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Purification and characterization of red beet (*beta vulgaris*) peroxidase

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ABSTRACT

Red beet (*Beta vulgaris*) is an important source of dietary having various bioactive compounds. In this study, a peroxidase was purified for the first time from native red beet (*Beta vulgaris*) in a single step using 4-aminobenzohydrazide affinity chromatography and characterized biochemically. The molecular weight of the purified enzyme was calculated approximately as 160 kDa by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and a single band was observed. As a result of the kinetic studies on the enzyme; optimum pH, optimum ionic strength, optimum temperature and stable pH were determined as 6.5, 0.7 M, 70 °C, 6.5, respectively for red beet (*Beta vulgaris L.*). Red beet (*Beta vulgaris*) peroxidase showed K_M and V_{max} values of 9.09 mM and 1.38 EU/mL.min for guaiacol/H_2O_2, respectively. Also, inhibitory effect of 4-aminobenzohydrazide on purified peroxidase enzyme was examined *in vitro* condition. The IC_{50} and K_i values were calculated as 0.047 and 0.78±0.17 mM, respectively.

KEYWORDS

Affinity chromatography;
Enzyme kinetics;
Enzyme purification;
Peroxidase;
Red beet (*Beta vulgaris*).

INTRODUCTION

Peroxidases (PODs, E.C 1.11.1.7) are widely found in different kinds of organisms and they have been implicated in a broad range of physiological and biochemical functions. Particularly in plants, wound healing, general stress response, synthesis of cell wall components, hormone regulation, and protection of tissue from physical damage and control of defense mechanisms against pathogens are some of the proposed functions[1-3]. Peroxidases are a member of the oxidoreductase family and they catalyze the H_2O_2-dependent oxidation reaction of a large variety of substrates containing aromatic structures[4, 5].

Peroxidase is an important enzyme having broader catalytic activity, high sensitivity and wide substrate specificity[6]. Also, its activity can be easily and accurately measured spectrophotometrically. These characteristics of peroxidase make it useful tool in a wide range of analytical, biomedical and industrial applications. Peroxidase is used commercially in biosensor construction[2], as components of kit for medical diagnosis[7],

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biotransformation of organic compounds[8], and treatment of waste water[9, 10]etc.

Peroxidases are widely distributed in plants, and until now, more than 150 plants have been examined to determine peroxidase activity. Although, they have been isolated and purified from a large number of sources, the main source of commercially available peroxidase is horseradish roots[11]. Horseradish peroxidase (HRP) has been studied for several decades and it is a highly investigated and characterized member of the peroxidase family. However, low thermostability and low reactivity in organic media of HRP[12] and higher cost of purification from the sources[13] are the main disadvantages, and these features limit industrial applications of its. For this reason, further investigation for an alternative source of peroxidase with higher activity, pH and thermo stability and degree of purification is the need of the day. It is well known that availability of peroxidase with higher stability and different substrate specificity of peroxidase purified from different sources would promote the development of new analytical procedures and/or potential applications[14]. Therefore, numerous investigations on the purification of peroxidase from alternative sources such as garlic (*Allium sativum*) bulb[1], oil palm (*Elaeis guineensis Jacq.*) leaf[7], leaves of Ipomoea palmetta[15], turnip (*Brassica napus*) hairy-root cultures[16], flowers of Cynara scolymus L.[17], broccolli (*Brassica oleracea Var Italica*)[18], apple (*Mallus pumilus*) fruits[19], sunflower roots[20], leaves of Ricinus communis[21], black gram (*Vigna mungo*) husk[22], cotton (*Gossypium hirsutum L.*)[23], Turkish black radish (*Raphanus sativus L.*)[24], edible mushroom *pleurotus eryngii*[25], wild endemic hemi-parasitic plant (*Viscum angulatum*)[26], Caribbean plant (*Euphorbia cotinifolia*)[27], papaya (*Carica papaya*) fruit[28], sweet gourd (*Cucurbita moschata Lam. Poiret*)[29],bamboo shoots[30], Jatropha curcas[31], leaves of *sapindus mukorossi*[32], pearl millet grains[33], fresh-cut *Zizania latifolia*[34], leaves of chard (*Beta vulgaris* subspecies *cicla*)[35]have been reported.

Red beet (*Beta vulgaris L.*) is the member of the *Chenopodiaceae*[36] and it has been used for food traditionally in many parts of the world. It has been also widely used commercially to produce natural pigment and red beet juice[37, 38]. Although this vegetable is generally cultivated for its large roots, the leaves also are utilizable. Red beet represents a highly renewable and cheap source of nutrients and it is consumed in many ways such as salad, pickles or cooking etc. It is well known, red beet (*Beta vulgaris*) has been used for a long time for their beneficial health effects[36], and it is an important source of dietary having various bioactive compounds. Ninfali and Angelino (2013) have reported a review focuses on *Beta vulgaris*. They have reviewed the biological and pharmacological activities, chemical composition and nutritional value of Swiss chard (*Beta vulgaris cicla*) and red beetroot (*Beta vulgaris rubra*). They also have reported that red beet (*Beta vulgaris*) contains secondary metabolites (betalains) which are show anticancer activity.

Some studies have been reported about peroxidase and red beet (*Beta vulgaris*). They have mostly related about inactivation of peroxidase or chemical components of red beet (*Beta vulgaris*) rather than peroxidase purification. Liu et al. (2010) have studied inactivation of peroxidase in red beet extract with continuous high pressure carbon dioxide[37], and Latorre et al. (2012) have studied inactivation of peroxidase in red beet by traditional and microwave blanching[39]. Rudrappa and coworkers have intensively studied on the purification of peroxidase from red beet (*Beta vulgaris L.*) hairy roots cultures. They have used hairy root cultures of red beet (*Beta vulgaris L.*), obtained after genetic transformation with *Agrobacterium rhizogenes*, to purify peroxidase[40, 41]. They have purified peroxidase with higher activity and thermostability from genetically transformed red beet (*Beta vulgaris L.*) hairy roots cultures using a combination of ammonium sulfate fractionation and ion exchange chromatography. However, genetically transformed cell cultures have some disadvantages *e.g.* slow growth rate, inconsistent product yield and genetic instability. So, we have purified and characterized a peroxidase from native red beet (*Beta vulgaris*) as a new source of peroxidase. To the best of our knowledge, there is no report on the purification of peroxidase from native red beet (*Beta vulgaris L.*) using affinity chromatography.

MATERIALS AND METHODS

Chemicals and apparatus

CNBr-activated-Sepharose 4B, L-tyrosine, 4-

amino benzohydrazide, guaiacol, H_2O_2, Coomassie Brilliant Blue R, potassium phosphate (monobasic), glycerol, and all other reagents used in the electrophoresis and protein assay were obtained from Sigma Aldrich. A freezer (Sanyo, Ultra Low, -86°C) was used to protect the purified enzyme and other chemicals. In the experiments, a UV-VIS spectrophotometer (Beckman Coulter Du730), an ultra turrax (Heidolph Silient Crusher M), a peristaltic pump (Ismatec), a pH-meter (SCHOTT CG840), a temperature controlled centrifuge (HermleZ 323K), a SDS–PAGE (Mini-Protean, Bio-Rad) and a temperature controlled circulating water bath (Grant LTD 6G -20 to 100°C) were used. All chemicals were of analytical grade.

Plant materials

The red beet (*Beta vulgaris*) used in this work, was obtained from a local market in Erzurum, Turkey. It was washed, drained and packed. Only the root was used for peroxidase purification. The samples were stored at -20 °C until use.

Preparation of the crude enzyme extract

The homogenate was prepared according to the procedure described previously by Kalın et al. (2014)[4]. For this purpose, firstly approximately 20 gram of red beet (*Beta vulgaris*) root was cut into small pieces, processed in a blender and was mixed with liquid nitrogen, carefully. After, using a mortar it was crushed until a fine powder was obtained. Then, 50 mL of KH_2PO_4 (0.3 M, pH: 7.0) solution was mixed to a slurry, and the slurry was homogenized with an ultra turrax for 1-2 min. After homogenization, using a temperature controlled centrifuge, the slurry was centrifuged at 16.000xg for 1 h at +4 °C. Finally, the precipitate was separated and discarded, and the supernatant, considered as crude extract, was stored at -20°C in small aliquots for further enzyme purification.

Purification of peroxidase by 4-amino-benzohydrazide affinity chromatography

A 4-amino-benzohydrazide affinity column material was prepared and red beet (*Beta vulgaris*) peroxidase enzyme was purified from the column according to the described previously published procedure[4].

SDS-Page (sodium dodecyl sulfate-polyacrylamide gel electrophoresis)

Using a SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), purification degree and molecular weight (M_w) of purified peroxidase were determined. For this purpose, purified enzyme sample containing approximately 20 µg of protein from the homogenous pool fractions was mixed with a sample loading buffer. Then it was loaded on the electrophoresis medium under reducing conditions. SDS–PAGE was performed as described[42]. Standard proteins (Catalog No: 26630 Page Ruler Broad Range Unstained Protein Ladder, 2×250 µL) were also applied to the electrophoresis medium. After completion of the electrophoresis, the protein bands were stained with silver staining[43].

Qualitative and quantitative protein determination

Qualitative and quantitative protein determination was performed on the eluates at 280 nm and 595 nm respectively, with a UV-VIS spectrophotometer. Protein concentration was determined according to Bradford's (1976) dye binding method[44]. Bovine serum albumin (BSA) was used as a standard protein.

Assay of *in vitro* peroxidase activity

Peroxidase activity was determined by a colorimetric assay using guaiacol substrate spectrophotometrically described in Kalýn et al. (2014)[4]. All routine assays and kinetic studies were performed in KH_2PO_4 buffer solution (0.1 M, pH 6.0). In brief, the enzyme activity was determined using 3 mL of reaction mixture containing 1000 µl of 1% H_2O_2 (22.5 mM) and 1000 µl of guaiacol (45 mM) as substrates, and 900 µL of phosphate buffer solution. Immediately, after addition of the enzyme, peroxidase activity was determined measuring the initial rate of oxidation of guaiacol. The oxidation of guaiacol to 3,3'-Dimethoxy-4,4'-biphenoquinone in the presence of H_2O_2 was monitored using a spectrophotometer at a wavelent of 470 nm. The changes in absorbance were monitored for 3 min at 25°C and one unit of enzyme was defined as the amount of enzyme able to catalyse the oxidation of 1 µmol of guaiacolmin^{-1} at 25 °C[4]. During the enzyme activity measurements, all experiments were performed under optimum conditions (25°C, 0.1 M, pH 6.0

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KH_2PO_4) unless otherwise indicated.

Determination of pH optimum and pH stability

For pH optimum measurement, 0.1 M KH_2PO_4 buffer solution with different pH levels (pH 5.0-8.0) were prepared. Then, the red beet (*Beta vulgaris*) peroxidase activity was determined in the presence of buffers of wide pH range (pH 5.0-8.0).

The pH stability was also investigated. For this purpose, enzyme activity was monitored for 108 h to determine the stable pH value of the enzyme. The remaining activities of the enzyme after it had been kept at +4°C for 108 h at various pHs was monitored by one measurement every twelve hours.

Determination of temperature optimum

To determine the effect of temperature on the red beet (*Beta vulgaris*) peroxidase activity, the purified enzyme was incubated for 5 min at different temperature ranging from 0 to 80 °C. All experiments were performed at optimum pH (predetermined) and during the experiments; temperature was controlled using a thermostatic water bath.

Effect of ionic strength

Different concentrations of KH_2PO_4 buffers (25 mM- 1 M) were used to determine the effect of ionic strength on red beet (*Beta vulgaris*) peroxidase activity at the predetermined optimal pH.

Kinetic constants for guaiacol and H_2O_2

To determine K_M and V_{max} values, a suitable amount of red beet (*Beta vulgaris*) peroxidase enzyme solution was incubated with five different concentrations of guaiacol (1.5- 15 mM) and a fixed saturated concentration of H_2O_2, and also incubated with five different concentrations of H_2O_2 (0.7- 6.0 mM) and a fixed saturated concentration of guaiacol. The K_M and V_{max} values were calculated from a plot of 1/V versus 1/ [S] according to the method of Lineweaver and Burk (1934)[45].

Inhibition Effects of 4-amino benzohydrazide

The inhibition effect of 4-amino benzohydrazide on the red beet (*Beta vulgaris*) peroxidase enzyme was determined following the procedure of Lineweaver and Burk (1934)[45]. To determine half maximal inhibitory concentration (IC_{50}) and K_i values, all experiments were performed according to the described detailed previously with a slight modification[4,5]. All experiments were performed in triplicate and for K_i value, result was given as mean±SD.

RESULTS AND DISCUSSION

Purification of peroxidase from affinity column

A peroxidase enzyme was purified from red beet (*Beta vulgaris*) with 4-amino benzohyrazide affinity chromatography. To purify the peroxidase, firstly, red beet (*Beta vulgaris*) homogenate was prepared. Secondly, a 4-amino benzohydrazide affinity column was prepared as described earlier[4], and red beet (*Beta vulgaris*) homogenate was loaded onto this affinity column. Then, a sufficient amount of washing buffer was used to wash the affinity column. Elution of the peroxidase enzyme from the affinity column was performed with an elution buffer. Finally, eluates were collected as 1.5 mL fractions and the absorbance and activity measurements of each eluates were performed separately at 280 and 470 nm, respectively. Quantitative protein determinations and activity measurements were performed on the crude extract and all eluted fractions. To the best of our knowledge, there is no report on the purification of peroxidase from native red beet (*Beta vulgaris L.*).

The peroxidase enzyme purification is summarized in TABLE 1.

The red beet (*Beta vulgaris*) peroxidase was purified to a high degree (196.9-fold). And it was purified had a specific activity of 1725 EU/mg and a yield of 38%. To purify peroxidase enzyme from the plants (Turkish blackradish (*Raphanus sativus L.*) and the turnip (*Brassica rapa L.*)), 4-amino benzohydrazide was used for the first time as an affinity ligand by Kalın et al. (2014)[4]. The Turkish blackradish (*Raphanus sativus L.*) peroxidase and the turnip (*Brassica rapa L.*) peroxidase were purified had a specific activity of 947.5 EU/mg, a yield of 10.6% with a factor of 40.3-fold and a specific activity of 9626.67 EU/mg, a yield of 9%, with a factor of 269.13-fold, respectively, by Kalın et al. (2014)[4]. The red cabbage (*Brassica oleracea var. capitata f. rubra*)

TABLE 1 : Levels of purification of red beet (*Beta vulgaris)*

Total Volume	*Activity (EU/mL)*	*Protein Quantity (mL)*	*Total Protein*	*Total Activity (mg/mL)*	*Specific Activity (mg)*	*Yield (%)(EU)*	*Purification Fold (EU/mg)*
*Homogenate*5mL	2.54	0.26	1.4	12.7	8.76	100	1
*Elution*5 mL	0.966	0.000561	0.0028	4.83	1725	38	24.7

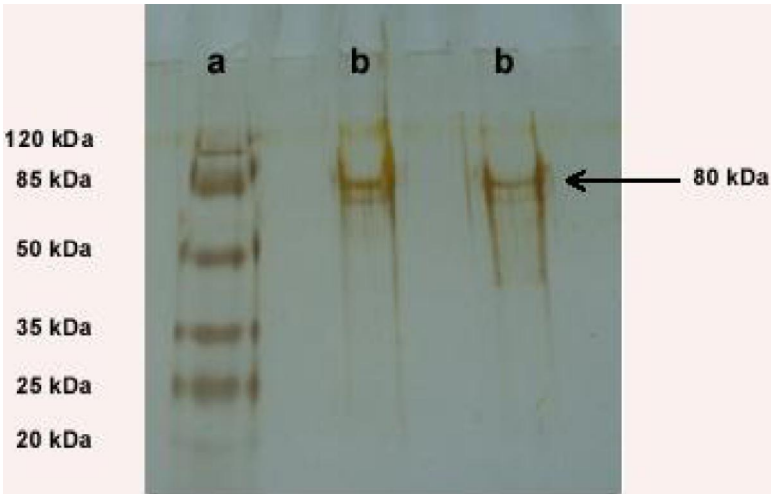

Figure 1a : SDS–PAGE of purified peroxidase: Column a: Standard proteins, Column b; Purified peroxidase from red beet (*Beta vulgaris*). The standard proteins used for SDS-PAGE were highly purified proteins ranging from 10 kDa to 250 kDa (Catalog No: 26630 Page Ruler Broad Range Unstained Protein Ladder, 2×250µL)

peroxidase was purified had a specific activity of 3350 EU/mg, a yield of 2.9% with a factor of 120.6 by Somtürk et al. (2014)[5]. The white cabbage (*Brassica oleracea var. capitata f. alba*) peroxidase was purified 24.7-fold with overall recovery of 4.3% and with a specific activity of 964.5 EU/mg by Erdem et al. (2015)[46]. Similarly, in our study, 4-amino benzohydrazide was used as an affinity ligand. Considering the previous studies, the purified red beet (*Beta vulgaris*) peroxidase has relatively good properties such as higher specific activity, pH stability, overall yield, purification factors etc.

Determination of molecular weight and purity

The activity of purified enzyme and also its homogeneity were checked by SDS–PAGE. The purified peroxidase enzyme from red beet (*Beta vulgaris*) when subjected to SDS-PAGE analysis showed only a single band (Figure 1a). The molecular weight of the enzyme was calculated from the Log of molecular weight ($LogM_w$) versus relative mobility (R_f), and it was found to be 160 kDa using the formula obtained from the standard graph (Figure 1b).

In the literature, a wide range of molecular weight has been reported for purified peroxidase from different sources. In previous studies have been reported that molecular weights of peroxidases are ranging from 20 to 95 kDa, depending on the purified source. The molecular weight value of red beet (*Beta vulgaris*) was higher than those reported for peroxidases from black cabbage (*B. oleracea var. acephala*) (95 kDa)[47], white cabbage (*Brassica oleracea var. capitata f. alba*) (73.2 kDa)[46], red cabbage (*Brassica oleracea var. capitata f. rubra*) (69.3 kDa)[5], Turkish blackradish (*Raphanus sativus L.*) (67.3 kDa), turnip (*Brassica rapa L.*) (65.8 kDa)[4], oil palm (*Elaeis guineensis jacq*) leaf (48 kDa)[7], fresh cauliflower (*Brassica oleracea L.*) buds (44 kDa)[48], edible mushroom (*Pleurotus eryngii*) (43 kDa)[25], horseradish (*Armoracia rusticana*) (37 kDa)[49]and olive (*Olea europaeaL.*) (20 kDa)[50].

Some Biochemical Properties

Optimum pH

The effect of pH on the activity of red beet (*Beta vulgaris*) peroxidase was studied by caring out the activity measurement of different pH in the range of 5.0–8.0. The red beet (*Beta vulgaris*) peroxidase

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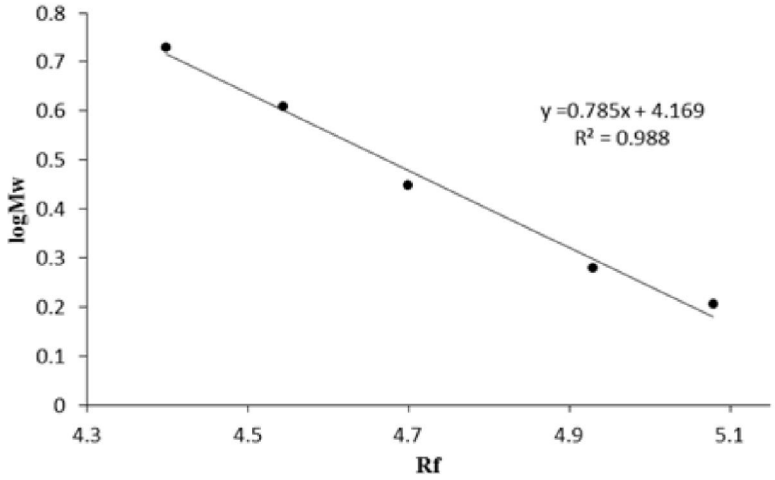

Figure 1b : Standard Log MW-Rf graph of peroxidase purified from red beet (*Beta vulgaris*) using SDS-PAGE.

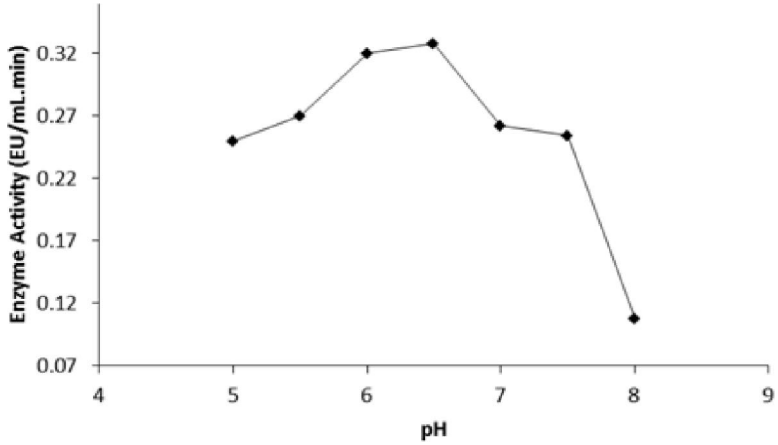

Figure 2 : The effect of pH on peroxidase activity

was exhibited a maximum at pH 6.5 in0.1 M KH_2PO_4 buffer solution and decreased slightly at lower and higher pHs values (Figure 2 and TABLE 2).

But more than 75% of activity is present in the pH range between 5.0 and 8.0. Similar optimum pH was reported for strawberry[51], Turkish black radish (*Raphanus sativus L.*), turnip (*Brassica rapa L.*)[4], and white cabbage (*Brassica oleracea var. capitata f. alba*)[46]. It is known that the active site of peroxidase is composed of ionic groups and the detachment and/or binding of heme prosthetic group to active site is pH dependent. In particular, at lower pHs, the heme group releases from the active site of enzyme[52]. And also at higher pHs, the activity of the enzyme is lost through chemical changes in heme prosthetic group[53]. The optimum pH values of purified peroxidases from different plants are mostly in the range between 4.0 and 7.5. The optimum pH for red pepper[54] is 4.0, for rice[55]*ricinus communis*[21], and cauliflower buds[48] it is 5.0, for soybean[56], Tomato[57], cabbage leaves (*Brasicca olearacea var. capitata*)[58] and coconut[59] it is 5.5, for fresh-cut Zizania latifolia it is 6.0[34], for olive[50] and red cabbage (*Brassica oleracea var. capitata f. rubra*)[5] it is 7.0. Although purified enzyme exhibited highest activity and stability at pH 6.5, it retained approximately 50% of the activity for 12 h.

pH stability

The pH stability of red beet (*Beta vulgaris*) peroxidase was also examined and stabile pH was found to be pH 6.5 (Figure 3 and TABLE 2). Figure 3 shows the pH stability profile for the purified peroxidase enzyme.

Using a KH_2PO_4 buffer solution at optimum pH, the specific activity levels were measured. Based on these activity measurements, the peroxidase activity was the highest at 0.7 M (TABLE 2).

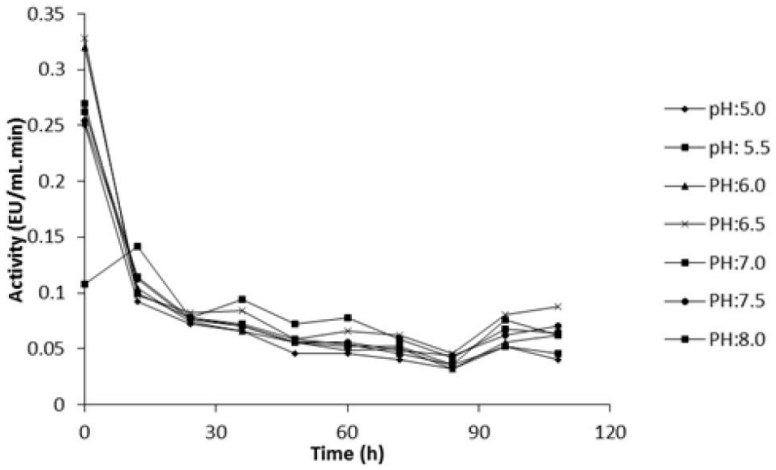

Figure 3 : Stable pH profile of peroxidase from red beet (B*eta vulgaris*)

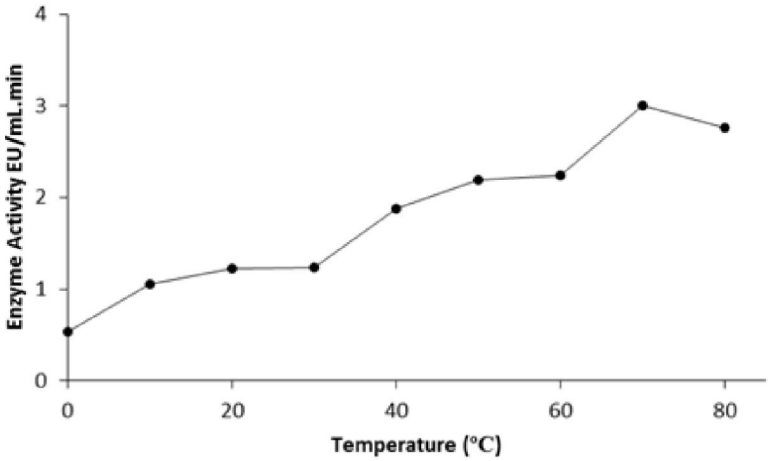

Figure 4 : The effect of temperature on red beet peroxidase activity

Optimum Temperature

Red beet (*Beta vulgaris*) peroxidase showed high optimum temperature. It is reported that peroxidase is one of the most heat stable enzyme in plants. Using guaiacol as substrate, the effect of temperature on purified red beet (*Beta vulgaris*) peroxidase enzyme activity was examined at temperatures ranging from 0 to 80°C (Figure 4).

It can be seen from Figure 4, the peroxidase enzyme activity increased with an increase in the temperature and the enzyme showed maximum enzyme activity at 70°C. And it can be also seen that the activity was slightly lost at 80°C. It is reported that the peroxidase enzyme have typically determined an optimum temperature in the range of 30 to 70°C, in previous studies. This optimum temperature value is higher than that reported for many peroxidases from different sources. The optimum temperature of peroxidase enzyme from cauliflower (*Brassica oleracea* L.) buds was reported as 25°C for pyrogallol, 30°C for guaiacol/ABTS substrates patterns, 45°C for 4-methyl catechol, and 50°C for catechol[48]. Turkish black radish (*Raphanus sativus* L.) and turnip (*Brassica rapa*L.) peroxidase showed an optimum temperature of 30°C, for guaiacol/H_2O_2 substrates[4]. Using same substrates (guaiacol/H_2O_2),

TABLE 2 : Kinetic properties of red beet (*Beta vulgaris)*

Kinetic properties	
Optimum pH	: 6.5
Stable pH	: 6.5
Optimum Temperature	: 70 °C
Optimum Ionic Strength	: 0.7 M
K_M (mM)	: 9.09
V_{max} (EU/mL.min)	: 1.38
IC_{50} (mM)	: 0.047
K_i (μM)	: 0.78±0.17
Inhibition type	: Non competitive

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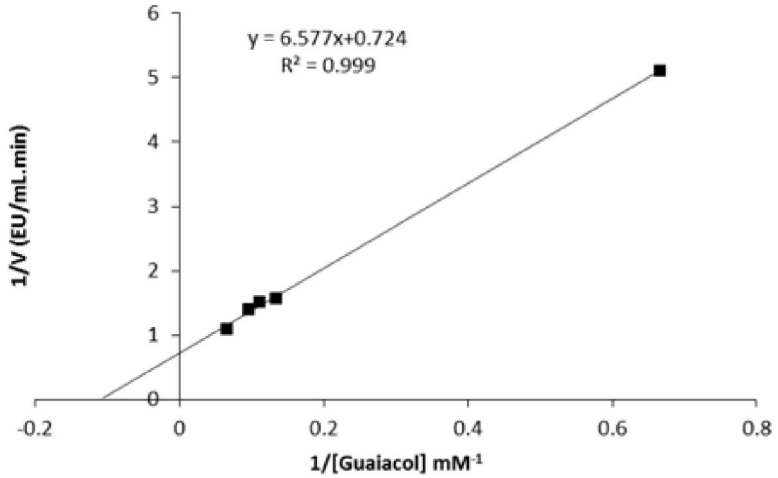

Figure 5 : Lineweaver-Burk plots for peroxidase purified from red (*Beta vulgaris*)

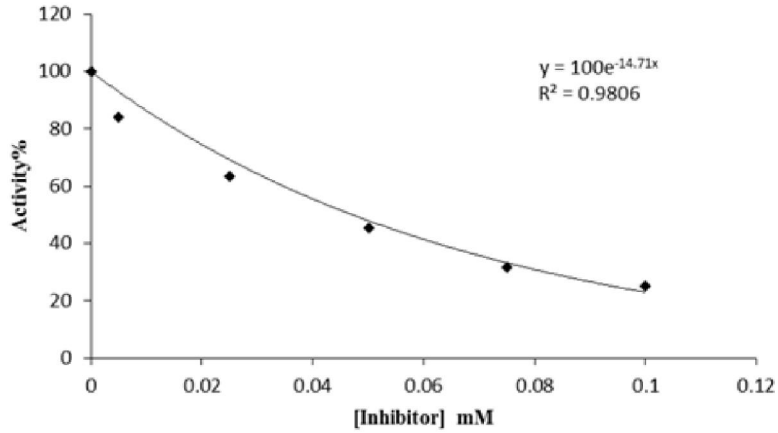

Figure 6 : The effect of 4-amino benzohydrazide on peroxidase activity

optimum temperature of fresh-cut *Zizania latifolia* peroxidase was determined to be 40°C[34]. Red cabbage (*Brassica oleracea var. capitata f. rubra*), edible mushroom (*Pleurotus eryngii*), *ricinus communis* and green asparagus peroxidases have optimum temperatures at 50, 60 and 70°C, respectively[5, 21, 25, 60].

Kinetic constants for guaiacol and H_2O_2

Michaelis constant (K_M) and maximum reaction velocity (V_{max}) values are well known characteristic values for an enzyme. K_M and V_{max} values of red beet (*Beta vulgaris*) were determined with guaiacol/hydrogen peroxide from Lineweaver-Burk graph (Figure 5).

During the experiments, the concentration of H_2O_2 was constant but guaiacol concentrations were changing, and vice versa. In this study, K_M value was found as 9.09 mM and V_{max} value was found as 1.38 EU/mL.min for red beet (*Beta vulgaris*) peroxidase. The K_M and V_{max} values for Turkish black radish (*Raphanus sativus L.*) peroxidase are 24.88 mM and 3.23 EU/mL.min, respectively[4], for red cabbage (*Brassica oleracea var. capitata f. rubra*) are 0.048 mM and 1.46 EU/mL/min[5], for white cabbage (*Brassica oleracea var. capitata f. alba*) are 3.19 mM and 0.2 EU/mL.min[46], for chard (*Beta vulgaris subspecies cicla*) leaves are 15.8 mM and 3840 EU/mL.min[35], for sweet gourd are 17.1 mM and 15.500 EU/mL.min[29], for edible mushroom (*Pleurotus eryngii*) are 0.2 mM and 188.68 U/mg[25], for cauliflower (*Brassica. oleracea L.*) buds peroxidase are 141.64 mM and 7.500 EU/mL.min[48].

Inhibition effects of 4-amino benzohydrazide

As it known, the most suitable parameters to determine the effect of a compound or an inhibitory are the IC_{50} and K_i values. The IC_{50} and K_i values were calculated to be 0.047 and 0.78±0.17 mM from the graph (Figure 6 and Figure 7), respectively for 4-

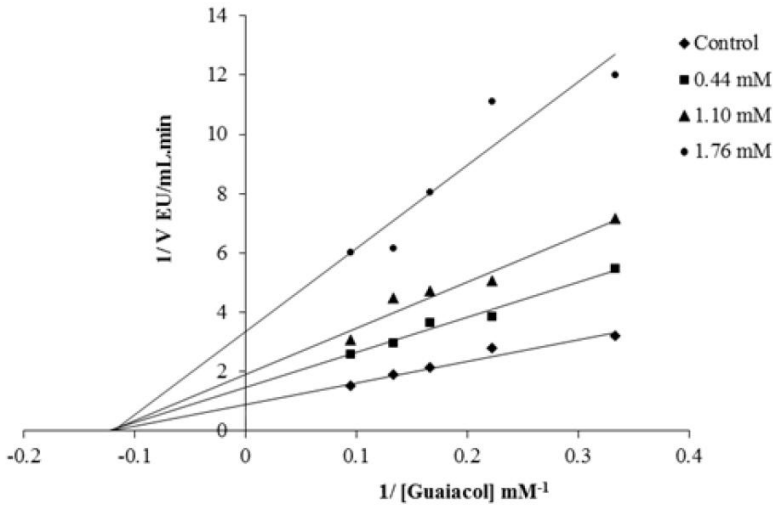

Figure 7 : Lineweaver-burk graph for determination of K_i constant

aminobenzohydrazide.

4-aminobenzohydrazide has also shown noncompetitive inhibition. Using the same ligand (4-amino benzohydrazide), the IC_{50} value was determined to be 0.726 mM for the Turkish blackradish (*Raphanus sativus L.*) peroxidase and 1.167 mM for the turnip (*Brassica rapa L.*) peroxidase. The K_i constants of the Turkish blackradish (*Raphanus sativus L.*) peroxidase and turnip (*Brassica rapa L.*) peroxidase were 0.5947 ± 0.0122 mM and 1.1033 ± 0.1016 mM, respectively[4]. In another study, for 4-amino benzohydrazide, the IC_{50} and K_i values were calculated to be 1.047 and 0.702±0.05 mM for red cabbage (*Brassica oleracea var. capitata f. rubra*) peroxidase[5]. And the IC_{50} and K_i values were calculated to be 0.517 and 0.994±0.453 mM for white cabbage (*Brassica oleracea var. capitata f. alba*) peroxidase, respectively[46]. In all these studies, 4-aminobenzohydrazide has shown noncompetitive inhibition.

CONCLUSIONS

The red beet (*Beta vulgaris*) peroxidase was purified with a high purification fold, a specific activity and a yield. Our study is the first report of peroxidase purified from native red beet (Beta vulgaris) using an affinity chromatography. Considering the previous studies, the purified red beet (*Beta vulgaris*) peroxidase has relatively good properties such as higher specific activity, pH stability, overall yield, purification factors etc. These properties make the purified peroxidase a good candidate for its possible applications in different areas.

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