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Original Article

Kinetic Studies with Crude Wild *Pear* Lipoxygenase at During Ripening and After Storage

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ABSTRACT

Crude wild ***pear*** lipoxygenase (LOX) from ripe Kurdistan wild ***pears*** was used in this study. Extracted crude LOX was assayed spectrophotometrically for raw, ripe wild ***pear*** lipoxygenase and after 7 day storage. The effects of different buffers and pHs, substrate preparations, temperature, inhibitors and metal ions on LOX activity were evaluated at three conditions. The enzyme was most active with Hcl-Tris buffer at a pH of 7.5 and a temperature of 40 °C. The best substrate was the first preparation with linoleic acid/Tween 20 at a ratio of 1:1. The kinetic parameters determined under the best conditions were a Km of 6.5 mM and a V_{max} of 0.9 unit/mg protein for raw condition, 5.8 mM and a V_{max} of 0.95 unit/mg protein for ripe condition and 5 mM and a V_{max} of 1.1 unit/mg protein obtained after storage. The enzyme was heat-labile. It was shown that crude wild ***pear*** LOX is fully active at room temperature (20-30 °C), while the best activity occurred at 35 °C for Lox after storage that activity of lipoxygenase increased up to 130%. The results showed that lipoxygenase inhibitory activity of chemical compounds and standard compound decreased in the order of ascorbic acid>nicotinic acid>benzoic acid>DL-*α*-tocopherol. Monovalent and trivalent ions are inhibitors of LOX and divalent ions except Z_ncl_2 are activators of LOX.

Key words: Wild ***pear***, Lipoxygenase, Crude, Kinetics, Substrate.

Introduction

Lipoxygenase (LOX, EC 1.13.11.12) are a group of enzymes that catalyze dioxygenation of fatty acids by use of molecular oxygen which contains one or more 1,4(Z)-pentadiene systems, yielding chiral (E, Z) conjugated hydroxy fatty acids (Veldink ***et al.***, 1998; Riley ***et al.***, 1996). Lipoxygenase does the dioxygenation of unsaturated fatty acids, yielding a hydroperoxy fatty acid product. The physiological role of lipoxygenase in plants is not clear, but its function is in wounding and other stress responses (Rosahl, 1996). Lipoxygenase activity is additionally the primary step within the pathway resulting in the formation of a variety of flavor and aroma compounds and has been shown to contribute to the development of off flavors in several vegetables throughout frozen storage (Barrett and Theerakulkait, 1995). Multiple isozymes of lipooxygenase exist in many tissues of plants that have differences in regiospecificity, pH

optimum, pI and enzymic properties. These isoenzymes have a single polypeptide chain with nearly 850 amino acids and an average of 96 kDa molecular weight (Grechkin, 1998). Metabolites of unsaturated fatty acids have a function in growth and development, plant senescence and response to diseases and wounding., LOX in fruits have a role in the formation of volatile flavor compounds (Veldink ***et al.***, 1998; Riley ***et al.***, 1996; Ealing, 1994). LOX activity in wild ***pear*** fruits was interfered both in membraneous systems (Thompson ***et al.***, 1990; Riley ***et al.***, 1996) and insoluble form (Ealing, 1994; Veldink ***et al.***, 1998). Enzyme activity has been assayed by colorimetric, manometric, polarographic, spectrophotometric and radiolabeled substrate monitoring assays (Thompson ***et al.***, 1990; Surrey, 1964; Riley ***et al.***, 1996; Veldink ***et al.***, 1998). Values of Km and V_{max} vary due to purity and assay differences (Thompson ***et al.***, 1990; Veldink ***et al.***, 1998). Product analysis has revealed a 96% product specificity of 9-***S***-hydroperoxy-trans-10, cis-12-octadecanoic acid (9S-HPOD) formation from linoleic acid with an 82% S enantiomeric excess. The 13-HPOD formation was 1% and racemic (Veldink ***et al.***, 1998). In the present study, crude wild ***pear*** LOX was used to investigate the effect of different substrate forms and assay buffers on enzyme activity and also the thermal stability of the enzyme.

Materials and methods

Raw wild ***pears*** and ripe wild ***pears*** of the cultivar Kurdistan were harvested and transported to the University of payame Noor, Kurdistan, on the same day for the study. Some of the ripe wild ***pears*** were stored (7 days) at room temperature (25 °C) to a table ripe stage before homogenizing. Tween 20, linoleic acid, $MgCl_2$, PVPP (polyvinylpyrrolidone), PMSF (phenylmethylsulfonyl fluoride) were purchased from Sigma Chem. Co. Tris ***N***-[Tris(hydroxymethyl) methyl]glycine, MES morpholinoethane sulfonic acid), EPPS (***N***-2-Hydroxyethylpiperazine-NÕ-3-propane sulfonic acid), MOPS (3-(***N***-Morpholino)propane sulfonic acid), Hepes (***N***-2-Hydroxyethylpiperazine-NÕ-2-ethane sulfonic acid) and sodium acetate were purchased from Aldrich Chem. Co.

Enzyme extraction: Wild ***pears*** were washed with water and distilled water. After cutting the endocarp, some thick rings were separated transversely from each fruit. Slices were dipped in a potassium phosphate buffer (50 mM) with a pH of 7.0. To preventing from tissue browning, 0.1% ascorbate was added. For analyzing mesocarp fractions, the epicarp was physically separated. A total of 500 g of ***pears*** at raw, ripe and after storage were blended by a milling process, separately. The seed flour was mixed with 800 mL of 50 mM sodium phosphate buffer, pH 7.5. The crude homogenate was subjected to mechanical stirring at 4 °C for 1 h sonicated using five pulses (30 S each) of a sonicator (Qsonica, LLC series XL-2000, USA), set at the power setting 5 W. Later, the homogenate was filtered through four layers of cheesecloths. The resulting suspension was centrifuged at 7,000 × g for 20 min. The pellet was discarded and the supernatant further treated with solid ammonium sulfate up to 60% of saturation and then the inactive supernatant eliminated by centrifugation at 7,000 × g for 20 min. The resulting precipitate was resuspended in a minimal amount of sodium phosphate buffer solution (50 mM pH 7.5) dialyzed twice against the same buffer for 20 h at 4 °C. The dialyzed extract was assayed for protein content by Bradford's method. Bovine serum albumin (BSA) was used as the standard. The homogenate was used for evaluation of the activity of lipoxygenase of raw wild ***pear*** (RW-LOX), ripe wild ***pear*** (RP-LOX) and LOX activity of wild ***pear*** after storage (ST-LOX).

Protein determination: The Bradford protein assay is used to measure the concentration of total protein of LOX (1976). The calibration curve was determined with IgG (bovine plasma gamma

globulin) as the standard.

pH optimum: The optimum pH of 6.5 used for linoleic acid and linolenic acid. The optimum pH of LOX of other sources is in a wide pH range from 5.5 to 9.5. pH optimum was determined in the presence of 0.1 M acetate buffer and at different pHs of the solutions (Elez-Martínez ***et al.***, 2005; Marcus ***et al.***, 1988; Jacobo-Velázquez ***et al.***, 2010; Robinson ***et al.***, 1995).

Temperature optimum: Effects of temperature (20-75 °C) on LOX activities were also determined. Given that wild ***pear*** grows in moderate and mild climates, a temperature optimum for LOX activity between 30 °C and 40 °C, as the one found in the present work, was expected. Since thermal processing or long exposure to high temperatures is likely to promote undesirable organoleptic changes in wild ***pear***, the use of high temperature is an effective strategy to partially inactivate LOX.

Preparation of substrate: preparation of Linoleic acid as substrate was done in three different forms. At first, 0.5 mL of Tween 20 was dissolved in 10 mL of 0.1 M Borate buffer with a pH of 9.0. Then 0.5 mL of linoleic acid was added simultaneously with mixing. Finally, 1.3 mL of 1 N NaOH was added. So after minutes, a clear transparent solution was obtained. After that, 90 mL of Borate buffer was added until the total volume was reached to 200 mL with water. Preparation of the second substrate was done by dissolving an equal amount of linoleic acid (0.5 mL) in 200 mL of methanol without using detergent or buffer. For third preparation, 0.5 mL of linoleic acid was in presence of 20 mL of methanol added to a mixture of 180 mL of Borate buffer and Tween 20 with the same ratios. Also, for the first substrate preparation, the linoleic acid to Tween 20 ratio was evaluated (1:0, 1:1, 1:1.5, and 1:2).

LOX assay: A jenway Spectrophotometer with 190-820 nm range and 2 nm bandwidth was used for determining of LOX activity. The assay method was done according to (Riley ***et al.***, 1996). Increasing of absorbance at 234 nm was followed for 5 min at 25 °C by addition of 1 mL substrate solution in a cuvette in the presence of 1 mL of the crude LOX extract and 1 mL of assay buffer. The buffer that used was Na-Acetate (0.1 M) with pH of 5.0; MES (150 mM) with pH of 6.0; MOPS (50 mM) with pH of 7.0; Hepes-KOH (0.2 mM) with pH of 7.5; EPPS-KOH (150 mM) with pH of 8.0; Tris-HCl (150 mM) with pH of 8.5; Borate (0.1 M) with pH of 9.0 and Glycine-KOH (50 mM) with pH of 9.6. Enzyme specific activity is the amount of enzyme-producing one unit change in absorbance in one minute. The same method of the assay was done with the first substrate preparation of 1:1.5 LA: Tween 20 ratio. So, the values of Vmax and Km were also determined with different concentrations of the substrate (1.6-72.4 mM).

Temperature activity and thermal stability determination: To determine the optimum temperature of LOX activity, the assay was carried out at a range of 25 °C until 80 °C in presence of Tris-HCL buffer (50 mM, pH 8.0) and 5 °C intervals. Thermal stability evaluated by incubation of enzyme solution in a water bath from 25 °C to 80 °C (30 min) and in the following, the mixture was kept at room temperature(10 min). Eventually, the residual LOX activity was assayed. Experiments were done in triplicates. For half-life (t 1/2) determination, incubation of enzyme solution (220 U/mg) was done up to 60 min in optimal conditions (temperatures of 25 °C, 30 °C, 35 °C, 40 °C, and 45 °C). For each 30 min interval, 20 µl of the sample was taken to evaluating residual activity. The amount of activity at the onset of the assay was considered as 100%. Finally, the results of residual activity were depicted against incubation time.

In vitro lipoxygenase-inhibitory assay: Assay for determination of inhibition of lipoxygenase activity was done and determined by a spectrophotometric method reported by (Yawer ***et al.***,

2007). The reaction mixture for assay, containing inhibition solution and lipoxygenase solution in 0.1 M phosphate buffer with a pH of 8.0. So they were incubated for 10 min at 25 °C. After that, the assay was initiated by addition substrate. The absorbance value was measured at 234 nm after 6 min. was standard inhibitor that used in the assay was quercetin. The percent inhibition of lipoxygenase activity was evaluated by the equation of Inhibition (%)=(1-A/B)×100, where A refers to enzyme activity without inhibitor and B is the activity in presence of inhibitor. The IC50 was determined as the concentration of LOX extracts required to inhibit lipoxygenase activity by 50%.

Tissue localization of LOX activity: Crude enzyme extract was prepared from isolated pericarp tissue and used for all the above assays. Also, wild ***pear*** skin, locular gel material, and whole fruit were used to compare tissue activity distribution.

Statistical analysis: Statistical procedures were performed using the statistical analysis system (SAS Institute, Cary, NC). The effect of LOX specific activity by different substrate preparations was determined by ANOVA with means separation by DuncanÕs Multiple Range test. Regression analysis of the double-reciprocal plot determined the best-fitting straight line. All measurements were made twice and the result reported was the mean of the two. The level of significance was 5% for all tests.

Effect of metal ions on enzyme activity: To the investigation of effects of metal ions on lipoxygenase activity of RW-LOX, RP-LOX, and ST-LOX, some chemical compounds such as monovalent ion (NaCl), Divalent ions (Z_nCl_2, $CaCl_2$, $FeCl_2$, and $MgCl_2$) and trivalent ion($Fecl_3$) were selected. The applied concentrations at which these ions were prepared in 50 mM Tris-HCL buffer at pH 7.5. Concentrations of metal ions that prepared were 0.1, 0.5 and 1 mM. Each mixture reaction was incubated at room temperature for 10 min and finally, the activity of RW-LOX, RP-LOX, and ST-LOX was measured, separately. A mixture assay without metal ions was considered as 100% activity.

Results and discussion

In this research, the assay buffers evaluated. So, the Tris-HCl (pH 7.5) was the best in terms of crude LOX specific activity (Figure 1). Buffers such as MOPS with a pH of 7.0 and MES buffer with pH of 6.0 were also suitable for the enzyme. On the other hand, Borate with a pH of 9.0 and Glycine-KOH with pH of 9.6 showed the lowest LOX activity. Optimal pH of 6.0-6.5 has been reported in several studies (Thomson ***et al.***, 1990; Lauriere ***et al.***, 1993; Ealing 1994). The reason for inappropriate Borate buffer as an inorganic buffer might be due to its pH (9.0). Between the five buffers that showed different pH values for the specific LOX activity (Figure 1), Tris-HCl buffer was chosen for the subsequent analyses.
The efficiency of the different substrate preparations for crude wild ***pear*** LOX is summarized in Table 1. The ratio of 1:1 linoleic acid: Tween 20 and 1:1 linolenic acid: Tween 20 ratio were the best of all evaluated, with the specific activity of 0.83 and 0.75 (Units/mg protein) value, respectively. It can be concluded from the results that when linoleic and linolenic acid can form micelles with sufficient amount of Tween 20, they are most suitable for the reaction, but very high or low concentrations of Tween 20 can cause problems in solubility and diffusion.

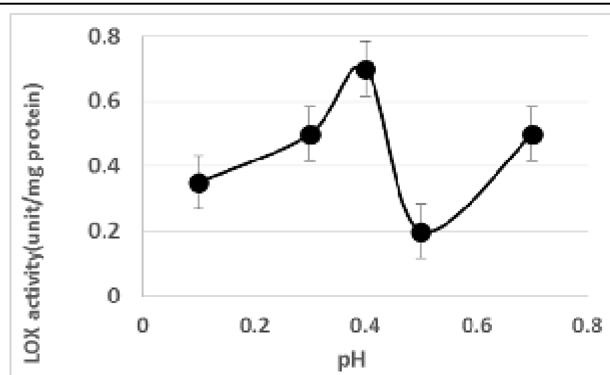

Figure 1. Effects of the different assay buffers (and pHs) on the activity of crude ***pear*** LOX

According to these results, preparation with the 1:1 linoleic acid: Tween 20 and linolenic acid: Tween 20 ratio was chosen for doing the analyses. Figure 2 shows the Michaelis-menton plot of the crude wild ***pear*** lipoxygenase.

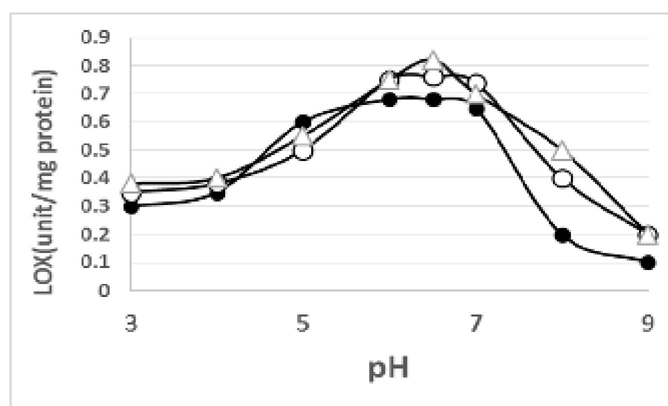

Figure 2. Effects of the different assay buffers (and pHs) on the activity of crude wild ***pear*** lipoxygenase. Linolenic acid (Immature (●), ripe(O), after storage (Δ)) in presence of linoleic acid:tween 20 as substrate

Table 1. Effect of substrate preparation methods on the crude wild ***pear*** LOX specific activity

Substrate preparation	Ratio	LOX activity (unit/mg protein)
Linoleic acid : tween 20	1 :0	0.72
Linoleic acid : tween 20	1 : 1	0.83
Linoleic acid : tween 20	1 : 1.5	0.79
Linoleic acid : tween 20	1 : 2	0.75
Linolenic acid : tween 20	1 :0	0.61
Linolenic acid : tween 20	1 : 1	0.75
Linolenic acid : tween 20	1 : 1.5	0.62
Linolenic acid : tween 20	1 : 2	0.58

Analyzing of Data was done to determine the best fitting theoretical hyperbolic curve. The kinetic parameters were estimated from analyzing of data. so, the K_m of the LOX obtained 6.5 mM and V_{max} was obtained as 0.9 unit/mg protein in immature wild ***pear***. The K_m in ripe wild ***pear*** was 5.8 mM and V_{max} was 0.95 unit/mg protein, although these parameters

reached to 5 mM and 1.1 unit/mg protein for k_m and V_{max} in wild ***pear*** after storage, respectively, in presence of linoleic acid as substrate (Table2).

Table 2. Kinetic parameters of lipoxygenase at raw, ripe and storage condition in presence of linoleic acid

Linoleic acid	Kinetics parameters				
	K_m (mM)	Vmax (unit/mg)	Catalytic efficiency	pH optimum	Temperature optimum
Immature pear	6.5	0.9	0.13	6.5	35 °C
Ripe pear	5.8	0.95	0.16	6.5	35 °C
Pear after storage	5	1.1	0.22	6.5	35 °C

The kinetic parameters were estimated in immature wild ***pear***, ripe wild ***pear*** and ***pear*** after storage in presence of linolenic acid as the second substrate. So, the K_m of the enzyme is 6.8 mM and V_{max} value of 0.81 unit/mg protein in immature wild ***pear*** and K_m of the enzyme in ripe wild ***pear*** obtained 6.2 mM and V_{max} value of 0.92 unit/mg protein and these kinetic parameters in wild ***pear*** after storage reached 5.4 mM and 0.99 unit/mg protein for K_m and V_{max}, respectively (Table 3). Previous studies have reported K_m values ranging from 0.015×10^{-4} M to 4.1 mM, and V_{max} values ranging from 0.186 mM /U to 7.4 mM /U (Thompson ***et al.***, 1990; Veldink ***et al.***, 1998; Biacs and Daood 1987). The values vary with the level of enzyme purity and difference in assay method.

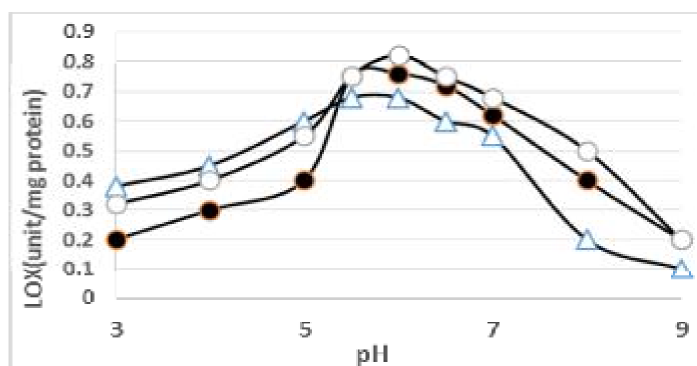

Figure 3. Effects of the different assay buffers (and pHs) on the activity of crude wild ***pear*** lipoxygenase. (Immature wild ***pear*** (●), ripe (O), after storage (Δ)) in presence of linolenic acid:tween 20 as substrate

Table 3. Kinetic parameters of lipoxygenase at raw, ripe and storage condition in presence of linolenic acid

Linolenic acid	Kinetics parameters				
	K_m (mM)	V_{max} (unit/mg)	Catalytic efficiency	pH optimum	Temperature optimum
Immature pear	6.8	0.81	0.11	6.5	35 °C
Ripe pear	6.2	0.92	0.14	6.5	35 °C
Pear after storage	5.4	0.99	0.18	6.5	35 °C

The assay of the enzyme in the form of crude was done with the buffer in optimum of pH and best substrate at room temperature. The heat stability of LOX at different temperatures is shown in Figure 4. At raw condition, crude lipoxygenase was rapidly inactivated at 70-80 °C, and during 2-3 min, the activity of enzyme reached to near zero. The enzyme was incubated at 70 °C for 1 min, after that, only 20% of the initial activity was retained, but after 7 min incubation, all of the activity was lost. On the other hand, incubation for 2 min at 60 °C caused only 40% activity loss. At 5 min, 20% of the activity of LOX remained at the same temperature. The assay for ripe condition, crude lipoxygenase was rapidly inactivated at 80 °C, and after 6-7 min, activity reached to near zero. Incubation of enzyme for 1 min at 70 °C, so, 65% of the initial activity has remained, but after 7 min incubation, the activity was zero. On the other hand, after incubation for 2 min at 60 °C, only 25% of activity missed. At 5 min, however, only 40% of the activity remained at the same temperature.

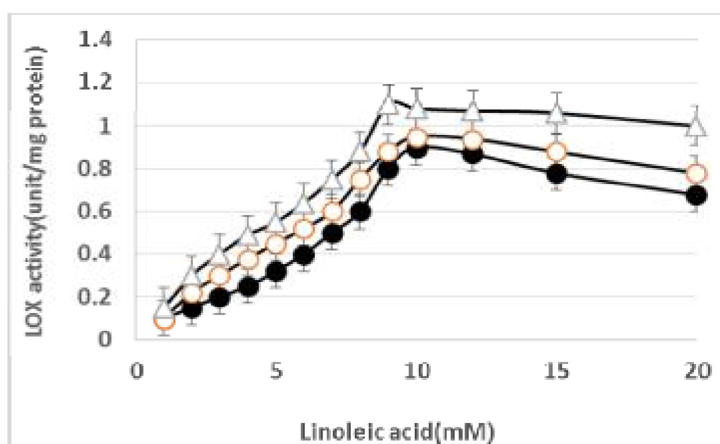

Figure 4. Michaelis-menton plot of crude Wild ***pear*** lipoxygenase with linoleic acid: tween 20 (1:1 ratio) as substrate and Tris-HCl (pH 7.5)

This assay was done for the condition that wild ***pear*** store for 7 days at room temperature. So, at this condition, crude lipoxygenase was inactivated at 80 °C and in 6-7 min all activity reached to near zero. The enzyme was applied 1 min at 70 °C after 1 min, so, 60% of the initial activity was retained, but when the time was increased to 7 min, the activity was zero. On the other hand, putting on for 2 min at 60 °C caused only 15% activity loss. At 5 min, however, only 50% of the activity remained at the same temperature. The optimum temperature for the activity of RW-LOX, RP-LOX, and ST-LOX are shown in Figure 5. It was shown that crude wild ***pear*** LOX is fully active at room temperature (20-30 °C), while the best activity occurred at 35 °C for Lox after storage that activity of lipoxygenase increased up to 130%. At 40 °C, the activity of LOX obtained at 100-110% and at 45°-80 °C, the activity of LOX decreased so, at 60° reached to 60-70% and at 80 °C reached to zero. In conclusion, crude LOX is active at room temperature range and more activity obtained up to 40 °C where it naturally acts on substrates. By increasing the storage time, the thermal effect on the LOX structure led to less decrease in the enzyme activity in compare to RP-LOX and RP-LOX, so the stability of enzyme increased by increasing time of storage up to 7 days. The inhibitory properties of benzoic acid, ascorbic acid and nicotinic acid and DL-α-tocopherol on ***pear*** lipoxygenase activity of RW-LOX, RP-LOX, and ST-LOX were compared

using linoleic acid as a substrate. All of the compounds exhibited affinity to a substrate-enzyme complex. Ascorbic acid was found to has the highest LOX inhibitory activity.

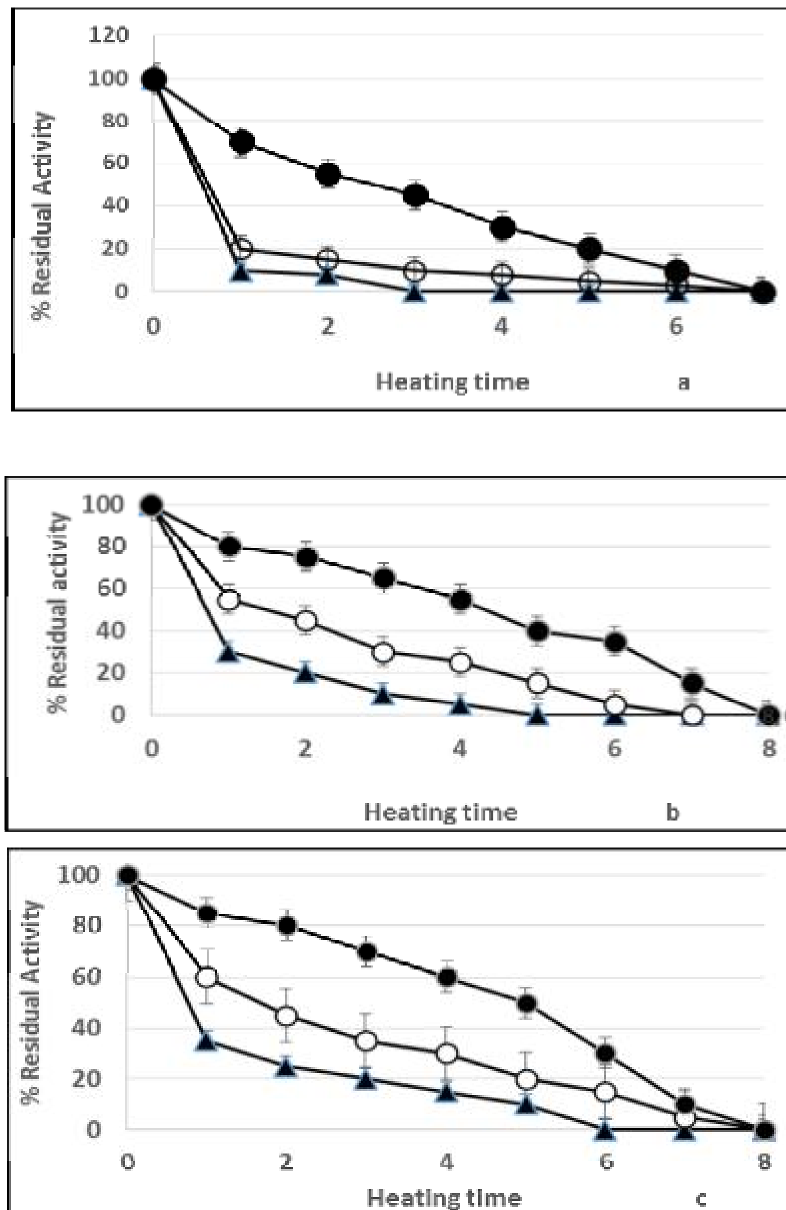

Figure 5. Thermal stability of crude lipoxygenade at Raw crude wile ***pear***(a), Ripe crude wile ***pear*** (b) and after storage(c). (60 °C (●), 70 °C (O) and 80 °C (Δ)

IC50 value was found 0.15, 0.13 and 0.11 mM for RW-LOX, RP-LOX and ST-LOX, respectively.

DL-α-tocopherol was found to have the lowest LOX inhibitor activity, 4.6, 4 and 3.7 mM for RW-LOX, RP-LOX, and ST-LOX, respectively. Lipoxygenase inhibitory activity of chemical compounds and standard compound decreased in the order of ascorbic acid>nicotinic acid> benzoic acid>DL-α-tocopherol (Table 4). Parallel lines were observed for inhibition of LOX by ascorbic acid in RW-LOX, RP-LOX, and ST-LOX. This acid showed the nature of

uncompetitive inhibitors. benzoic acid and nicotinic acid and DL-α-tocopherol were non-competitive inhibitors. Since some metal ions are known to be required as coenzymes or as cofactors for various enzymes such as LOX, we tested the effects of various metal ions on the activity of RW-LOX, RP-LOX ,and ST-LOX.

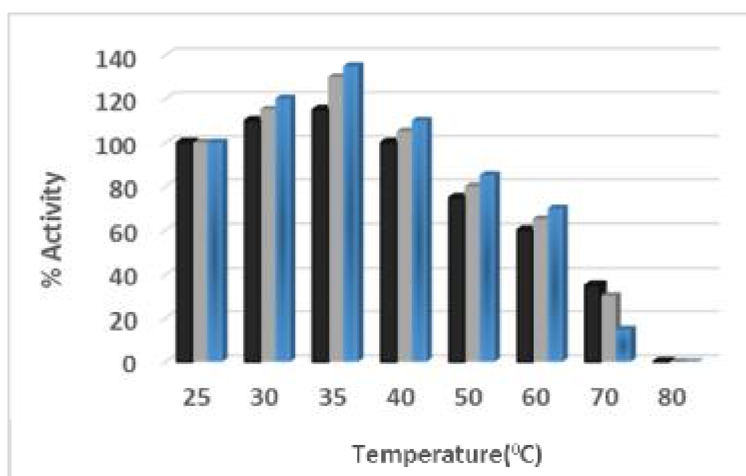

Figure 6. The optimum temperature for activity of lipoxygenase in presence of linoleic acid at Raw crude wile ***pear*** (a), Ripe crude wile ***pear*** (b), and after storage (c)

Each metal ion (0.1, 0.5 and 1 mM) in the form of chloride salt was added to the assay mixture before the enzyme assay, separately. Our results about metal ions showed that Divalent ions such as $CaCl_2$, $FeCl_2$,and $MgCl_2$ are activators of lipoxygenase, specifically at RW-LOX and RP-LOX. So, at 0.1 mM of $CaCl_2$, $FeCl_2$ and $MgCl_2$, the activity of LOX increased ,and reached 135, 148, and 128% in comparison to control (without metal ions). With increasing the concentrations of $CaCl_2$, $FeCl_2$,and $MgCl_2$ up to 0.5 and 1 mM, LOX activity decreased, but their activities were upper than control. LOX activity of RW-LOX, RP-LOX and ST-LOX in presence of 0.1, 0.5 and 1 mM of Nacl decreased, so for RW-LOX reached to 70, 60 and 40%, and for RP-LOX reached to 80, 71 and 55% and for ST-LOX reached to 85, 76 and 62%, respectively.

Table 4. Lipoxygenase inhibitory activity of some chemical compounds for lipoxygenase activity at raw, ripe and storage condition in presence of linoleic acid

Inhibitors	Source	IC50 (mM)	Type of inhibition
Ascorbic acid	Raw wile pear	0.15	un-competetive
	Ripe wild pear	0.13	un-competetive
	Wild pear after storage	0.11	un-competetive
Nicotinic acid	Raw wile pear	0.45	non-competitive
	Ripe wild pear	0.42	non-competitive
	Wild pear after storage	0.39	non-competitive
Benzoic acid	Raw wile pear	3	non-competitive
	Ripe wild pear	2.5	non-competitive
	Wild pear after storage	2	non-competitive
DL-α-tocopherol	Raw wile pear	4.6	non-competitive
	Ripe wild pear	4	non-competitive
	Wild pear after storage	3.7	non-competitive

Maximum of activity of lipoxygenase obtained for wild ***pear*** after storage in presence of $FeCl_2$ (0.1 mM) and minimum of activity of lipoxygenase obtained for RW-LOX in presence of Nacl (1 mM). In according to our results, monovalent and trivalent ions are inhibitors of LOX and divalent ions except of $ZnCl_2$ are activators of LOX. The activation of lipoxygenase of wild ***pear*** in the presence of metal ions was in the order of $FeCl_2$>$CaCl_2$> $ZnCl_2$>Nacl>$FeCl_3$ and these activation was in order of ST-LOX>RP-LOX>RW-LOX. Other researchers confirmed our results, so, Soybean LOX activity has been inhibited by Mg^{2+} and Mn^{2+}, whereas its activity was increased vigorously by $FeCl_2$ (Aberomand ***et al.***, 2013).

Table 5. Effect of metal ions on LOX activity at raw, ripe and conditions after storage of wild pear in presence of linoleic acid as substrate

Metal ions	Source	Relative activity (%) Nacl (0.1 mM)	Relative activity (%) Nacl (0.5 mM)	Relative activity (%) Nacl (1 mM)
Nacl	Raw wild pear	70	60	40
	Ripe wild pear	80	71	55
	Pear after storage	85	76	62
$ZnCl_2$	Raw wild pear	82	77	54
	Ripe wild pear	88	81	63
	Pear after storage	94	85	69
$CaCl_2$	Raw wild pear	135	115	92
	Ripe wild pear	146	122	98
	Pear after storage	155	136	108
$FeCl_2$	Raw wild pear	148	132	115
	Ripe wild pear	161	148	122
	Pear after storage	168	151	134
$MgCl_2$	Raw wild pear	128	112	88
	Ripe wild pear	138	119	95
	Pear after storage	146	125	105
$FeCl_3$	Raw wild pear	58	46	38
	Ripe wild pear	68	55	43
	Pear after storage	77	61	52

Conclusion

Genarally, crude of wild ***pear*** LOX is optimum at pH 6.0 in the MES buffer (25 ^oC). Similarly, the best substrate used was first preparation with linoleic acid and Tween 20 with proportion of 1:1.5. Assays of kinetic study of LOX revealed a K_m with value of 4.198 mM and a V_{max} with value of 0.84 mM/min. The optimum of activity of LOX obtained at room temperature (20-30 ^oC). The activity of enzyme at 80 ^oC after 2 min was diminished. Most of the activity was seen in the pericarp tissue. These results might be very useful while wild ***pear*** pericarp tissue is known as a LOX source in biotechnological applications. Among the studied metal ions, $FeCl_2$ had the maximum effect on the RW-LOX, RP-LOX and ST-LOX activity at 0.1 mM concentration, and, $FeCl_3$ strongly decreased the LOX activity at the same concentration. Consequently, it seems that these ions have varying effects on the oxygenating activity of LOX or the binding of fatty acid substrate to the active site (Marvian-Hosseini and Asoodeh, 2017). At the concentration of 1.0 mM, $ZnCl_2$ inhibited enzyme activity of about 20% compared with the control.

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