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Research Article

VALORIZATION OF SUN MELON PEEL WASTE: PURIFICATION AND CHARACTERIZATION OF PEROXIDASE

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ABSTRACT
Peroxidase from Sun melon, was purified by ion exchange chromatography and its biochemical characterization has been done in the present study. Purification fold achieved was 22.56. The enzyme was characterized for its effect on pH, temperature, metal ions, organic solvents and kinetic parameters has also been evaluated using guaiacol and H_2O_2 as substrates. Sun melon peroxidase has shown its optimum activity at pH 6 and at temperature 25°C. The values for km for Sun melon peroxidase were 0.216 mM for guaiacol, respectively. The enzyme was found to be resistant against all concentration of urea. Also, it has been observed that the Sun melon peroxidase was found to be slightly affected against commonly used organic solvents such as ethanol, methanol, acetone, acetonitrile and n-propanol. These properties of Sun melon peroxidase suggests that it can be a promising enzyme for various analytical as well as industrial applications.

Keywords: *Peroxidase, Sun Melon Fruit, Purification, Characterization*

INTRODUCTION
Sun melon, *Cucumis melo* also known as melon, is a species of Cucumis that has been developed into many cultivated varieties. The tropical fruit which is native to Southeast Asia belongs to Rutaceae family. It is fleshy with characteristic aroma and taste. Due to its notable pharmacological effects, it is considered to be one of the valuable fruits amongst the people. The fruit has outstanding medicinal uses and since an era employed as major components in *Ayurveda.* The fruit is found to contains anti-hyperlipidemic agents which can be comparable to atorvastin in animals which has been beneficial in cardiovascular health (G.Adebayo-Gege, 2022) the curbitacin B also has anti-inflammatory responses. Seasonal availability of *sun melon* fruit is mostly responsible for lack of awareness about its health benefits. The fruit comprises of ample amount of antioxidants required for proper functioning of the human body. *Sun melon* fruit juice serves various health benefits and its pleasant aroma and taste makes it a better choice for its consumption Researchers have widely reported the proximate composition as well as bioactive constituents in *sun melon*, however the knowledge regarding the composition of vastly occurring enzymes from *sun melon* juice is rarely reported. Peroxidases are amongst one of those enzymes which plays a key role in the functioning of many fruits and vegetables studied so far. Peroxidases (PODs, EC 1.11.1.7) are the enzymes which oxidize various compounds in presence of hydrogen peroxide. They are ubiquitously present in the nature. Peroxidases plays a major role in plant growth and development, wound healing, auxin metabolism, fruit ripening and disease resistance. Non-animal peroxidases are categorized into three classes on the basis of sequence homology and location: Class I intracellular PODs (e.g., bacterial peroxidase, yeast cytochrome C peroxidase and ascorbate peroxidase), Class II fungal PODs (lignin and manganese peroxidase) and class III consists of plant PODs such as Horse raddish peroxidase (Welinder, 1992).Class III peroxidase are single chain proteins which are often glycosylated, and found to oxidize a wide range of phenolic compounds such as guaiacol, pyrogallol, catechol, catechin etc. High catalytic activity of these peroxidases makes their way in various food processing and industrial applications as well as clinical analysis purpose (Al-sanaidy *et al.,*

2011). High substrate specificity of peroxidase makes the enzyme useful for waste water treatment as well as in industrial effluent treatment (Wagner *et al.,* 2002; Hussain *et al.,* 2008).
Sun melon fruit has tremendous amount of peroxidase enzyme and hence the present study focuses on purification and characterization of this enormously available enzyme in sunmelon. No reports are till now available on the study of peroxidase from *sun melon*, hence the work has been proposed to intervene into the objectives of purifying and characterizing one of the industrially valuable enzyme. Peroxidases are most commonly used in the construction of biosensors as well as in various analytical applications such as ELISA. Some of the other applications of the peroxidases includes determination of lipid peroxidation value in cell membranes and in meat food products, in catalyzing the polymerization and precipitation of aqueous phenols and decolorization of bleach plant effluent by hydrogen peroxide.

MATERIALS AND METHODS

2.1 Materials:

Firstly, the sun melon was collected from nearby fruit market from Kalyan, Maharashtra, India. Fully ripe sunmelon fruit is yellowish in colour with yellowish white pulp. Hence the stage of the fruit was decided on the basis of yellowish outer covering and was utilized for the further studies. Guaiacol, and hydrogen peroxide were purchased from M/s SRL Pvt. Ltd., Mumbai, India. All the other chemicals used were of reagent grade.

2.2. Peroxidase extraction:

The fruit was washed thoroughly first the peel of the fruit was removed and weighed and was suspended in 1:3 proportions (w/v) of 10mM of cold sodium phosphate buffer of pH 7.0. The mixture was then blended and the mixture was then filtered using a muslin cloth. And the filtered extract was taken as the crude extract of the fruit (enzyme extract). The conditions were maintained throughout 4°C.

2.2 Peroxidase Assay:

Assay was performed using colorimeter at 470nm.The reaction mixture included 0.05 M phosphate buffer (2.73ml), 1% hydrogen peroxide (0.1) ml, 4% guaiacol (0.15) ml. After the enzyme sample was added. The reaction was monitored for 120sec and the activity was determined from the linear part of the curve. One unit of peroxidase activity is defined as the change in absorbance of 0.001 min^{-1}.

2.3 Peroxidase purification:

Sun melon POD crude was subjected to ammonium sulphate at 0-80 % concentration for salting out. The enzyme precipitate was then collected by centrifuging at 5000 rpm for 10 mins and then dissolved in 10mM sodium phosphate buffer. Removal of excess salt is perquisite as it then interferes with the purification runs. Dialysis was performed using 0.02 M phosphate buffer for 24 hrs. And the dialyzed sample was then assayed for POD activity.
Ion Exchange Chromatography was followed after ammonium sulphate precipitation. Amberlite IR 120 resin was used for purifying the enzyme using a glass column. The resins were activating using 20Mm phosphate buffer then added into the column. The 20mM phosphate buffer with 8.8 % NaCl was used as the elution buffer,5ml of dialyzed sample was added and fraction were collected in the Eppendorf tubes at fraction time 1ml/min and 1ml/2min.The assay was done and pooled later on.
Proteins were determined using Folin's method using BSA as standard (Lowry, 1951).

2.4 pH optima determination:

pH optima for peroxidase enzyme was determined by performing enzyme assay using following buffers of the pH ranging from 4 to 8: 50mM acetate buffer (pH: 4,5,6), 50mM Phosphate buffer (pH: 7) and 50mM Tris- HCL buffer (pH: 8&9). The reaction mixture was comprised of buffers with respective pH, Guaiacol and H2O2 as substrates. The experiment was performed in triplicates.
pH stability profile was investigated by incubating the purified enzyme with buffers of different pH for 1hr and residual activity was calculated using the given formula:

$$Residual\ activity\ \% = \frac{Ct}{C0} \times 100$$

2.5 Temperature optima determination:
Optimum temperature of the purified enzyme was determined by incubating the reaction mixture at varied temperature ranges from 15 to 75°C. In order to determine thermal stability the enzyme solution was incubated at various temperatures (15-75 °C) for 30 minutes. Enzyme was then cooled in ice water for 5 minutes and then assayed for residual enzyme activity.

2.6 Effect of substrate concentration
Different concentrations of guaiacol were made and their effect on enzyme activity was analyzed.

2.7 Effect of metal ions
Purified enzyme was pre-incubated for 2hrs at 25°C with various metal ions (Cu2+, Ba2+, Mn2+, Fe2+ and Mg2+) following residual activity measurement. Assays were repeated three times.

2.8 Effect of various compounds:
Purified enzyme (50 ng) was incubated for 2hrs at 25°C with various compounds (Urea, EDTA, SDS, citric acid and oxalic acid). After incubation, the residual activity was assayed as described above. All experiments were repeated three times.

2.9 Effect of organic solvents
Purified enzyme was incubated in various organic solvents (25%, v/v) for 5hrs at 25°C with constant shaking on rocking platform shaker. After incubation, residual activity was measured as described above. Assays were repeated three times.

RESULTS AND DISCUSSION

3.1. Purification of peroxidase
Peroxidase from *Sun melon* fruit pulp was extracted as crude extract and purified through successive steps of ammonium sulphate fractionation followed by dialysis to remove excess salt and the sample was further purified by ion exchange chromatography. The purification chart for *sun melon* peroxidase is given in Table 1**.** Peroxidase was purified to 22.6 folds and specific activity 12121.2 Units/mg protein.

Table 1: Stepwise purification profile of sun melon POD

Step	total protein (mg)	total activity (U)	specific activity (U/mg)	fold purity
crude	33.5	18,000	537.3	
dialyzed	97.5	5,70,000	5846	10.8
ion exchange	19.8	2,40,000	12121.2	22.6

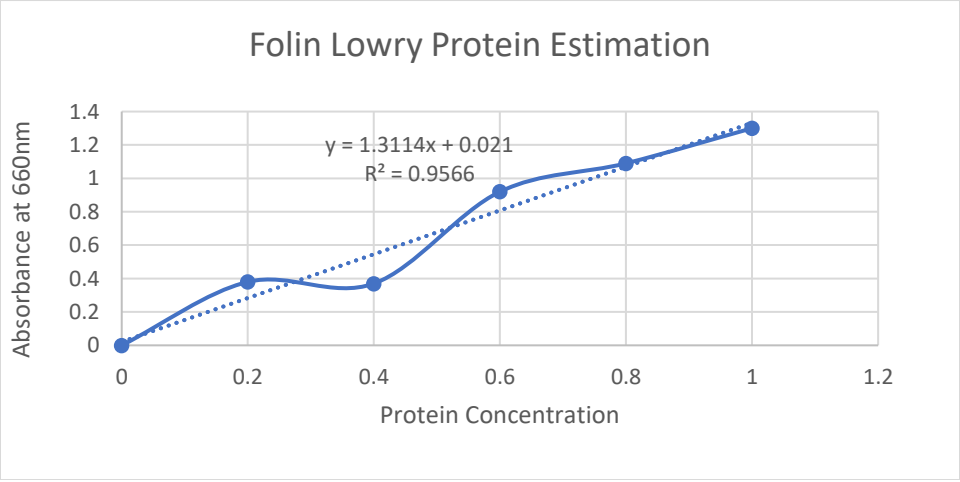

Figure 1: Folin Lowry Protein Estimation

3.2. pH optima and pH stability determination:

pH is a determining factor in the expression of enzymatic activity as it alters the ionization states of amino acid side chains or the ionization of the substrate (Voet and Voet, 1990). The pH optima for *sun melon* peroxidase was shown in fig. 2. It was clearly observed that *sun melon* peroxidase is showing optimum at pH 6.0.

Sun melon peroxidase was found to be stable in only pH of buffer 6.0 retaining almost 100% of its activity (Fig.2). Extreme acidic pH of 4.0 retained only around 25% of the enzyme activity and similar inhibitory effect was seen at extreme basic pH of 8.0 of the peroxidase activity was observed. Lopez and Burgos, (1995) have reported that the heme group present in the peroxidase's active site was pH dependent and gets released easily at very low or high pH values leading to the loss of enzyme activity. Reaction catalysis and enzyme substrate binding depends upon the nature of active site, as the active site is composed of various ionic groups which maintains the proper enzyme confirmation (Whitaker, 1994).

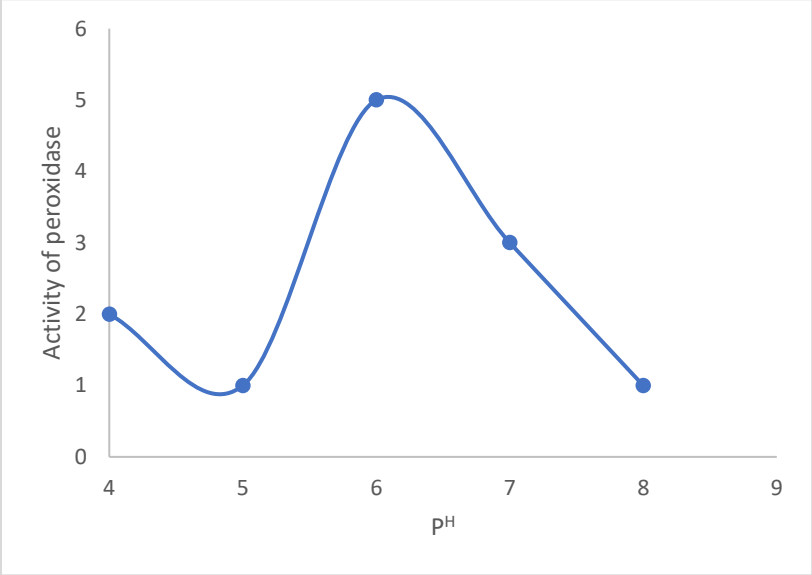

Figure 2: pH optima for sun melon peroxidase

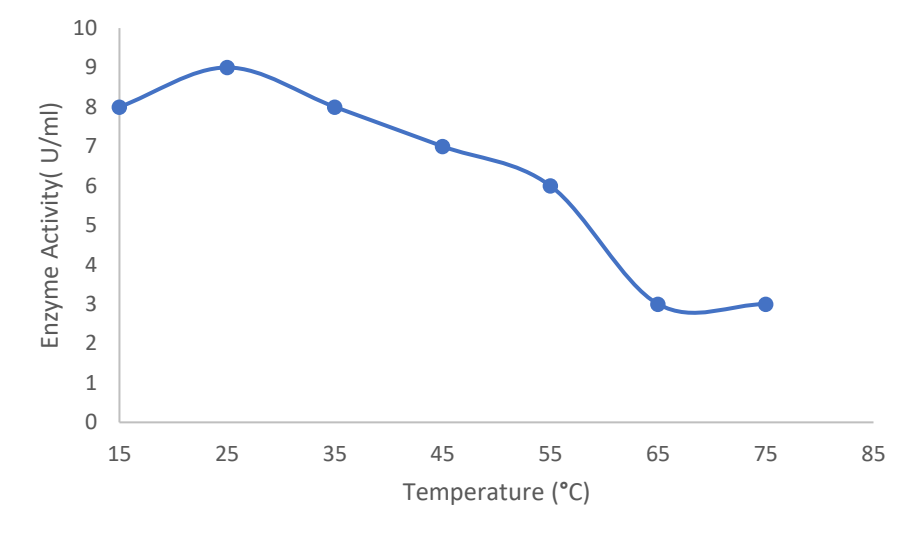

Figure.3. Determination of Temperature optima of Sun melon peroxidase

3.3. Temperature activity profile

From the present study it has been observed that the optimum temperature for *sun melon* peroxidase is 25°C (Fig.4). Optimum temperatures of some plant peroxidases fall in the similar ranges, Suzuki *et al.,* (2006) has found the temperature optima of 10-30°C for buckweed seed peroxidase. Turnip peroxidase has the temperature optima of 35°C. Variability has been observed in the temperature optima profiles of various plant peroxidases. A. sativum, S. melongena, peroxidases have temperatures optima 35 ◦C, 84 °C, respectively. (Marzouki *et al.,* 2005; Vernvawal *et al.,* 2006).

3.4. Effect of substrate concentration on enzymes

The purified *sun melon* peroxidase has shown typical Michalis-Menton kinetics for both Guaiacol (Fig.6) Km and Vmax values were obtained from substrate saturation curve by interpolating the values of substrate concentration and against enzyme activity values.

Km values for *sun melon* peroxidase has been found to be 0.216mM for guaiacol substrates respectively. These values were found to be lower than those reported for green peas (Km values for guaiacol/ H_2O_2 – 10.8 mM (pH 5.0) and 10.8 mM (pH 6.0) for C1 and C2, isozymes) and turnip roots (3.7 mM). The general consideration is lower the km value higher is the affinity of the enzyme for substrate. Hence, it has been observed that *sun melon* peroxidase showed higher substrate affinity as compared to other peroxidases previously reported.

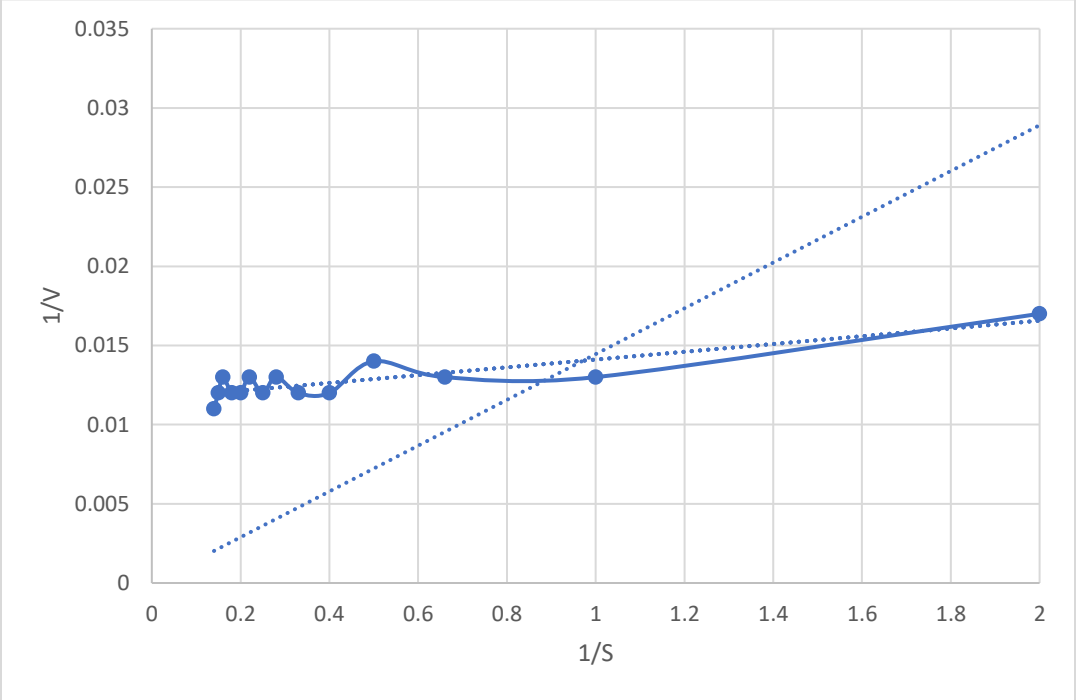

Figure.4. Effect of substrate concentration (Guaiacol)

3.5. Effect of various compounds

Compounds such as EDTA (10 mM, 20 mM) and oxalic acid (5 mM) has shown increased enzyme activity in concentration dependent manner. It was found that the enzyme was resistant against urea of all concentrations, EDTA 1mM, oxalic acid 10Mm, and SDS at 0.5 and 4 %. Such high tolerance against urea has also been reported for *M. charanha* peroxidase (Feng Cai *et al.,* 2012). Oxalate 20mM and citric acid 20 mM has complex inhibitory effect on peroxidase activity.

Table 2. Effect of various compounds on enzyme activity

Inhibitors (1hr incubation)	Residual activity %
control	100±3.4
2mM urea	98.4±8.0
4mM	95.1±3.5
8mM	97.6±2.3
5mM Citric Acid	92.7±20.7
10mM	91.9±12.7
20mM	70.7±17.3
EDTA 1mM	98.4±3.5
10mM	103.3±0.6
20mM	101.6±4.1
5mM Oxalic Acid	101.6±4.1
10mM	95.9±2.3
20mM	39.8±19.5
0.5% SDS	99.2±0.0
2%	78.0±4.6
4%	94.3±2.3

3.6. Effect of Metal ions

Sun melon peroxidase was incubated with various metal ions, the residual activity was shown in table 3. POD was found to be increased by Mn++, Mg++,Ba++,Fe++, and Cu++ metal ions. As sun melon peroxidase has shown increased activity in presence of these metal ions indicates its significant applicability in various industrial processes.

Table 3. Effect of Metal ions on peroxidase activity

Metal ions (10mM)	Residual activity %
Control	100±0
Ba	165.9±0
Mg	152.1±0
Fe	101.4±0
Mn	170.5±0
Cu	175.1±0

3.7. Effect of organic solvent

Sun melon peroxidase was found to be affected significantly against various organic solvents (Table 4). Around 90% of activity was found to be reduced during 5hrs of incubation with ethanol and methanol, acetone, acetonitrile and n-propanol. All of these solvents has shown inhibitory effect by decreasing the activity of peroxidase.

Table 4. Effect of organic solvents on peroxidase activity

organic solvents	Residual activity %	polarity index	solubility in water %
Control	100±9.43		
n-propanol	3.33±4.71	4	
Acetone	0±0	5.1	100
methanol	6.67±0.00	5.1	100
Ethanol	16.67±4.71	5.2	100
acetonitrile	0±0	5.8	100

CONCLUSION

Peroxidases are gaining attention because of its wide applicability in the fields of biotechnology, biochemistry, molecular biology, animal physiology, etc. Peroxidase from *sun melon* was found to be unique due to its high activity, excellent pH stability, stabilities in presence of various metal ions and various other compounds makes this enzyme a choice of enzyme for industrial purpose. Optima values for pH and temperature was found to be 6 and 25°C respectively. Peroxidase activity was found to be activated by divalent cations such as Mn++, Pb++. Organic solvents such as ethanol, methanol, acetone, acetonitrile and n-propanol remarkably affected the activity of *sun melon* peroxidase. Future work can be done for the cloning and sequencing the structural gene of this enzyme, leading to high level production and expression of enzyme.

REFERENCES

Al-Marzouki S., Roberts I., Marshall T., Evans S. (2005). The effect of scientific misconduct on the results of clinical trials: a delphi study. *Contemp. Clin. Trials,* **26**, 331–337.

Al-Senaidy, A. M., & Ismael, M. A. (2011). Purification and characterization of membrane-bound peroxidase from date palm leaves (*Phoenix dactylifera L.*). *Saudi Journal of Biological Sciences,* **18**, 293–298.

Badam, L., Bedekar, S. S., Sonawane, K. B., & Joshi, S. P. (2002). *In vitro* antiviral activity of *sun melon* (*Aegle marmelos* Corr) upon human coxsackie viruses B1-B6. *Journal of Communication Disorders,* **34**, 88.

Bradford, M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of dye binding. *Analytical Biochemistry,* **72**, 248–254.

Cai F., OuYang C., Duan P., Gao S., Xu Y., Chen F. (2012). Purification and characterization of a novel thermal stable peroxidase from Jatropha curcas leaves. *Journal of Molecular Catalysis B: Enzymatic,* **77**, 59– 66

Chopra, R. (1982). Indigenous drugs of India Calcutta. Academic publishers.

Ciou, J. Y., Linb, H. H., Chiang, P. Y., Wang, C. C., & Charles, A. L. (2011). The role of polyphenol oxidase and peroxidase in the browning of water caltrop pericarp during heat treatment. *Food Chemistry,* **127**, 523–527.

Das, M. K., Sharma, R. S. & Mishra, V. (2011). A novel cationic peroxidase (VanPrx) from a hemi-parasitic plant (*Viscum angulatum*) of Western Ghats (India): Purification, characterization and kinetic properties, *Journal of Molecular Catalysis B: Enzymatic,* **71**, 63–70.

Deepa, S. S., & Arumughan, C. (2002). Purification and characterization of soluble peroxidase from oil palm (Elaeis guineensis Jacq.) leaf. *Phytochemistry,* **61**, 503–511.

Duarte-Vázquez, M. A., Whitaker, J. R., Rojo-Domínguez, A, García- Almendárez, B. E., & Regalado, C. (2003). *Journal of Agricultural Food Chemistry,* **51**, 5096–5102.

Feng, C., Chao, O. Y., Peipei, D., Shun, G, Ying, X., & Fang, C. (2012) Purification and characterization of a novel thermal stable peroxidase from Jatropha curcas leaves. *Journal of Molecular Catalysis B: Enzymatic,* **77**, 59– 66

Gupta, A. K., & Tandon, N. (2004). Reviews on Indian medicinal plants, Volume 1 *(Indian Council of Medicinal Research, New Delhi).* 312
Hajra, P. K., Nair, V. J., & Daniel, P. (1997). Flora of India. Botanical Survey of India Calcutta 4, 264.
Husain, Q., & Husain, M. Applications of redox mediators in the treatment of organic pollutants by using oxidoreductive enzymes: A review. *Critical Reviews in Environmental Science and Technology, 38*, 1–42.
Lopez, A., & Burgos, J. (1995). Peroxidase stability and reactivation after heat treatment and manotherosonication. *Journal of Food Science,* **60**, 451–455.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) "Protein measurement with the Folin phenol reagent," Journal of Biological Chemistry, **193**(1), pp. 265–275.
Marquez, O., Waliszewski, K. N., Oliart, R. M., & Pardio, V. T. (2008). Purification and characterization of cell wall-bound peroxidase from vanilla bean. *LWT-Food Science Technology,* **41**, 1372–1379.
Omumi, A., Morshedi, D., & Samadi, A. (2001). Isolation of a novel stable peptide from cultivated Raphanus sativus with peroxidase activity. *World Journal of Microbiology & Biotechnology,* **17**, 827–828.
Suzuki, T., Honda, Y., Mukasa, Y., Kim, S.J. (2006). Characterization of peroxidase in buckwheat seed. *Phytochemistry.* **67**, 219–224.
Takase, H., Yamamoto, K., Hirano, H., Saito, Y, & Yamashita, A. (1994). Pharmacological profile of gastric mucosal protection by marminutes and nobiletin from a traditional herbal medicine, *Aurantii fructus immaturus. The Japanese Journal of Pharmacology,* **66**, 139.
Thongsook, T., & Barrett, D. M. (2005). Reactivation of broccoli peroxidases: Structural changes of partially denatured isoenzymes. *Journal of Agricultural Food Chemistry, 53,* 3206–3214.
Vernwal S.K., Yadav R.S.S., Yadav K.D.S. (2006). Purification of a peroxidase from solanum melongena fruit juice. Indian J. Biochem. Biophys. **43** (2006) 239–243.
Voet, D., & Voet, J. G. (1990). Mechanisms of enzyme action. In Woolsey (Ed.), Biochemistry (1st ed) (pp. 344). New York: Viley.
Wagner, M., & Nicell, J. A. (2002). Detoxification of phenolic solutions with horseradish peroxidase and hydrogen peroxide. *Water Research*, **36**, 4041–4052.
Welinder, K. G. (1992). Superfamily of plant, fungal and bacterial peroxidases. *Current Opinion in Structural Biology*, **2**, 388–393.
Whitaker, J. R. (1994). Effect of pH on enzyme catalysed reactions. In Principles of enzymology for the food science (2nd ed.) (pp. 271–300). New York: Marcel Dekker.