عنوان المشروع باللغة العربية	
عنوان المشروع باللغة الإنجليزية	Unexplored stability of Date palm (Phoenix dactylifera) peroxidase: Comparison with Horseradish peroxidase (HRP)
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التخصص الدقيق للمشرف الرئيس	Proteins
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المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات العليا	12 months
Abstract or synopsis of the proposal (200 words or less):	Horseradish peroxidase (HRP, EC 1.11.1.7) is an important heme-containing enzyme belonging to the class III of plant peroxidases that have been studied for more than a century. This class of enzymes has Fe(III) protoporphyrin IX as the prosthetic group, which plays an important role in their catalytic mechanism. The structural features of HRP include four disulfide bonds, two Ca^{2+} binding sites located distal and proximal to heme and eight glycans [1–4]. The heme cavity is the catalysis center of the enzyme. The most widely studied peroxidase is isoform C from horseradish roots (Armoracia rusticana) mainly due to its many diverse uses in biotechnology such as fine chemicals' synthesis, medical diagnostics and bioremediation [5, 6]. Nevertheless, its further industrial application is greatly limited by its low thermostability and low reactivity in organic media. HRP is also prone to suicide inactivation by the H_2O_2 substrate, which is particularly problematic in high-value applications such as diagnostics and biosensors [7] as well as in wastewater treatment [8]. Enzyme stabilization will continue to be a key issue in biotechnology. Both storage (and shelf- life) and operational stabilities can determine the usefulness of enzyme based products [9]. Several studies have tried to increase the stability of HRP by modern approaches [10], including immobilization, site-directed mutagenesis, chemical modification and cross-linking [11–14]. However, in many cases, simple traditional methods such as the addition of low molecular weight solutes and choosing optimized buffer system could be very promising for increasing the stability of enzymes. Stabilizing effects of several small molecules such as salts, sugars and polyols have been proved for different enzymes including HRP [15]. Trehalose, sucrose, maltose, glycerol and inositol are some of small molecules which have been used to raise the Tm of proteins and prevent their unfolding process [16]. It is also well established that the type and concentration of buffer can effectively influence the enzyme stability [17]. In this study, with respect to the importance of HRP stabilization, we have investigated the stabilizing effect of potassium phosphate and Tris buffer systems in two different concentrations. In addition, the effect of some additives on the thermal and H_2O_2 stability of HRP was investigated. Glycosylation is one of the structural factors, believed to affect stability of HRP toward elevated temperatures and H_2O_2 concentration [18].

Hypothesis or scientific justification of the proposal	Industrial application of peroxidases is greatly limited by its low thermostability and low reactivity in organic media. Moreover, peroxidases are prone to suicide inactivation by the H2O2 substrate, which is particularly problematic in high-value applications such as diagnostics and biosensors as well as in wastewater treatment. Also, previous studies showed increase in the stability of horseradish peroxidase (HRP) by modern approaches, including immobilization, site-directed mutagenesis, chemical modification and cross-linking.Stabilizing effects of several small molecules such as salts, sugars and polyols have been proved for different enzymes including horseradish peroxidase (HRP). In addition, small molecules like trehalose, sucrose, maltose, glycerol and inositol have been used to raise th e Tm of proteins and prevent their unfolding process . Any improvement in the stability of the enzyme will greatly enhance its applications.To the best of my knowledge, thermal stability and secondary structure determination has not been studied for date palm peroxidase. Thus, it is prudent to explore stability of peroxidase purified from novel source (Phoenix dactylifera) date palm and to compare its stability and properties with HRP. Thermophilic nature of date palm prompted us to hypothesize that date palm peroxidase has more stability under various conditions of salts, sugars and organic solvent in comparison to HRP.
Specific objectives	Objective 1: Purification/Cloning and purification of Peroxidase from date palm Objective 2: Effects of various osmolyte on date palm peroxidase and HRP (horseradish peroxidase) Objective 3: Effects of organic solvent on peroxidases Objective 4: Effect of Chemical denaturant on peroxidases
Methodology & Major Techniques to be used	Purification of date palm peroxidase: Fresh mature leaflets from date palm (Phoenix dactylifera L.) will be collected from King Saud University campus garden. After limb and spines removal, leaflets will be washed thoroughly in distilled water and cut into small pieces. Leaflet pieces were frozen in liquid nitrogen and ground to a fine powder using a prechilled grinder for 2–4 min. Ground tissues were stored at −70 °C until used. After homogenization of leaflets, the enzyme will be purified by a combination of anion exchange and size exclusion chromatography using an FPLC-System (AKTA-purifier GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The fractions showing activity will be pooled and will use as enzyme source for further studies. Peroxidase activity assay: Peroxidase activity will be measured spectrophotometrically using guaiacol as substrate. The increase in the absorption as a result of the formation of the oxidized product (tetraguaiacol) will be measured at 470 nm.

	SDS-PAGE Analysis: Enzyme purity and molecular weight will be analyzed by SDS–PAGE in a Mini Protean III Electrophoresis Cell (Bio-Rad), with 12% resolving and 4% stacking gel. Proteins staining will be done using the Coomassie Blue staining technique and molecular weight will be estimated by comparison to molecular weight markers (Amersham Biosciences). Thermal and pH stability: Thermal stability of HRP and date palm peroxidase will be determined at different temperature and pH. After the incubation, tubes will be chilled on ice and the residual activity will be measured spectrophotometrically. Enzyme activity without thermal treatment will marked as 100%. Thermal stability will also determine in different buffers with different concentrations and in the presence of osmolyte (glycerol, arginine, salts, metal ions, trehalose and sorbitol as stabilizers (20%, w/v). The control reaction was carried out without any additives. Protein Aggregation Assay: Turbidometry (Absorbance at 360 nm) and ThT assay will be employed to know the possible aggregation/amyloid formation under above mentioned condition of stress (High temperature, extreme pH and presence of metal ions) and in the presence of osmolyte. Protein tertiary structure analysis (Fluorescence measurement): Total Fluorescence and tryptophan specific fluorescence of proteins will be targeted to know the conformational change under these conditions. Hydrophobicity of proteins will be measured using ANS fluorescence. Secondary structure and Tm determination of peroxidases (Circular Dichroism analysis): Secondary structure (alpha helix, β-sheet, random coil) content and change in their conformation under various condition of stress and osmolyte will be used to characterize stability of peroxidase. Further, Tm value is an indication of stability will be calculated under all these stability and unstable conditions. Techniques employed for this study: 1. Chromatography (Size Exclusion and ion-exchange Chromatography) 2. SDS-PAGE Analysis 3. Peroxidase activity 4. Fluorescence Measurement 5. Circular Dichroism (CD) 6. UV-Visible
Availability of Samples	YES
If the answer is no, kindly justify	
Availability of	YES

Chemicals	
If the answer is no, kindly justify	
Availability of Instruments	YES
Availability of Ethical Approval (if needed)	YES
Recent References	[1] K.G. Welinder, J.M.Mauro, L. Norskov-Lauritsen, Biochem. Soc. Trans. 20 (1992) 337–340. [2] T.L. Poulos, Curr. Opin. Biotechnol. 4 (1993) 484–489. [3] A.M. English, G. Tsaprailis, Adv. Inorg. Chem. 43 (1995) 79–125. [4] A. Henriksen, O. Mirza, C. Indiani, K. Teilum, G. Smulevich, K.G. Welinder, M. Gajhede, Protein Sci. 10 (2001) 108–115. [5] B. Ryan, N. Carolan, C.Ó. Fágáin, Trends Biotechnol. 24 (2006) 355–363. [6] N.C. Veitch, Photochemistry 65 (2004) 249–259. [7] A. Borole, S. Dai, C.L. Cheng, M. Rodriguez Jr., B.H. Davison, Appl. Biochem. Biotechnol. 113 (2004) 273–285. [8] M. Wanger, J.A. Nicell, Water Res. 36 (2002) 4041–4052. [9] J.Y. Feng, J.Z. Liu, L.N. Ji, Biochimie 90 (2008) 1337–1346. [10] E.Y Shami, A. Rothstein, M. Ramjeesingh, Trends Biotechnol. 7 (1989) 186–190. [11] G. DeSantis, J.B. Jones, Curr. Opin. Biotechnol. 10 (1999) 324–330. [12] D. Garcia, J.L. Marty, Appl. Biochem. Biotechnol. 73 (1998) 173–184. [13] O. Ryan, M.R. Smyth, C.Ó. Fágáin, Enzyme Microb. Technol. 16 (1994) 501–505. [14] E. Miland, M.R. Smyth, C.Ó. Fágáin, Enzyme Microb. Technol. 19 (1996) 63–67. [15] A.S. Carvalho, A.M. Santos, M.T. Neves-Petersen, S.B. Petersen, M.R. Aires-Barros, E.P. Melo, Biopolymers 75 (2004) 173–186. [16] C.Ó. Fágáin, Enzyme Microb. Technol. 33 (2003) 137–149. [17] L. Haifeng, L. Yuwen, C. Xiaomin,W.Zhiyong,W.Cunxin, J. Therm. Anal. Calorim. 93 (2008) 569–574.