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Full Length Research Paper

The effect of heavy metals on peroxidase from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers

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Peroxidases (EC 1.11.1.7; donor: hydrogen peroxide oxidoreductase, POD) are part of a large group of enzymes. In this study, peroxidase, a primer antioxidant enzyme, was partial purified with 2.49 fold and 29.3% efficiency from Jerusalem artichoke (*Helianthus tuberosus* L.) by ammonium sulphate precipitation and dialysis purification steps. The specific activity of enzyme was calculated as 612.1 EU/mg. The substrate specificity of peroxidase was investigated using 2-methoxyphenol (guaiacol)/hydrogen peroxide (H_2O_2) substrate pattern. Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) values were calculated from Lineweaver-Burk graph for this substrate pattern. The enzyme had K_m values of 0.263 and 1.143 mM for guaiacol and H_2O_2, respectively. The enzyme had V_{max} of $33.3x10^5$ and $0.213x10^5$ EU/mL.min for guaiacol and H_2O_2, respectively. Also, the *in vitro* effect of some heavy metals such as iron ($Fe^{2+}$and Fe^{3+}), cobalt (Co^{2+}), strontium $(Sr^2)^+$, zinc (Zn^{2+}), mercury (Hg^{2+}), nickel (Ni^{2+}), aluminium (Al^{2+}) and lead (Pb^{2+}) on POD from Jerusalem artichoke (*H. tuberosus*) was evaluated. These heavy metals inhibited POD acticity. IC_{50} values, represents the inhibitor concentration required for obtaining 50% of inhibition of peroxidase. The above mentioned metals had IC_{50} values of 12.58, 9.48, 12.59, 24.51, 13.57, 7.32, 10.57, 18.69 and 6.00 mM for Fe^{2+}, Fe^{3+}, Co^{2+}, Sr^{2+}, Zn^{2+}, Hg^{2+}, Ni^{2+}, Al^{2+} and Pb^{2+}, respectively.

Key words: Jerusalem artichoke, *Helianthus tuberosus*, peroxidase; enzyme, metal ions, inhibition.

INTRODUCTION

Peroxidase (POD) is a heme protein, which is a member of oxidoreductases [E.C.1.11.1.7] and catalyses the oxidation of a wide variety of organic and inorganic substrates in the presence of hydrogen peroxide (Banci, 1997; Yemenicioğlu et al., 1998; Köksal and Gülçin, 2008). Peroxidases are widely distributed in living organisms including microorganisms, plants and animals. POD and catalase are two major systems for the enzymatic removal of H_2O_2 and peroxidative damage of cell walls is controlled by the potency of antioxidative peroxidase enzyme system (Sreenvasulu et al., 1999; Velikova et al., 2000). This enzyme is one of the key enzymes controlling plant growth and development. POD is involved in various cellular processes including construction, rigidification and eventual lignifications of cell walls, protection of tissue from damage and infection by pathogenic microorganisms (Farrel et al., 1989; Sakharov et al., 2000). This enzyme participates in the formation of lignins in the secondary cell walls during normal growth (Sato et al., 1993; Pedreno et al., 1995) and in the formation of phenolic polymers such as lignins, suberins, etc when plants are infected or wounded (Dixon and Palva, 1995; Köksal and Gülçin, 2008). POD is also widely used as an important reagent for clinical diagnosis and microanalytical immunoassay. Some applications for POD have been suggested in the medicinal, chemical and food industries (Kwak et al., 1996). Peroxidase has been used for food processing as an indicator of enzyme's stability (Keleş, 1986) and biotransformation of organic molecules (Adam et al., 1999; Gülçin and Yıldırım, 2005). Because of its broader catalytic activity, a wide range of chemicals can be modified using POD. Also, it can be used for other applications such as synthesis of various aromatic compounds, removal of phenolics from waste waters and the removal of peroxides from foodstuffs, beverages and industrial wastes (Torres et al., 1997). POD is also related to quality of plant commodities, particularly the flavour, in both raw and processed foods. POD activity is also correlated to fruit ripening as has been shown in a number of cases and it is also involved in enzymatic browning, either or together with polyphenol oxidase activity. A precise understanding of the implication of

POD in these mechanisms is an essential step towards a more efficient control of these undesirable reactions, particularly in heat-processed products, which frequently contain residual peroxidase activity (Cardinali, et al., 2007; Köksal and Gülçin, 2008).

Heavy metal pollution occurs in many industrial wastewaters such as those produced by metal plating facilities, mining operations, battery manufacturing processes, the production of paints and pigments, ammunition, ceramic and glass industries. These heavy metals are not biodegradable and their presence in streams and lakes leads to bioaccumulation in living organisms, causing health problems in animals, plants, and human beings (Oliver, 1997; Argun and Dursun, 2008).

Lead is ubiquitous and can be found naturally occurring in many different foods as metallic lead, inorganic ions and salts. Lead has no essential function in man, but has a number of adverse effects and young children and the developing foetus which are considered to be at most risk from its toxic effects (WHO, 1995). Mercury has a wide variety of effects, and range in value from therapeutic agents to lethal chemicals. Exposure to mercury vapor over periods of months or years can result in chronic poisoning (Harrison, 2002).

Aluminum and its compounds are used in the paper, glass and textile industries as well as in food additives. Raised concentrations of aluminium in water used for renal dialysis can lead to toxic effects (Harrison, 2002). Zinc is an essential element for all plants and animals. It is necessary for the correct function of various enzyme systems (Festa et al., 1985). Cobalt is used in the manufacture of alloys and in nuclear technology (Delves et al., 1996). Cobalt compounds are also included in trace element supplement preparations for ruminants. The cobalt concentrations in vegetables and other foods have been found to be between < 0.01 and 0.83 mg/kg (Ministry of Agriculture, 1985; Delves et al., 1996). Nickel salts are widely used in industry for plating and as pigments. Alloys of nickel are used in storage batteries, coins, cooking utensils and other products. Fats and oils are hydrogenated using a nickel catalyst. Nickel has not been shown to be an essential nutrient for humans, but is considered to be a normal constituent of the diet (Ministry of Agriculture, 1985; Harrison, 2002). The human body absorbs strontium as if it were calcium. Due to the elements being sufficiently similar chemically, the stable forms of strontium do not pose a significant health threat, but the radioactive strontium can lead to various bone disorders and diseases, including bone cancer (Reginster et al., 2005).

The ferrous state of iron (Fe^{2+}) accelerates lipid oxidation by breaking down hydrogen and lipid peroxides to reactive free radicals via the Fenton reaction. Fe^{2+} ion is the most powerful prooxidant among the various species of metal ions (Gülçin, 2006a). However, ferric (Fe^{3+}) ions also produce radicals from peroxides although the rate is tenfold less than that of ferrous (Fe^{2+}) ions (Gülçin, 2006b).

Living organisms require trace amounts of some heavy metals, including iron, cobalt, copper, manganese, molybdenum, vanadium, strontium, and zinc, but excessive levels can be detrimental to the organism. Other heavy metals such as mercury, lead and cadmium with the exception for the latter are toxic metals. They have no known vital or beneficial effect on organisms, and their accumulation over time in the bodies of mammals can cause serious illness (Duffus, 2002).

Jerusalem artichoke (*Helianthus tuberosus* L.) is native to North America and is presently cultivated in Europe, Asia and Australia. In Turkey, Jerusalem artichoke (*H. tuberosus*) is cultivated in all the regions. The tubers accumulate high levels of polysaccharides during their growth. On a dry weight basis, the tubers contain 68 - 83% fructans, 15 - 16% proteins, 13% insoluble fibre and 5% ash (Fleming and GrootWassink, 1979). Remarkably, its tubers do not contain starch. It has traditionally been used as food and animal feed and, more recently, as a raw material for the industrial production of fructose and fructans (Kosaric et al., 1984). In this study the *in vitro* effect of some heavy metals such as Fe^{2+}, Fe^{3+}, Co^{2+}, Sr^{2+}, Zn^{2+}, Hg^{2+}, Ni^{2+}, Al^{2+} and Pb^{2+} on POD from Jerusalem artichoke (*H. tuberosus*) tubers was invesigated.

MATERIALS AND METHODS

Plant materials

Jerusalem artichoke (*H. tuberosus*) was obtained from a local market in Erzurum, Turkey. It was washed, drained, packed in polyethylene bags and stored at -83°C until required.

Preparation of Jerusalem artichoke (*H. tuberosus*) homogenate

The homogenate preparation procedures for POD were adapted from the methods described by Sakharov et al. (2002). For this purpose, 50 g Jerusalem artichoke (*H. tuberosus*) tubers were taken from frozen storage (-83°C) and ground in a mortar in the presence of liquid nitrogen. This powder then mixed with 150 mL of phosphate buffer (pH 7.0, 0.1 M) and subsequently the collard slurry was centrifuged at 13.000 x g for 30 min at 4°C (Gülçin, 2002; Gülçin et al., 2005). The pellet was discarded (Gülçin et al., 2005).

Ammonium sulphate precipitation and dialysis

The crude extract was subjected to ammonium sulphate fractionation and the precipitate in the 20 - 70% saturation range was collected by centrifugation (30 min at 13000 x g). The precipitate was suspended in about 2 mL phosphate buffers (pH 7.0, 0.1 M) and dialyzed for 12 h at 4°C against 1 L of the buffer (Köksal and Gülçin, 2008).

Peroxidase activity assay

The POD activity in the Jerusalem artichoke (*H. tuberosus*) sample

Table 1. Levels of purification of POD from Jerusalem artichoke (*H. tuberosus*) peroxidase obtained after the application of different purification steps leading to the improvement in the activity of enzyme.

Purification step	Total volume (mL)	Enzyme activity (EU/mL.min)	Total enzyme activity (EU/mL.min)	Protein (mg/mL)	Total Protein (mg)	Specific activity (EU/mg)	Yield (%)	Purification fold
Homogenate	20	26840	536800	0.109	2.180	246.2	100	-
$(NH_4)_2SO_4$ precipitation	8	37480	299840	0.095	0.760	394.5	34.9	1.60
Dialysis	11	35500	390500	0.058	0.638	612.1	29.3	2.49

was measured using guaiacol substrate. Initial rates of free radical formation for substrate were monitored at maximum wavelength of 470 nm. The changes in absorbance were read for 3 min using a double beam UV-VIS spectrophotometer (CHEBIOS s.r.l.). Enzyme activity assay method was as described previously (Yoo and Kim, 1988; Gülçin et al., 2005). Briefly, an aliquot of enzyme sample (25 µL) was added to a mixture of 1 mL 22.5 mM H_2O_2, 1 mL 45 mM guaiacol, and final volume of this mixture was adjusted to 3 mL by addition of phosphate buffer (pH 7.0, 0.1 M). The change in the absorbance at above wavelength monitored for 3 min at 20°C. One unit of peroxidase activity was defined as 0.01 ΔA_{470} per min (Halpin et al., 1989; Gülçin et al., 2005).

Quantitative protein determination

Quantitative protein determination was achieved by absorbance measurements at 595 nm according to Bradford's method (1976), with bovine serum albumin as standard (Gülçin et al., 2004).

RESULTS AND DISCUSSION

Extraction of POD was carried out in 0.1 M phosphate buffer (pH 7.0). Then the enzyme was precipitated with $(NH_4)_2SO_4$. Several precipitations with solid $(NH_4)_2SO_4$ between 0 - 10%, 10 - 20%, 20 - 30%, 30 - 40%, 40 - 50%, 50 - 60%, 60 - 70% and 70 - 80% were conducted to find the optimum saturation point. POD activity of the precipitate from 20 - 70% $(NH_4)_2SO_4$ was found to have the highest activity and this saturation point was used in all the extraction processes. Following ammonium sulfate precipitation, the enzyme extract was dialyzed. The degree of purification of POD was 2.49-fold after dialysis (Table 1).

POD activity was assayed using guaiacol/H_2O_2 substrate pattern. The rate of the reaction was measured in terms of the increase in absorbance. To determine the Michaelis-Menten constant (K_m) and maximum velocity (V_{max}), POD activities were measured with variable substrate concentrations in the standard reaction mixture. The K_m and V_{max} of POD for each substrate was calculated from a plot of 1/V and 1/[S] by the method of Lineweaver and Burk (1934). As can be seen in Figure 1, the enzyme had K_m values of 0.263 and 1.143 mM for guaiacol and H_2O_2, respectively. Also, the enzyme had V_{max} of 33.3×10^5 and 0.213×10^5 EU/mL.min for guaiacol and H_2O_2, respectively.

The K_m value for guaiacol is lower than that of H_2O_2, suggesting the higher affinity of POD for guaiacol when compared with H_2O_2. K_m value was found as 141.64 mM for cauliflower peroxidase by using guaiacol substrate

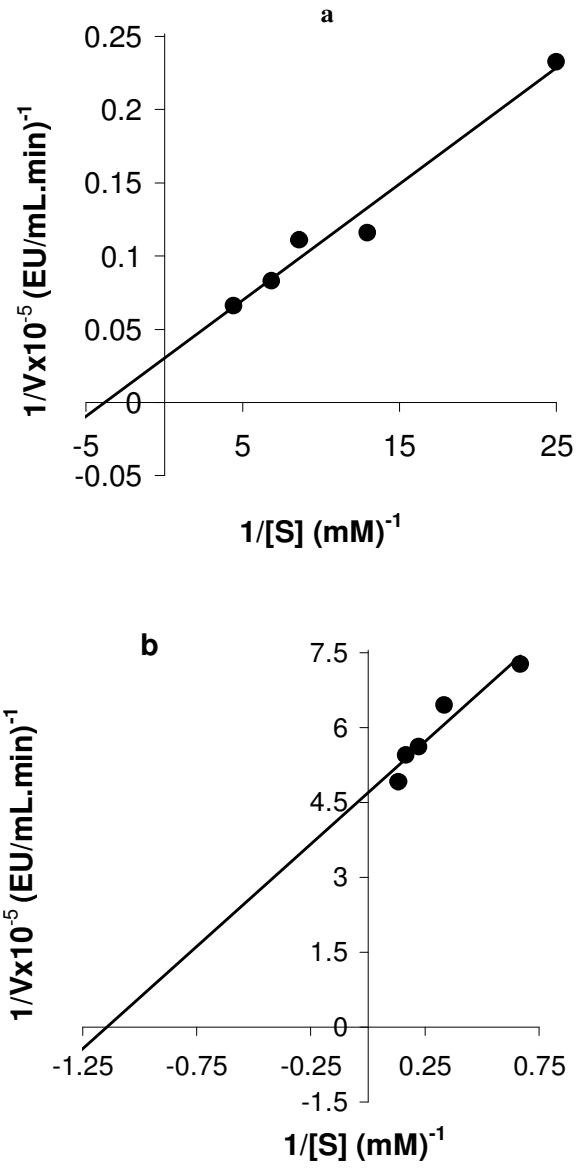

Figure 1. The Lineweaver-Burk plots for 2-methoxyphenol (guaiacol) (a) and hydrogen peroxide (H_2O_2) (b) substrates.

Table 2. IC_{50} values of different heavy metals on peroxidase from Jerusalem artichoke (*H. tuberosus*)

Heavy metals	EC_{50} values (mM)
Fe2+	12.58
Fe^{3+}	9.48
Co^{2+}	12.59
Sr^{2+}	24.51
Pb^{2+}	6.00
Hg^{2+}	7.32
Ni^{2+}	10.57
Al^{2+}	18.69
Zn^{2+}	13.57

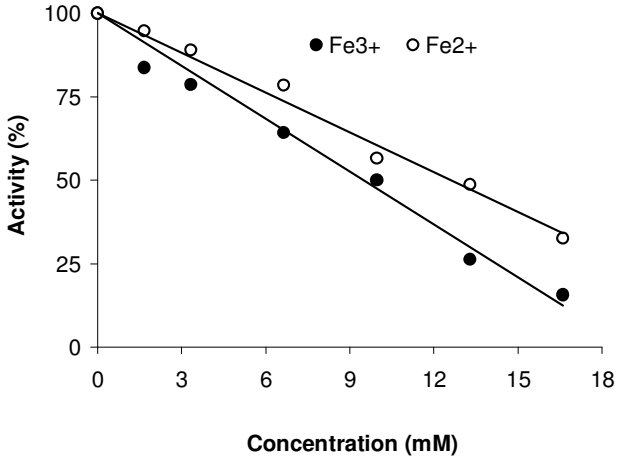

Figure 2. The effect of ferrous (Fe^{2+}) and ferric ions (Fe^{3+}) on POD from Jerusalem artichoke (*H. tuberosus*) tubers.

(Köksal and Gülçin, 2008). In another study, this value was found as 5.5 mM for *Brassica oleracea* L. *var.* Acephala peroxidase by using guaiacol substrate (Gülçin and Yıldırım, 2005). They were higher than that of Jerusalem artichoke (*H. tuberosus* L.) tubers K_m. The lower K_m value indicates higher substrate affinity. The results obtained from present study showed that Jerusalem artichoke tuber POD has greater substrate affinity for guaiacol than above mentioned plants. Likewise, Jerusalem artichoke (*H. tuberosus* L.) tubers POD had higher V_{max} values than the enzyme from both plants. When the guaiacol used as substrate, *B. oleracea* L. *var.* Acephala and cauliflower demonstrated V_{max} values as 5000 and 7500 EU/mL.min, respectively. However, Jerusalem artichoke (*H. tuberosus* L.) tubers POD had V_{max} value as $33.3x10^5$ EU/mL.min using the same substrate.

Inhibition effects of many substances such as medical drugs, various metals, anions and pesticides have been reported in the literature (Casini et al., 2002; Beydemir and Gülçin, 2004; Ekinci et al., 2007). Many chemicals

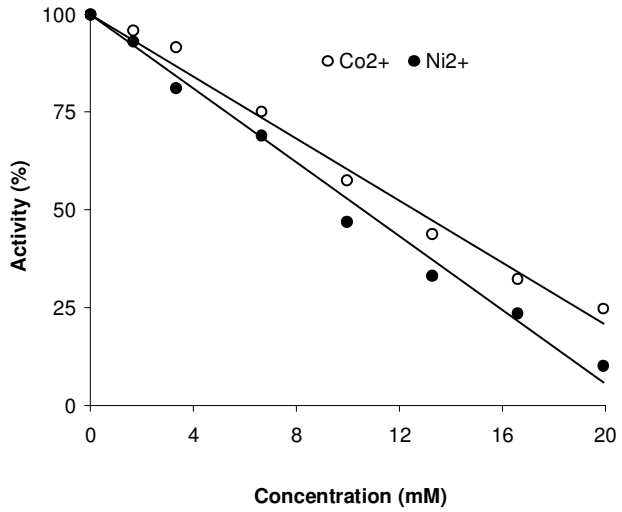

Figure 3. The effect of cobalt (Co^{2+}) and nickel ions (Ni^{2+}) on POD from Jerusalem artichoke (*H. tuberosus*) tubers.

affect metabolism by changing normal enzyme activity, particularly inhibition of a specific enzyme and the effects can be dramatic and systemic (Christensen et al., 1982). Hence, heavy metals have various toxicological effects on living organisms. For example, it has been reported that heavy metals, such as mercury and cadmium, exert a toxic action in a synergistic fashion with salinity (Christensen et al., 1982; Vitale et al., 1999). Also, it has been expressed that the binding of heavy metals with membrane transport ligands can alter their catalytic function (Rainbow and Dallinger, 1993; Vitale et al., 1999).

In the present study, Fe (II) and (III), Co (II), Sr (II), Zn (II), Hg (II), Ni (II), Al (II) and Pb (II) were chosen for investigation of their inhibitory effects on POD from Jerusalem artichoke (*H. tuberosus*). IC_{50} parameters of these metals for peroxidase from Jerusalem artichoke (*H. tuberosus*) were determined. It was found that the heavy metals were potent inhibitors of peroxidase from Jerusalem artichoke (*H. tuberosus*). As can be seen in Table 2 and Figures 2 - 5, IC_{50}values of 12.58 and 9.48 mM for Fe^{2+} and Fe^{3+} (Figure 2), 12.59 and 10.57 mM for Co^{2+} and Ni^{2+} (Figure 3), 18.69, 24.51 and 13.57 mM for Al^{2+}, $Sr^{2+}$and Zn^{2+} (Figure 4), 7.32 and 6.00 mM for Hg^{2+} and Pb^{2+} (Figure 5). Pb^{2+} was found to be an effective inhibitor for POD from Jerusalem artichoke (*H. tuberosus*) in used heavy metal examples. Also, Sr^{2+} exhibited the lowest inhibitory effect on POD from Jerusalem artichoke (*H. tuberosus*).

In conclusion, living organisms require trace amounts of some heavy metals, including iron, cobalt, copper, manganese, molybdenum, vanadium, strontium, and zinc, but excessive levels can be detrimental to the organism, as they can inhibit a large number of enzymes.

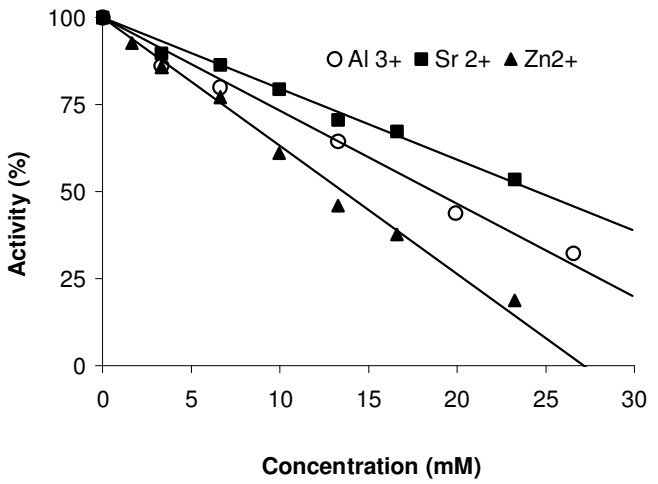

Figure 4. The effect of aluminium (Al^{2+}), strontium ions (Sr^{2+}) and zinc (Zn^{2+}) on POD from Jerusalem artichoke (*H. tuberosus*) tubers.

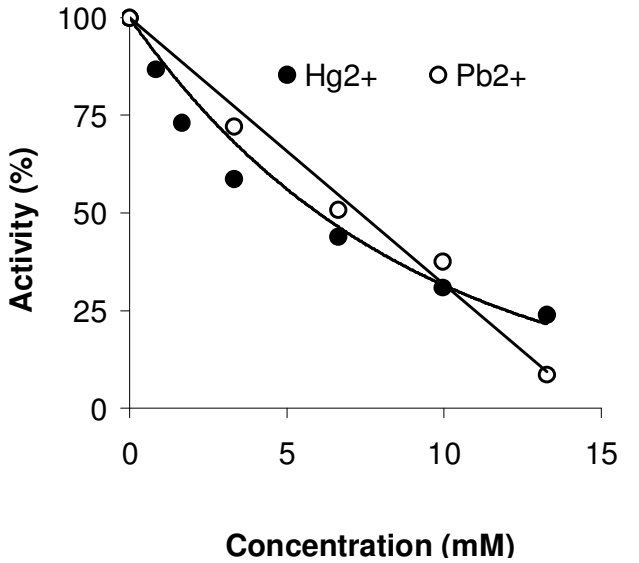

Figure 5. The effect of mercury (Hg^{2+}) and lead (Pb^{2+}) on POD from Jerusalem artichoke (*H. tuberosus*) tubers.

These heavy metals inhibited POD from Jerusalem artichoke (*H. tuberosus*).

REFERENCES

Adam W, Lazarus M, Saha-Moler CR, Weichold O, Hoch U, Scherier P (1999). Biotransformations with peroxidases. Adv. Biochem. Eng. 63: 74-108.

Argun ME, Dursun S (2008). A new approach to modification of natural adsorbent for heavy metal adsorption. Bioresour. Technol. 99: 2516-2527.

Banci L (1997). Structrual properties of peroxidases. J. Biotechnol. 53: 253-263.

Beydemir S, Gülçin İ (2004). Effects of melatonin on carbonic anhydrase from human erythrocytes *in vitro* and from rat erythrocytes *in vivo*. J. Enzym. Inhib. Med. Chem. 19: 193-197.

Bradford MM (1976). A rapid and sensitive method for the quantition of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-251.

Cardinali A, Sergio L, Di Venere D, Linsalata V, Fortunato D, Conti A, Lattanzio V (2007). Purification and characterization of a cationic peroxidase from artichoke leaves. J. Sci. Food Agric. 87: 1417-1423.

Casini A, Scozzafava A, Mincione F, Menabuoni L, Supuran CT (2002). Carbonic anhydrase inhibitors: Synthesis of water soluble sulfonamides incorporating a 4-sulfamoylphenylmethylthiourea scaffold, with potent intraocular pressure lowering properties. J. Enzym. Inhib. Med. Chem. 17: 333-343.

Christensen GM, Olson D, Riedel B (1982) Chemical effects on the activity of eight enzymes: A review and a discussion relevant to environmental monitoring. Environ. Res. 29: 247-255.

Delves HT, Diaper SJ, Oppert S, Prescott-Clarke P, Periam J, Dong W, Colhoun H, Gompertz D (1996). Blood levels in the United Kingdom have fallen substantially since 1984. Br. Med. J. 313: 883-884.

Dixon RA, Palva NL (1995). Stress-induced phenylpropanoid metabolism. Plant Cell. 7: 1085-1097.

Duffus JH (2002). Heavy Metals-A meaningless term? Pure Appl. Chem. 74: 793-807.

Ekinci D, Beydemir S, Kufrevioglu OI (2007). *In vitro* inhibitory effects of some heavy metals on human erythrocyte carbonic anhydrases. J. Enzym. Inhib. Med. Ch. 22: 745-750.

Farrel RL, Murtagh KE, Tien M, Mozuch MD, Kirk TK (1989). Physical and enzymatic properties of lignin peroxidase isoenzymes from *Phanerochaete chrysosporium*. Enzym. Microbiol. Technol. 11: 322-328.

Festa MD, Anderson HL, Dowdy RP, Ellersick MR (1985). Effect of zinc on copper excretion and retention in men. Am. J. Clin. Nutr. 41: 285-292.

Fleming SE, GrootWassink JWD (1979). Preparation of highfructose syrup from the tubers of the Jerusalem artichoke. CRC Crit. Rev. Food Sci. Nutr. 12: 1-28.

Gülçin I, Kufrevioglu OI, Oktay M (2005). Purification and characterization of polyphenol oxidase from nettle (*Urtica dioica* L.) and inhibitory effects of some chemicals on enzyme activity. J. Enzym. Inhib. Med. Chem. 20: 297-302.

Gülçin İ (2002). Determination of antioxidant activity, characterization of oxidative enzymes and investigation of some in vivo properties of nettle (*Urtica dioica*). PhD Thesis, Atatürk University, Erzurum, Turkey. pp. 45-48.

Gülçin İ, Yıldırım A (2005). Purification and characterization of peroxidase from *Brassica oleracea* var. Acephala. Asian J. Chem. 17: 2175-2183.

Gülçin İ (2006b). Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid). Toxicology, 217 (2-3): 213-220.

Gülçin İ (2006a). Antioxidant and antiradical activities of L-Carnitine. Life Sci. 78(8): 803-811.

Gülçin İ, Beydemir Ş, Büyükokuroğlu ME (2004). In vitro and in vivo effects of dantrolene on carbonic anhydrase enzyme activities, Biol. Pharm. Bull. 27: 613-616.

Halpin B, Pressey R, Jen J, Mondy N (1989). Purification and characterization of peroxidase isoenzymes from green peas (*Pisum sativum*). Food Sci. 54: 644-659.

Harrison N (2002). Inorganic contaminants in food. Food Standards Agency, in Contaminants in Food London.

Keleş F (1986). Gıda işlemede peroxidazlar (In Turkish). Gıda, 11: 117-123.

Kwak SS, Kim SK, Park IH, Liu JR (1996). Enhancement of peroxidase activity by stress-related chemicals in sweet potato. Phytochemistry, 43: 565-568.

Kosaric N, Cosentino GP, Wieczorek A, Duvnjak Z (1984). The Jerusalem artichoke as an agricultural crop. Biomass, 5: 1-36.

Köksal E, Gülçin İ (2008). Purification and characterization of peroxidase from cauliflower (*Brassica oleracea* L.) buds. Protein Peptide Lett. 15(4): 320-326.

Lineweaver H, Burk D (1934). The determination of enzyme dissociation constants. J. Am. Chem. Soc. 56: 658-661.
Ministry of Agriculture (1985). Fisheries and Food, 'Survey of aluminium, antimony, chromium, cobalt, indium, nickel, thallium and tin in food', Food Surveillance Paper No. 15, London, The Stationery Office,
Oliver MA (1997). Soil and human health: a review. Eur. J. Soil Sci. 48: 573-592.
Pedreno MA, Ferrer MA, Gaspar T, Munoz R, Barcelo A (1995). The polyfunctionality of cell wall peroxidases avoids the necessity of an independent H_2O_2-generating system for phenolic coupling in the cell walls. Plant. Peroxidases Newsl. 5: 3-8.
Rainbow PS, Dallinger R (1993). Metal uptake, regulation and excretion in freshwater invertebrates. In: Dallinger R, Rainbow PS, editors. Ecotoxicology of metals in invertebrates. Boca Raton, FL: Lewis Publishers, 119-131.
Reginster JY, Seeman E, De Vernejoul MC, Adami S, Compston J, Phenekos C, Devogelaer JP, Diaz Curiel M, Sawicki A, Goemaere S, Sorensen OH, Felsenberg D, Meunier PJ (2005). Strontium ranelate reduces the risk of nonvertebral fractures in postmenopausal women with osteoporosis: treatment of peripheral osteoporosis (TROPOS) study. J. Clin. Metab. 90: 2816-2822.
Sakharov IY, Blanco MKV, Sakharova IV (2002). Substrate specificity of African oil palm tree peroxidase. Biochemistry (Moscow) 67: 1043-1047.
Sakharov IY, Castillo JL, Areza JC, Galaev IY (2000). Purification and stability of peroxidase of African oil palm *Elaies guineensis*. Bioseperation, 9: 125-132.
Sato Y, Sugiyama M, Gorecki RJ, Fukuda H, Komamine A (1993). Interrelationship between lignin deposition and the activities of peroxidase isoenzymes in differentiating tracheary elements of Zinnia. Planta, 189: 584-589.
Sreenvasulu N, Ramanjulu S, Ramachandra-Kini K, Prakash HS, Shekar-Shetty H, Savithri HS, Sudhakar C (1999). Total peroxidase activity and peroxidase isoforms as modified by salt stress in two cultivars of fox-tail millet with differential salt tolerance. Plant Sci. 141: 1-9.
Torres F, Tinoco R, Vazquez-Duhalt R (1997). Bio-catalytic oxidation of polycyclic aromatic hydrocarbons in media containing organic solvents. Wat. Sci. Technol. 36: 37-44.
Velikova V, Yardanov I, Edreva A (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. Plant Sci., 151: 59-66.
Vitale AM, Monserrat JM, Castilho P, Rodriguez EM (1999). Inhibitory effects of cadmium on carbonic anhydrase activity and ionic regulation of the estuarine crab Chasmagnathus granulata (Decapoda, Grapsidae). Comp. Biochem. Phys. C:122: 121-129.
World Health Organization (WHO) (1995) Inorganic lead, Environmental Health Criteria 165, Geneva, World Health Organization.
Yemenicioğlu A, Özkan M, Cemeroğlu B (1998). Partial purification and thermal characterization of peroxidase from okra (*Hibiscus esculentum*). J. Agr. Food Chem., 46: 4158-4163.
Yoo WI, Kim SS (1988). Purification and characterization of an anionic isoperoxidase from Korean-radish root. Korean Biochem. J. 21: 207-213.