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Peroxidase from infected fruit of *Solanum* sp. grown in Nsukka

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Abstract

In this study, we characterized the activity of peroxidase a quality control enzyme from the infected fruit of *Solanum* sp. Peroxidase was purified to homogeneity by ammonium sulfate precipitation, dialysis, ion exchange chromatography and size exclusion chromatography. The molecular weight of the native enzyme was 63000 da. The enzyme was shown to have two iso-enzymes with distinct optimum pH of 4.5 and 7.0 and optimum temperature of 40 and 70^{0}C. The purified enzyme had broad substrate specificity with o-dianisidine being the ideal substrate. Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, $Cu^{2+,}$ Al^{3+} were shown to be activators of the enzyme, while the peroxidase activity was severely inhibited by $Co^{2+.}$

Keywords: Enzyme; Characterization; Infected fruit; *Solanum spp*; Peroxidase

Introduction

Peroxidase (EC. 1. 11. 1.7), represents a huge family of heme-containing enzyme that catalyze the oxidation of various electron donor substrates such as phenol, and aromatic amines in the presence of hydrogen peroxide (Khatun *et al*., 2012). They are widely distributed in plants, microorganisms and animals, where they catalyze the reduction of hydrogen peroxide to water rendering it harmless under normal and stress conditions (Bania and Mahanta, *et al.,* 2012),

Peroxidases have previously been purified from plant sources such as *Moringa oleifera* leaves (Khatun *et al*., 2012), *Nicotiana tabaccum,* (Bania and Mahanta, 2012), juice of *Beta vulgaris* (Chaurasia *et al*., 2013), *Camellia sinensis* (Shah *et al*., 2014), apple and orange seeds (Zia *et al*., 2011). It has wide industrial applications which include the oxidation of organic compound (Fatima *et al*., 2007), clinical diagnosis and micro analytical immunoassay (Chaurasia *et al.,* 2013), de-colourization of waste (Jadhav *et al*., 2009), removal of peroxides from food stuffs and industrial wastes (Kim and Yoo, 1996) and bleaching of synthetic dyes (Osuji *et al*., 2014).

Solanum sp. fruit belongs to the family of *Solanaceae* with over 1000 species worldwide (Agoreyo *et al*., 2012). It is a highly valued constituent of Nigerian foods (leaves and fruits) and indigenous medicine that are either eaten raw or cooked (Edem *et al*., 2009). It is not just consumed daily but preferred when fresh. But, it is susceptible to worm infestation, this leads to the generation of agro waste that litters our environment.

Hence, this study was aimed at the extraction and characterization of peroxidase from infected fruit of *Solanum* sp. as a way of converting the agro waste to a useful material of enzyme production.

Materials and methods

Infected*Solanum spp*fruit was harvested from a farm in Edem-ani, Nsukka L. G. A. of Enugu state. Guaiacol, pyrogallol, o-dianisidine and ascorbic acid were products of Sigma-Aldrich. DEAE cellulose (Hannover), sephadex G75 was product of Pharmacia (Sweden). All other chemicals and reagents were of analytical grade.

Methods

Extraction of peroxidase was carried out according to the method of Eze *et al*. (2010), with slight modification of pH. The infected fruit (100g) was washed, sliced and homogenized by grinding with mortar and pestle in 1000ml of 0.1M phosphate buffer pH7.0. The homogenate was filtered with double layered cheesecloth and Whatman's No.

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1 filter paper. The filtrate was centrifuged with palmer VS130000 centrifuge at 4000 ×g 1h. The supernatant was collected and stored at 4^0C as crude enzyme.

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Purification of peroxidase

The filtrate was brought to 60% ammonium sulphate saturation followed by stirring for 4h. Then, the solution was centrifuged at 4000 ×g 1h. The pellet was dissolved in the buffer and stored at 4^0C for further studies.

Dialysis of peroxidase

The precipitated peroxidase solution was dialyzed against the working buffer (0.1M phosphate buffer, pH7.0) for 12h, with four changes of the buffer after every 3hr.

DEAE-cellulose ion exchange chromatography

Further purification was carried out by ion exchange chromatography using diethylaminoethyl cellulose column as described by Kelley and Reddy (1986), with slight modification. The enzyme solution was applied to a DEAE-cellulose column, gel bed 30 cm (1.8cm × 40 cm), previously equilibrated with 0.1M phosphate buffer. The column was washed with 50ml of the same buffer, and eluted stepwise from the column with 50ml 0.1M of phosphate buffer containing 0.05, 0.10, 0.15, 0.20 and 0.25M NaCl. Aliquot, 0.4 ml were collected from each fractions and tested for peroxidase activity as described above. Fractions with highest activity were pooled and stored for further studies.The pooled fractions were applied to sephadex G75 column (Pharmacia; 32 ml of gel; 1.8 by 28 cm gel bed) equilibrated with 0.1 M phosphate buffer. A total of 29 fractions of 2.5 ml each were collected at the flow rate of 0.5ml/min and both enzyme activity as well as protein content was assayed for each fraction as described above.

Enzyme activity and protein determination

The enzyme activity was assayed according to the method of Eze (2012). The assay mixture contained 2.0 ml of 0.1M phosphate buffer pH 7.0, 0.3 ml guaiacol, 0.4 ml crude enzyme and 0.2ml of 30% H_2O_2 peroxidase activity was monitored by change in absorbance due to oxidation of guaiacol in the presence of H_2O_2 using Jenway 6303 UV/VIS spectrophotometer at wavelength of 470nm. The protein concentration was measured according to the method described by Lowry *et al*. (1951) using bovine serum albumin as the standard protein.

Characterization of peroxidase

Molecular weight determination

The molecular mass of the purified peroxidase was determined by gel filtration on sephadex G75 column (1.8 × 40 cm). The column was calibrated with gel filtration standard (α-lactoalbumin (14200 da), trypsinogen (21500da), egg albumin (45000 da), ovalbumin (45700 da), bovine serum aalbumin (66000 da) and haemoglobin (68000 da)) obtained from Bio-Rad Laboratories, Richmond, Calif. Void volume (Vo) of the column was measured using dextran blue. Elution volume (Ve) and Ve/Vo was defined as Rf value. The Log MW vs Rf graph obtained from the above standards was used to determine the native molecular mass of the purified peroxidase.

Optimum pH and temperature of peroxidase activity

Peroxidase activity was assayed at various pH values using the following buffers, sodium – acetate buffer adjusted to pH from 3.5 – 6.0; Tris-HCl buffer 7.5 – 9.0 and sodium phosphate pH from 6.5 – 8.0 at intervals of pH level 0.5. The optimum temperature was determined by assaying for peroxidase activity at different temperatures of 30 - 100^{o}C.

Substrate specificity

The specificities of various hydrogen donor substrates (*o*-dianisidine, guaiacol, ascorbate and pyrogallol) were studiedin the presence of hydrogen peroxide. Different concentrations of the substrate ranging from 0.1- 1.0 mg/ml of each substrate was added to the reaction media and peroxidase activity assayed. The michealis constant (*km*) and maximum catalytic velocity (*Vmax*) were extrapolated from

their respective Lineweaver-Burk plots.

Effect of metal ion concentration

The effect of some metal ions (Na^{+}, Cu^{2+}, Ca^{2+}, Mg^{2+}, Al^{3+}, K^{+}, Mn^{2+} and Co^{2+}) on the enzyme activity was carried out according to the method of Shah *et al*., (2014). The purified peroxidase was incubated for 60 min at 30^{0}C with different metal ions such as Na^{+}, Cu^{2+}, Ca^{2+}, Mg^{2+}, Al^{3+}, K^{+}, Mn^{2+} and Co^{2+}at various concentration. Their effect was determined by assaying for the peroxidase residual activity after 60 min.

Results and discussion

Peroxidase activity was detected in the crude extract of infected *Solanum* sp. fruit solution. The purification of peroxidase from *Solanum* sp. was summarized in Table I.

Ammonium sulphate saturation of 60% was obtained for the enzyme, yielding 0.671mg/ml of protein and specific activity of 248.88U/mg. These data indicated the appropriateness of the salt in precipitating peroxidase. Some researchers had used ammonium sulphate for plant peroxidase precipitation

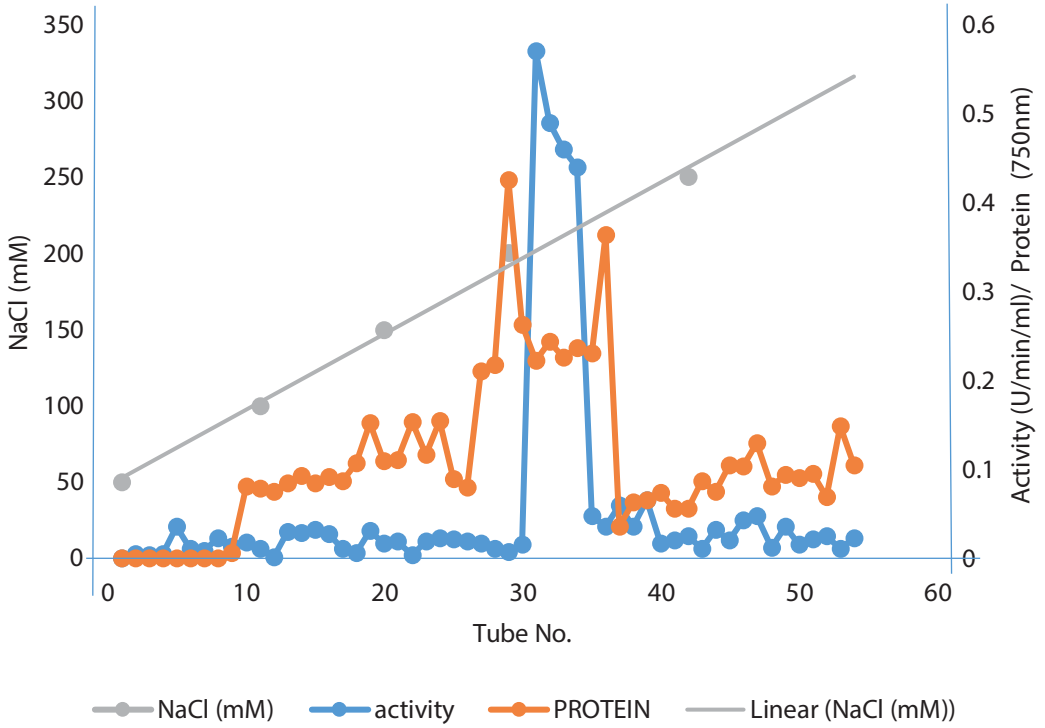

Fig. I. Ion exchange chromatography using DEAE-cellulose column (40×1.3) cm for purification peroxidase from infected *Solanum* sp.

Table I. The purification table of peroxidase extracted from infected *Solanum* sp.

Purification step	Volume (ml)	Enzyme activity (U/ml)	Protein concentration (mg/ml)	Specifi activity (U/mg)	Total activity (U)	Purification fold	Percentage yield (%)
Crude extract	1000	99	0.205	482.93	99000	1	100
80% $(NH_4)_2SO_4$	250	167	0.671	248.88	41750	0.515	42.17
Dialysis	100	171	0.264	647.72	17100	1.34	17.27
DEAE-cellulose	30	136	0.226	601.77	4080	1.246	4.121
Sephadex G-75	30	144	0.246	585.36	4320	1.212	4.363

(Diao *et al*., 2014; Zia *et al*., 2011; and Pandey and Dwivedi, 2011). The ammonium sulphate saturation obtained from this study (60 %) was low in comparism to the work of Cai *et al*., (2012). They obtained 85% ammonium sulphate saturation in their work on *J. curcas* leaves peroxidase. Similarly, peroxidase obtained from Tartary buckwheat shoots was precipitated at 80% saturation (Mikami *et al*., 2013). On the other hand, the result of ammonium sulphate concentration that precipitated the peroxidase obtained in this study (60%) was higher than the result of peroxidase precipitation from apple and orange seeds, which were reported to be 50% (Zia *et al.*, 2011). *L. leucocephala* peroxidase was also precipitated at 50% ammonium sulphate saturation (Pandey and Dwivedi, 2011).Increase in the protein concentrations at 60% ammonium sulphate saturation as observed in this study is in accordance with the report of Copeland and Robert (2000), that proteins can be precipitated at high concentration of ammonium sulphate. Also, the high specific activity (248.88U/mg) was an indication that the salt did not cause serious damage to the protein structure at 60% saturation which would have led to loss in peroxidase activity. Ammonium sulphate precipitation is an ideal and initial useful method of enzyme concentration and purification (Bhatt, 2011).

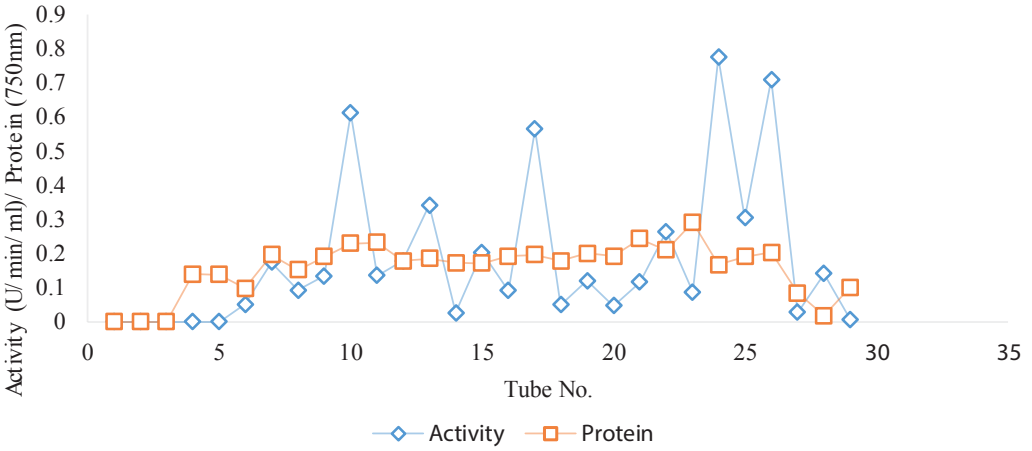

Fig. 2. Sephadex-G75 gel filtration elution profile for infected *Solanum* sp.

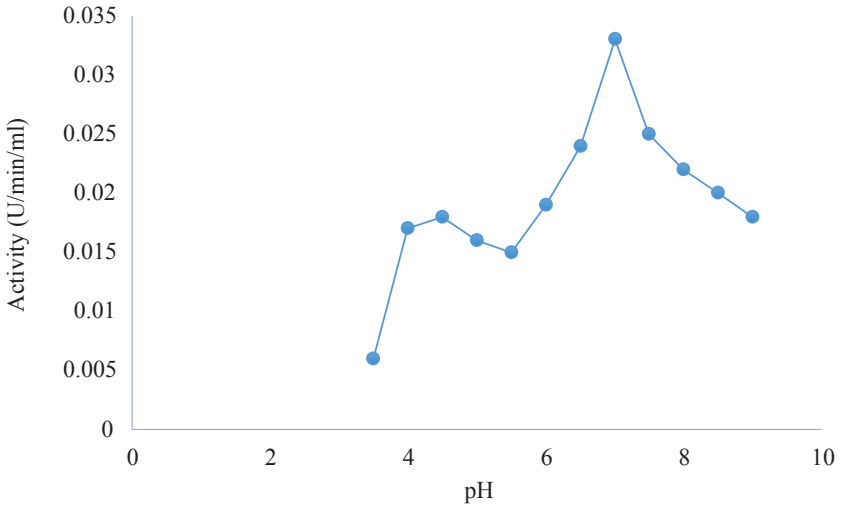

Fig. 3. Effect of pH on peroxidase activity of infected *Solanum* sp. at 30°C

The precipitated enzyme was desalted using dialysis. After this purification step, the enzyme was purified to 1.34 fold and 17.27 % as shown in table 1, with concomitant rise of the specific activity to 647.72U/mg. The process has been previously employed in the purification of peroxidase of plant origins as reported by Pandey and Dwivedi (2011) on *L. leucocephala* peroxidase, Zia *et al*. (2011) on apple and orange seeds, Osuji *et al.* (2014) on garlic and Mohamed *et al.* (2011) on *F. sycomorus*. The increase observed in the activity and specific activity (Table I) showed that the purification step aided in removal of the excess salt residues and other compounds that would have inhibited or interfered with the enzyme active site.

DEAE-cellulose ion exchanger was used for further chromatographic purification of the dialyzed enzyme. Its chromatograph is shown in figure 1. This technique purifies biomolecules based on the nature of charges present on the enzyme's active site amino acids contains, since enzymes are made of amino acids (Bhatt, 2011). Mohamed *et al.* (2011) reported the use of DEAE-sepharose in purifying plant peroxidase, which produced seven protein fractions as shown in their chromatograph. Peroxidase was purified from the leaves of *C. sinesis* by DEAE-cellulose chromatography (Shah *et al*., 2014). The specific activity of the DEAE cellulose purified peroxidase was 601.77 U/mg and purification fold of 1.246 as shown in Table I.

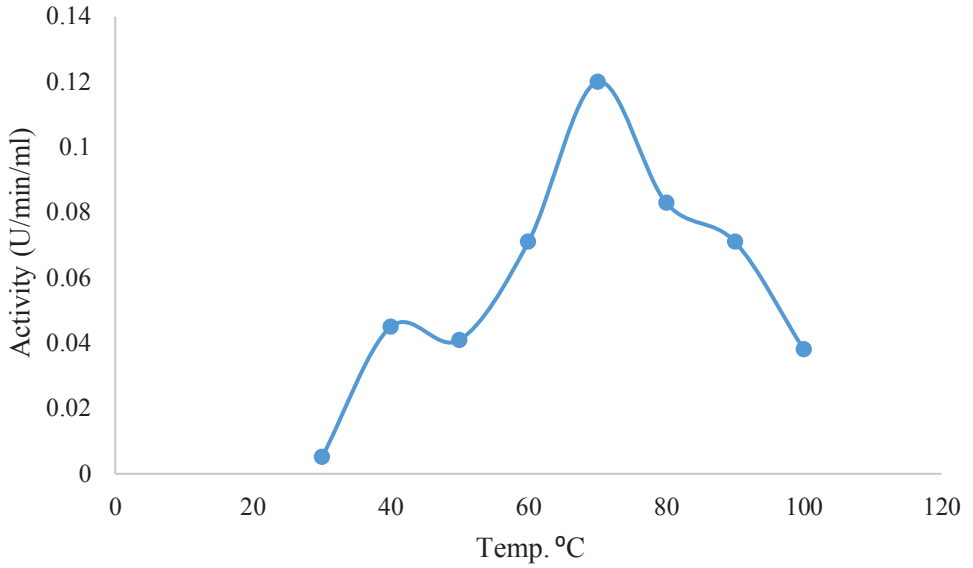

Fig. 4. Effect of temperature on peroxidase activities from infected *Solanum* sp. at pH 7.0

Table II. Kinetic parameters (*Km and Vmax*) of peroxidase at 30°C and pH 7.0

Enzyme	substrate	Km (mg/ml)	Vmax (U/min/ml)	Specificity (Vmax/Km)
INGE	*o*-dianisidine	0.930	58.82	63.24
INGe	Guaiacol	1.319	34.48	26.14
INGE	Ascorbate	2.100	55.55	26.45
INGE	Pyrogallol	2.55	27.02	10.59

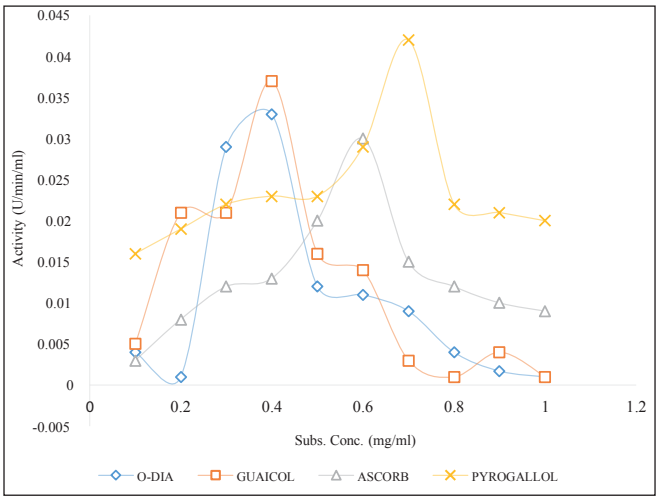

Fig. 5. Effect of *o*-dianisidine, guaiacol, ascorbate and pyrogallol on the activity of peroxidase extracted from infected *Solanum* sp. at temperature of 30°C and pH 7.0

process. Similarly, Moulding *et al.* (1988) observed an increase in the specific activity of peroxidase after gel filtration.

Molecular weight determination

The molecular weight of purified peroxidase was determined using gel filtration on sephadex-G75. Based on gel filtration chromatography on sephadex G75 column, the apparent molecular weight of the purified native peroxidase was estimated to be 63000 da.

Optimum pH and temperature

The purified enzyme had a pH optimum of 7.0 as shown in figure 3. Peroxidase from other plant sources were reported to have pH optimum of 6.5 (Shah *et al.*, 2014); and 5.5 (Vernwal *et al.*, 2006). Similarly, the enzyme showed

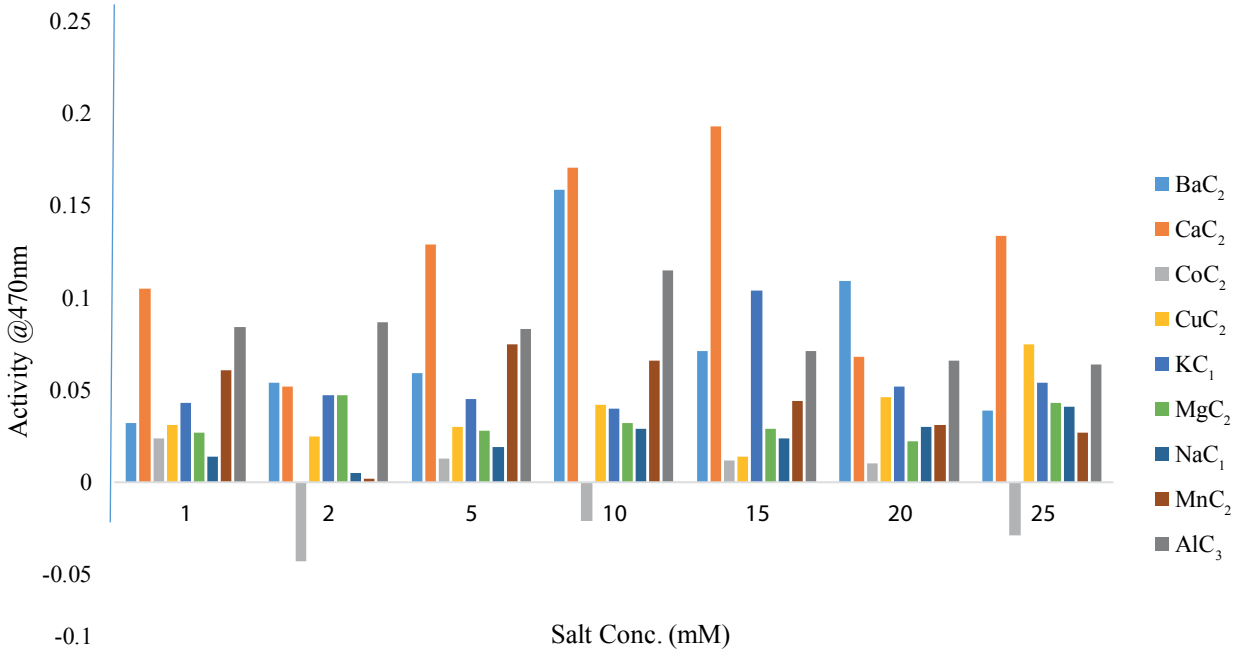

Fig. 6. Effect of ion concentration on peroxidase activity extracted from infected *Solanum* sp. at 30°C and pH 7.0

The fractions pooled after DEAE cellulose step were loaded on sephadex G75 column to polish the enzyme. Fig. 2 shows the chromatograph of the gel filtration step. After the gel filtration process, purification fold of 1.212 and 43.63% recovery was obtained for the peroxidase (Table I). Zia *et al.* (2011) obtained 8.34 fold for apple seed peroxidase and 30.64 for orange seed peroxidase respectively after gel filtration

optimum temperature of 70°C (figure 4). The high optimum temperature of peroxidase (70°C) indicated that the peroxidase could had been induced as a result of heat and other harsh environmental conditions. This report is comparable with the high optimum temperature (80°C) obtained from *S. melongena* peroxidase (Vernwal *et al.*, 2006). Also, 60°C was obtained for peroxidase purified from the leaves of *J. curcas* (Cai *et al.*, 2012). The optimum

temperature of characterized peroxidase from *C. sinensis* was reported to be 30^0C (Shah *et al*., 2014) was low when compared to the report of this study.

For the substrate specificity study, the *Km* and specificity values (Table II) of each substrate assayed were determined at optimum temperature and pH. Among the four substrates assayed o-dianisidine and guaiacol showed high affinity towards the peroxidase with the *Km* values of 0.930 and 1.319 mg/ml. The *Km* values obtained for ascorbate and pyrogallol were higher than those *o*-dianisidine and guaiacol, revealing low affinity of the substrate towards the peroxidase. The peroxidase showed to have broad substrate specificity toward many phenolic compounds used. Ogana and Eze, (2015) reported *km* and *Vmax* values of 3.3u/ml and 0.12mM for *o*-dianisidine peroxidase extracted from *Gongronema latifolium*. The *Km* and *Vmax* for o-dianisidine was 25mM and 0.75U/min for peroxidase partially purified from garlic (Osuji *et al*., 2014). Kim and Lee (2005) reported the *Km* and *Vmax* values of 1.18mM and 0.032 U/ml/min for *o*-dianisidine.

Effect of metal ions on peroxidase activity

The peroxidase was incubated with different concentrations of metal ions and the residual activity was represented in figure 5. The metal ions assayed were observed to enhance peroxidase activity except for Co^{2+}, which inhibited peroxidase activity in all the concentrations monitored. The effect of the ions concentrations were observed to be concentration dependent (fig. 6), with corresponding increase in enzyme residual activity at 25mm. $Mg^{2+,}$ $Mn^{2+,}$ $Cd^{2+,}$ Cu^{2+} and Al^{3+} enhanced peroxidase activity, while Co^{2+}, Ca^{2+}, and Zn^{2+}, inhibited peroxidase from the peels of *C. reticulata*. Decrease in peroxidase activity by Co^{2+} was also observed by Onsa *et al*., (2004). Marqueza *et al*., (2008) reported that Ca^{2+} exerted an inhibitory effect on peroxidase. Similarly, Bhatti *et al*., (2006), reported the inhibition of chick pea peroxidase by $Co^{2+}Al3^{+}$ and Mn^{2+}**.** Mikami *et al*., (2013) discovered that $Fe^{3+}$and Al^{3+} activated peroxidase purified from the shoot of Tartary buckwheat. Ca^{2+} enhanced peroxidase activity of *F. sycomorus* source while Mn^{2+} inhibited the peroxidase (Mohammed *et al*., 2011).

Conclusion

In this report, we have described the purification and characterization of peroxidase from worm infected fruit of *Solanum* sp. The native molecular weight of the peroxidase was 63000 da., and optimum temperature and pH of 70^oC and pH 7.0 respectively. The *Solanum* sp. peroxidase showed broad specificity on *o*-dianisidine, guaiacol, pyrogallol and ascorbate, with *o*-dianisidine showing highest specificity (0.930 mg/ml). All metal ions assayed, activated the enzyme while Co^{2+} severely inhibited the enzyme activity**.** These properties recorded from peroxidase extracted from infected *Solanum* sp. are of great industrial advantage since there are within the mild conditions. Hence, infected *Solanum* sp. could be regarded as a cost effective source of peroxidase.

References

Agoreyo BO, Obansa ES and Obanor EO (2012), **C**omparative, nutritional and phytochemical analyses of two varieties of *solanum melongena***,** *Science World Journal* **7**(1): 5-8.

Bania I and Mahanta R (2012), Evaluation of peroxidases from various plant sources, *International Journal of Scientific Research Publication* **2**(5): 1-5.

Bhatt SM (2011), Enzymology and Enzyme Technology, S. Chand and Company Ltd., New Delhi,

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

Chaurasia PK, Bharati SL, Singh SK, Ma R and Yadav SS (2013), A potential peroxidase obtained from the juice of Beta vulgaris (Beet), *American Journal of Food Science and Technology* **1** (3); 30-35.

Copeland and Robert A (2000), Enzymes: a practical introduction of structure, mechanism and data analysis 2nd Ed. John Wiley and sons'Inc., New York, pp 188-196.

Diao M, Ayekoue BN, Dibala CI Dabonne Sand Dicko HM (2014), Purification and characterization of sweet potato peroxidase, *Journal of Animal and Plant Science* **22**(2): 3419-3432.

Edem CA, Dounmu MI, Bassey FI, Wilson C and Umoren P (2009), A comparative assessment of the proximate composition, ascorbic acid and heavy metal content of two species of Garden egg (*Solanum gilo* and *Solanum aubergine*), *Pakistan Journal of Nutrition* **8**(5): 582-584.

Eze SOO (2012), Kinetic analysis of the thermostability of peroxidase from African oil bean (*Pentaclethra macrophylla Benth*) seed, *Journal of Biochemistry and Technology* **4**(1): 459-463.

Eze SOO, Chilaka FC and Nwanguma BC (2010), Studies on thermodynamics and kinetics of thermo-inactivation of some quality-related enzymes in white yam (*Dioscorea rotundata*), *Journal of Thermodynamics and Catalysis* **1**: 104-109.

Fatima A, Husain Q and Khan RH (2007), A peroxidase from Bitter gourd (*Momordicacharantia*) with enhanced stability against organic solvent and detergent: A comparison with horseradish peroxidase, *J. Mol. Cataly. B; Enzymes* **47:** 66-71. DOI:org/10.1016/ j.molcatb. 2007.03.010

Jadhav UU, Dawkar VV, Telke AA and Govindwar SP (2009), Decolourisation of direct blue (GLL) with enhanced Lignin peroxidase enzyme production in *Comanonas Sp*, *Journal of Chemical Technology and Biotechnology* **84:** 126-132.

Kelley RL and Reddy CA (1986), Purification and characterization of glucose oxidase from ligninolytic culture of P. chrysosporium, *Journal of Bacteriology* **166** (1): 269-274. DOI: 10.1128/jb.166. 1.269-274.1986

Khatun S, Md A, Md RK, Pervin F, Absar N and Rosma A (2012), Peroxidase from *M. oleifera* leaves, *Bio Research* **7**(3): 3237-3251.

Kim YH and Yoo JY (1996), Peroxidase production from carrot hairy root cell culture, *Enzyme Microbial Technology* **18**: 531-535. DOI:org/10.1016/0141-0229(95)00168-9

Kim SS and Lee DJ (2005), Purification and characterization of a cationic peroxidase C in Raphanus *sativns*, *Journal ofPlant Physiology* **162**: 609-617. DOI:org/10.1016/j.jplph. 2004.10.004

Lowry OH, Rosebrough NJ, Farr AL and Randell RJ (1951), Protein measurement with the folin phenol reagent, *The Journal of Biological Chemistry* **193**(1): 265-275.

Ma´rqueza O, Waliszewskia KN, Oliarta RM and Pardiob VT (2008), Purification and characterization of cell wall-bound peroxidase from vanilla bean, *LWT- Food Science and Technology* **41**(8): 1372–1379. DOI:org/10.1016/j.lwt. 2007.08.017

Mikami D, Kurihara H, Takahashi K, Suzuki T and Morishita T (2013), Effects of metal ions on the activity and stability of peroxidase in Tartary buckwheat shoots,*Journal of AgriculturalChemistry and Environment* **2**(3): 59-64.

Mohamed SA, Abdel-Aty AM, Mohamed BH, Mohamed OE and Fahmy AS (2011), Ficus sycomorus latex: a thermostable peroxidase, *African Journal of Biotechnology* **10**(76): 17532-17543.

Moulding PH, Singleton DE, McLellan KM and Robinson DS (1988), Purification and heat stability of Cox apple pulp peroxidase isoenzymes, *International Journal of Food Science and Technology* **23:** 343-351. DOI:org/10.1111/j.1365-2621.1988.tb00588.x

Ogana J and Eze SOO (2015), Partial purification and characterizaton of peroxidase extracted from *Gongronema latifolium***,** *American-Eurasian Journal of Scientific Research* **10**(4): 221-227.

Onsa GH, Saari NB, Selamat J and Bakar J (2004), Purification and characterization of membrane-bound peroxidases from *metroxylon sagu*, *Food Chemistry* **85**: 365-376.

Osuji AC, Eze SOO, Osayi EE and Chilaka FC (2014), Bio-bleaching of industrial important dyes with peroxidase partially purified from garlic, *The Science World Journal* **1:** 8. DOI:org/10.1155/2014/183163

Pandey VP and Dwivedi N (2011), Purification and characterization of peroxidase from *L. leucocephala*, a tree legume, *Journal of Molecular Catalysis B: Enzymatic* **68**: 168-173. DOI:org/10.1016/ j.molcatb.2010.10.006

Shah S, Borah D and Yadav RNS (2014), Extraction and characterization of peroxidase from *Camellia sinensis*. *Procedure of National Academy of Science, India, Section B: Biological Science* **84** (2): 343-348.

Vernwal SK, Yadav RSS and Yadav KDS (2006), Purification of a peroxidase from *S. melongena* fruit juice, *Indian Journal of Biochemistry and Biophysics* **43**: 239-243.

Zia MA, Kousar M, Ahmed I, Igbal HMN and Abbas RZ (2011), Comparative study of peroxidase purification from apple and orange seeds, *African Journal of Biotechnology* **10**(33): 6300-6303.