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Studies on the Activity of Peroxidase in the Crude Extracts of Sweet Potato (*Ipomea batatus*) Cauliflower (*Brassica oleracea*), Soyabeen (*Glycine max*) and Sourlime (*Citrus amantifolia*)

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Abstract

Peroxidases were screened for specific enzyme activity in the extracts from sweet potato, cauliflower, soybean and sourlime, using acidic phosphate buffered saline (pH 6.5). The peroxidases activities were recorded using guaiacol and H₂O₂ by spectrophotometric absorbance. Enzyme concentrations were optimized as 0.30 mL, (1:5), 0.20 (1:15), 0.10(1:15), 0.20 (1:10) for sweet potato, cauliflower, soybean and sourlime peroxidases respectively, The maximum activities of sweet potato (0.080000), cauliflower (0.890DU), soybean (0.0710DU) and sourlime (0.0890DU) peroxidase were observed at the pH of 6.5, 6.5, 5.5 and 5.0 respectively. The soybean peroxidases was found more thermostable and against an elevation of 1°C in temperature, the drop in sweet potato, cauliflower, soyabeen and sourlime peroxidase activity was of the order of 0.006, 0.007, 0.004 and 0.010, respectively. The total protein values for sweet potato, cauliflower, soybean and sourlime extracts were found to be 2.38, 2.050, 1.9 and 10.8 percent respectively.

Key words: Peroxidase, *Ipomea batatus* (sweet potato), *Brassica Oleracea* (cauliflower), *Glycine max* (soybean), *Citrus amantifolia* (sourlime), Guaiacol, H₂O₂, crude suspension, spectroscopy.

Introduction

Peroxidase (Ubiquitous oxidative metabolizing heamoprotein (E. C. 1.11.1.7) are among the most extensively studied enzyme with a long history of mechanistic and spectroscopic investigations, catalyze the oxidation of number fo organic substances by using the oxygen of hydrogen peroxide (Krell, 1991).

Peroxidases are widely distributed as an auxiliary enzyme in plants, animals, fungi, bacteria (Loew and Dupuis, 1996), microbes, spermatozoa and mammalian species (Harris and Loew, 1996). Peroxidase have been implicated in numerous physiological processes in plants, including lignification biosynthesis (Fry, 1986), degradation of indole 3-acetic acid, in biosynthesis of ethylene (Calderon *et al.*, 1992) in regulation of plant growth (Campa, 1991), in respiration, in stress response, in defense reaction against pathogen and in wound healing (Asada 1992) in structural protein (Brownleader and Dey, 1993) and formation of cutin (Biles *et al.* 1993).

Peroxidase has a wide range of application in health science, food industry and in diagnostic purposes. (Kwak *et al.* 1995. POD, being the most heat stable enzyme in the plant is used as an index of blanching procedures in food industry (Chuang and Chen, 1988). Pod labelled antispecies globulins have a tremendous application in enzyme linked immunosorbent assays (ELISA) world wide while pod labelled antibodies are employed in western blot assay too (Davidson and Sittman, 1994). The commercial horse adish peroxidase available in Pakistan is imported from abroad at considerably higher prices, where as in fact the enzyme can be isolated from human rich soil (Bollag *et al.* 1987) in Pakistan, colossal amounts of the decaying plant material in soil at the premissis of fruit & vegetable markets have no other utility but to exacerbate environmental pollution of the urban establishments. The ingernius use of such debris and rummage for isolating peroxidase would

turn out as a sustainable and incredibly cheaper source of a much useful enzyme. This may as well go a long way to retrench unabated soil pollution. Having such replenishment established, the manufacturing costs of ELISA diagnostic kits may also be cut down to cost effective ranges.

Materials and Methods

Crude enzyme extracts: The vegetables were procured from the local vegetable market and washed thoroughly in distilled water. After through cleansing, the vegetables were sliced into small bits. One hundred grams of chopped pieces were added to 400 mL of distilled water and thoroughly homogenized for 15 to 20 minutes. The contents were then shifted to centrifuge tubes and spun at 3000 r.p.m for 15 minutes. The supernatant was removed carefully from the sediment, cellular debris and filtered.

The crude enzyme extract was heated in small flask at 65°C for 3 minutes in a water bath (preset) and than cooled promptly by placing in ice box to selectively inactivate the contaminating traces of the catalase present in the extracts. After the accomplishment of thermal inactivation, the final extract was stored under refrigeration condition at 4°C till use.

Buffered substrate solution: Buffer solution was prepared by using guaiacol (1ml) Phosphate buffer having pH 6.5 (46.68 mL) and H₂O₂ 35 percent (0.32 mL). The contents were thoroughly mixed together by placing on a vortex agitator. For standardization blank solution was also prepared.

Different dilution of crude enzyme extract (1:5, 1:10, 1:15, 1:20, 1:25 and 1:40) were prepared to check the enzyme activity in different species.

Enzyme Assay: Above mentioned dilution of different

species were checked in order to obtain the maximum enzyme activity by using spectrophotometric method at 470 nm upto a reaction period of 5 minutes. Enzyme activity was also checked by using variable volume (0.02, 0.04, 0.06, 0.08, 0.10, 0.20, 0.30, 0.40) of enzyme extracts and at varying time intervals (20 to 280 sec). Further investigations were made to check the maximum enzyme activity at varying pH (by using citrate phosphate buffer) having a varying range of 3.0, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 8.0, 9.0, 10.0 and at temperature 40, 56, 64, 72, 80, 88, 90°C respectively.

The total protein in crude enzyme extracts was determined by Biuret method. The tube containing standard Biuret reagent and BSA were incubated at 37°C for 15 minutes. The tubes were cooled and optical densities were recorded at 540 nm (Gornall *et al.* 1949).

Results

The study of optical densities showed that 1:5, 1:15, 1:15 and 1:10 dilutions were selected for sweet potato, cauliflower soybean and sourlime peroxidase respectively for subsequent studies on peroxidatic activity. The result showed that the maximum enzyme activity was shown by soybean peroxidase (Table 1). The following concentration 0.20, 0.30, 0.20 and 0.10 ml of sweet potato, cauliflower, soybean and sourlime extracts yielded the maximum absorbance indicating optimal enzyme activity as 1.400, 1.280, 0.542, 0.503 respectively (Table 2). Concerning reaction time the enzyme activity consistently increased (0.075-1.99 of sweet potato, 0.054-1.600 of cauliflower, 0.055-0.910 of soybean and 0.151-0.580 of sourlime) with in the time interval of 20 to 280 sec (Table 3). Regarding effect of pH on peroxidatic function, maximum activities of sweet potato, cauliflower, soybean and sourlime was found to be 0.80, 0.089, 0.071 and 70.065 respectively at pH 6.5, 6.5, 5.5 and 5.0 (Table 4). The effect of thermal treatment showed that against an elevation of 1°C temperature, the drop in sweet potato, cauliflower, soybean and sourlime peroxidase activity was of the order of 0.006, 0.007, 0.004 and 0.010 respectively. These results showed that soybean peroxidase was the most thermostable enzyme among the others. The corresponding total protein values wer recorded as 2.38, 2.05, 1.9 and 10.8 percent for sweet potato, cauliflower, soybean and sourlime.

Discussion

During the measurement of enzyme activity in various dilution vs. time interval it was found that as the dilution factor increases the enzyme activity decreases gradually due to decrease in enzyme concentration. In case of measurement of enzyme activity a varying concentration of peroxidase there was found a general trend of increase in peroxidase activity with the increase in enzyme concentration (increasing volume).

During the measurement of enzyme activity at various time interval a constant trend of increase in the enzyme activity with the increase of reaction period was found. The amount of peroxidase activity in plants varies in relation with anatomical location, age of tissue/plant, state of being fresh or stored. During the present investigation the peroxidase was found to be greater in stem of cauliflower. This result is in accordance with the findings of Rosoff and Cures (1949) and Vamos-Vigyazo *et al.* (1980) who also reported greater peroxidase activity in stem of cauliflower. In case of sweet potato maximum enzyme activity was found in the cells of sweet potato, these results is in concordance with the investigation of Kim and Kim (1996). In case of soybean the greatest enzyme activity was found to be present in the husk of soybean as reported by Griffing and Fowke (1985) that soybean peroxidase is found primarily in the cell wall and at the tonoplast of the cell while in case of sourlime, peroxidase activity was found to be greatest in the peel and completely absent in the juice of sour lime due to the extreme acidic nature of the juice. These results are in accordance with the work of Willimott and Wokes (1926) and Cabrera and Lima (1982) who studied the peroxidase activity in different parts of citrus fruit and observed that peroxidase activity is greatest in the peel and in the internal layers of peel.

While studying the effect of pH on the enzyme activity it was concluded that the enzyme activity increased gradually with an increase in pH and at certain pH, the enzyme showed it maximum activity and then activity decreased again with increasing pH and ultimately at a very high pH it became inactive (Reed., 1975). In concern with the probable effect of pH on the enzyme activity it was derected that beyond the range of 6.0 to 7.0 the peroxidase activity varied drastically. These results are in accordance with the finding of Soda *et al.* (1991). It may be deciphered by stating that the acidification causes a change

Table 1: Average rates of enzyme activity (absorbance per min) for various dilutions of crude sweet potato cauliflower, soybean and sourlime extracts.

Dilutions	Rate of sweet potato pod activity	Rate of cauliflower pod activity	Rate of soybean pod activity	Rate of sourlime pod activity
1:5	0.3330	1.5080	1.3726	0.4401
1:10	0.0226	0.9066	0.6182	0.4778
1:15	0.0284	0.3704	0.2664	0.3028
1:20	0.0242	0.2392	0.0970	0.1644
1:25	0.0172	0.1366	0.0520	0.1348
1:30	0.0154	0.1194	0.0450	0.0712
1:40	0.0128	0.0524	0.0199	0.0600

Table 2: Absorbance values of optical densities on varying concentration.

Volume	Optical densities			
	Sweet potato	Cauliflower	Soybean	Sourlime
0.02	0.063	0.161	0.031	0.081
0.04	0.139	0.070	0.069	0.185
0.06	0.201	0.120	0.089	0.285
0.08	0.282	0.150	0.159	0.420
0.10	0.321	0.324	0.291	0.503
0.20	1.400	0.601	0.542	1.850
0.30	1.999	1.280	1.999	1.999
0.40	1.999	1.820	1.999	1.999

Table 3: Absorbance values of the selected volume of crude sweet potato, cauliflower, soybean and sourlime extracts at various time intervals.

Time (sec)	Sweet potato	Cauliflower	Soybean	Sourlime
20	0.075	0.054	0.055	0.151
40	0.175	0.139	0.120	0.210
60	0.318	0.230	0.201	0.322
80	0.461	0.356	0.301	0.360
100	0.620	0.501	0.349	0.382
120	0.810	0.652	0.398	0.430
140	1.010	0.780	0.448	0.441
160	1.200	0.110	0.449	0.490
180	1.410	1.270	0.543	0.503
200	1.620	1.310	0.624	0.510
220	1.700	1.340	0.680	0.521
240	1.850	1.380	0.781	0.525
260	1.990	1.490	0.790	0.541
280	1.990	1.600	0.910	0.580

Table 4: Effect of pH peroxidase activity of crude sweet potato, cauliflower, soybean and sourlime extracts.

pH	Crude extract			
	Sweet potato	Cauliflower	Soybean	Sourlime
3.0	0.052	0.031	0.049	0.077
4.0	0.065	0.041	0.052	0.086
4.5	0.069	0.062	0.063	0.087
5	0.074	0.075	0.065	0.089
5.5	0.076	0.084	0.071	0.088
6	0.078	0.086	0.069	0.087
6.5	0.080	0.089	0.068	0.086
7	0.077	0.088	0.055	0.065
8	0.060	0.056	0.049	0.054
9	0.044	0.043	0.032	0.046
10	0.031	0.032	0.025	0.035

Table 5: Effect of temperature on peroxidase activity (absorbance) of crude sweet potato, cauliflower, soybean and sourlime extracts.

Temperature	Crude extract			
	Sweet potato	Cauliflower	Soybean	Sourlime
40	0.430	0.360	0.235	0.562
48	0.352	0.141	0.170	0.513
56	0.321	0.085	0.151	0.390
64	0.299	0.071	0.110	0.361
72	0.270	0.052	0.091	0.020
80	0.081	0.021	0.080	0.019
88	0.052	0.011	0.052	0.015
92	0.035	0.009	0.031	0.013

in protein from native state to reversible denatured state, hence affecting enzyme activity due to altered haemoprotein interaction. Once the haemoprotein interaction is disturbed, there is a loss of protein stability. Sourlime showed maximum enzyme activity at pH 5.0 while soybean showed maximum enzyme activity at pH 5.5. These results are in concordance with the findings of Sessa and Anderson (1981) who studied that soybean showed maximum peroxidase activity at pH 5.5 with guaiacol. The activity of sourlime and soybean decreased in alkaline medium having pH 7 to 8 and at pH 10 the soybean became inactive which are in accordance with the findings of Tauber (1949). During this research work it was found that peroxidase is the most thermostable enzyme as compared to other enzymes, this result is in accordance with the findings of Ivakin and Grushin (1990), Rosoff and Cruess (1949) and Cruess *et al.* (1944) who demonstrated that peroxidases were the most thermostable enzyme found in plants. Ivakin and Grushin (1990) studied changes in peroxidase activity in relation to treatment temperatures (25-85°C) and found a strong positive correlation between heat resistance of the plants and peroxidase thermostability. Using guaiacol as a substrate, peroxidase was found to have a greater thermal resistance than measured with other substrates which in accordance with the work of Gazaryan and Lagrimini (1996) who reported that guaiacol peroxidase was found to have greater thermal resistance than the peroxidase measured with other substrates.

References

- Asada, K., 1992. Ascorbate peroxidase-a hydrogen peroxide-scavenging enzyme in plants. *Physiologia Plantarum*, 85: 235-241.
- Biles, C.L., M.M. Wall, M. Waugh and H. Palmer, 1993. Relationship of Phytophthora fruit rot to fruit maturation and cuticle thickness of New Mexican-type peppers. *Phytopathology*, 83: 607-611.
- Bollag, J.M., C.M. Che, J.M. Sarkar and M.J. Loll, 1987. Extraction and purification of a peroxidase from soil. *Soil Biol. Biochem.*, 19: 61-67.
- Brownleader, M.D. and P.M. Dey, 1993. Purification of extensin from cell walls of tomato (hybrid of *Lycopersicon esculentum* and *L. peruvianum*) cells in suspension culture. *Planta*, 191: 457-469.
- Cabrera, A.M. and H. Lima, 1982. Peroxidase activity during fruit development in the so called acidic fruits of citrus fruits. *Proc. Inst. Soc.*, 1: 13-17.
- Calderon, A.A., J.M. Zapata, M.A. Pedreno, R. Munoz and A.R. Barcelo, 1992. Levels of 4-hydroxystillbene-oxidizing isoperoxidases related to constitutive disease resistance in *in vitro*-cultured grapevine. *Plant Cell Tissue Org. Cult.*, 29: 63-70.
- Campa, A., 1991. Biological roles of plant peroxidases: Known and potential function. *Peroxidases Chem. Biol.*, 2: 25-50.
- Chuang, W.J. and A.O. Chen, 1988. Purification and characterization of soybean peroxidase. II. Characterization of soybean peroxidase isoenzyme. *Food Sci. China*, 15: 1226-1226.
- Cruess, M.V., E.G. Balog, H.R. Friar and M. Lew, 1944. Sulfiting to improve vegetable for dehydration. *Food Packer*, 25: 31-33.
- Davidson, V.L. and D.B. Sittman, 1994. *Biochemistry*. 3rd Edn., Shanghai People's Publishing House, USA.
- Fry, S.C., 1986. Cross-linking of matrix polymers in the growing cell walls of angiosperms. *Annu. Rev. Plant Physiol.*, 37: 165-186.
- Gazaryan, I.G. and L.M. Lagrimini, 1996. Purification and unusual kinetic properties of a tobacco anionic peroxidase. *Phytochemistry*, 41: 1029-1034.
- Gornall, A.G., C.J. Bardawill and M.M. David, 1949. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.*, 177: 751-766.
- Griffing, L.R. and L.C. Fowke, 1985. Cytochemical localization of peroxidase in soybean suspension culture cells and protoplasts: Intracellular vacuole differentiation and presence of peroxidase in coated vesicles and multivesicular bodies. *Protoplasma*, 128: 22-30.
- Harris, D.L. and G.H. Loew, 1996. Identification of putative peroxide intermediates of peroxidases by electronic structure and spectra calculations. *J. Am. Chem. Soc.*, 118: 10588-10594.
- Ivakin, A.P. and A.A. Grushin, 1990. Thermostability of peroxidase in relation to heat resistance of cabbages and tomatoes. *Fiziologiya Biokhimiya Kul'turnykh Rastenii*, 22: 463-468.
- Kim, S.H. and S.S. Kim, 1996. Carbohydrate moieties of three radish peroxidases. *Phytochemistry*, 42: 287-290.
- Krell, H.W., 1991. Peroxidase: An Important Enzyme for Diagnostic Test Kits. In: *Biochemical, Molecular and Physiological Aspects of Plant Peroxidases*, Lobarsewski, J., H. Greppin, C. Penel and T. Gaspar (Eds.), University M.Curie-Sklodowska/University of Geneva, Lublin, Poland/Geneva, Switzerland, pp: 469-478.
- Kwak, S.S., S.K. Kim, M.S. Lee, K.H. Jung, I.H. Park and J.R. Liu, 1995. Acidic peroxidases from suspension-cultures of sweet potato. *Phytochemistry*, 39: 981-984.
- Loew, G. and M. Dupuis, 1996. Structure of a model transient peroxide intermediate of peroxidases by ab initio methods. *J. Am. Chem. Soc.*, 118: 10584-10587.
- Reed, G., 1975. Oxidoreductase. In: *Enzymes in Food Processing*, Reed, G. (Ed.). Academic Press, USA.
- Rosoff, H.D. and W.V. Cruess, 1949. The oxidase of cauliflower. *J. Food Sci.*, 14: 283-297.
- Sessa, D.J. and R.L. Anderson, 1981. Soybean peroxidases: Purification and some properties. *J. Agric. Food Chem.*, 29: 960-965.
- Soda, I., T. Hasegawa, T. Suzuki and N. Ogura, 1991. Purification and some properties of peroxidase from kiwifruit. *Agric. Biol. Chem.*, 55: 1677-1678.
- Tauber, H., 1949. *Enzyme Chemistry*. John Wiley and Sons, New York, pp: 118-181.
- Vamos-Vigyazo, L., J. Farkas and E. Babos-Szebenyi, 1980. A study into some properties of peroxidase in vegetables. *Acta Alimentaria Academiae Scientiarum Hungaricae*, 9: 11-21.
- Willimott, S.G. and F. Wokes, 1926. Oxidising enzymes in the peel of *Citrus* fruits. *J. Biol. Chem.*, 20: 1008-1012.