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Original article

An efficient methodology for the purification of date palm peroxidase: Stability comparison with horseradish peroxidase (HRP)

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ABSTRACT

In the present study, Peroxidase from date palm (*Phoenix dactylifera*) leaves was purified to homogeneity by three-step procedure including aqueous two-phase system, hydrophobic and Ion-exchange chromatography. The enzyme migrated as single band on SDS-PAGE giving molecular weight of 68 ± 3 kDa. The purification factor for purified date palm peroxidase was 68 with high 41% yield. Enzymatic assays together with far-UV circular dichroism (CD), intrinsic and extrinsic fluorescence studies were carried out to monitor the structural stability of date palm and horseradish peroxidase (HRP) against various pH and temperatures. Activity measurements illustrated different pH stability for date palm and HRP. Both peroxidases are more susceptible to extreme acidic conditions as suggested by 4 & 15 nm red shift in date palm and HRP, respectively. Secondary structure analysis using far UV-CD exhibited predominance of α-helical (43.8%) structure. Also, pH induces loss in the secondary structure of date palm peroxidase. Thermal stability analysis revealed date palm peroxidase is more stable in comparison to HRP. In summary, date palm peroxidases could be promising enzymes for various applications where extreme pH and temperature is required.

1. Introduction

Peroxidases (EC 1.11.1.7) are enzymes that catalyze the H_2O_2 dependent oxidation of a wide variety of organic and inorganic substrates. They are ubiquitous and found in animals, plants, algae, fungi and bacteria (Pandey et al., 2017). In plants, they are involved in various physiological processes including plant growth, differentiation and development (Laura, 2004). Studies have suggested that peroxidases played a role in auxin metabolism, lignification, suberization, cross-linking of cell wall components, self-defense against pathogens and senescence (Monica et al., 2000; Den Herder et al., 2007).

Apart from their biological functions, plant peroxidase has attracted industrial attention due to their usefulness in multiple applications including clinical diagnosis and laboratory experiments such as in (ELISA) enzyme immunoassay kits (Azevedo et al., 2003). Some novel applications suggested include decolorization of waste, synthesis of several different aromatic chemicals and polymeric materials, treatment of wastewater containing phenolic compounds, and removal of peroxides from foodstuffs and industrial wastes (Hamid, 2009; Regaldo et al., 2004; Mohan et al., 2005). They are also involved in the treatment of cancer (Jeong et al., 2010) and preparation of biosensor (Lomillo et al., 2005; Xu et al., 2014).

The most widely used and commercially available peroxidase is Horseradish peroxidase (HRP) isolated from root of horseradish (Amoracia rusticana L.). Horseradish peroxidase C isoenzyme comprises a monomeric polypeptide of 308 amino acid residues including only one tryptophan, with a molecular weight of ~44 kDa (Welinder, 1976). The native enzyme is generally considered to contain a prosthetic ferriprotoporphyrin IX (heme group) buried in the central region of the protein, two calcium atoms proximal and distal to the heme group, four disulfide bridges between

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cysteine residues 11–91, 44–49, 97–301 and 177–209, and a buried salt bridge between Asp99 and Arg123 (Berglund et al., 2002). Although accounting for 90% of the world production of peroxidases, its industrial application is greatly limited by its low thermostability and low reactivity in organic media (Asad et al., 2011). Horseradish peroxidase is also prone to suicide inactivation by the H_2O_2 substrate (Asad et al., 2011), which is particularly problematic in high value applications such as diagnostics and biosensors. Thermal studies of HRP has been earlier reported under various conditions (Bamdad et al., 2014; Pina et al., 2001).

Several peroxidases from tropical palm-trees (Elaies guineensis, Roystonea regia, Trachycarpus fortunei and Chamaerops excelsa) have been isolated and characterized (Wantanabe et al., 2010; Sakharov et al., 2000; Sakharov et al., 2001; Caramyshev et al., 2006). Soybean seed-coat and peanut peroxidase are structurally and functionally stable enzymes and exhibit higher thermal stability within a broad pH range and also in the presence of denaturing agents in comparison to horseradish peroxidase (Bernardes et al., 2015). Although horseradish peroxidase is a stable enzyme, availability of peroxidases with higher stability may promote the development of new analytical methods and potential industrial processes. However, these enzymes have been rarely reported from date palm which is widely distributed in Saudi Arabia. In the present study, peroxidase from date palm leaves was purified to homogeneity and its kinetic and structural properties were studied in comparison with horseradish peroxidase.

2. Materials and methods

2.1. Materials

Leaves of female 'Khalas' date palm (*Phoenix dactylifera*) tree, were freshly collected from palm grove in Governorate of Al-Kharj, Saudi Arabia. 2,2′-azino-bis (3-ethylbenzthiazoline-6-sul fonic acid) (ABTS) and Guaicol were purchased from Sigma and Fluka respectively. All other chemicals were of analytical grade.

2.2. Methods

2.2.1. Preparation of date palm leaves

Fresh date palm (*Phoenix dactylifera)* leaves were cut from the plant and rinsed thoroughly with distilled water. After getting dried with blotting paper, the leaflets were cut into small pieces and the tip part was removed. Leaflets pieces were further frozen in liquid nitrogen and grinded to fine powder by grinder for 2–4 min. Ground tissues were stored at −80 °C until used for further purification.

2.2.2. Isolation of peroxidase from date palm leaves

For proteins extraction, six hundred grams of ground tissues powder from date palm leaves were homogenized with three liters of cold extraction buffer (50 mM sodium phosphate buffer pH 6.5) in a blender for 5 min. Extract was filtered on 2 layers of cheesecloth to remove suspended solid particles. Then, 3% (w/v) insoluble Polyvinylpolypyrrolidone (PVPP) was suspended in the filtrate with constant stirring in ice bath for 30 min. Further, filtrate was centrifuged at 3220 xg for 20 min at 4 °C. Finally, 0.2 mM Phenyl Methyl Sulfonyl Fluoride (PMSF) was added to the clear supernatant to obtain clear solution of crude extract and used for further purification.

2.2.3. Purification of peroxidase from date palm leaves

2.2.3.1. 1st step-aqueous two-phase system. To remove the colored compounds and concentrate the large volume of crude solution, an aqueous two-phase system was used. Briefly, 14% (w/v) solid Poly Ethylene Glycol (PEG-6000) and 10% (w/v) solid ammonium sulfate ($(NH_4)_2SO_4$) was added to the crude extract. The entire mixture was stirred thoroughly for sufficient time on ice bath and then poured into a separatory funnel. The two phases were allowed to come to equilibrium, over 24 h at 4 °C. Top and bottom phases were separated and volumes were measured. Aliquots of the phases were taken for enzyme assay and protein concentration was measured. The bottom phase which contain peroxidase was centrifuged (3220 × *g*) for 15 min at 4 °C and the clear supernatant was collected for next purification steps.

2.2.3.2. 2nd step-hydrophobic interaction chromatography. Butyl Sepharose medium (GE Healthcare, Cat. No. 17-0980-01) was packed into a XK (2.6 × 20 cm) column to form a packed bed. The bed was equilibrated with 2 column volumes of sodium phosphate buffer (50 mM, pH 6.5) containing 2 M $(NH_4)_2SO_4$. Further, crude extract generated by aqueous two-phase system was treated with ammonium sulfate (2M), and subjected to column using a peristaltic pump. Following, column was washed with the same equilibration buffer (5 column volume). Protein was eluted by decreasing $(NH_4)_2SO_4$ concentration and monitored at 280 nm. Fractions containing peroxidase activity were pooled and concentrated (Amicon, 10 kDa cutoff). Subsequently, the purity was tested using 4–15% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). After hydrophobic interaction chromatography, dialysis was used to remove ammonium sulfate and to exchange buffer from the eluted peroxidase fractions. The concentrate was dialyzed against 10 mM Tris buffer pH 8.0 for 24 h with constant stirring on magnetic stirrer at 4 °C.

2.2.3.3. 3rd step-ion exchange chromatography. Purification of date palm peroxidase was completed using a HiTrap Q HP 5 ml column (GE Healthcare, Cat. No. P8375). The column was first equilibrated with 5 column volumes of Tris–HCl buffer (10 mM, pH 8.0). Dialyzed sample was loaded on to the column, following washed out by equilibration buffer to remove unbound proteins. Bound proteins were eluted with a linear, (0–1 M NaCl), salt gradient in the same buffer using a flow rate of 1 ml min^{-1}. The fractions with peroxidase activity were collected and the purity was verified using 4–15% SDS-PAGE. The purified date palm peroxidase was stored at −20 °C for further analysis and characterizations.

2.2.4. Peroxidase assay and protein determination

Activity of date palm was measured via continuous spectrophotometric rate determination process according to the method of Lobarzewski et al. (1990) by using Guaiacol as substrate. The increase in the absorption as a result of the formation of the oxidized product, tetraguaiacol, was measured at 470 nm (ε470 nm = 26.6 mM^{-1} cm^{-1}). The protein concentration at different stages of date palm peroxidase purification was determined by the method of Bradford (1976), using a commercial Bradford assay reagent (Bio-Rad) with bovine serum albumin (BSA) as a standard.

2.2.5. Purity and molecular weight determination by SDS-PAGE

Purity of date palm peroxidase at multiple purification stages and their molecular weight was analyzed by SDS-PAGE. The electrophoresis was conducted using a Mini-protein tetra cell with 4–15% polyacrylamide slab gradient gel (Bio-Rad, Cat. No. 456-1083). The gel was run using standard Laemmli (1970) sample buffer and Tris/glycine/SDS running buffer. Protein bands were stained with Coomassie brilliant blue. Molecular weight was estimated by plotting the logarithm of protein molecular weight vs the relative mobility (Retention Factor Rf) of the protein (Rf = distance migrated by the protein/distance migrated by the dye front).

2.2.6. Effect of pH on stability of peroxidases

The effect of pH on the enzymatic activity of date palm and horseradish peroxidases were determined by measuring the oxidation of ABTS in a set of buffers at various pH values ranging from pH 2.2 to 10.0. The buffers used were at a concentration of 50 mM as the following: glycin–HCl (pH 2.2); sodium acetate (pH 3.0–5.0); sodium phosphate (pH 6.0 and 7.0); Tris–HCl (pH 8.0 and 9.0); and sodium bicarbonate (pH 10.0).

2.2.7. Effect of pH on tertiary structure of date palm and horseradish peroxidase

Conformational changes of date palm and horseradish peroxidases on the tertiary structure level were monitored by tryptophan fluorescence under different pH values. Proteins were prepared at different pH values ranging from 2 to 10 in 25 mM buffer. Tryptophan fluorescence emission was recorded from 305 to 400 nm after excitation of tryptophan at 295 nm.

2.2.8. Effect of pH on secondary structure of date palm peroxidase

pH induced changes in the secondary structure of date palm peroxidases was measured using far UV-CD spectroscopy. Protein (0.2 mg/ml) was incubated with buffers of different pH range 2–10 and spectra were collected between 190–250 nm.

2.2.9. Thermal stability of date palm and HRP

Activity: The enzymes were incubated in its optimum pH at 75 °C up to 60 min and at 90 °C up to 30 min. At the end of the required time, the enzymes were cooled in an ice bath and brought to room temperature and their residual activity was measured. Residual activity was assayed under standard conditions of ABTS oxidation.

Far UV-CD analysis: To compare secondary structures of peroxidase, Far-UV CD spectra in the range of 190–260 nm were recorded and the percentage of different secondary structures was estimated using CDNN software package (version 2.1) provided by Applied Photophysics. For thermal stability, Far-UV CD spectra in the range of 200–250 nm were recorded by raising the temperature from 20 to 94 °C.

3. Results

3.1. Purification of peroxidase from date palm leaves

Peroxidase from date palm leaves was purified to homogeneity by a 3-step procedure including aqueous two phase system, hydrophobic interaction and Ion-exchange chromatography. Aqueous two phase system aided in the removal of pigments from the crude extract. A typical elution profile of date palm peroxidase purification scheme is shown in Figs. 1 and 2. A summary of purification procedure and specific information on the degree of purification obtained at each step appears in Table 1. The purification factor for purified date palm peroxidase was 68 with high 41% yield. The purity of the enzyme was analyzed by SDS–PAGE (Fig. 3) revealed single band with a molecular weight of 55 kDa.

3.2. Effect of pH on stability of peroxidases

The results of enzymatic activity at different pH values for date palm and horseradish peroxidases are shown in Fig. 4. The purified date palm peroxidase demonstrated a maximum activity ranges between pH 3.0–5.0, with optima at around pH 4.5. While the higher activity of horseradish peroxidase was exhibited between pH 5.0–7.0, with optimum activity at pH 6.0.

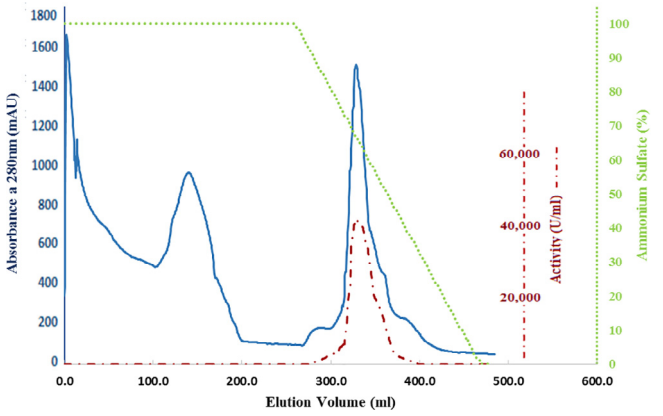

Fig. 1. Hydrophobic interaction chromatogram of date palm peroxidase purification on Butyl Sepharose column. Total protein of 121.25 mg from aqueous two-phase separation fraction was loaded onto Butyl Sepharose column (size: 2.6 × 20 cm) pre-equilibrated with 50 mM sodium phosphate buffer (pH 6.5) containing 2 M $(NH_4)_2SO_4$. Bound proteins were eluted using 2–0 M linear gradient of $(NH_4)_2SO_4$. The flow rate was 2 ml min^{-1}. Protein elution profile was monitored at 280 nm.

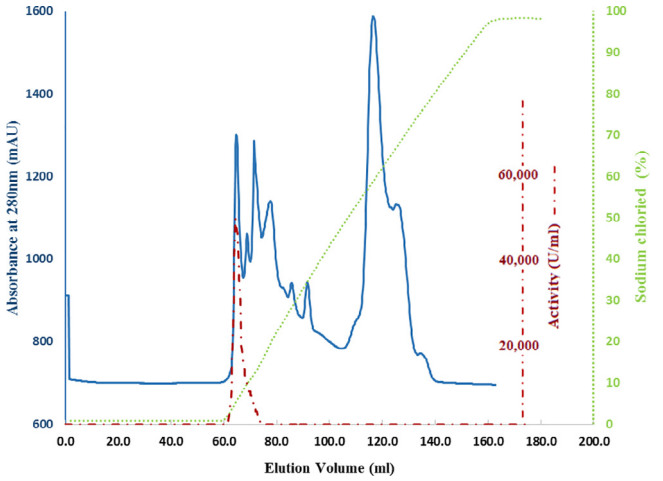

Fig. 2. Anion exchange chromatographic profile of date palm peroxidase purification on Q Sepharose column. Active fraction from Butyl Sepharose was loaded onto Q Sepharose column equilibrated with 10 mM Tris-HCl buffer, pH 8.0. The NaCl linear gradient was maintained between 0–1 M. The flow rate was 1 ml min^{-1}. Protein elution profile was monitored at 280 nm.

3.3. Effect of pH on tertiary structure of peroxidases

In order to investigate the effect of pH on the conformation of date palm and horseradish peroxidases, tryptophan fluorescence spectra of peroxidases were recorded at different pH (Fig. 5). The results depicted emission maxima of both peroxidases are not changes between 4 and 10 pH values. However, under extreme acidic conditions (pH 2–3), date palm peroxidase and HRP experiences a red shift of 4 and 15 nm, respectively. These results indicate that under extreme acidic pH medium, tryptophan environment in both peroxidases change as a result of lost their tertiary structures.

3.4. Effect of pH on secondary structure of date palm peroxidases

pH induced conformational changes in date palm peroxidase was also analysed by far UV-CD (Fig. 6). Far UV-CD measurement measure changes in the secondary structure of proteins under acidic and alkaline conditions. Result illustrated date palm contain

Table 1
Purification steps of date palm peroxidases.

Step	Protein conc. (μg/ml)	Total protein (mg)	Peroxidase activity (U/ml)	Total activity (U)	Specific activity (U/mg)	Yield (%)	Purification fold
Aqueous tow phase system	97 ± 4.59	121.25	$2.14 \times 10^3 \pm 59.74$	2.67×10^6	2.20×10^4	100	1
Butyl sepharose	133 ± 8.81	4.92	$62.20 \times 10^3 \pm 1122.16$	2.30×10^6	46.70×10^4	85	21
Q-sepharose	369 ± 28.37	0.73	$558.85 \times 10^3 \pm 18034.68$	1.11×10^6	151.241×10^4	41	68

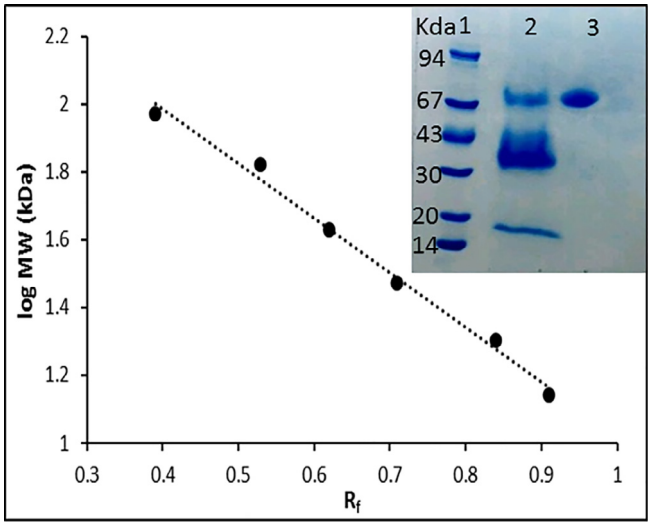

Fig. 3. Standard curve of molecular weight markers using SDS-PAGE for molecular weight estimation of date palm peroxidase. Standard proteins used for the calibration were phosphorylase b (94 kDa), Bovine serum albumin (67 kDa), Ovalbumin (43 kDa), Carbonic anhydrase (30 kDa), Soybean trypsin inhibitor (20 kDa), α-Lactalbumin (14 kDa). (Rf = distance migrated by the protein/distance migrated by the dye front). (Inset) Photograph of SDS-Polyacrylamide slab gel electrophoresis of various fractions obtained during the purification of date palm peroxidase. Lane 1, Marker proteins; Lane 2, eluted protein fractions from Butyl Sepharose column; Lane 3, purified date palm peroxidase from Q Sepharose column. Bands were visualized using Coomassie Brilliant Blue R-250 staining.

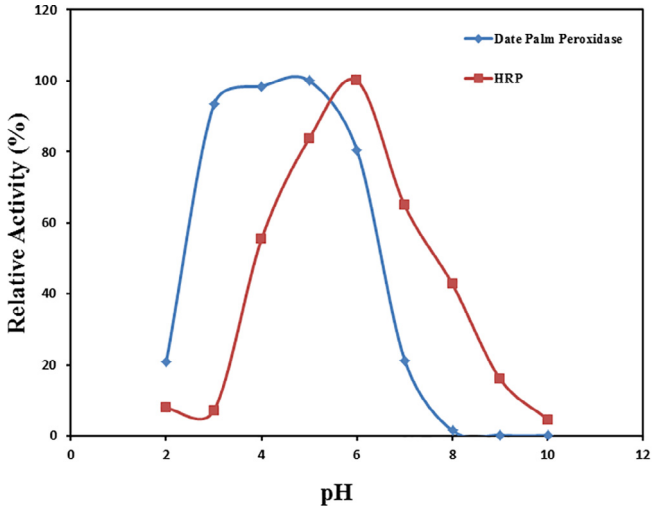

Fig. 4. pH stability of date palm and horseradish peroxidases. Remaining activity was carried out with ABTS as substrate, after 24 h incubation at 25 °C in series of buffers at various pH values ranging from pH 2.2 to 10.0.

alpha-helical structure at physiological pH 7.0. With increase in pH (8–10) towards alkaline, date palm retain its secondary structure while acidic conditions (pH 2–3) induces significant conformational changes and formation of cross- β structure was observed as evident by shift in the peak from 222 to 225 nm.

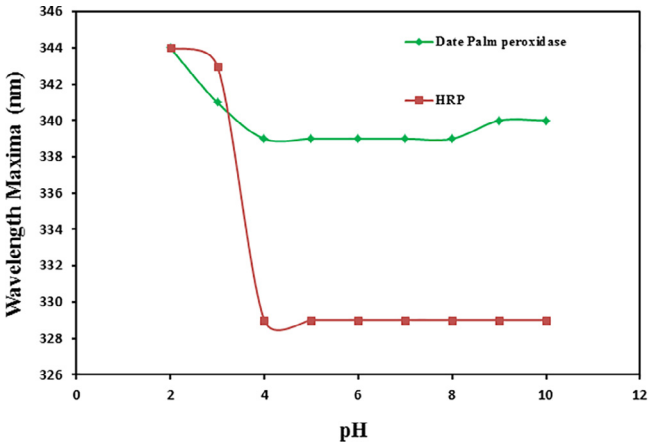

Fig. 5. Effect of pH on tertiary structure of date palm and horseradish peroxidases: Wavelength of emission maxima changes in tryptophan fluorescence at different pH values ranging from 2 to 10.

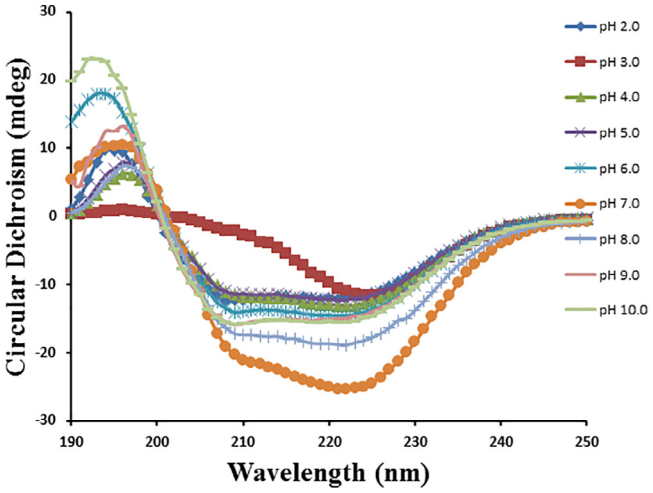

Fig. 6. Effect of pH on secondary structure of date palm peroxidase: Far UV-CD in the range of 190–250 was measured for date palm peroxidase incubating under different pH range 2–7. The protein concentration used was 0.2 mg/ml.

3.5. Secondary structure analysis of peroxidases: Circular dichroism (CD) studies

Circular Dichroism has proven to be a useful tool to study protein structure. In the far-UV- CD, the peptide bond is the principal absorbing group and studies in this region can give information on the secondary structure (Kelly et al., 2005). Fig. 7 showed far-UV-CD spectra of date palm at physiological pH and temperature (7.4 and 25 °C). The backbone of CD spectrum for both peroxidases are characteristic of a protein with predominance of α-helix-rich structure, as date palm peroxidase exhibited two minima at 210 and 222 nm and maxima at 198 nm, likewise, horseradish peroxi-

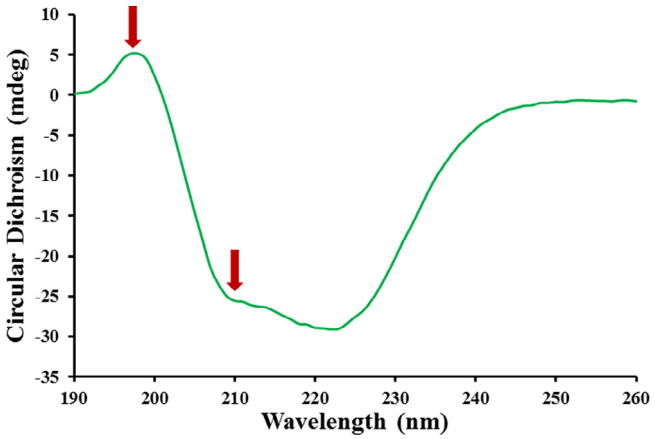

Fig. 7. CD spectra of date palm peroxidase: Far UV-CD spectra of date palm peroxidase was measured under physiological conditions (10 mM Tris-HCl buffer pH 7.0 at 25 °C). A protein concentration of 0.2 mg ml^{-1} was used.

dase (data not shown) exhibited the same characteristics of α-helical structure. A detailed of comparative secondary structure contents estimation using the CDNN software package are shown in Table 2. On the basis of the analysis of CDNN software, α-helix, β-sheet and β-turn were quantified and compared. Importantly, α-helix is significantly higher in date palm peroxidase (43.8%) than HRP (33.8%), while other structures like β-sheet and random coils sharing common pattern.

3.5.1. Thermal stability determination

Activity results illustrating the thermal stability of date palm and horseradish peroxidase are shown in Fig. 8a and b. After incubation at 75 and 90 °C for different time intervals, the results showed that date palm peroxidase was fairly maintained its stability at temperature of 75 °C during 60 min (Fig. 8a), while at 90 °C, it retained more than 40% of its original activity with in 10 min. and continued to maintain approximately 30% of its initial activity with increasing time of incubation (Fig. 8b). In case of horseradish peroxidase, related results showed considerable loss of peroxidase activity due to inactivation at 75 °C and almost complete inactivation occurred at 90 °C. These results suggested that date palm peroxidase possesses better thermal stability than horseradish peroxidase.

Changes in the far-UV CD spectra of date palm and horseradish peroxidases at different temperatures are shown in Figs. 9. When the temperature raised to 94 °C, only marginal changes are seen in the CD spectra of date palm peroxidase (Fig. 9a); whereas, significantly changes in CD spectra of horseradish peroxidase was observed (Fig. 9b), indicating that date palm peroxidase secondary structure is quite stable while the heating promotes significant alteration in secondary structure of horseradish peroxidase.

4. Discussion

In the present study, a peroxidase was purified from date palm leaves and its structural and kinetic aspect properties were studied. Peroxidase from date palm leaves was purified to homogeneity by a 3-step procedure including aqueous two phase system, hydrophobic Interaction and Ion-exchange chromatography. Like many plant extract, date palm leaves extract contain a high concentration of plant cell secondary metabolites which must inactivate and remove before the development of a purification procedure (El-Hadrami and Al-Khayri, 2012). These compounds can cause browning of the crude extract, hinder the recovery of the enzymes and strongly lower the yield (Al-Senaidy and Ismael, 2011; Altunkaya and Gokmen et al., 2011). Our purification protocol aided PVPP to avoid undesirable effects of polyphenolic compounds. Laing et al. (2004) employed the same procedure for removal of polyphenolic components from plant tissues. However, many studies have demonstrated the suitability of aqueous two phase system for the successful extraction of enzymes from plant sources (Vilter and Jordan, 1989). Aqueous two phase system, namely PEG-$(NH_4)_2SO_4$, was employed here as a primary purification step, to improving the degree of enzyme purification and achieved efficient pigments removal. The ratio of 14/10 PEG/$(NH_4)_2SO_4$% (w/v) was selected as reported by Sakharov et al. (2000). Further, downstream processing including hydrophobic interaction and ion-exchange chromatography resulted in purification of peroxidase with 68-fold increase in specific activity and 41% yield. Our purification strategy was advantageous over previously reported procedure from for purification of peroxidase from

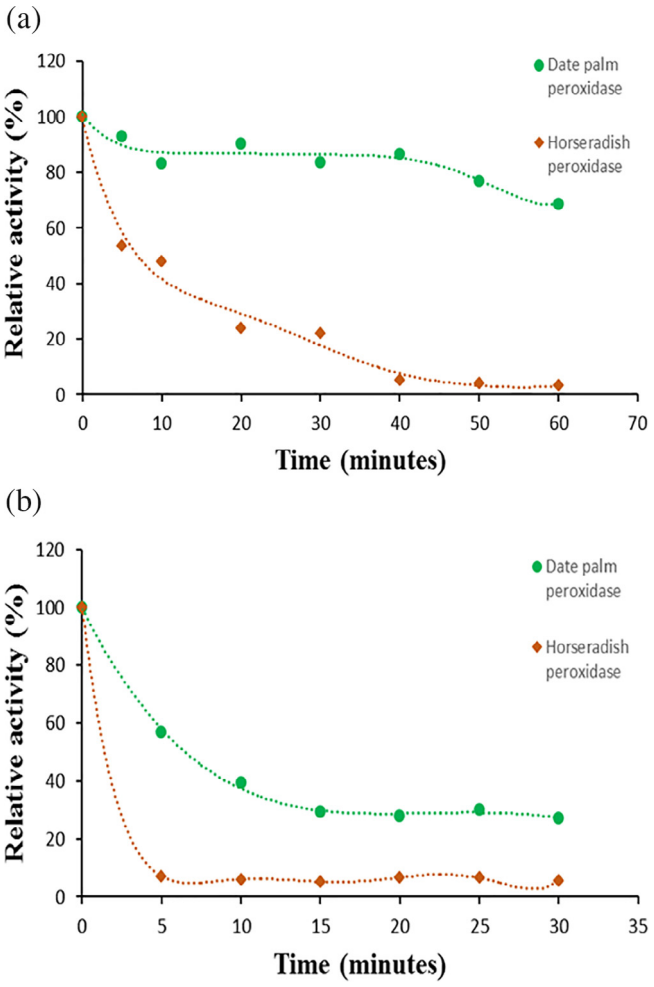

Fig. 8. Thermal stability of date palm and horseradish peroxidases incubated at (A) 75 °C and (B) 90 °C in different time intervals. Remaining peroxidase activities were determined using ABTS as substrate under the standard test conditions.

Table 2
The percentage content of the secondary structures of date palm and Horseradish peroxidase in neutral pH at 27 °C estimated from CD spectra between 190–260 nm using CDNN software.

Secondary structures	Date palm peroxidase	Horseradish peroxidase
Helix	43.8%	33.8%
Anti parallel β-sheet	5.9%	8.5%
Parallel β-sheet	6.4%	8.2%
β-turn	14.9%	16.9%
Random coil	26.5%	30.2%

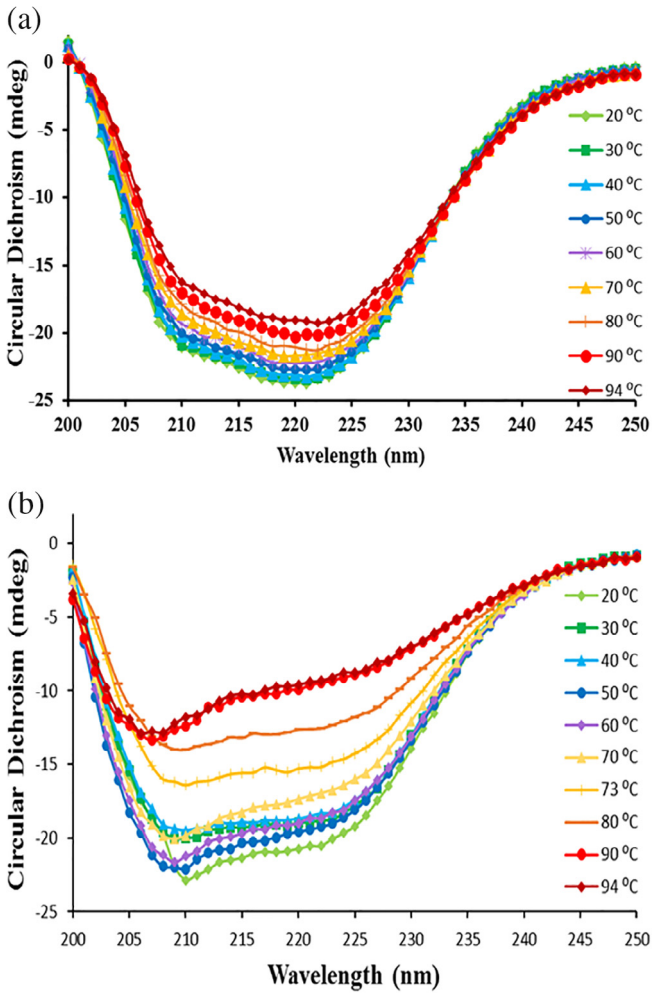

Fig. 9. CD spectra in the in the far-ultraviolet spectral region of (A) date palm peroxidase and (B) horseradish peroxidase at different temperatures ranging from 20 to 94 °C. A protein concentration of 0.2 mg ml^{-1} prepared in 10 mM Tris-HCl buffer pH 7.4 was used.

Eichhornia crassipes leaves (23.58-fold with 18.58% yield) (Arise et al., 2016), date palm leaves (17 purification fold with 5.8% yield) (Al-Senaidy and Ismael, 2011) and royal palm leaves (73-folds with 12.5% recovery) (Sakharov et al., 2001).

The molecular weight of purified date palm peroxidase was estimated to be approximately 68 kDa by SDS–PAGE (Fig. 3). This value was slightly higher than those published for peroxidase from other plant sources (Al-Senaidy and Ismael, 2011; Sakharov et al., 2001); possibly due to higher glycosylation degree of date palm peroxidase. However, molecular weights of peroxidases from various plant sources have been reported to be range from 30 to 60 kDa, and the differences observed are attributed to post-translational modifications of the polypeptide chain including the number and composition of glycan chains present in plant peroxidases (Duarte-Vazquez et al., 2007).

It is well known that the optimal conditions for the catalysis by different peroxidases are not identical. Their activity largely depends upon environmental factors like pH and temperature. Date palm peroxidases demonstrated optimum pH at 4.5, which was lower than the corresponding value (pH 6.0) for horseradish peroxidase (Fig. 4). In general, peroxidases purified from various sources showed pH optima mostly in the region of 4.5–6.5 (Arise et al., 2016; Sarika et al., 2015; Al-Senaidy and Ismael, 2011), which is consistent with our results.

The effect of pH on the tertiary structure of date palm and horseradish peroxidases (Fig. 5) showed stability in the pH range of 4–10 as suggested by constant emission maximum wavelength. Both peroxidases undergoes red shift under extreme acidic conditions. Red shift of emission maxima illustrated tryptophan residues became exposed to more polar environment as a result of changes in the tertiary structure of date palm and HRP. Red shift of 5 nm is produced in date palm while HRP displayed 15 nm, suggesting higher tertiary structure stability of date palm peroxidase than HRP. The pH induced conformational changes has been observed in many enzymes including peroxidase (Kourash et al., 2004).

Further, secondary structure stability against various pH values demonstrated loss of secondary structure and formation of cross-β structure under acidic conditions (Fig. 6) implying date palm has propensity to aggregate. Proteins are susceptible to aggregation and amyloid formation has been reported earlier (Khan et al., 2013)

Circular Dichroism is one of the most rapid, sensitive techniques for determining structures and monitoring the structural changes occurring in proteins. Far-UV CD spectra backbone in Fig. 7 showed both peroxidases displayed negative minima peak at 208 and 222 nm and positive peak between 191–193 nm, suggesting predominance of alpha helix structural conformation. Quantitative estimation of secondary structure in date palm peroxidase (Table 2) contains a considerable amount of α-helix structure, 43.8%, compared to horseradish peroxidase which under the same experimental conditions has 33.8%. Although of predominance of α-helical structures in both peroxidases, there was minor contribution of β-structure, like parallel β-sheet, anti-parallel β-sheet and β-turn in these enzymes. The values obtained for both peroxidases are typical of different heme peroxidases as it has been previously established that the proportion of α-helix of peroxidases is higher than that of β-sheet by using different methods such as circular dichroism (Strickland et al., 1968), Fourier transform infrared spectroscopy (Holzbaur et al., 1996) and crystallography (Gajhede et al., 1997), where the structure of heme peroxidases from plants sources is formed by 10–11 α-helices (ca. 30–40% of the total secondary structure) linked by loops and turns, while the β-strand structures are a minor component (Banci, 1997).

Thermal stability of enzymes depends on the assay conditions, especially incubation time and pH. Peroxidases show highly variable thermal stability which is attributed to their particular enzyme structure (Marquez et al., 2008). Date palm leaf peroxidase exhibited very high resistance to heat even at temperature as high as 75 °C while HRP lost its activity gradually (Fig. 8). Enzyme activity is correlated with their structure as it is evident that secondary structure of date palm peroxidase is more stable against thermal denaturation and exhibit higher activity (Fig. 9a) while HRP lost their secondary structure and function (Fig. 9b). High thermal stability of date palm peroxidase might be attributed to its more alpha-helical content and different folding pattern associated with hydrogen bonding and disulphide linkages (Liu and Wang, 2003). Also, high thermal stability exhibited by date palm peroxidase probably due to the presence of sugars moiety in their structure.

5. Conclusion

In conclusion, in the present study, a comparative investigation on kinetics and structural properties of date palm and horseradish peroxidases has been performed. This study is important at least from two points of view: First, date palm peroxidase can be easily purified with our optimized purification procedure with high purity and yield assurances modern future research which needed high purity of enzyme. Secondly, stabilizing studies may clarify the impact of denaturants such as acidic/basic pH conditions, and

high temperatures on the functionality/efficiency of the enzyme molecule. There was limited data on the conformational changes and structural characteristics of date palm peroxidase, our finding illustrated secondary and tertiary structure of date palm peroxidase is more resistant towards conformational changes under acidic pH and high temperature.

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