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Purification, characterization and kinetic properties of partially purified lipoxygenase, extracted from peanut seeds (*Arachis hypogaea*)

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ABSTRACT

Background: Lipoxygenases (LOX) are found widely in plants, fungi and animals. These enzymes catalyze the hydroperoxidation of polyunsaturated fatty acids containing cis, cis-1,4-pentadiene moieties. Furthermore, plants contain multiple isoforms of the LOX enzyme which differs in their biochemical properties. **Objectives:** The present study was carried out to isolate, purify and characterize LOX from peanut seeds. **Methods:** The concentrated dialyzed cell free extract was fractionated using ammonium sulfate followed by ion exchange chromatography using DEAE-cellulose. SDS-PAGE was performed for the molecular weight determination. **Results:** Purification of peanut LOX indicates that peanut LOX has three isoenzymes with molecular masses: 113, 113 and 95 KD. The isoenzymes exhibited optimum pH of LOX- 1 and 2 at pH 6 while the optimum pH of LOX -3 was at pH 8. While, the three isoenzymes have the same optimum temperature; 20^{O}C. The Vmax and the Km values of the three isoenzymes were estimated. Our data showed that the LOX activity was irreversibly inhibited by α-chymotrypsin to about 66% as compared to the control. While, atropine had no effect on LOX activity. The heating of peanut at 80^{O}C for 2 and 10 min and at 90^{O}C for 2 min inhibited the LOX activity by about 71.4 %, 84 % and 90.8 %, respectively. While, the heating of peanut at 80^{O}C for 10 min extend the shelf- life of peanut.

INTRODUCTION

Lipoxygenases (linoleate:oxygen oxidoreductase, LOX, EC; 1.13.11.12) are a class of widespread dioxygenases that catalyze the addition of oxygen to the polyunsaturated fatty acids that contain a (1Z, 4Z)-pentadiene system [(1)]. Animal

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LOXs initiate the cascade of arachidonic acid, which is a source of several bioregulators in a number of cellular responses such as prostaglandins, lipoxins, thrombaxanes, leuktrienes and hepoxilins [(2)]. Some products of LOX reaction, such as dihydroxy fatty acid leuktriene B4 are ligands for G- protein coupled receptors and nuclear hormone receptors. These receptors regulate the inflammation and lipid homeostasis pathways [(2)].

Although these enzymes are widely distributed in the animal and plant kingdoms, they are particularly abundant in grain legume seeds and potato tubers [(3, 4)]. The plants contain multiple isoforms of the LOX enzyme which differs in terms of its substrate preference, optimal pH, product formation and stability [(5)]. The positive effect of the added lipoxygenase during the production of white bread is well established. In addition to the overall dough rheology, lipoxygenase is also an effective bleaching agent [(4)]. Lipoxygenase from different sources catalyzes oxygenation at different points along the carbon chain, referred to as "positional" or "regio" specificity. Such specificity has significant implications for the metabolism of the resultant hydroperoxides into a number of important secondary metabolites [(6)]. The Plant lipoxygenases are particularly abundant in grain legume seeds (beans and peas) and potato tubers [(6)]. LOXs have a role in the production of the volatile molecules that can positively or negatively influence the flavor or aroma of many plant products [(7)]. Higher plants produce multiple isoforms of LOX enzyme. These isozymes differ in terms of their optimal pH of catalysis, substrate preference, regiospecificity and the ability to bleach carotenoid pigments [(6)]. A key role for some LOX isoforms is in the generation of fatty acid hydro eroxides destined for jasmonic acid (JA), which triggers gene activation during wound response in plants. The fatty acid hydroperoxides generated by the activity of LOX are potentially deleterious to membrane function by causing increased rigidity [(6)].

Most data concerning the structure and the mechanism of LOX originate from soybean LOX1 because this enzyme is abundant in the seeds. Lipoxygenase has food-related applications in bread making and aroma production while has negative implications for color, off-flavor and antioxidant status of plant based foods. In many applications, it is possible to use plant extracts, but such extracts usually contain multiple enzymes that may reduce polyunsaturated fatty acid substrate availability or metabolize the hydroperoxide products thereby modulating lipoxygenase action. This fact has led to understand the activities and properties of plant and other lipoxygenases as food processing additives and to remove selectively specific lipoxygenase from plants based on such understanding [(4,6)]. Therefore, our study was carried out to characterize the peanut lipoxygenase at the biochemical level. The purification steps of peanut LOX and its biochemical characterization were also reported. In addition, the effect of atropine and α-chymotrypsin, as drugs, on the partially purified peanut lipoxygenase were also determined.

2- Materials and methods

2.1. Plant material

Peanut was brought from the local market at Alexandria, Egypt and stored in dry place at 4°C.

2.2. Chemicals

Linoleic acid, Tween 20, bovine serum albumin, DEAE-Cellulose, ammonium sulphate, sodium dodecyl sulfate-sephadex, and standard protein markers for electrophoresis were purchased from Sigma–Aldrich (St. Louis, MO). All the

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used reagents and chemicals were of analytical grade.

2.3. LOX assay

The activity of LOX was determined by continuous monitoring of the formation of conjugated dienes at 20°C [8]. The mixture contained (2.98-X) ml of Tris-HCl buffer (pH 7) since, X is the volume of enzyme plus 20µl of sodium linoleate (substrate). After each addition, the mixture was stirred with a few strockes of plastic puddles. The reference cuvette contained no enzyme. The absorption was recorded at 234 nm as function of time and the activity was determined from the slope of the linear portion of the curve. One unit of LOX activity is defined as the amount of enzyme catalyzing the formation of 1 µmol of the hydroperoxide per min.

2.4. Protein determination

Protein content was determined by modified Lowry's method using bovine serum albumin as standard protein [9].

2.5. LOX purification

A LOX has been purified according to the method of liegenthart et al. [10].

All steps of LOX purification were performed at 4°C. The first step is the preparation of crude enzyme where, about 300 g of peanut were grounded using cold mortar and rapidly homogenized in Tris-HCl buffer (0.1 M, pH 7) containing KCl (1:10 w/v). The homogenate was filtered through sterilized muslin and centrifuged at 17,000 rpm for 20 min at 2°C and the supernatant (crude enzyme) was kept at -80°C. The second step is the preparation of the protein using ammonium sulfate fractionation (20%, 40% and 65%). The solid ammonium sulfate was added to the crude enzyme to obtain 20% saturation and centrifuged at 18,000 rpm and at 2 °C. The precipitate was dissolved in 25 ml of Tris-HCl buffer (0.1M, pH 7) and dialyzed against the same buffer. While, the supernatant was saturated to 45% with (NH4)2 SO4 and centrifuged at 18,000 rpm for 20 minutes at 2°C. The precipitate was dissolved in 25 ml of Tris-HCl buffer (0.1M, pH 7) and dialyzed against the same buffer. The supernatant fluid was saturated to 65% with (NH4)2SO4 and then centrifuged for 20 minutes at 18,000 rpm. The precipitate was dissolved in 25 ml Tris-HCl buffer (0.1M, pH 7) containing KCl and dialyzed against the same buffer. The protein contents and enzyme activities of each precipitate were determined. The third step is the ion exchange chromatography using DEAE-cellulose. The specific activity per mg protein for each fraction was determined. The fraction which contained higher LOX activity was chromatographed on DEAE-cellulose ion exchange chromatography (column size =2cm×15 cm) and eluted with a linear KCl gradient (0 - 0.5 mM) dissolved in Tris-HCl buffer (0.1M, pH 7) at flow rate 50 ml/h. Active fractions were collected and concentrated using ultrafiltration.

SDS-PAGE and gel staining

SDS-PAGE was performed according to the method of Laemmli [11] using 10 % polyacrylamide gel. Standard protein markers were used as reference.

Determination of enzyme kinetics

The optimum pH and optimum temperature as well as the Steady state kinetic constants (Michaelis constant; Km) and maximum velocity (Vmax) of the parially purified LOX were determined.

Inhibition of partially purified peanut LOX activities

The partially purified peanut LOX was incubated with 2 mM of either atropine or α- chymotrypsin, for 5 min. at 20°C. Then

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the drugs inhibitory effect was determined by detecting the activity for 2 min., spectrophotometrically. In the control sample, the enzyme was replaced by identical volume of buffer.

The IC50 value of α- chymotrypsin, defined as the drug concentration necessary to give 50% enzyme inhibition, was calculated where 15 mg partially purified peanut LOX was incubated with different concentration of α- chymotrypsin (0.08-14 mM) for 10 min at pH 7. Then, 20 µl of sodium linoleate (66 µM) was added and the values of Vmax and Km were determined. The relationship between the concentration of α- chymotrypsin and LOX activity was plotted as percentage of control (enzyme activity without α-chymotrypsin). Steady state kinetic constants (Michaelis constant) and maximum velocity were determined in the absence and presence of α- chymotrypsin (0.24 mM).

Reversibility test: The reversibility or irreversibility of LOX inhibition was examined by dilution test that was carried out by incubating 15 mg protein of the partially purified peanut LOX with 2 mM of α- chymotrypsin, separately. LOX activities were measured after dilution (12.5-fold) with Tris - HCl buffer (0.1 M, pH 7) and compared with the inhibition of LOX with the same concentration of α-chymotrypsin without dilution.

2.6. Short-time heat treatments of peanut

Peanut granules were divided into six groups. Group 1 was heated at 70ºC for 2 min, group 2 was heated at 70ºC for 10 min, group 3 was heated at 80ºC for 2 min, heated group 4 was at 80ºC for 10 min, group 5 was heated at 90ºC for 2 min and group 6 was heated at 90ºC for 10 min. After heat treatments, crude peanut LOX was prepared, LOX activities of the six groups were determined for seven months and the mean of the activities were plotted.

2.7. Statistical analysis

Each parameter was tested in triplicate. Conventional statistical methods were used to calculate means and standard deviations. Statistical analysis (ANOVA) was applied to the data to determine the significant differences ($P< 0.05$). To determine the significant differences between the levels of the main factor, Tukey's multiple range test was applied between means. Statistical data analysis was undertaken using the statistical software Prism, version 3.

3. Results and discussion

3.1. Purification of peanut LOX

Through ammonium sulfate fractionation, the fraction which contained high activity of lipoxygenase was 45-65 %. The enzyme activity in this fraction was 192.666 µ mol/mg protein and the protein content per 1ml was 1.2 mg. The yield was 80 % and the purification fold was 2.44 (Table 1). The separation of peanut LOX on DEAE-cellulose ion-exchange chromatography showed that the activity of the enzyme was recovered in three peaks (Fig. 1, and Table 1). The SDS-PAGE analysis of the eluted materials (peak 1, 2 and 3 of DEAE-cellulose) showed three bands (Fig. 2, lane 1, 2 and 3) and their molecular masses were about 113, 113 and 95 KD, respectively. The equal mixture of peak 1, 2 and 3 gave two bands (a and b). This indicates that peanut LOX has three isoenzymes (1, 2 and 3), with molecular masses: 113, 113 and 95 KD, respectively.

3.2. The optimum pH and optimum temperature of peanut LOX

The specific activities of LOX -1, -2 and -3 were measured at different values

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of pH ranged from (5 - 9). The isoenzymes exhibited optimum pH of LOX- 1 and 2 at pH 6 while the optimum pH of LOX -3 at pH 8 (Fig. 3). The specific activities of LOX -1, -2 and -3 were measured over the temperature range 10-80ºC. The data showed that the three isoenzymes have the same optimum temperature, 20ºC (Fig. 4).

3.3. Kinetic parameters

Linoleic and linolenic acids are known to be the best substrates for lipoxygenase in plants [1, 12]. In the present study, linoleic acid was used for the determination of peanut LOX activity. Figure 5 shows the relationship between the substrate concentration and LOX activity and Figure 6 shows the linweaver-Burk Plot analysis of LOX -1, -2 and -3. Vmax of LOX -1, -2 and -3 are 48, 59.5 and 81.96 nmol hydroperoxy linoleate /mg protein /min, respectively. While the Km of LOX -1, -2 and -3 are 10.25, 12.5 and 5.04 µM respectively.

A LOX extract from the fungus *Fusarium proliferatum* was also partially purified by ammonium sulfate precipitation at 0-40% of saturation [13]. They reported a 2.7-fold increase in the purification of LOX activity. Moreover, a partially purified extract from the alga *Chlorellapyrenoidosa* was obtained by ammonium sulfate precipitation at 0-42% of saturation [13]. On the other hand, the purification of lipoxygenase from olive fruit (*Olea europaea L.*) achieved 65-fold purification with a yield of 18%. Its specific activity reached 34,350 Units/mg protein when linoleic acid was used as substrate. The SDS-PAGE analysis of olive LOX showed a single band with a molecular mass of 98 KD [1]. Olive LOX showed its maximum activity at pH 6.0. The activity of olive LOX remained high (> 50%) between pH 5.0 and 6.5 [1]. Olive LOX had better affinity for Linoleic (Km= 82.44 µM) than for the linolenic acid (Km= 306.26 µM).

The purification of soluble tomato lipoxygenase could be achieved giving a 28 % yield and a specific activity of 267 nkat./mg protein with Km= 4.1 mM and Vmax= 7.4 mmol/mg protein/min. [14]. While, the plasma membrane bound LOX from soybean cotyledons had molecular mass of about 92 KD and exhibited maximal activity in the alkaline borate buffer pH range (7.5-10). Also, its specific activity was 24.2 µ mol/mg protein/min and the purification fold was 21.2 [15]. On the other hand, Perraud [13] reported that the LOX extract of *Geotrichum candidum* exhibited two major activities at pH 8 and 3.75 using linoleic acid as substrate. At pH 3.75, the enzyme extract displayed the highest specific activity using linolenic and linoleic acids as substrates (Km= 0.067 mM and Vmax= 71 mmol/mg protein/min). Whereas, at pH 8.00 the maximal LOX activity was obtained using arachidonic and linoleic acids (Km= 1.05 mM and Vmax=59 mmol/mg protein/min).

3.4. Inhibition studies

Our data showed that the LOX activity was inhibited by α- chymotrypsin to about 66% as compared to the control. While, atropine had no effect on LOX activity (Fig.7 & 8). The inhibition of LOX by α-chymotrypsin was time dependent (Fig. 9). The degree of inhibition of LOX as function of α-chymotrypsin concentration (0.08-14 mM) was also determined (Fig. 10). The present study showed that the inhibition of LOX by α-chymotrypsin was concentration dependent and the IC50 value equal 0.26 mM.

3.4.1 Reversibility of LOX activity by α-chymotrypsin

The LOX activity of the control test was 0.688 nmol/mg protein/min. However, the LOX activities in the presence of α-chymotrypsin (2mM) without dilution and after dilution were 0.23 and 0.25 nmol/mg protein/min (66.5 % and 63.6% of

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inhibition), respectively. This indicates that the inhibition of LOX by α-chymotrypsin was irreversible. It has been reported that α- chymotrypsin hydrolyzes peptide bonds on the carboxyl side of the aromatic residues; phenyl alanine, tyrosine and tryptophan [16].

3.4.2. Inhibition type

The LOX activity was measured at different concentrations of sodium linoleate (66-600 µM) in the absence and presence of 0.24 mM α-chymotrypsin (Fig. 11). The results showed that α-chymotrypsin inhibited LOX activity and this inhibition changed the Vmax value. The Vmax of the control was 0.491 nmol/mg protein/min, while the Vmax in the presence of α- chymotrypsin was 0.311 nmol/mg protein/min, respectively. However, α- chymotrypsin did not change the Km value (66.3 µM). These results indicated that α- chymotrypsin inhibited LOX by a noncompetitive inhibition (Fig. 12). The kinetic constants, Ki, were 421 µM, 63.6 % and 36.4 %, respectively. Alpha chymotrypsin is one of the most well-known anti-inflammatory enzymes, the proteolytic activitiy of α-chymotrypsin has been suggested to be vital for the control of inflammation by clearing inflammatory debris [17, 18]. There are several reports advocating the use of this proteolytic enzyme for the treatment of the inflammatory disorders [18]. The anti-inflammatory effect of α- chymotrypsin may be attributed due to the stimulation of neutrophil apoptosis, inhibition of neutrophil migration at the inflammatory site, inhibition of bradykinin synthesis in addition to decreased vascular permeability [19-21].

Several non-steroidal anti-inflammatory compounds such as glaphenine, oxyphenbutazone and sodium dichlofenac showed inhibitory effect on LOX [22]. In addition SK and F 86002 [5-(4-pyridyl-6-(4-flourophenyl-2-,3-dihydroimmidazo (-2,-1-b) thiozole] and manolidal exhibited inhibition of LOX [23]. Also, indomethacin and other non-steroidal anti-inflammatory drugs such as flurbiprofen and aspirin reduced the concentration of PGE2 and TXB2 but not LTB4 [24].

3.5. Lipoxygenase activity of untreated and heat treated peanut grains

The activity of LOX in the homogenate of untreated and peanut heat treated (at different temperature where; 70°C, 80°C, 90°C for 2 min and 10 min) are recorded in Table 2 and Fig. 13. The data showed that the initial activity of the untreated peanut LOX was 0.678 nmol/mg protein/min. While, the initial activities of the heat treated peanut LOX at 70°C, 80°C, 90°C for 2 min were 0.41, 0.356, and 0.11 nmol/mg protein/min, respectively. However, the initial activity of the heat treated peanut LOX at 70°C, 80°C, 90°C for 10 min were 0.205, 0.194 and 0.063 nmol/mg protein/min, respectively. The present data demonstrated that the heating of peanut at 80°C for 2 and 10 min and at 90°C for 2 min inhibited the LOX activities by about 71.4 %, 84 % and 90.8 %, respectively. In addition, the heat treatment showed that the best taste of peanut and the best color of peanut kernels were at 80°C for 10 min and at 90°C for 2 min. So, we hypothesize that the short heat treatment inactivated LOX and extend the shelf- life of peanut.

Branch et al., [25] reported that peanuts lost 46 % of LOX activity after a 90s exposure to 79°C, and the oil extracted from untreated peanuts exhibited higher peroxide and fatty acid values than the oils extracted from heat treated peanuts. Heat treated peanuts at 79°C for 90s exhibited a longer shelf-life than untreated [26]. Buranasompob [27] demonstrated that the oils extracted from walnut kernels exhibited significantly greater peroxide values than the oils extracted from short

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heat treated walnut kernels. Also, it has been demonstrated that the short time heat treatment at 55 or 60ºC for 2 or 10 min do not enhance the development of the oxidative rancidity during accelerated storage of walnut kernels [26, 28]. Henderson et al., [29] reported that LOX in pea flour lost 100 % activity after 25 min exposure to 65 or 70ºC and after 15 min exposure to 80ºC. Kermasha and Metche [30] reported that french beans lost almost 100 % LOX activity after a 150s exposure to 96ºC. They also reported that air drying is effective method to inactivate LOX and suggested that oxidation of unsaturated fatty acids in dried stored French beans may result from auto oxidation rather than enzyme activity. Furthermore, Zacheo et al., [31] reported that LOX in almonds remained unchanged after 10 min exposure to 40ºC, while LOX was inactivated by increasing the temperature. They also found that LOX in almonds lost 100 % of its activity after exposure to 80ºC. In addition, Zacheo et al., [31] reported that LOX activity in almond kernels remains unaltered or increased with aging time and that in-shell almonds did not exhibit significant changes in peroxide values after 2 years of storage at room temperature.

The peanut or groundnut (*Arachis hypogaea*), is a species in the *legume Fabaceae* native to South America, Mexico and Central America [32]. It is an annual herbaceous plant. Peanuts are rich source of protein, containing low amounts of the essential amino acids cysteine and methionine but high in lysine [33]. Peanuts contain substantial quantities of polyunsaturated fatty acids while, peanut oil is a mainly monounsaturated fat (50%), 97% of which is oleic acid. Saturated fatty acids compose 13% of peanut fat; where (74%) of which is palmitic acid and (16%) is stearic acid. Therefore, peanuts susceptible to oxidative and hydrolytic rancidity [34]. On the other hand, peanuts and nuts in general have antioxidants and other chemicals that may provide health benefits [35]. Many researches showed peanuts rival the antioxidant content of many fruits. Roasted peanuts rival the antioxidant content of blackberries and strawberries which are far richer in antioxidants than apples, carrots or beets [36]. Peanuts contain high concentrations of antioxidant polyphenols, primarily a compound called p-coumaric acid [37]. The roasting can increase peanuts' p-coumaric acid levels [36].

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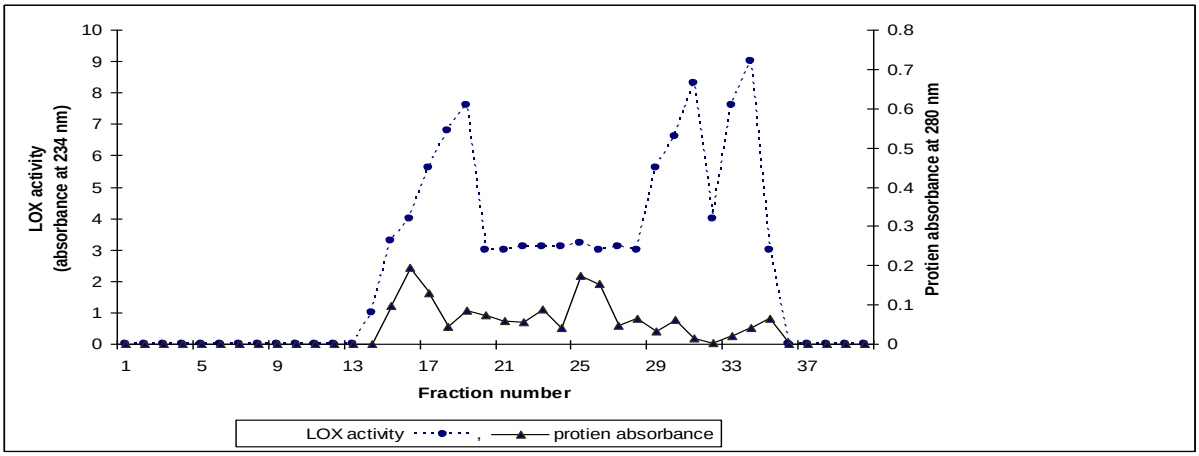

Fig.1. Purification of Peanut LOX on DEAE-Cellulose. Protein were eluted first with 50 ml of Tris-HCl buffer (0.1 M, pH 7), Followed by 500 ml of a linear gradient (zero - 0.5M KCl). The eluent was collected in fractions of 10 ml and assayed for LOX activity.

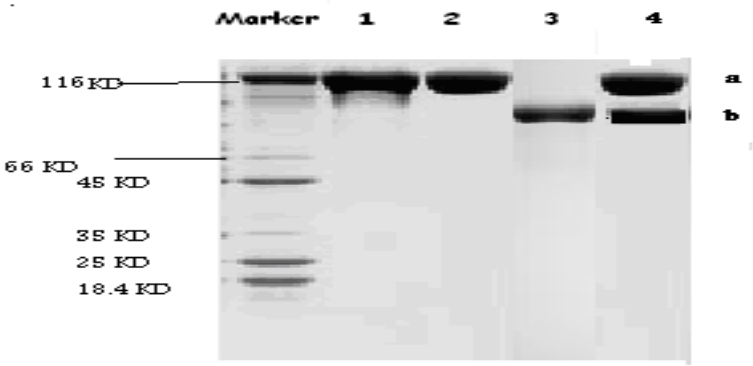

Fig. 2. SDS-PAGE of peanut LOX. Molecular mass markers: β galactosidase (116 KD), bovine serum albumin (66 KD), oval albumin (45 KD), lactate dehydrogenase (35 KD), restriction endonuclease (25 KD), lysozyme (18.4 KD). Lane 1, the soluble LOX fractions (peak 1) which was eluted from the anion exchange chromatography (~ 113 KD), lane 2, the soluble LOX fractions (peak 2) which was eluted from the anion exchange chromatography (~ 113 KD), lane 3, the soluble LOX fractions (peak 3) which was eluted from the anion exchange chromatography (~ 95 KD), lane 4 include the mixture of 3 fractions and they migrated as double band one representing 2 isoenzymes with the same molecular weight.
* SDS-PAGE of proteins, stained by Coomassie Brilliant Blue.
* Molecular mass markers (14 – 116 KD), ((Fermentase, Canada).

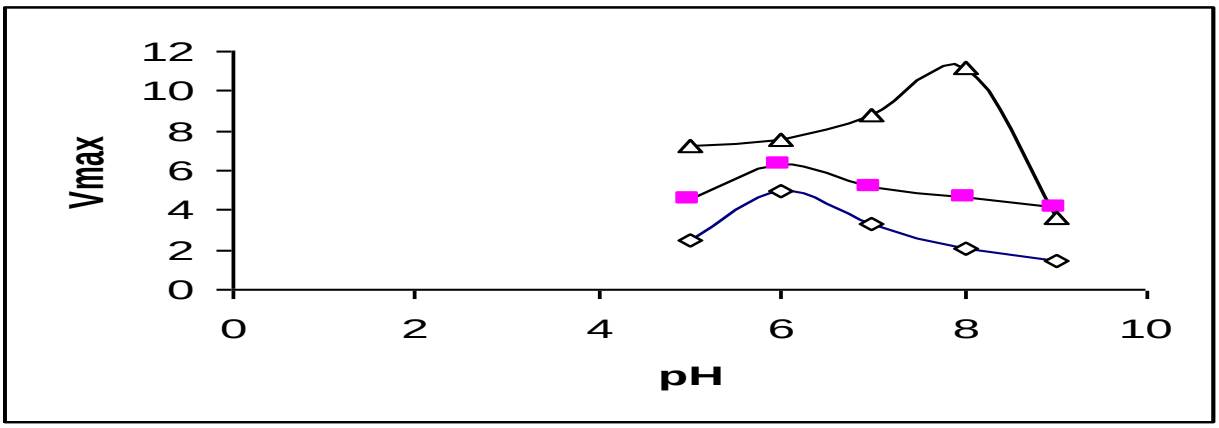

Fig.3. The optimum pH-values of LOX isoenzymes. The activity of LOX 1, 2 and 3 were measured at different values of pH ranged from (5 – 9) and the reaction mixtures contained 15 mg protein / assay, 66 µM sodium linoleate as substrate. Each point represents the mean of triplicate experiments. (V_{max}=nmol/mg protein/min).

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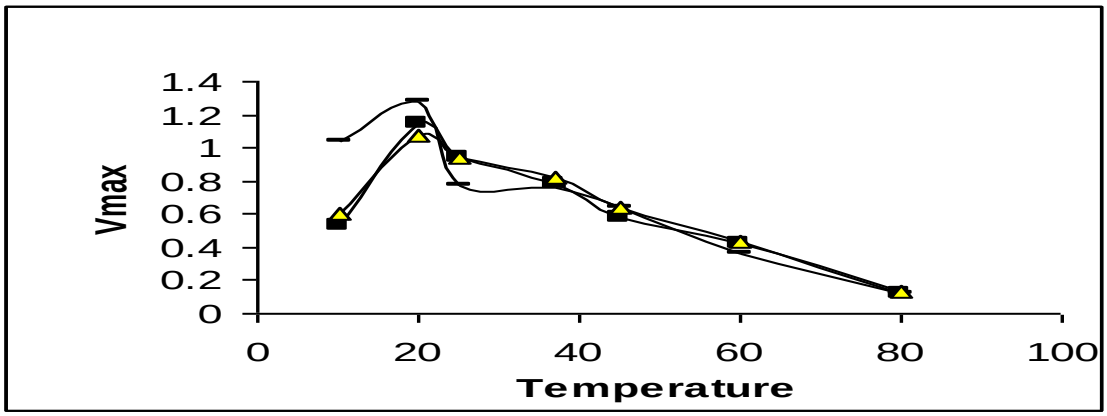

Fig.4. Heat stability of LOX - 1 (-), LOX -2 (■), LOX -3 (△). LOX 1, 2 and 3 were heated at different temperature (10-80 ˚C) for 10 min and the values of Vmax were determined under standard conditions. The reaction mixtures contained 15 mg protein / assay, 66 µM sodium linoleate as substrate. Each point represents the mean of triplicate experiments. (V_{max}=nmol/mg protein/min).

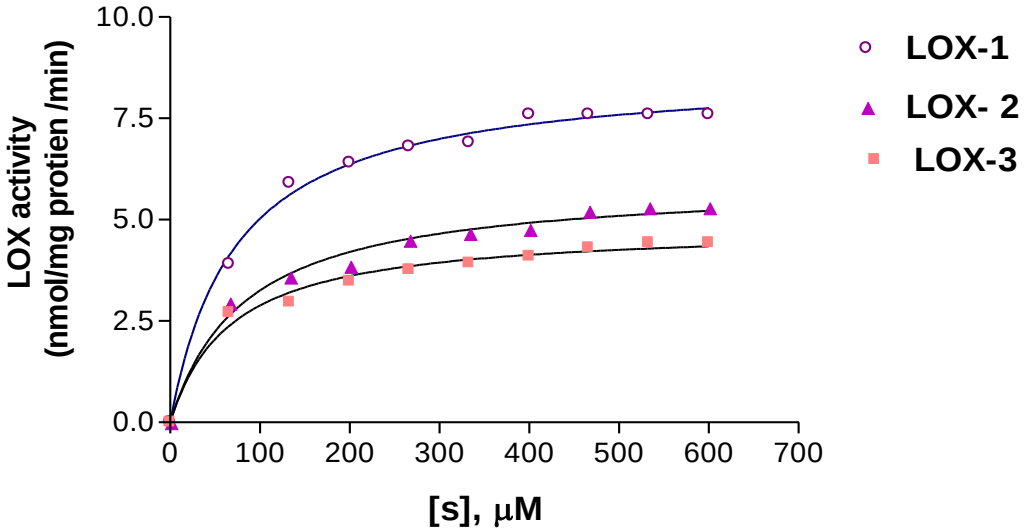

Fig.5. Plots of V against [S], LOX-1 (o), LOX-2 (▲), LOX-3 (■). 15 mg protein of each LOX-1, 2 and 3, were separately incubated with different concentrations of sodium linoleate (the concentrations ranging from 66-600 µM). Each point represents the mean of triplicate determinations.

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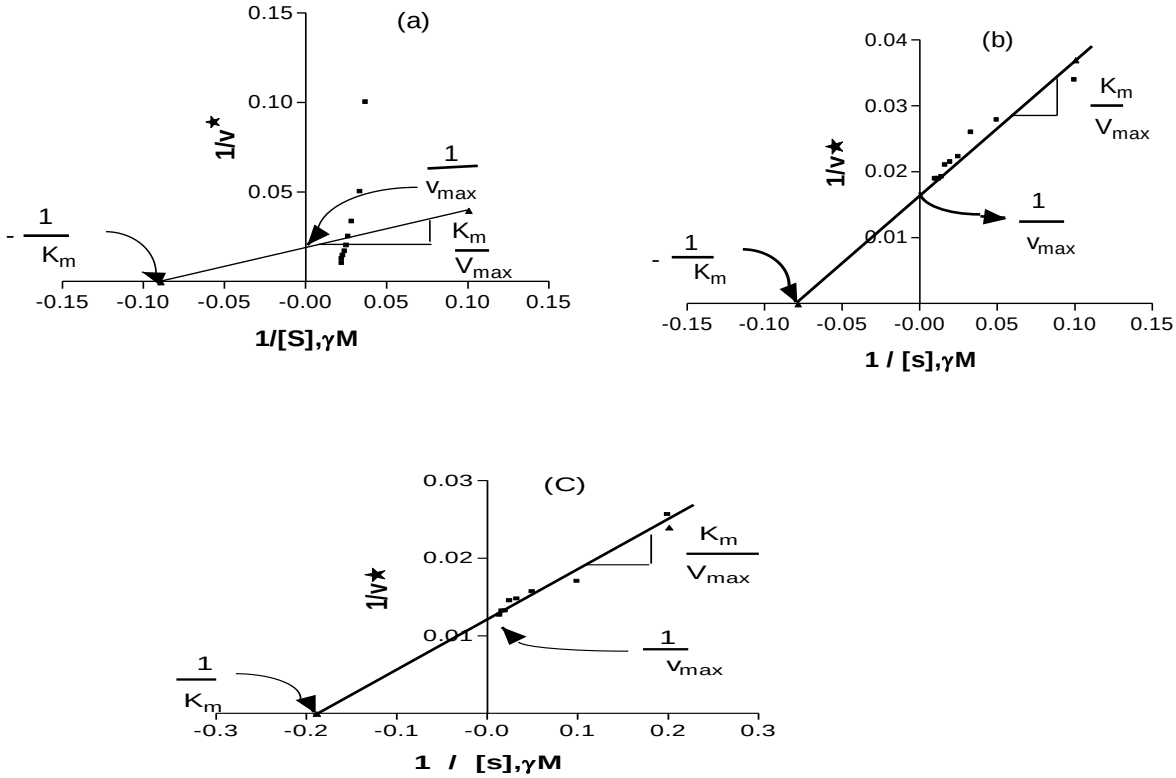

Fig.6. Lineweaver-Burk plot analysis of LOX 1 (panel a), LOX 2 (panel b), LOX 3 (panel c). Purified LOX 1, 2 and 3 (15 mg protein / assay) were incubated with sodium linoleate (66-600 µM). Each point represents the mean of triplicate determinations.
1/v* = nmol/mg protein/min.

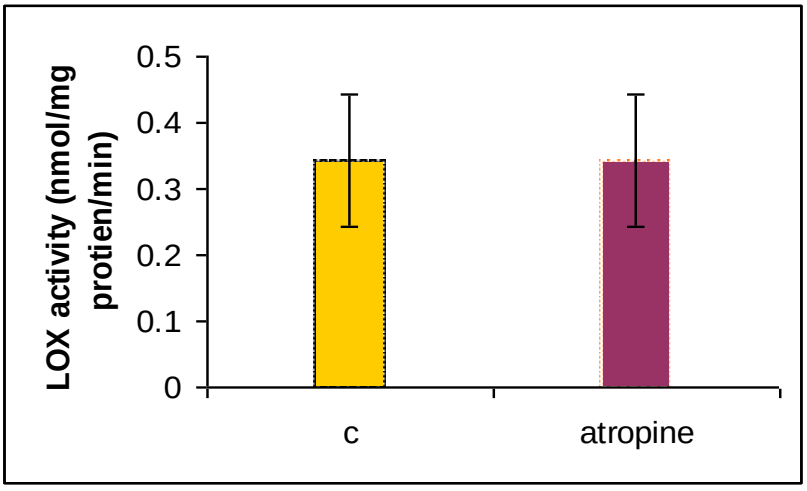

Fig.7. Effect of atropine on LOX activities. Peanut LOX (15mg protein / assay) was incubated with 2 mM of atropine for 5 min at 20^0 C. prior to the addition of 66 µM Sodium linoleate as specific substrate and compared to control, C. Each point represents the mean of triplicate experiments.

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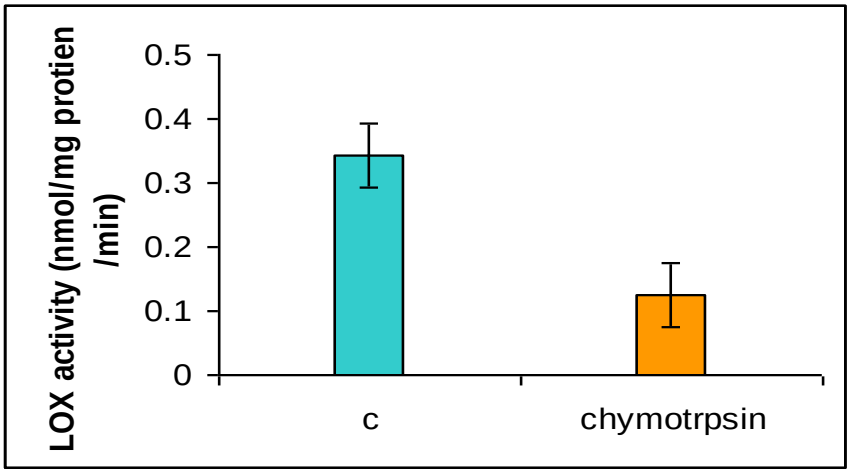

Fig.8. Effect of α- chymotrypsin on LOX activity. Peanut LOX (15mg protein / assay) was incubated with 2 mM of α- Chymotrypsin for 5 min at 20^0 C. prior to the addition 66 µM Sodium linoleate as specific substrate and compared to control, C. Each point represents the mean of triplicate experiments.

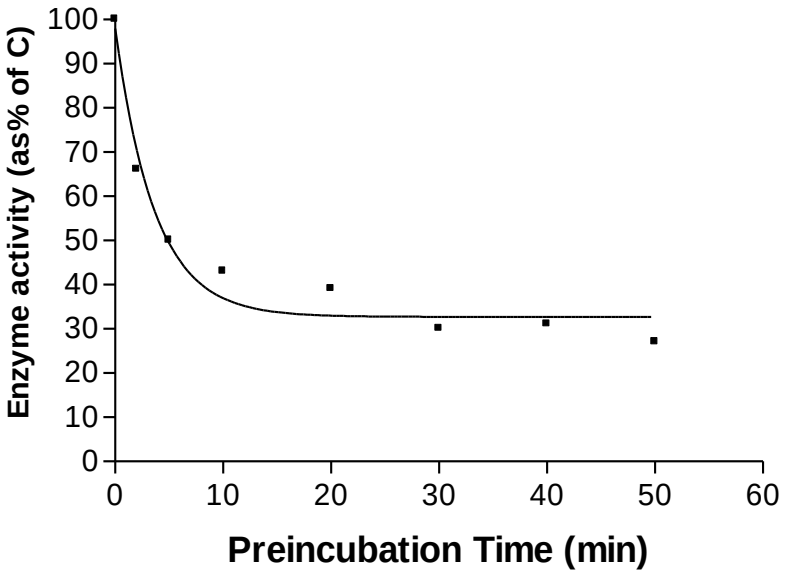

Fig. 9. Time course of the inhibition of LOX by α- chymotrypsin. Peanut LOX (15mg protein / assay) was incubated with 0.24 mM of α- chymotrypsin for different period. After the preincubation period, LOX activity was determined as described before using 66 µM Sodium linoleate as specific substrate and compared to control, C. Each point represents the mean of triplicate experiments.

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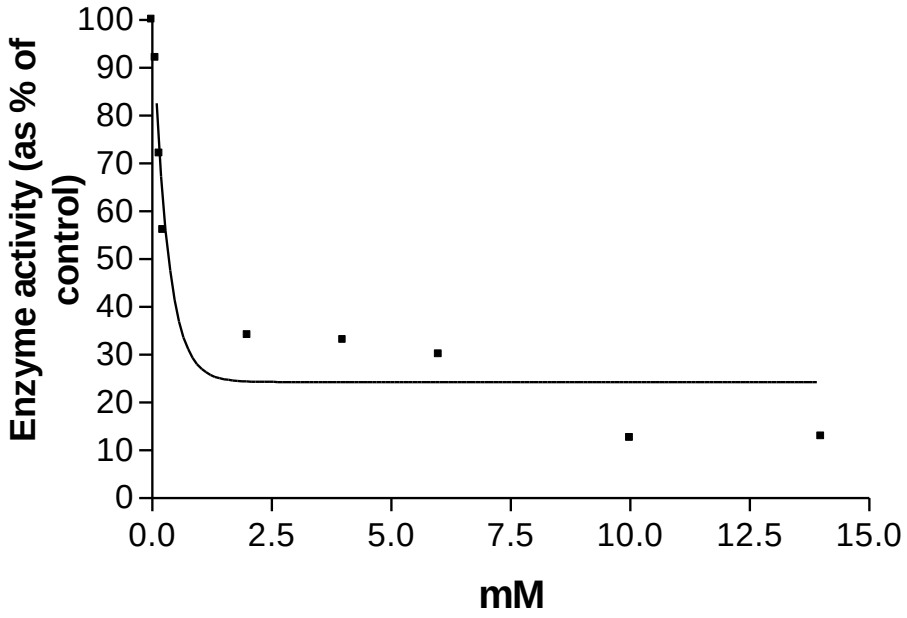

Fig.10. Effect of α- chymotrypsin concentrations on LOX activity. Peanut LOX (15 mg protein/assay) was incubated with α-chymotrypin in the concentration range 0.08 mM-14 mM for 10 min at 20 ˚C prior to the addition of 66 μM sodium linoleate to assay for activity. The results were compared with control that had been incubated for the same time in the absence of α-chymotrypin. Each point represents the mean of triplicate experiments.

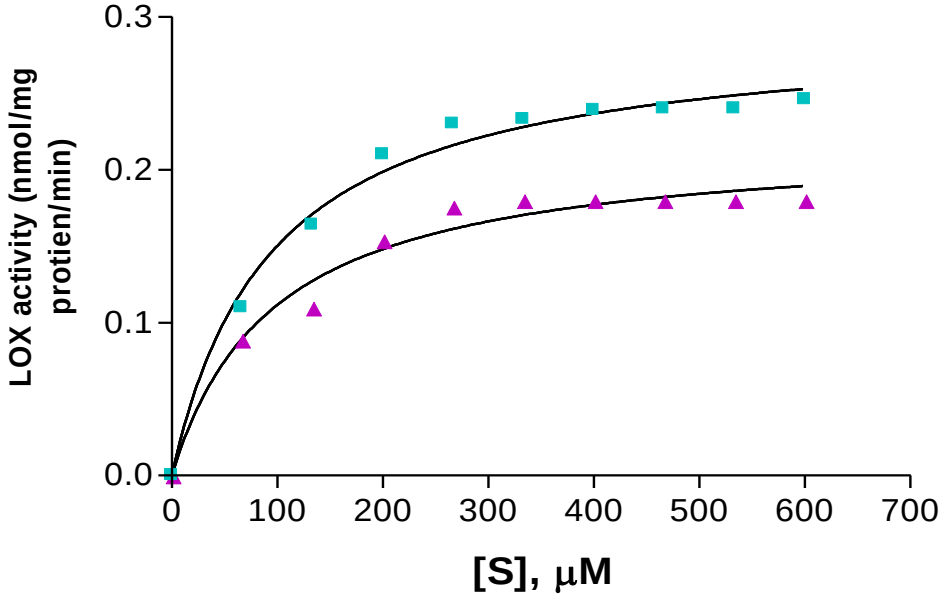

Fig.11. Plots of V against [S] in the absence (control, C [■[ ) and presence of α-chymotrypsin (▲). Peanut LOX (15 mg protein / assay) was preincubated with 0.24 mM of α-chymotrypsin for 10 min at 20 ˚C prior to the addition of sodium linoleate (66 - 600 μM). Each point represents the mean of triplicate determinations. (v = nmol / mg protein per min).

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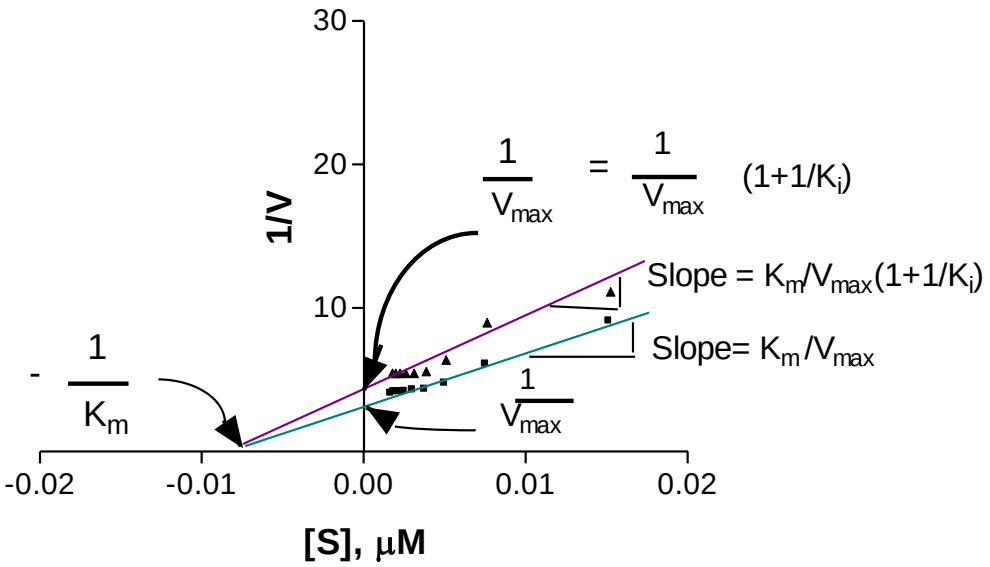

Fig.12. Lineweaver-Burk plot analysis of LOX inhibition by α-chymotrypsin. Peanut LOX (15 mg protein / assay) was preincubated with 0.24 mM of α-chymotrypsin for 10 min at 20 ˚C prior to the addition of sodium linoleate (66 - 600 µM). Each point represents the mean of triplicate determinations. (*v* = nmol / mg protein per min).

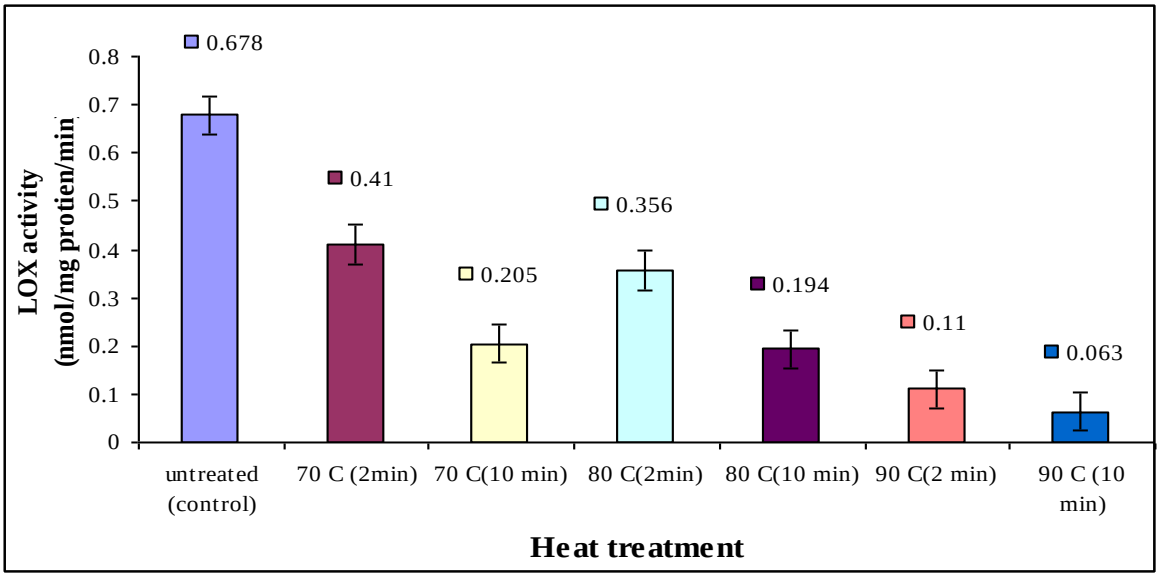

Fig.13. LOX activity of untreated and heat treated peanut grains. 15 mg of peanut LOX of each control and heat treated were incubated with 66 µM of sodium linoleate at pH 7 and LOX activity was determined as described before.

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Table 1
Purification summary of LOX from peanut (300 g)

Stage of purification	Vol. ml	Protein content		LOX activity		Purify	Yield
		mg/ml	total	S.A*	Total	Fold	(%)
1- Crude extract	**300**	**2.44**	**732**	**78. 69**	**575. 99**	**1**	**100**
2- Ammonium sulfate 45%	**20**	**1.2**	**24**	**192. 67**	**462. 40**	**2.44**	**80**
3-DEAE-Cellulose							
Isoenzyme 1	**70**	**1**	**70**	**672**	**47040**	**8.54**	**81.6**
Isoenzyme 2	**50**	**0.76**	**38**	**550**	**20900**	**6.99**	**36.2**
Isoenzyme 3	**30**	**0.93**	**27.9**	**372**	**10378.*8***	**4.72**	**18**

S.A*= specific activity, Enzyme activity are given as μ moles of hydroperoxylinoleate/ mg protien/min

Table (2): LOX activity of untreated and heat treated peanut grains

		LOX activity		
Temperature	**Time of heating**	**S.A***	**A◈ as % of C•**	**% of inhibition**
70 ^{0}C	2 min	0.41± 0.01	60.4	39.6
	10 min	0.205± 0.03	30	70
80^{0}C	2 min	0.356± 0.01	2.55	47.5
	10 min	0.194± 0.02	28.6	71.4
90^{0}C	2 min	0.11± 0.01	16	84
	10 min	0.063± 0.01	9.2	90.8

C• (control) = 0.678 nmol/mg protein/min.
S.A*= specific activity of LOX (nmol/mg protein/min).
A◈ = LOX activity as % of control.