@futa.edu.ng Phone: +2348034261870

ABSTRACT

Lipoxygenase (LOX) is an enzyme that catalyses the step-wise oxygenation of polyunsaturated fatty acids (PUFAs) to form fatty acid hydroperoxides within living tissues. In the present study, lipoxygenase was isolated from defatted and whole (full fat) conophor nut (*Tetracarpidium conophorum*) and purified to homogeneity using 60% ammonium sulphate precipitation, DEAE-sephadex A50 and sephadex G-200 chromatography. The effects of pH and temperature on the activity and stability of LOX were investigated. The optimum pH and temperature of the purified lipoxygenase were 5.0 and 50 ^{o}C, respectively. Lipoxygenase activity was stable at 40 ^{o}C and 50 ^{o}C after an hour of incubation. The enzyme was stable at pH values of 5.0 and 6.0. The activity of the enzyme was enhanced by Ca^{2+} while Zn^{2+}, Fe^{2+} and Cu^{2+} inhibited its activity. The K_m and V_{max} values were 40.38 µM and 125 µmol/min/mg proteins, respectively. The molecular weight was estimated to be 67.83 kDa by SDS-PAGE. The findings reveal that conophor nut can be a cheap source of industrial lipoxygenase, which could be exploited in various biotechnological applications. The enzyme's thermostability and adaptation in slightly acidic medium are factors that can help boost its acceptability in food systems.

Keywords: Conophor nut, Lipoxygenase, Activity, Physicochemical properties, Molecular weight

INTRODUCTION

Conophor plant (*Tetracarpidium conophorum*) has been identified as being an integral part of cocoa and kola plantations, as a pervasive climber with high medicinal properties (Enujiugha, 2008). It is widely cultivated for the production of nutritious nuts which are commonly cooked and consumed with boiled corn during the harvest season (Enujiugha, 2003). The conophor plant is a major component of the agro-ecological landscape of southern rainforest zones of Nigeria. Enujiugha (2017) has rightly observed that the forests and agro-forestry landscape of some African countries are richly blessed with a wide range of wild and underutilised oil-bearing seeds and nuts which have not been evaluated for their nutritional potentials and or toxicity risks. Some of the underexploited oilseeds that are found to be nutrient dense with a good compliment of bioactive components include melon seed (Babatuyi et al., 2022; Enujiugha et al., 2023), conophor nut (Talabi et al., 2023) and African oil bean seed (Enujiugha and Agbede, 2000; Oyinloye and Enujiugha, 2019). *Tetracarpidium Conophorum* nut has been reported to be used as a male-fertility agent (Ajaiyeoba and Fadare, 2006). *T. conophorum* is known among local consumers in western Cameroon as kaso or ngak and serves as an edible nut eaten between meals (Tchiegang *et al.*, 2007). Conophor nut contains antinutritional factors such as oxalates, phytates and tannins which may constitute a limitation to its widespread utilisation. However, the presence of significant amounts of macro-nutrients such as protein, crude fibre, oil/fat and carbohydrate in *Tetracarpidium conophorum* has been reported (Enujiugha and Ayodele-Oni, 2003). The nut is noted for its high oil content which has been found to be unstable during frying owing to its high content of polyunsaturated fatty acids, especially linolenic acid (Oyinloye and Enujiugha, 2017). Two isolectins, Agglutin I and Agglutin II were isolated and characterized from the seed extract of the plant (Animashaun *et al*, 1994).

In Nigeria, conophor plant is found in south west Nigeria where it is cultivated primarily for the nuts which are cooked and widely consumed as snacks (Enujiugha, 2008). The leaves serve as male fertility agents and are usually employed in the treatment of dysentery (Sofowara, 1993). The oil from the nut is occasionally used as a source of energy for growing seedlings and has been observed to be useful in the formulation of wood varnish, stand oil and vulcanized oil (Ajaiyeoba and Fadare, 2006). *T. Conophorum* seed kernels which are noted for their high lipid contents are often eaten as nibbles in Cameroon (Tchiégang *et al*., 2007). Previous studies focused on the high nutrient potentials of the seeds as well as their functional properties (Akpuaka and Nwankwo, 2000; Enujiugha, 2003) and antimicrobial activity of the extracts and fractions of the nut (Ajaiyeoba and Fadare, 2006). There is, to our knowledge, currently no known work on the presence and behaviour of lipoxygenase in the nuts.

Lipoxygenase (LOX) (EC 1.13.11) is an enzyme which is found in a wide variety of plant and animal tissues in more than 60 species, and catalyses the step-wise oxygenation of polyunsaturated fatty acids (PUFA) to yield fatty acid hydroperoxides in tissues (Eskin *et al.*, 1977). They are present in abundance in grain legume seeds (beans, lentils and peas) as well as in potato tubers (Casey *et al*., 1996). The soybean lipoxygenases were the first to be characterized and are named sequentially beginning with soybean lipoxygenase 1 (LOX1), which was also the first lipoxygenase isozyme to have its three dimensional X-ray crystal structure solved (Minor *et al*., 1996).

Lipoxygenase is utilised extensively in food-related applications, such as in bread making (Casey *et al*., 1997) and aroma production (Whitehead *et al*., 1995), while at the same time showing negative implications for colour, off-flavour and antioxidant activity in plant-based food products (Casey *et al*., 1996). Lipoxygenase in vegetative tissues provides hydroperoxide substrates that are mostly metabolized to compounds that play remarkable roles in plant defence

(Pfeiffer *et al*., 1992) or pea (Forster *et al*., 1999). Although tree nuts and oilseeds have been used for food since dawn of civilisation, the potentials of most tree nuts and oilseeds have not been fully exploited. In the present study, lipoxygenase was isolated from defatted and whole (full fat) conophor nut (*Tetracarpidium conophorum*) with the specific objectives of purifying it and characterizing its quality and behaviour.

MATERIALS AND METHODS

Plant material collection and preparation

Conophor nuts were purchased from a local market Ago-Alao in Ipele, Owo local government Area, Ondo State. Nigeria. The nuts were carefully cracked, sliced to about 1-2 cm thickness with a kitchen knife and dried at 30 ^{o}C for 6 h (to avoid denaturing the enzyme).This was milled into powder (fine flour) using laboratory hammer mill and kept at 4 °C prior to further analysis.Three hundred grams (300 g) of the flour was defatted with n-hexane using Soxhlet Extractor. The flours of the defatted and non-defatted seeds were stored in dried plastic containers tightly covered at 4 ^{o}C until used.

Chemicals and reagents

Linolenic acid, Tween 20, Ammonium sulphate, Sephadex G-200, Phosphate titrate, SDS, acrylamide, Coomassie brilliant blue R250, Tris –HCl buffer, Bovine Serum Albumin (BSA), Calcium chloride, Magnesium chloride, Copper chloride, Zinc chloride, Iron chloride, Sodium acetate, Hydrogen Chloride and Sodium Hydroxide were all purchased from Sigma Chemical Company (USA). All reagents and chemicals used were of analytical grade.

Preparation of crude enzyme

Extraction of crude Lipoxygenase was performed as described by Clemente *et al.,* (2000). Defatted and non - defatted conophor flours (15 g eah) were extracted in each case with 150 mL of 50 mM sodium phosphate buffer, pH 6.8 with constant stirring for 2 h. the mixture was separated by muslin cloth and the residue discarded. The filtrate was centrifuged at 6000 rpm for 30 min at 4 ^{o}C and the supernatant obtained was stored in aliquots at 4 ^{o}C.

Lipoxygenase activity assay

A stock solution of 10 mM sodium linoleate and Tween 20 was prepared using the method of Axelrod *et al.,* (1981) with some modifications. LOX activities in the sample extracts were determined using UV-Visible spectrophotometer. The standard assay mixture consisted of 3 mL of sodium phosphate buffer (50 mM, pH 6.8), 25 µL of substrate solution (10 mM linolenic acid), and an appropriate volume of the enzyme solution (0.5ml). The reaction was initiated by addition of the enzyme solution; and the subsequent formation of both hydroperoxides and ketodienes was followed by monitoring the change in absorbance at 234 nm for 3 min (Extinction value =2.5 x 10^4 M^{-1} cm^{-1}) at 25 °C. One unit of LOX activity was defined as the amount of enzyme catalyzing the formation of 1µmol of the hydroperoxide per min under standard assay conditions. Protein concentration was determined by the method of Lowry *et al.,* (1951). Bovine serum albumin (BSA) was used as standard.

Enzyme purification procedures

Ammonium sulphate precipitation

The supernatant (crude enzyme) was gradually brought to 40-60% saturation with ammonium sulphate at 4 ^{o}C with constant gentle stirring for 1h on magnetic stirrer for the complete dissolution of the ammonium sulphate. The precipitate was then centrifuged at 6,000 rpm for 30 min using refrigerated centrifuge. The pellet obtained was dissolved in 5 ml buffer and introduced into dialysis bag for dialysis against 50 mM potassium phosphate buffer (pH 6.8) for 24 hrs with three changes of buffer at 4 ^{0}C. The dialysate was assayed for enzymatic activity and protein concentration was determined.

Ion exchange chromatography

Further purification of lipoxygenase was carried out using DEAE-sephadex A50. The dialysate was loaded onto a DEAE-sephadex A50 column (2.5x 40 cm). Fractions of 5ml were collected until no protein was detected in the flow through fractions. The bound proteins were eluted using linear gradient of 1 M sodium chloride in 50 mM sodium phosphate pH 6.8, at a flow rate of 1 mL/min. Absorbance of the fractions were determined and LOX activity was then assayed in fraction containing protein. The fractions containing LOX activity were pooled and concentrated using 4 M sucrose solution at 4 ^{0}C.

Gel filtration

The concentrated enzyme solution was applied on a sephadex G-200 column (1.5x75 cm) equilibrated with 50 mM sodium phosphate buffer pH 6.8. The protein was eluted with the same buffer at a flow rate of 20 mL/h. Absorbance of the fractions was measured at 280 nm and fractions were assayed for LOX activity. The fractions containing enzymatic activity were pooled and used for further experiment.

Molecular weight determination

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out following the method of Laemmli (1970). The standard protein marker consisted of: (I) Phosphorylase b, 103.14 kDa; (II) Bovine serum albumin, 81.35 kDa; (III) Ovalbumin, 47.05 kDa; (IV) Carbonic anhydrase, 34.17 kDa; (V) Soybean trypsin inhibitor, 27.26 kDa and (VI) Lysozyme, 17.67 kDa. Gels were stained with a 0.05% (w/v) Coomassie brilliant Blue G-250 solution. Molecular mass of LOX was determined using molecular protein markers.

Effect of pH on lipoxygenase activity and stability

The pH-activity profile of lipoxygenase was determined spectrophotometrically by applying various pH values under conditions of constant ionic strength with linolenic-Tween 20 substrate. The buffer systems (50 mM) used were as follows: sodium acetate pH 4-5; sodium phosphate, pH 6.0-7.0; Tris-HC1, pH 8-10. Stock linolenic-Tween 20 was prepared with appropriate buffer prior to the assay. The initial substrate concentration was 2.56 mM linolenic while 0.1 mL of enzyme preparation was used. The pH stability of lipoxygenase was investigated by incubating 0.25 mL aliquots of enzyme preparation in 2.5 mL of 50 mM buffer solutions of various pH values. After l h incubation at 4 ^{o}C, l mL samples were removed and assayed spectrophotometrically.

Effect of temperature on lipoxygenase activity and stability

The effect of temperature on activity and stability of LOX was determined according to Greiner *et al.,* (2001). The effect of temperature on the activity of purified lipoxygenase was determined in the temperature variation ranging from 30 ^{o}C to 80 ^{o}C using the standard LOX assay. The thermal stability was investigated, whereby the purified enzyme was incubated for 2 h at different temperatures. At intervals of 20 min, aliquot of 0.5 ml of the incubated enzyme was assayed for residual LOX activity.

Effect of metal ions on lipoxygenase activity

LOX was incubated with 1 mM of different metals ions solution was mixed with 2ml of enzyme solution for 5 min at room temperature (25 ºC). A 0.5 ml of the mixture was withdrawn and assayed according to standard assay procedures. The metals ions used were: Ca^{2+}, $Mg^{2+,}$ Zn^{2+}, Fe^{2+} and Cu^{2+}. The treated enzyme was assayed as described above.

Determination of K_m and V_{max}

K_m and V_{max} were determined by measuring the LOX activity at varied concentrations of linolenic acid 0-125 µM. Kinetic parameters were calculated from the Lineweaver-Burk plot (Sanni et al., 2019). The enzyme activity was determined according to the standard assay procedure.

RESULTS AND DISCUSSION

Lipoxygenase activity values were 60.0µmol/min/ml and 39.11 µmol/min/ml for defatted and non-defatted samples, respectively; while the protein concentration obtained were 19.72 mg/ml and 25.4 mg/ml for defatted and non-defatted sample, respectively. Since defatted crude extract LOX activity was higher than the non-defatted further experiments proceeded on the defatted sample. The summary of the result for purification of lipoxygenase from conophor nut was presented in Table 1. The single band obtained during purification in Figures 1 and 2 clearly suggests that the enzyme consists of one form of lipoxygenase which shows striking similarities to those obtained with immature English Pea lipoxygenase (Chen and Whitaker, 1986) and commercial soybean lipoxygenase (Stevens *et al*., 1970). The three step purification procedures resulted in a 6-fold purification of the conophor lipoxygenase with 11.4% recovery; this is lower compared to the recovery of lipoxygenase activity in banana leaf which was 11.7% with 83.9-fold purification (Kuo, *et al*., 2006). However, this is higher when compared with the overall purification recovery performance of lipoxygenase from *Pleurotus ostreatus* (Kuribayashi *et al*., 2002).

Table 1: Purification Table of lipoxygenase

Purification Step	Enzyme Activity (µmol/min/mL)	Protein Conc. (mg/ml)	Total Protein (mg)	Total Activity (U)	Specific Activity (U/mg)	Yield %	Fold
CE	60	19.72	2958	9000	3.04	100	1
ASP	58.22	18.4	828	2619.9	3.16	29.1	1.03
IEC	81.77	7.95	206.7	2126.0	10.28	23.6	3.38
GF	102.66	5.68	56.8	1026.6	18.07	11.4	5.94

CE= Crude extract, ASP= Ammonium Sulphate Precipitation, IEC= Ion Exchange Chromatography, GF= Gel Filtration.

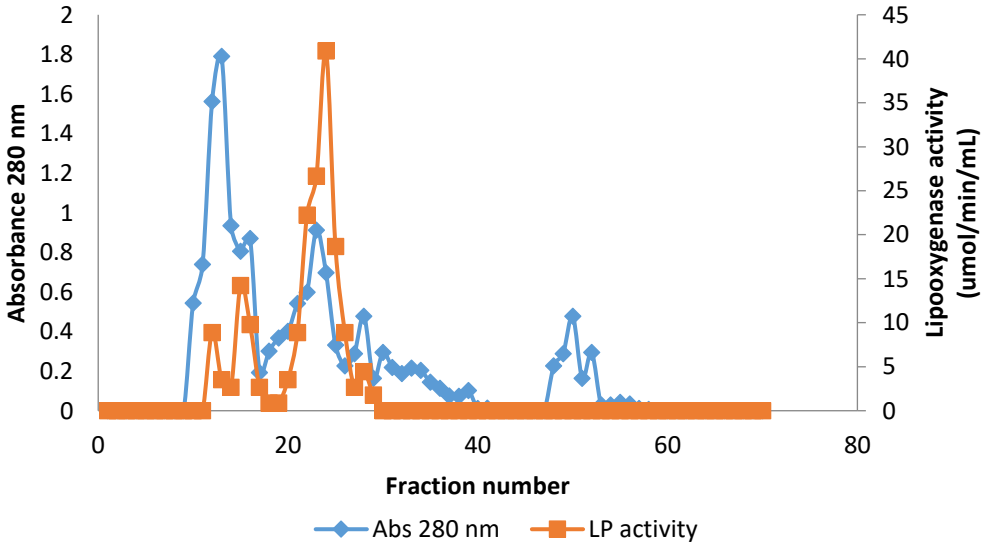

Figure 1: Chromatogram of Lipoxygenase on ion exchange column (2.5x 40 cm).

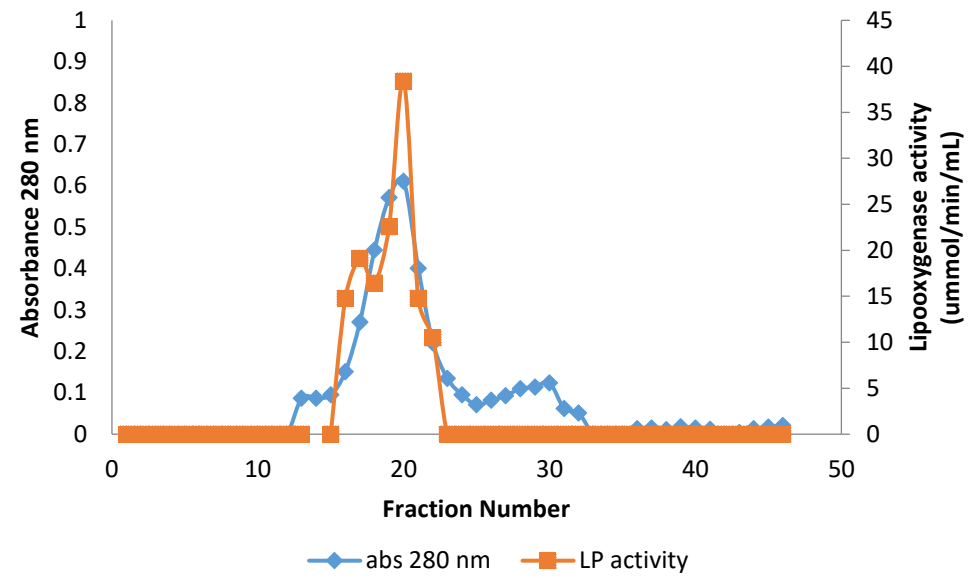

Figure 2: Elution profile of Lipoxygenase on Gel filtration column (1.5x75 cm) of Sephadex G200.

The optimum pH of the enzyme was pH 5.0 as shown on Figure 3. About 43% relative activity was obtained at pH 3.0 with a gradual increase in enzymatic activity as the pH increase given the optimum at pH 5.0. However a marked activity was observed between pH 5.0 – pH 7.0 with 85% and 74% relative activity at pH 6 and pH 7.0 respectively (Figure 4). The result of this study is similar to values obtained for lipoxygenase isozymes partially purified from soybean leaves all leaf ages (Christopher *et. al,* 1972). Optimum pH for lipoxygenase activity of conophor nut is somewhat close to that of broad bean of pH 5.6 (Clemente *et al.,* 2000), dried green pea seed pH 5.5 (Forster *et al*., 1999), but lower in comparison to eggplant pH 7.0 (Lopez-Nicolas *et al*., 2001). Wallace and Wheeler (1979) also reported an optimum pH of 6.0-6.5 for all lipoxygenase isoenzymes. The two isoenzymes of LOX from *Lasiodiplodia theobromae* have optimum activity at pH 6.0 (Patel *et al.*, 2014).

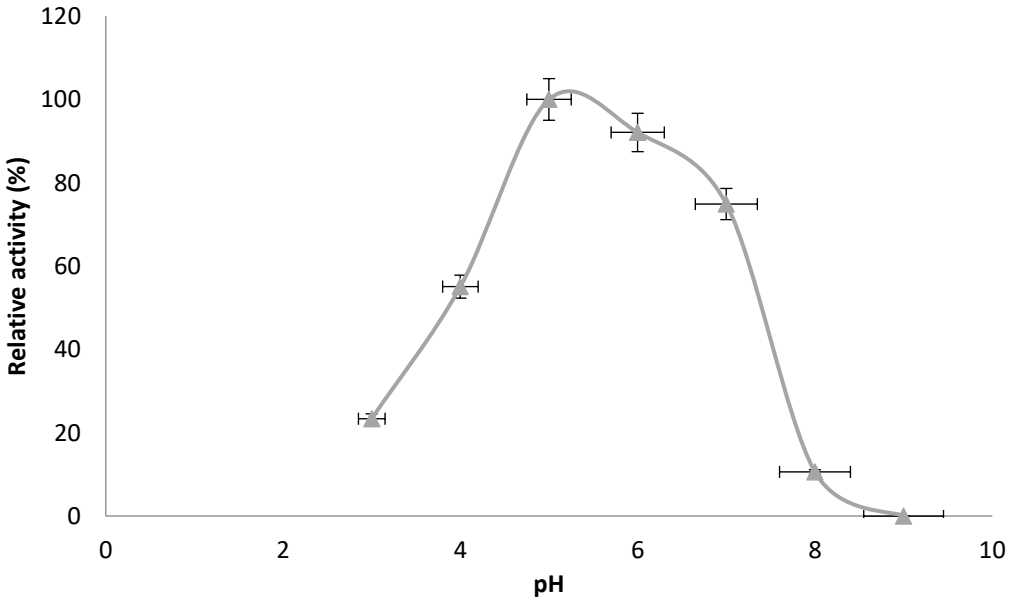

Figure 3: Effect of pH on activity of purified Lipoxygenase. Optimum activity was expressed at 100%

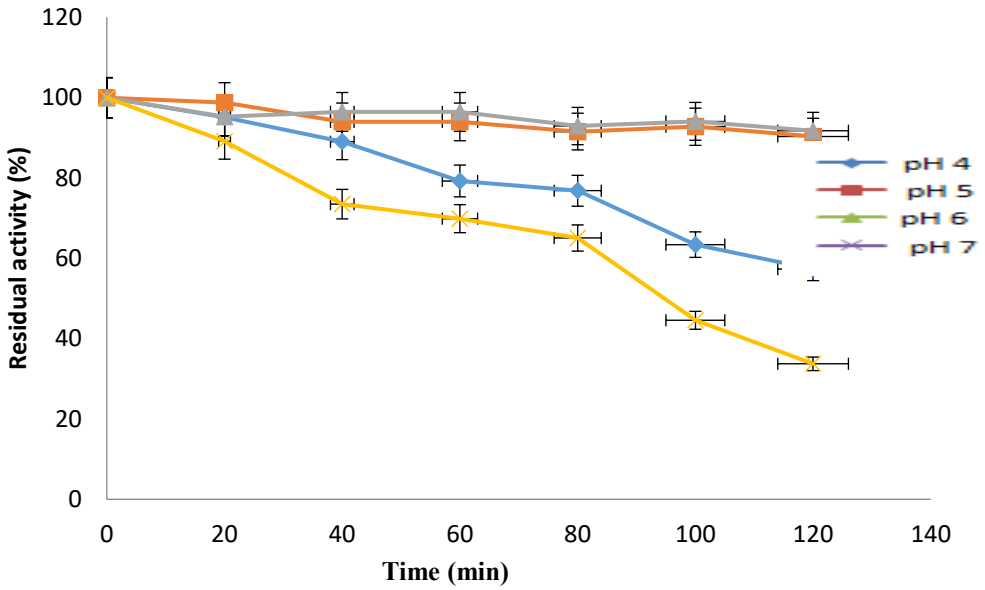

Figure 4: Stability of purified Lipoxygenase as affected by pH. Optimum activity was expressed as 100%

The optimum temperature for LOX was 50 ºC, while relatively high lipoxygenase activity values of 60.7% and 69.6% were observed at 30 ºC and 40 ºC respectively as shown on Figure 5. Lipoxygenase activity was stable at 40 ºC and 50 ºC after an hour of incubation (Figure 6). At higher temperatures, there was rapid loss in the enzymatic activity. This result is similar to that of sweet corn with 50 ºC (Yamamoto et al., 1970) and LOX1 from *Lasiodiplodia theobromae* with optimum temperature of 50 ºC (Patel *et al.*, 2014). This result is somewhat lower than the purified LOX from thermophilic fungus with optimum temperature at 55 ºC (Li *et al.*, 2001), and higher than that of purified lipoxygenase from banana leaf (40 ºC). Also the LOX optimal temperature of sea algae (*E. intestinalis*) was 35 °C as reported by Kuo *et al.*, (2006) as well as for both millet gill (Hsu and Pan, 1996) and trout gill (Hsieh *et al*, 1988) which was 20 °C. This result reveals that LOX from conophor is more thermal stable when compared with that of soybeans (Ludikhuyze *et al.*, 1998),

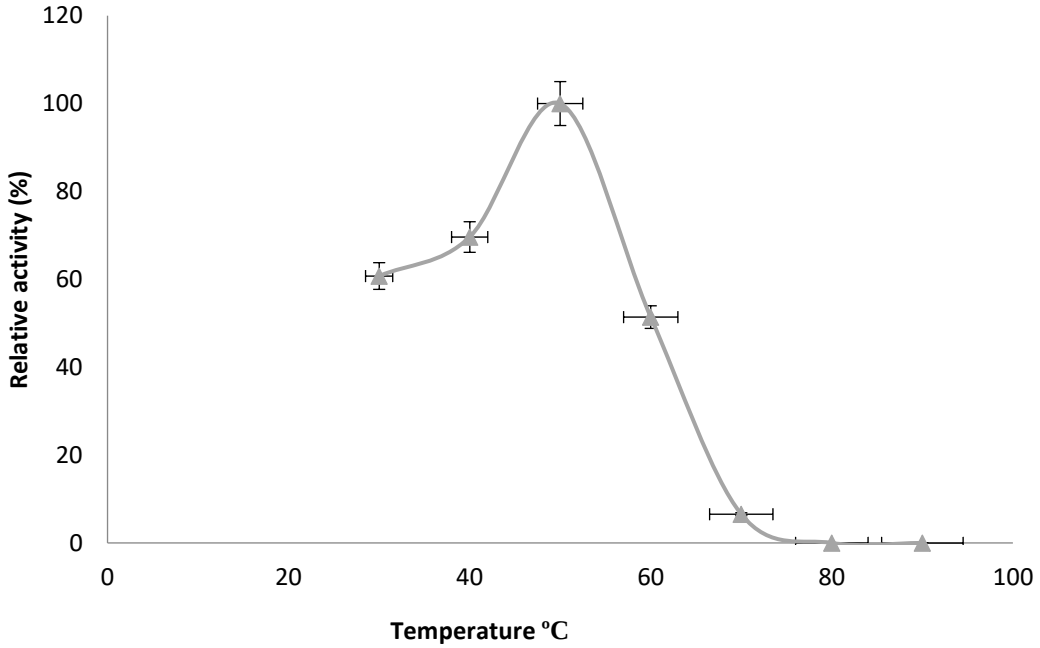

Figure 5: Thermal effect on the activity of purified Lipoxygenase. Optimum activity was expressed as 100%.

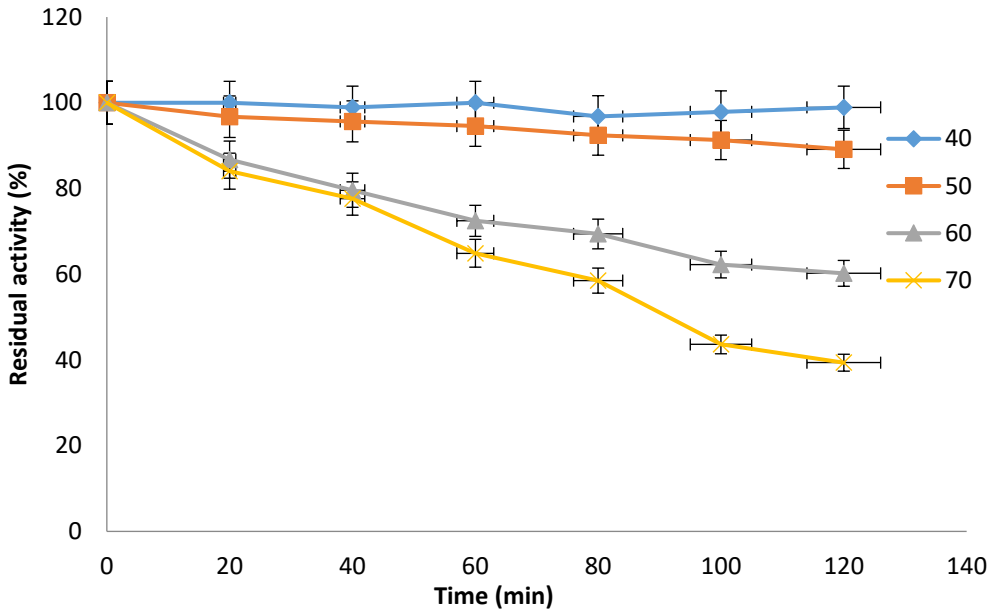

Figure 6: Thermal stability of purified Lipoxygenase. Optimum activity expressed as 100%.

Ca^{2+} enhanced Lipoxygenase activity with relative activity of 110%, and Mg^{2+} did not significantly affect the activity of the enzyme; however, the activity was inhibited by Zn^{2+}, Fe^{2+} and Cu^{2+} with 52%, 33% and 25% remaining activity, respectively as shown on Figure 7. Calcium Chloride had a strong activating effect on the LOX activity with 110% relative activity, similar to soybean isozyme-1 and isozyme-2 which were activated by Ca^{2+} although, Ca^{2+} inhibited peanut isozyme-1 and soybean isozyme-3 (Sander *et al.*, 1975). This result is similar to that observed for lipoxygenase isoenzymes from different sources (Yamamoto et al, 1970; Truong et al., 1982).

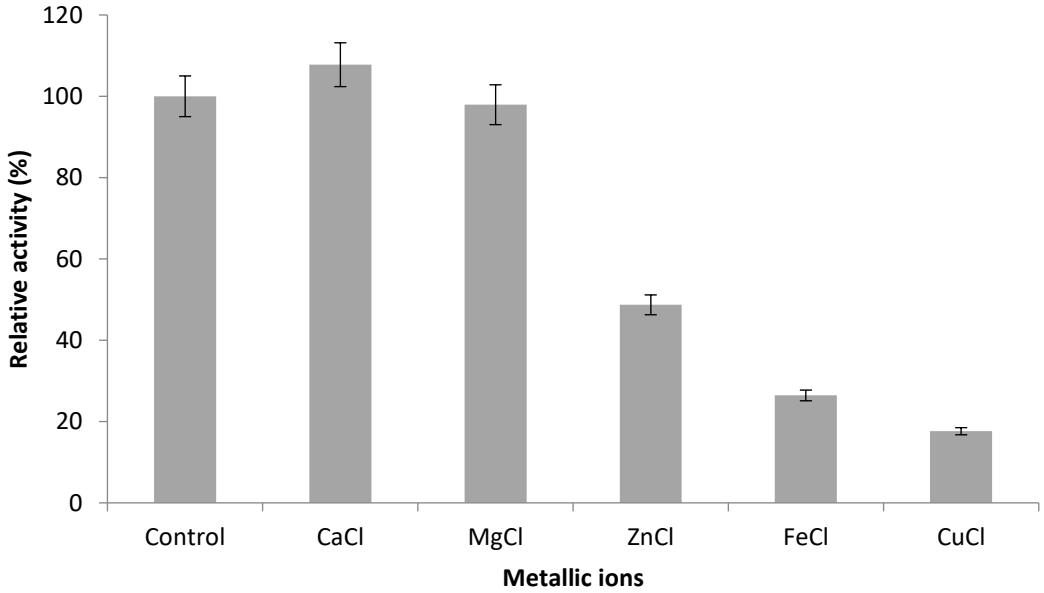

Figure 7: Effect of metallic ions on the purified Lipoxygenase activities.

The Lineweaver–Burk plot of the Lipoxygenase activity is presented on Figure 8. It shows that the Kinetic constants, K_m and V_{max} values for the purified Lipoxygenase were determined to be 40 mM and 125 µmol/min, respectively. The Kinetic behavior of the purified conophor nut lipoxygenase was studied and the reaction of linoleic acid by conophor follows Michaelis-Menten kinetics (Axelrod *et al*., 1981). Much lower values of 0.15 mM and 2.4 µmol/min. were reported by Kuo *et al*., (2006) for purified LOX from banana leaf. Kuribayash *et al.* (2002) also reported that the K_m and V_{max} of LOX from *Pleurotus ostreatus* were 0.13 mM and 23.4 µmol/min/mg, respectively. However, soybean LOX-1 K_m was 0.02 mM (Hall *et al.*, 2004), while for canola seed (0.2 mM) (Khalyfa *et al*., 1990), for tomato 0.52 mM (Todd, *et al*., 1990), for a land plant *Phaseolus vulgaris* L. it was 1.4 mM (Sicilia, *et al.*, 2005), and 2.8 mM for broad bean (Al-Obaidy and Siddioi,1981).

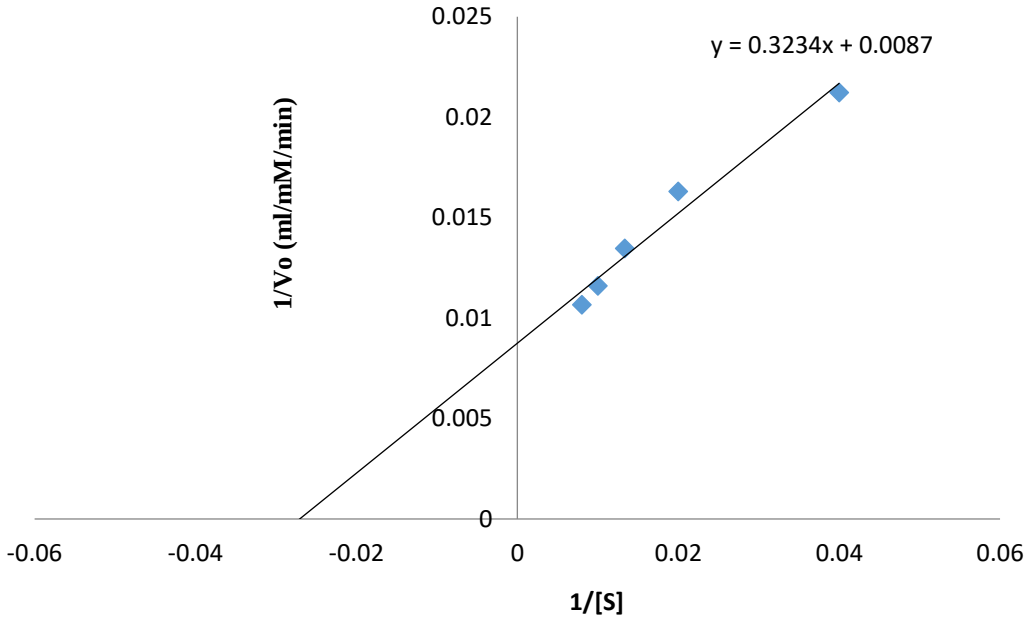

Figure 8: Lineweaver-Burk plot of Lipoxygenase activity

Polyacrylamide gel electrophoresis in the presence of a surfactant SDS produced a single protein band as represented on Figure 9. The molecular weight of the purified lipoxygenase from conophor nut was estimated to be 67.83 kDa by SDS-PAGE. This result is similar to that of purified papaya LOX (~68 kDa) and Pea LOX-1 (67 kDa) obtained by ultracentrifugation (Eriksson and Svensson, 1970) and cowpea L-1(Truong *et al.,* 1982). Purified LOX of cucumber and brinjal were each reported to share similarity of 95 kDa, respectively (Vozzo, 2002) and that of Olive fruit was reported as 98 kDa (Lorenzi *et al*., 2006), tomato fruit (95 kDa), pea seeds (93 kDa) (Forster *et al.*, 1999), and 97 kDa for mung bean LOX (Aanangi *et al.,* 2016). Pearl millet lipoxygenase isozymes had molecular masses of 83 kDa, 77 kDa and 73 kDa (Babitha *et al*., 2004). Generally, most of the plant sources contain lipoxygenases with molecular weights in the range of 94-104 kDa (Shibata *et al.*, 1994).

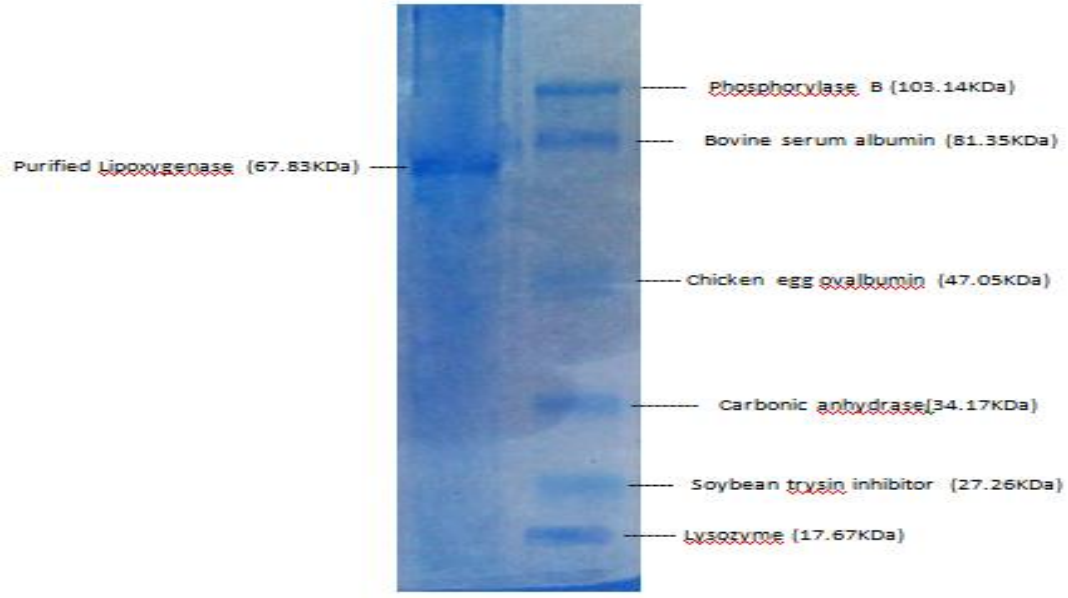

Figure 9: Electrophorectogram of purified lipoxygenase. The protein which was analyzed on 10% Bis –Tris gel showed a single band.

CONCLUSION

The results of the present study have highlighted the great potentials of lipoxygenase and its activity in conophor nut (*Tetracarpidium conophorum*). The purified enzyme showed high reactivity to linolenic acid, with optimal pH at 5.0 and optimum temperature 50 ºC. About 5.94 purification fold and 11.4% recovery were achieved. Ca^{2+} enhanced the lipoxygenase activity while Zn^{2+}, Cu^{2+} and Fe^{2+} inhibited the enzyme activity, significantly. The molecular weight of conophor nut lipoxygenase was found to be 67.83 kDa. Therefore, it could be deduced from the results obtained that

this underutilized nut is a potential source of lipoxygenase for various industrial purposes.

COMPETING INTERESTS

The authors declare that there are no competing financial interests.

REFERENCES

Aanangi R. Kotapati K.V., Palaka B.K., Kedam T., Kanika N.D, and Ampasala D.R., (2016). Purification and characterization of lipoxygenase from mung bean (*Vigna radiate L.*) germinating seedlings. *3 Biotech,* 6:113.

Ajaiyeoba EO, Fadare D.A, (2006).Antimicrobial potential of extract and fraction of the African Walnut – *Tetracarpidium. Conophorum. African Journal of Biotechnology*, **5**(22):2322-2325.

Akpuaka M.U, Nwankwo E.(2000). Extraction, analysis and utilization of a drying oil from *Tetracarpidium conophorum*. *Bioreource. Technology,* **73**: 195-196.

Al-Obaidy, H. M., Siddioi, A. M. (1981) Properties of broad bean lipoxygenase. *Analytical Biochemistry*, ***46***, 622-625.

Animashaun T, Togun RA, Hughes, CR (1994). Characterization of isolectins in *Tetracarpidium conophorum* seeds (Nigerian Walnut). *Glycoconjug. J*. **11**:299-303.

Axelrod, B., Thomas, T. C. and Laakso, S. (1981). Lipoxygenase from soybeans. EC 1.13.11.12 Linoleate: oxigenoxidoreductase. *Methods in Enzymology*, **71**: 441-451.

Babatuyi, C. Y., Oyetayo, V. O., and Akinyosoye, F. A. (2022). Chemical composition and functional properties of *Citrullus vulgaris* fermented with mutant and non-mutant strains of *Bacillus subtilis* to produce ogiri. *FUDMA Journal of Sciences*, **6**(6), 31–43. https://doi.org/10.33003/fjs-2022-0606-1102

Babitha, M. P., Prakash, H. S., and Shetty, H. S. (2004). Purification and properties of lipoxygenase induced in downy mildew resistant pearl millet seedlings due to infection with *Sclerospora graminicola*. *Plant Science*, 166: 31–39.

Casey R, Domoney C, Forster C, Robinson D, Wu Z. In: Fenwick GR, Hedley C, Richards RL, Khokhar S, editors.,(1996). The significance of plant lipoxygenases to the agrifood industry in agrifood quality: an interdisciplinary approach. The Royal Society of Chemistry, pp. 127–30.

Casey R., Angelino S.A, Hamer RJ, van Hartingsfeld W, Heidekamp F, vander J, (1997). Lipoxygenases and breadmaking. In Proceedings of the first European symposium on enzymes and grain processing, pp.188–94.

Chen, A. O.; Whitaker, J. R, (1986), Purification and characterization of a lipoxygenase from immature English peas. *Journal of Agricultural and Food Chemistry*, ***34***, 203-211.

Christopher, J.P., Pistorius ,E., Axelrod ,B (1972). Isolation of a third isoenzyme of soybean lipoxygenase. *Biochem .Biophys. Acta*, **284**, 54-62.

Clemente, A., Olias, R., and Olias, J.M. (2000).Purification and Characterization of Broad Bean Lipoxygenase Isoenzymes. *Journal of Agricultural and Food Chemistry*.**48:**1070-1075.

Enujiugha, V.N. (2003). Chemical and functional characteristics of conophor nut. *Pakistan Journal of Nutrition*, **2**(6), 335-338

Enujiugha V.N (2008). *Tetracarpidium conophorum* conophor nut (pp.378-379) In: The Encyclopedia of Fruit and Nuts (J. Janick & R. E. Paull, eds.). CABI International, Oxfordshire, UK.

Enujiugha, V.N. (2017). Improving agricultural productivity through increased local biodiversity exploitation and food composition database management. *Journal of Biodiversity, Bioprospecting and Development*, **4**(1), 162. doi:10.4172/2376-0214.1000162.

Enujiugha V.N., Adeyemo M.B. and Adisa A.M. (2023). Nutritional and safety implications of consuming melon seeds and impacts on international trade: a review. *Food and Humanity*, **1**, 241-249.

Enujiugha, V.N., and Agbede, J.O. (2000). Nutritional and anti-nutritional characteristics of African oil bean (*Pentaclethra macrophylla* Benth) seeds. *Applied Tropical Agriculture*, **5**(1), 11-14

Enujiugha V.N. and Ayodele-Oni O. (2003). Evaluation of nutrients and some antinutrients in lesser known under-underutilised oilseeds. *Int. J. Food Sci. Technol*. **38**: 525-528.

Eriksson C.E, Svensson, S. G., (1970). Biochem. Biophys. Acta.198, 449

Eskin NAM, Grossman S, Pinsky A. (1977). Biochemistry of lipoxygenase in relation to food quality. CRC Crit Rev Food Sci Nutr; 9:1–40.

Forster C, North H, Afzal N, Domoney C, Hornostaj A, Robinson D.S, (1999). Molecular analysis of a null mutant for pea (*Pisum sativum* L.) seed lipoxygenase 2. Plant Mol Biol, 39:1209–1220.

Greiner, R., Muzquiz M., Burbano C., Cuadrado C., Pedrosa, M.M. and Goyoga C. (2001). Purification and characterization of a phytate –degrading enzyme from germinating fababeans (Viciafaba var. Almeda).*Journal of Agricultural and Food Chemistry*.49(5): 2234-2240.

Hall, C. E.; Husson, F.; Kermasha, S. (2004). Characterization of an enriched lipoxygenase extract from *Aspergillus niger* in terms of specificity and nature of flavor precursors production. *J. Mol. Catal. B: Enzymatic 29*, 201-209.

Hsieh, R. J.; German, J. B.; Kinsella, J. E. (1988). Lipoxygenase in fish tissue: some properties of 12-lipoxygenase form trout gill. *J.Agric. Food Chem* , **36**, 680-685.

Hsu, H. H.; Pan, B. S. (1996), Effects of protector and hydroxyapatite partial purification on stability of lipoxygenase from grey mullet gill. *J. Agric. Food Chem*.***44***, 741-745.

Khalyfa, A.; Kermasha, S.; Alli, I. (1990). Partial purification and characterization of lipoxygenase of canola seed (*Brassica napus* var. Wester). *J. Agric. Food Chem*., ***38,*** 2003-2008.

Kuo, J. M., Hwang, A., Yeh, D. B., Pan, M. H., Tsai, M. L., & Sun Pan, B. (2006). Lipoxygenase from banana leaf: Purification and characterization of an enzyme that catalyzes linoleic acid oxygenation at the 9-position. *Journal of Agricultural and Food Chemistry.* **54:** 3151–3156.

Kuribayashi, T., Kaise,H., Uno, C., Hara, T.,Hayakawa, T. and Joh, T. (2002). Purification and Characterization of Lipoxygenase from Pleurotusostreatus. *Journal of Agricultural Food Chemistry*.**50:**1247-1253

Laemmli, U.K. (1970). Cleavage of structural proteins during assembly of the head of bacteriophage T4.Nature.**227**, 680-685.

Li, D., Lui, Z. and Lu, J. (2001). Purification and Characterization of Lipoxygenase from the thermophilic fungus. Mycological Research. **105**(02): 190-194.

Lopez-Nicolas, J. M., Perez-Gilabert, M. andGracia-Carmona, F. (2001). Eggplant lipoxygenase (*Solanum melogena*): product characterization and effect of physicochemical properties of linoleic acid on the enzymatic activity. *Journal of Agricultural and Food Chemistry*, **49,** 433–438.

Lorenzi, V., Maury, J., Casanova, J. and Berti, L. (2006). Purification, product characterization and kinetic properties of lipoxygenase from olive fruit (Oleaeuropaea L.). Plant Physiology and Biochemistry. **44**: 450–454

Lowry,.H., Rosebrough, N. I.,Farr, L. and Randall, R. J.(1951) Protein determination.. *Journal Biology Chemistry*.193 -295.

Ludikhuyze, L., Broeck, V. D., Weemaes, C. and Hendrickx, M. (1998). Effect of combined pressure and temperature on soybean Lipoxygenase. Journal Agricultural Food Chemistry. 40(10): 4074-4080

Oyinloye, A.M., and Enujiugha, V.N. (2017). Evaluation of the suitability of *Tetracarpidium conophorum*, *Pentaclethra macrophylla* and *Citrullus vulgaris* seed oils for some fried products. *La Rivista Italiana delle Sostanze Grasse*, 94, 117-124.

Oyinloye, A.M., and Enujiugha, V.N. (2019). Antioxidant properties of African oil bean (*Pentaclethra macrophylla* Benth) seed phenolics as influenced by extraction solvents and heat treatments. *Applied Tropical Agriculture*, **24**(1), 42-48.

Patel D.D., Patel R.R., and Thakkar V.R., (2015). Purification, Characterization and Application of Lipoxygenase Isoenzymes from *Lasiodiplodia theobromae. Appl Biochem Biotechnol,* 175:513–525.

Pfeiffer TW, Hildebrand DF, Tekrony DM,(1992). Agronomic performance of soybean lipoxygenase isolines. Crop Sci; 32:362–87.

Sanders, T. H., Pattee, H. E. and Singleton, J. A. (1975). Lipoxygenase Isozymes of Peanut. Lipids. 10(11):681-685.

Sanni, D. M., Lawal, O. T., and Enujiugha, V. N. (2019). Purification and characterization of phytase from *Aspergillus fumigatus* isolated from African giant snail (*Achatina fulica*). *Biocatalysis and Agricultural Biotechnology*, **17**, 225-232.

Shibata D, Slusarenko A, Casey R, Hildebrand D, Bell E., (1994). Lipoxygenases. Plant Mol Biol Rep; 12 (CPGN Suppl.): 41–2.

Sicilia, F.; Mattei, B.; Cervone, F.; Bellincampi, D.; Lorenzo, G. D.(2005). Characterization of a membrane-associated apoplastic lipoxygenase in *Phaseolus* V*ulgaris* L. *Biochim. Biophys. Acta 1748*, 9-19.

Sofowara, A., (1993). Medicinal Plants and Traditional Medicine in African.Spectrum book. 2nd Ed, pp: 10-158

Stevens,F.C, Brown,D.M., Smith E.L., (1970). Arch.biochem.Biophys.136, 413.

Talabi, J.Y., Oguntoyinbo, O.O. and Enujiugha, V.N. (2023). Serum lipid profile and organ histology of albino rats fed on conophor (*Tetracarpidium conophorum*) nut oil-based diets. *IPS Journal of Nutrition and Food Science*, **2**(1), 9-12. DOI: https://doi.org/10.54117/ijnfs.v2i1.18

Tchiegang C, Kapseu C and Parmenter M, (2007). Chemical composition of oil from *Tetracarpidium conophorum* (Mull.Arg) Hucth and Dalz) nuts. *J of Food Lipids* **8**(2): 95-102.

Todd, J. F.; Paliyath, G.; Thompson, J. E. (1990). Characteristics of a membrane-associated lipoxygenase in tomato fruit. *Plant Physiol. 94*, 1225-1232.

Truong V. D. Evelyn M.T, Mendoza T (1982). Purification and characterization of Two Lipoxygenase from cowpea, *J. Agric. Food chem*. **30** ;54-60.

Vozzo, J.A. (2002). Tropical Tree Seed Manual. Agriculture Handbook No. 721. US Department of Agriculture (USDA), Washington DC.

Wallace J.M. Wheeler E.L, (1979).Two lipoxygenases isoemzymes and activator in wheat germ: *Phytochemistry Res*., 18,389-393.

Whitehead I.M., (1995). Industrial use of soybean lipoxygenase for the production of natural green note flavour compounds. *Cereal Foods World*; 40:193–7.

Yamamoto, A, Yasumoto, K & Mitsuda, H. (1970), Isolation of lipoxygenase isozymes & comparison of their properties, *Agric. Biol. Chem*. 34:1169.