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Isolation and Characterization of Lipoxygenase from Sponge Gourd (*Luffa aegyptiaca*)

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ABSTRACT : Lipoxygenase (Linoleate: oxygen oxidoreductase EC.1. 13. 11. 12), a dioxygenase catalyzing the hydroperoxidation of fatty acids containing a cis,cis- 1,4-pentadiene structure was isolated from defatted whole and dehulled seeds of *Luffa aegyptiaca* ,a local plant found growing wildly in waste lands and rubbish dumps. Several properties of the enzyme including effect of pH, substrate concentration and heat treatment were investigated and lipoxygenase activity was determined spectrophotometrically at 234nm.

Optimal activity was found at pH 6 and 7 and a linoleic acid concentration of 3.0mM and 3.5mM for the whole and dehulled seeds respectively. The values of Michalis-menten constant (km) and Vmax were determined as 1.563mM and 344.8 units ml^{-1} mm^{-1} for the whole seed and 1.724mM and 2083 units ml^{-1} mm^{-1} for the dehulled seed. The energy of activation for both the whole and dehulled seeds were obtained as 28.3 kJ mol^{-1} and 10.8 kJ mol^{-1} respectively. Lipoxygenase activity was found to increase at the temperature 60 ^{o}C and 50 ^{o}C for the whole seed and the dehulled seed respectively.

Key words: *Luffa aegyptiaca,* Lipoxygenase, Kinetic characterization.

Introduction

The need to adequately feed a growing population has led to the utilization of unexploited plants as sources of food for either human consumption or as livestock feeds.

In Nigeria, getting the required daily allowance of food is a major problem primarily due to the poor economic status. Hence the search for cheap sources of the different forms of food and scientists are getting more involved in determining their nutritive value.

Luffa aegyptiaca is a local plant found growing wildly in waste lands and rubbish dumps, usually needing little or no cultivation. The seeds of Luffa aegyptiaca have thus been of interest for quite some time. The seeds are oil seeds having 44.8% oil comprising mostly of polyunsaturated fatty acids. The protein content of the deffated seed was found to be 39.0%. Therefore the seeds of *Luffa aegyptiaca* presented a high nutritive value and thus potential for livestock feed. However, due to the high percentage of the oil and high linoleic acid content (46.2%) (1), the seeds of *luffa aegyptiaca* would be highly susceptible to rancidity due to the presence of the enzyme Lipoxygenase (LOX).

Lipoxygenase (linoleate:oxygen oxidoreductase, (EC 1.13.11.12) is a dioxygenase catalyzing the hydroperoxidation of fatty acids containing a cis,cis- 1,4-pentadiene structure, e.g. linoleic, linolenic, and arachidonic acids. The enzyme is apparently ubiquitous in eucaryotes. In animals, the hydroperoxides produced from arachidonic acid by different LOX form precursors of important classes of chemical messengers such as leukotrienes and lipoxins (2). In plants, no clearly defined physiological role has been demonstrated for LOX, but the hydroperoxides formed from linoleic and linolenic acids are further metabolized and produce several substances with pronounced physiological activity. Roles for LOX in plant growth and development, senescence, wound responses, and resistance against pathogens and pests have all been proposed (3).

Lipoxygenase (linoleate: oxygen oxidoreductase (E.C. 1.13. 11. 12) are very relevant to food plants because their action destroys the essential polyunsaturated fatty acids producing hydroperoxides that can decompose to form derivatives with undesirable flavour (4) and rancidity of oils.

The present study is aimed at isolating the enzyme Lipoxygenase from *luffa aegyptiaca* seeds and characterising it for adequate knowledge to assist in the processing of the oil from the seed. The paper therefore reports the properties and thermal stability of *Luffa aegyptiaca* lipoxygenase.

Materials and Methods

Plant material collection

Whole fruit of *luffa aegyptiaca* were freshly harvested from nearby bushes near the University-Lagos-State University, Ojo, Lagos. The seeds were removed and dehulled. (Soaked to make the coat softer and then manually dehulled).

Both whole seeds and dehulled seeds were sundried and blended into fine powder and stored in dried plastic containers tightly covered in a deep freezer until used.

Defatting of Luffa Seeds and Enzyme Extraction

Samples of ground whole and dehulled seeds of *luffa aegyptiaca* were defatted with hexane and the enzyme extracted with sodium phosphate buffer (0.1M, pH 6.5) at 0^{o}C by the method of Truong Van Den *et al* 1982 [(5).] The crude lipoxygenase extract was filtered, centrifuged and treated with ammonium sulphate (42.9% saturation). The precipitate was collected into sodium phosphate buffer (0.05M, pH 7) and then further diluted with 50mls distilled water and stored at -20^{o}C until it is ready for Lipoxygenase assay.

Physicochemical properties of Lipoxygenase

The effect of pH (range 5-9), temperature (50^{o}C- 70^{o}C) and thermal inactivation on lipoxygenase activity was determined using linoleic acid as substrate. Lipoxygenase activity was determined spectrophotometrically at 234nm.

Determination of effect of substrate concentration on Enzyme activity

The effect of substrate concentration (0.25- 4.00mM) on LOX was determined using linoleic acid as a substrate. LOX activity was determined spectrophotometrically at 234nm.

Enzyme kinetics

The Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) were determined using Lineweaver-Burk equation.

Lipoxygenase Assay

Lipoxygenase activity was determined spectrophotometrically by measuring the increase in absorbance due to the formation of conjugated diene hydroperoxide at 234nm (6). The assay was performed at 30^{o}C with a recording Varian series 634 spectrophotometer. A unit of lipoxygenase activity corresponds to a change in absorbance of 0.001min^{-1}.

Protein Determination

The protein concentrations in the enzyme extract were determined by the method of lowry *et al* (1951) [7] using BSA (Bovine serum albumin) as standard.

Results And Discussion

Enzyme activity

A unit of lipoxygenase activity is the activity that produces an increase of 0.001 absorbance at 234nm/min/ml of enzyme.

$$\text{Lipoxygenase activity} = \frac{\Delta A234}{0.01 \times 0.1 \text{ ml}} = \text{unit/ml/min}$$

where 0.1ml is the amount of enzyme extract used in the reaction

Effect of pH on Lipoxygenase Activity

Lipoxygenase activity values were calculated from plots of absorbance versus time. Table 1 shows the activity of lipoxygenase of *Luffa aegyptiaca* at different pH ranging from 5-9 for whole (W) and dehulled (D) seeds at a specific substrate concentration. This is also graphically represented in Fig. 1.

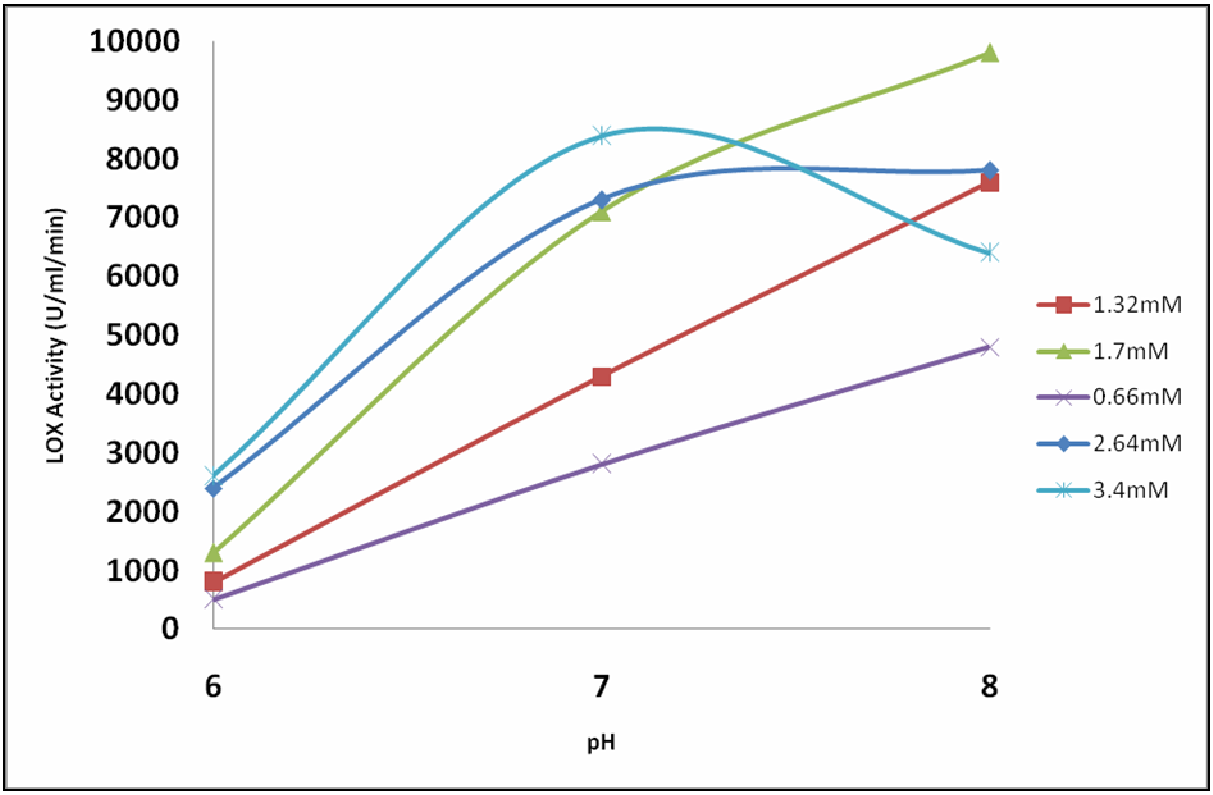

Fig 1: Lipoxygenase activity at different pH

The result shows that increasing the pH from 5 to 8 increased the activity of lipoxygenase in the dehulled seed while it falls drastically at pH 9. The optimal pH for lipoxygenase of dehulled seeds of *luffa aegyptiaca* is at pH 7.0. The whole seed has a pH optimal at pH 6.0. The activity of lipoxygenase of whole seed dropped at pH 7.0. Lipoxygenase isoenzyme are classified as type 1 or type 2 on the basis of the p H optima and the positioned specificity of the hydroperoxides formed (8).

Type 2 lipoxygenase generally have pH optima of 6-7 and therefore most of the lipoxygenases found in plants e.g. peas and cowpea are considered type 2 (9; 10). Type 1 lipoxygenase has optimum activity at pH 9.0 to 10.0 and the original enzyme has been crystallized from soybeans (11). Thus the lipoxygenase of *luffa aegyptiaca* can be classified as type 2 lipoxygenase. The difference observed in the pH between the whole seed and dehulled seeds would have been responsible to the presence of the hull of the seed.

Table 1: Lipoxygenase activity of the whole and dehulled seeds of *Luffa aegyptiaca* at different pHs.

	Lipoxygenase activity units ml^{-1} min^{-1} at different Ph				
Ph	5	6	7	8	9
Whole seed	600	1000	600	800	400
Dehulled seed	500	800	1600	1400	400

Effect of Substrate concentration on Lipoxygenase Activity

Table 2 shows the lipoxygenase activity at various substrate concentrations for whole and dehulled seeds of L. aegyptiaca. Lipoxygenase activity was found to be maximal at linoleic acid concentration of 3.00mM and 3.50mM for the whole and dehulled seeds respectively. At linoleic acid concentrations higher than the observed maximal, lipoxygenase activity decreased considerably (Fig. 2).

Table 2: Lipoxygenase activity at various substrate concentrations for whole and dehulled seeds of *Luffa aegyptiaca*.

Substrate concentration S (mM)	Lipoxygenase activity (units ml^{-1} min^{-1})		1/S	$1/Vx10^{-4}$	
	Whole seed	Dehulled seed		W	D
	pH 6.0	pH 7.0			
0.25	100	880	4.0	100	11.4
0.50	2600	680	2.0	3.8	14.7
1.00	1760	1000	1.0	5.7	10.0
1.50	200	800	0.67	50.0	12.5
2.00	240	1120	0.50	41.7	8.9
2.50	240	1600	0.40	41.7	6.3
3.0	460	1080	0.33	21.7	9.3
3.50	60	2320	0.29	166.7	4.3
4.00	200	1080	0.25	50.0	9.3

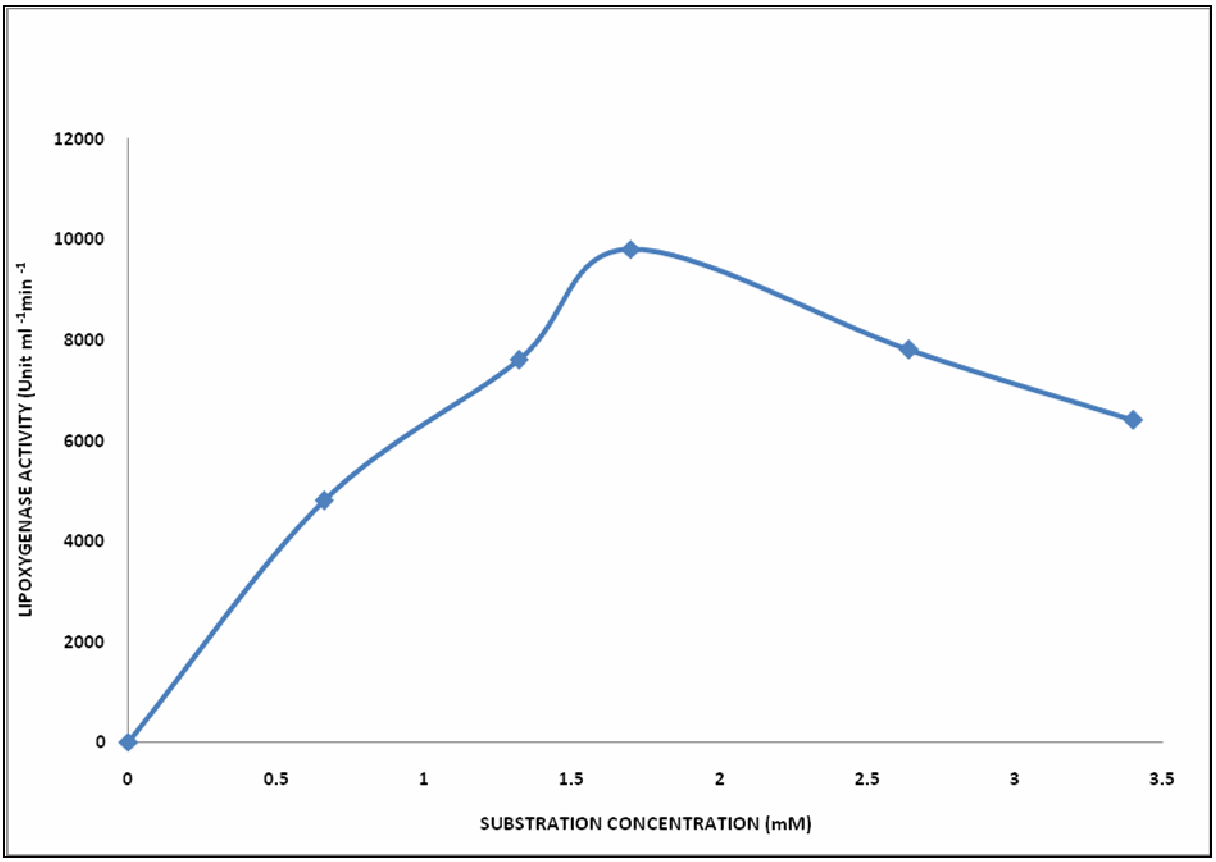

Fig 2: Effect of substrate concentration on Lipoxygenase activity

The great difference in lipoxygenase activity in the whole and dehulled seed at different linoleic acid concentration can be attributed to the presence of the hull which makes up about one-third of the total weight of the whole seed.

Winged bean lipoxygenase is recorded as having maximal activity at linoleic acid concentration of 2.64mM which is low compared to what obtains in *L. aegyptiaca.* Hence the substrate concentration required for maximum activity in *L. aegyptiaca* is greater than that of winged bean, this suggests that lipoxygenase in the former seed has greater affinity for linoleic acid than that of the latter seed.

Since km is the substrate concentration at which the reaction rate is half of its maximal value i.e ½ Vmax, and *L. Aegyptiaca* lipoxygenase has a greater affinity for substrate than winged bean lipoxygenase then lipoxygenase in the former seed requires more substrate than that in the latter seed for reaction to occur at maximum rate.

Effect of substrate concentration on enzyme kinetics, Michaelis Menten Constant

Lipoxygenase activity shown in Table 2 was plotted against substrate concentration at the optimal pH of 6 and 7 for whole seed and dehulled seeds of Luffa aegyptiaca respectively (Fig. 2). The results showed that the activity increases with linoleate concentration until maximum substrate concentration was achieved. The characteristic hyperbolic curve was obtained. From the double reciprocal (Lineweaver-Burk) plot of 1/v against 1/s (Fig. 3), the Michaelis constant Km are 1.563mM and 1.72mM for the whole and dehulled seeds respectively; while the values obtained for Vmax are 344.8 units and 2083.3 units respectively.

The Lineweaver-Burk plot is regarded inappropriate for estimating K_m values because it gives a misleading impression of the experimental errors (12). For small values of V, small errors in V lead to enormous errors in 1/V but for large values of V, the same small errors in V lead to barely noticeable errors in 1/V

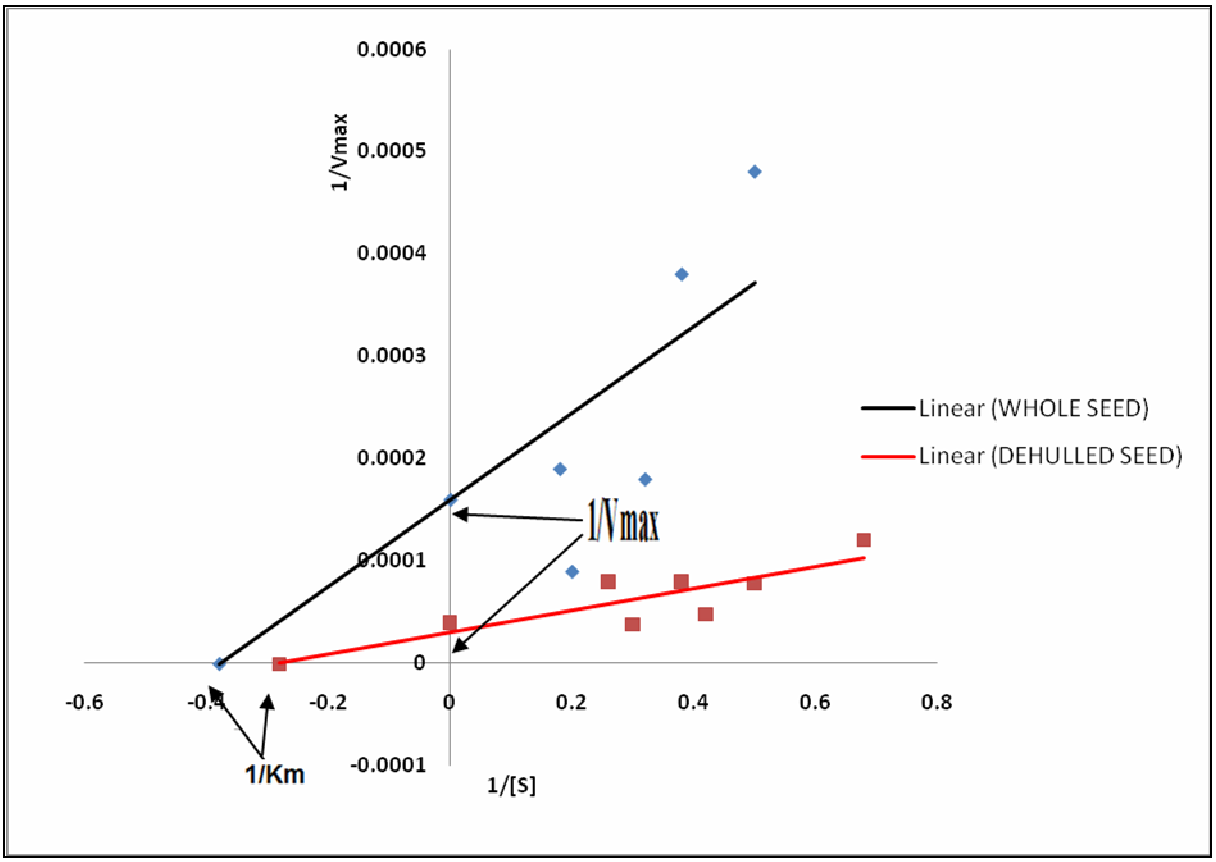

Fig 3: Lineweaver-Burk plot for Lipoxygenase activity of whole and dehulled seeds of *Luffa aegyptiaca*

Thermal Inactivation of Lipoxygenase

Lipooxygease activities (whole and dehulled) are shown on Table 3 for enzyme extracts heated at various temperatures of 50°C, 60°C, 65°C and 70°C. The heated extracts were assayed (in duplicate) for lipoxygenase activity using 2.5Mm pH6.0 and 3.5Mm pH7.0 linoleic acid for the whole and dehulled seeds respectively.

At 50°C lipoxygenase activity increased progressively with time for both the whole as well as dehulled seeds respectively. However at 60°C, 65°C and 70°C, activity of lipoxygenase was gradually reduced with time (Fig. 4). For the dehulled seed, loss of 67% activity occurred within 10mins at 60°C and 83% loss activity at same heating time at 65°C. The lipoxygenase of *luffa aegyptiaca* is quite unstable when compared to winged bean lipoxygenase studied by Truong *et al* (1982b)[9] and Gordon and Mtebe, 1987 [13].

However, the lipoxygenase activity of the whole seed appears to be more stable than the dehulled seed. The plausible explanation is that the lipoxygenase of the dehulled seed has been more exposed. The sensitivity of lipoxygenase isoenzymes to heat depends both on the source of lipoxygenase and on the type of isoenzyme.

The thermal stability of lipoxygenase from other sources has been investigated. Trong and Mendoza (1982) [10] have investigated the thermal stability of cowpea lipoxygenase isoenzyme type 1 and type 2. They reported that 1 and 2 differed markedly in thermal stability. The type 1 lipoxygenase retained 80% of its original activity after 30 minutes at 50°C while the L-2 lost more than 80%. At 60°C, L-2 had no activity after 2mins, but 30minutes was required for L-1 to lose its activity at this temperature. The thermal stability of the enzyme extract in this study therefore shows close similarities with the lipoxygenase L-2 isoenzyme described by Truong and Mendoza above.

The log of Lipoxygenase activity versus heating time is graphically represented in Fig. 5

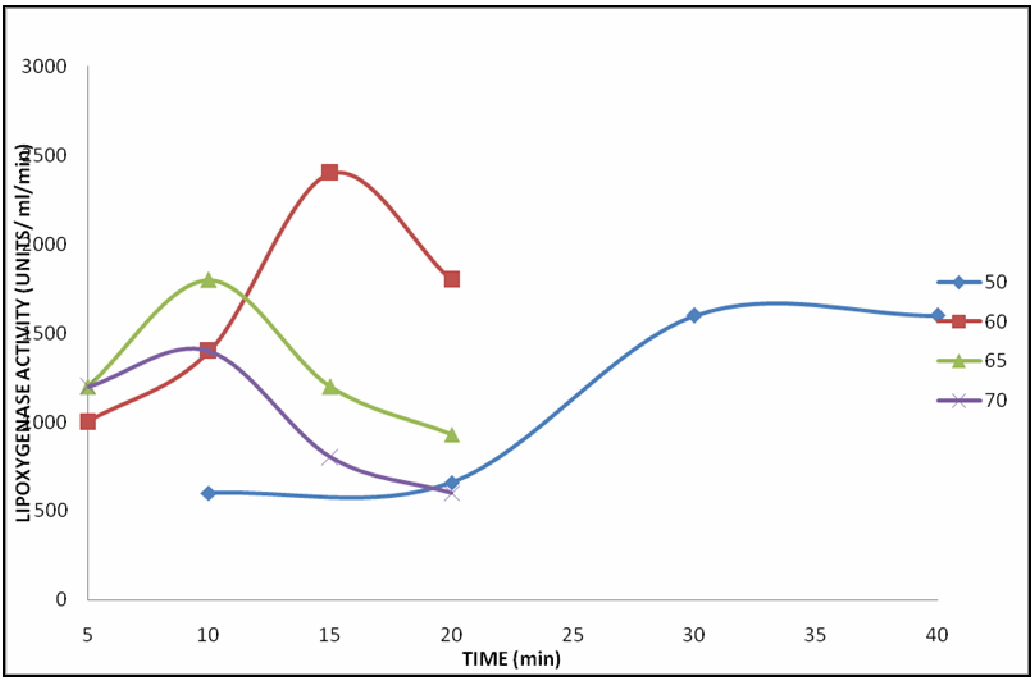

Fig 4: Thermal inactivation of lipoxygenase of *luffa aegyptiaca*

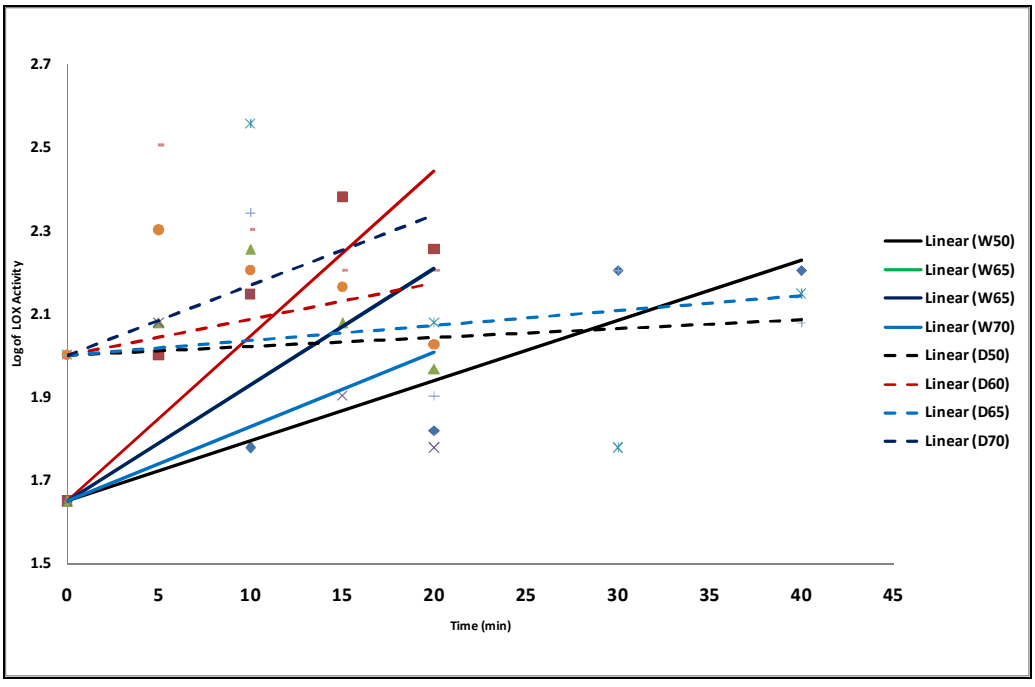

Fig 5: Log of Lipoxygenase activity versus heating time

Table 3: Lipoxygease activities of whole and dehulled seeds of *Luffa aegyptiaca* at varying temperature and time.

Heating Temp (^oC)	Heating Time (mins)	Lipoxygenase Activity (units ml^{-1} min^{-1})		% Activity		Log of Activity	
		W	D	W	D	W	D
Unheated	Control	140	480	100	100	2.146	2.681
	10	600	360	42.9	75.0	1.778	2.556
50	20	660	120	47.1	25.0	1.820	2.079
	30	1600	60	114.3	12.5	2.204	1.778
	40	1600	140	114.3	29.2	2.204	2.146
	5	1000	200	71.4	41.7	2.000	2.301
60	10	1400	160	100.0	33.3	2.146	2.204
	15	2400	146	171.4	30.4	2.380	2.164
	20	1800	106	128.6	22.1	2.255	2.025
	5	1200	220	85.7	45.8	2.079	2.342
65	10	1800	80	128.6	16.7	2.255	1.903
	15	1200	160	85.7	33.3	2.079	2.204
	20	930	120	66.4	25.0	1.968	2.079
	5	1200	320	85.7	66.7	2.079	2.505
70	10	1400	200	100.0	41.7	2.146	2.301
	15	800	160	57.1	33.3	1.903	2.204
	20	600	160	42.9	33.3	1.778	2.204

Conclusion

It can be concluded from the results of this work that *Luffa aegyptiaca* lipoxygenase, a type -2 enzyme, is a relatively active enzyme with maximum activity at a linoleic acid concentration of 3.00mM and 3.50Mm (whole and dehulled seeds respectively).

L.aegyptiaca lipoxygenase is a relatively more stable enzyme than that of winged bean since less of its activity is lost under specified conditions and hence requires tough conditions (e.g longer heating time at high temperature) for inactivation.

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