REFERENCES

1. Clowes A.E.E., Tatham, A.S., Beeching, J.R. and Shewry, P.R. Characterization of cassava root protein. CBN. 1994; Vol II: 716-27.
2. Sermsuvitayawong, K. The structure and properties of the isozymes of cassava linamarase. Master's Thesis. Department of Biochemistry, Faculty of Science, Mahidol University, 1991.
3. Chueskul, S. Cassava Hydroxynitrile lyase. Master's Thesis. Department of Biochemistry, Faculty of Science, Mahidol Univerity; 1995.
4. Booth, R.H. 1976. Storage of fresh cassava (*Manihot esculenta*). Postharvest deterioration and its contrl. Exp. Agric. 12: 103-111.
5. Rickard, J.E. 1985. Physiological deterioration of cassava roots. J. Sci. Food Agric. 36: 167-176.
6. Rickard, J.E. and Coursey, D.G. . 1981. Cassava storage. Part I: storage of fresh cassava. Trop. Sci. 23: 1-32.
7. Tanaka, Y., Data, E.S., Hirose, S., Taniguchi, T. and Uritani, I. 1983. Biochemical changes in secondary metabolites in wounded and deteriorated cassava roots. Agric. Biol. Chem. 47: 693-700.
8. Welinder, K.G. Superfamily of plant, fungal and bacterial peroxidase. Curr. Opin Struc. Biol. 1992; 2: 388-393.
9. Bowler, C., van Montagu, M. and D. Inze. Superoxide dismutase and stress tolerance. Annu. Rev. Plant Physiol. Plant. Mol. Biol. 1992; 43: 83-116.
10. Yu, Q., Rengel, Z. Drought and salinity differentially influence activities of superoxide dismutase, in narrow-leafed lupins. Plant Science 1999; 142: 1-11.

11. Mittler, R. and Zilinskas, B.A. Purification and characterization of pea cytosolic ascobate peroxidase. Plant Physiol. 1991; 97: 962-968.

12. Bunkelman, J.R. and Trelease, R.N. Ascorbate peroxidase. Plant Physiol. 1966; 110: 589-598.

13. Nakano, Y., Asada, K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 1981; 22: 867-880.

14. Asada, K. Ascorbate peroxidase. Physiologia Plantarum 1992; 85: 235-241.

15. Chen, G.-X., Asada, K. Hydroxyurea and p-aminophenol are the suicide inhibitors of ascorbate peroxidase. J. Biol. Chem. 1990; 265(5): 2775.

16. Ohya, T., Morimura, Y., Saji, H., Mihara, T. nd Ikaw, T. Purification and characterization of ascorate peroxidase in root of Japanese radish. Plant Science 1997; 125: 137-145.

17. Elisa, M.R., Borraccino, G. and Dipierro, S. Soluble ascorbate peroxidase from potato tubers. Plant Science 1992; 85: 17-21.

18. Eberhardt, t.L., Bernards, M.A., He, L., Devin, L.B. and Lewis, N.G. Signification in cell suspension cultures of *Pinus taeda*. J. Biol. Chem. 1993; 28: 21088-21099.

19. Hoyle, M.C. High resolution of peroxidase-indoleacetic acid oxidase isozymes from horseradish by isoelectric focusing. Plant Physiol. 1977; 60: 787-793.

20. Worthington Biochemical Corperation. Available from: http://www.worthington-biochem.com/priceList/P/Peroxidase.html

21. Takahama, U. and Egashira, T. Peroxidase in vacuoles of *Vicia faba* leaves. Phytochemistry 1991; 30: 73-77.

22. Keller, B. Structural cell wall proteins. Plant Physiol. 1993; 101: 1127-1130.

23. Cooper, J.B., Varner, J.E. Cross-linking of soluble extension in isolated cell wall. Plant Physiol. 1984; 76: 414-417.

24. Dey, M.P., Cantelides, A., Davies, G., Trevan, M. and Brownleader, M.D. Implications of potato peroxidase in cell wall architecture. Available from http://www.unique.ch/LABPV/books/per-appl/poster.html. [Accessed 1997].

25. Nakamura, W. and Nozy, Y. Studies on the biosynthesis of lignin. J. Biochem. 1967; Vol. 62: No. 3.

26. Sornwatana, T. and Chulavatnatol, M. Peroxidase isozymes from parenchyma of cassava. Presented in 25th Congress on Science and Technology of Thailand, Pitsanulok: 1999; B-071.

27. Wititsuwannakul, T. Wititsuwannalul, D., Sattayasevaha, B. and Pasitkul, P. Peroxidase from *Hevea Brasiliensis* bark. Phytochemistry 1997; 44: 2237-241.

28. Lagrimni, L.M. and Rothsteins, B.S. Peroxidase-induced witting in trangenic tobacco plants. Plant Cell 1990; 2: 7-18.

29. Nozu, Y. Studies on the biosynthesis of lignin. J. Biochem. 1967; vol. 62: 519-529.

30. Engvall, E. and Permann, P. Enzyme-linked immunosorbent assay (ELISA). Immunochemistry 1971; 8: 871-874.

31. Boonsiri, P. Peroxidase in Thai vegetables. Master's Thesis. Department of Biochemistry, Faculty of Science, Mahidol University, 1985.

32. Xiaoya, X. Spring greens peroxidase and its application. Master's Thesis. Department of Biochemistry, Faculty of Science, Mahidol University, 1987.

33. Glick, D. Peroxidase Assay. Methods of Biochemical Analysis. 1955; Vol I: 382-389.

34. Chance, B. and Kirk, T.K. Peroxidase assay by spectrophotometric measurements of the disappearance of hydrogen donor or the appearance of their colored oxidation products. Methods Enzymol. 1955; 2: 769-775.

35. Show calculations wherever calculations are needed. Available from http://www.mrs.umm.edu/~goochv/CellBio/labs/enz.html.

36. Kvaratskhelia, M., Winkel, C. and Thornely, R.N.F. Purification and characterization of a Noval Class III peroxidase isozyme from tea leaves. Plant Physiol. 1997; 144: 1237-1245.

37. Nozu, Y. Studies on the biosynthesis of lignin. J. Biochem. 1967; 62(5): 519-529.

38. Nada, M.S., Stojanov, M., Spasic, S. and Berkers, I. Spectrophotometric determination of serum uric acid by an enzymatic method with 2, 2;-azino-di(3-ethylbenzthiazoline-6-sulfonate). Clin. Chim. Acta 1981; 116: 117-123.

39. Ruengprapavut, S. and Chulavatnatol, M. A new chemiluminescent assay for linamarin. IUBMB Life 1999; 88: 219-223.

40. Tams, J.W. and Welinder, K.G. Mild chemical deglycosylation of horseradish peroxidase yields a fully active, homogeneous enzyme. Analytical Biochemistry 1995; 228: 48-55.

41. Tigier, H.A., Quesada, M.A., Heredia, A. and Valpuesta, V. Partial deglycosylation of an anionic isoperoxidase from peach seed-effect on enzyme activity, stability and antigenicity. Physiol. Plant 1991; 83: 144-148.

42. Sanchez-Romero, C., Garcia-Gomez, M.L., Pliego-Alfaro, F., and Heredia, A. Effect of partial deglycosylation on catalytic characteristics and stability of an avocado peroxidase. Physiol. Plant 1994; 92: 97-101.

43. Pharmacia LKB Biotechnology. Affinity Chromatography Principle and Method. 1993; 65-66.

44. Bradford, M.M. A rapid and sensitive method for determination of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

45. Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. Protein measurement with the folin phenol reagent. J. Biol. Chem. 1951; 193: 265-275.

46. Herzog, V. and Fahimi, H.D. A new sensitive colorimetric assay for peroxidase using 3, 3′-diaminobenzidine as hydrogen donor. Anal. Biochem. 1973; 55: 554-562.

47. Laemmli, U.K. Cleavage of structure protein during assembly of head of bacteriophage-T_4. Nature 1970; 227: 680-685.

48. Cameo, M.S. and Blaquier, J.A. An androgen controlled specific proteins in rat epididymis. J. Endocrnol. 1976; 69: 47-55.

49. Biorad Mini IEF Laboratory Manual. Bio-Rad company. 1990.

50. Tams, J.W. and Welinder, K.A. Mild chemical deglycosylation of horseradish peroxidase yields a fully active, homogeneous enzyme. Analytical Biochemistry 1995; 228: 48-55.

51. Dobois, M., Grills, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. Colorimetric method for determination of sugars and related substances. Anal. Chem. 1956; 28: 350-356.

52. Biorad Mini IEF Gel Manual. Bio-Rad company 1990.

53. Zacharius, R.M., Zell, T.E., Morrison, J.H. and Woodlock, J.J. Glycoprotein staining following electrophoresis on acrylamide gels. Anal. Biochem. 1969; 30: 148-152.

54. Rattanapume, P. and Surachittanont, W. Purification of peroxidase from *Hevea*l *brasillensis*. Presented in the 25th Congress on the Science Society of Thailand. Pitsanuloke: 1999; B-023.

55. Gazaryan, I.G. and Lagrimini, L.M. Purification and unusual kinetic properties of a tobacco anionic peroxidase. Phytochemistry 1996; 41: 1029-1034.

56. Suriyaprom, K. Characterization of peroxidase from cassava leaves. Master's Thesis. Department of Biochemistry, Faculty of Science, Mahidol University; 2000.

57. Zaton, A.M.L. and de Aspura, E.O. Horseradish peroxidase inhbition by thiouracil. FEBS Letters 1995; 374: 192-199.

58. Riquelme, A. and Cardemil, L. Two cationic peroxidase from cell walls of Aravcaria Aravcana seeds. Phytochemistry 1995; 39: 29-32.

60. Henorjks, T., Wijsman, H.J.W. and van Loon, L.C. Petunia peroxidase a isolation, purification and characteeristics. J. Biochem. 1991; 199: 139-146.

61. Christensen, J.H., Bauw, G., Welindern, K.G., Van Montagu, M. and Boerjan, K. Purification and characterization of peroxidases correlated with lignification in Poplar Xylem. Plant Physiol. 1998; 118: 125-135.

62. Shigeo, A. et al. Isolation and Properties of basic isoenzymes horseradish peroxidase. J. Biochem. 1981; 90: 489-496.

63. Nair, A.R. and Showalter, A.M. Purification and characterization of wound-inducible cell wall cationic peroxidase from carrot roots. Biochem. Biophysical Res. Commun. 1996; 226: 254-260.

64. Maranon, M.J.R. and van Huystee, R.B. Plant peroxidase: interaction between their prosthetic groups. Phytochemistry 1994; 37: 1218-1219.

65. Thamwarich, W. Study of enzyme peroxidase in *Hevea* bark tissue. Master's Thesis. Department of Biochemistry, Faculty of Science, Mahidol University; 1995.

66. Sreenivasula, N., et al. Total peroxidase activity and peroxidase isoforms as modified by salt stress in two cultivars of fox-tail millet with differential salt tolerance. Plant Science 1999; 141: 1-9.

67. Helfried, T., et al. Lignin peroxidase H_2 from phanerochaete chrysosporium: Archives of Biochemistry and Biophysics. 1990; 279: 158-166.

68. Suthiphongchai, T. Molecular characterization of non heme bromoperoxidase from Gracilaria species. Doctor of Philosophy Thesis. Department of Biochemistry, Faculty of Science, Mahidol University; 1993.

69. Hu, C. and van Huystee, R.B. Role of carbohydrate moieties in peanut peroxidases. Biochem. J. 1989; 263: 129-135.

70. Bernard, M.A. et al. Biochemical characterization of the suberization-associated anionic peroxidase of potato. Plant Physiol. 1999; Vol 121: 135-145.

71. Yaiyen, S. and Eksittikul, T. Partial purification and characterization of peroxidase in cassava root plantlet. Presented on 25th Congress on Science and Technology of Thailand. Pitsanulok: 1999; B-065.

72. Welinder, K.G., et al. Structure and organ specificity of an anionic peroxidase from *Arabidopsis thaliana* cell suspension culture. FEBS Letters 1996; 398: 243-247.

73. Eduardo Silva, et al. Enzymatic generation of triplet acetone by deglycosylated Horse-radish peroxidase. Archives of Biochemistry and Biophysics 1990; 276, No.2, 527-530.

APPENDICES

APPENDIX: A

Preparation for non-denaturing polyacrylamide gel electrophoresis

1. Stock reagents

30% Acrylamide, 0.8% bis-acrylamide, 100 ml

acrylamide 29.2 g

N,N-methylene-bis-acrylamide 0.8 g

Adjusted volume to 100 ml with distilled water

1.5 M Tris-HCl pH 8.8

Tris(hydroxymethyl)-aminomethane 18.17 g

Adjusted pH to 8.8 with 1 M HCl and adjusted volme to 100 ml with distilled water

2M Tris-HCl pH 8.8

Tris(hydroxymethyl)-aminomethane 24.2 g

Adjusted pH to 8.8 with 1 M HCl and adjusted volume to 100 ml with distilled water

0.5 M Tris-HCl pH 6.8

Tris(hydroxymethyl)-aminoethane 6.06 g

Adjusted pH to 6.8 with 1 M HCl and adjusted volume to 100 ml with distilled water

1 M Tris-HCl pH 6.8

Tris(hydroxymethyl)-aminoethane 12.1 g

Adjusted pH to 6.8 with 1 M HCl and adjusted volume to 100 ml with distilled water

Solution B (1.5 M Tris-HCl pH 8.8)

2 M Tris-HCl pH 8.8	75 ml
Distilled water	25 ml

Solution B-SDS (1.5 M Tris-HCl pH 8.8, 0.4% SDS)

2 M Tris-HCl pH 8.8	75 ml
10% SDS	4 ml
Distilled water	21 ml

Solution C (0.5 Tris-HCl pH 6.8)

1 M Tris-HCl pH 6.8	50 ml
Distilled water	50 ml

Solution C-SDS (0.5 M Tris-HCl pH 6.8, 0.4% SDS)

1 M Tris-HCl pH 6.8	50 ml
Distilled water	50 ml

5 x Sample buffer

1 M Tris-HCl pH 6.8	3.1 ml
Glycerol	5 ml
1% Bromphenol blue	0.5 ml
Distilled water	1.4 ml

Non-denaturing electrophoresis buffer, 1 litre

(25 mM Tris, 192 mM glycine)

Tris(Hydroxymethyl)-aminomethane	3.03 g
Glycine	14.40 g

Dissolved in distilled water to 1 litre without pH adjustment.

(Final pH should be 8.3)

SDS electrophoresis buffer, 1 litre

(25 mM Tris, 192 mM glycine, SDS 0.1%)

Tris (hydroxymethyl)-aminomethane	3.03 g
Glycine	14.40 g
SDS	1 g

Dissolved in distilled water to 1 litre without pH adjustment

(Final pH should be 8.3).

2. Preparation of Non-denaturing PAGE

Reagent		Stacking gel	Separating gel
		3%	5%
30% Acrylamide, 0.8% Bis	(ml)	1	1.3
1.5 M Tris-HCl, pH 8.4	(ml)	-	2
0.5 M Tris-HCl, pH 6.8	(ml)	2.5	-
Distilled water	(ml)	6.5	4.68
10% Ammonium persulpate	(µl)	25	10
100% TEMED	(µl)	5	5
Final volume	(ml)	10	8

3. Preparation of SDS-PAGE

Reagent		Stacking gel	Separating gel
		3%	10%
30% Acrylamide, 0.8% Bis	(ml)	1	5.3
1.5 M Tris-HCl, pH 8.8	(ml)	-	4
0.5 M Tris-HCl, pH 6.8	(ml)	2.5	-
10% SDS	(ml)	0.1	0.16
Distilled water	(ml)	6.4	6.5
10% Ammonium persulpate	(µl)	2.5	50
100% TEMED	(µl)	5	10
Final volume	(ml)	10	16

4. Protein staining

After electrophoresis the gel was stained for protein in 0.2% Brilliant blue R-250 in 50% methanol and 10% acetic acid for 2-4 hr at room temperature with moderate shaking. Destaining was performed by immersing the gel overnight in a solution 25% ethanol and 7% acetic acid until the background of the gel was clear.

APPENDIX : B

Preparation for isoelectric focusing gel electrophoresis

Mononer-ampholyle solution

30% Acrylamide solution	0.9	ml
1.0% Bis-acrylamide solution	1.25	ml
Ampholyte pH 3-10	0.243	ml
Distilled water	1.39	ml
50% sucrose	1.186	ml
TEMED	2	µl
0.02 M $(NH_4)_2S_2O_8$	39.5	µl

Fixative solution, 100 ml

Sulfosalicylic acid	4	ml
Trichloroacetic acid	12.5	g
Methanol	30	ml

Immerse gel in the solution for 30 min.

Staining solution, 100 ml

Ethanol	27	ml
Acetic acid	10	ml
Coomassie brilliant blue R-250	0.04	g
$CuSO_4$	0.5	g
Distilled water	63	ml

Dissolve the $CuSO_4$ in water before adding the alcohol. Either dissolve the dye in alcohol or add it to the solution at the end. Immerse the gel in the stain for approximately 1-2 hrs.

Destaining solution

First destaining solution

Ethanol	12	ml
Acetic acid	7	ml
$CuSO_4$	0.5	g
Distilled water	81	ml

Dissolve the cupric sulfate in water fore adding the alcohol.

Immerse the gel in two or three changes of this solution until the background is nearly clear.

Second destaining solution

Ethanol	25	ml
Acetic acid	7	ml
Distilled water	68	ml

Immerse the gel in this solution to remove the last traces of stain and $CuSO_4$.

APPENDIX : C

Preparation for phenol-sulfuric acid (PAS) staining solution

Fixative solution

Ethanol	200	ml
Glacial acetic acid	20	ml

Adjust volume to 500 ml with distilled water

Schiff's reagent

1. Dissolve 10 g of basic fuchsin in 21 ml of distilled water with heating. Cool in icd-bath to 50°C (not below 40°C).

2. Add 200 ml of 1 N HCl. Mix, and cool to 25°C.

3. Add 17 g of sodium metabisulfige. Mix, and let sit overnight at 4°C in the dark.

4 Add 20 g of HCl wash charcoal, and centrifuge charcoal to avoid contact with filter paper. Filter the supernatant through glass wool to remove remaining charcoal, the filtrate should be clear and colorless.

5. Stored in a brown bottle at 4°C.

0.7% Periodic acid solution

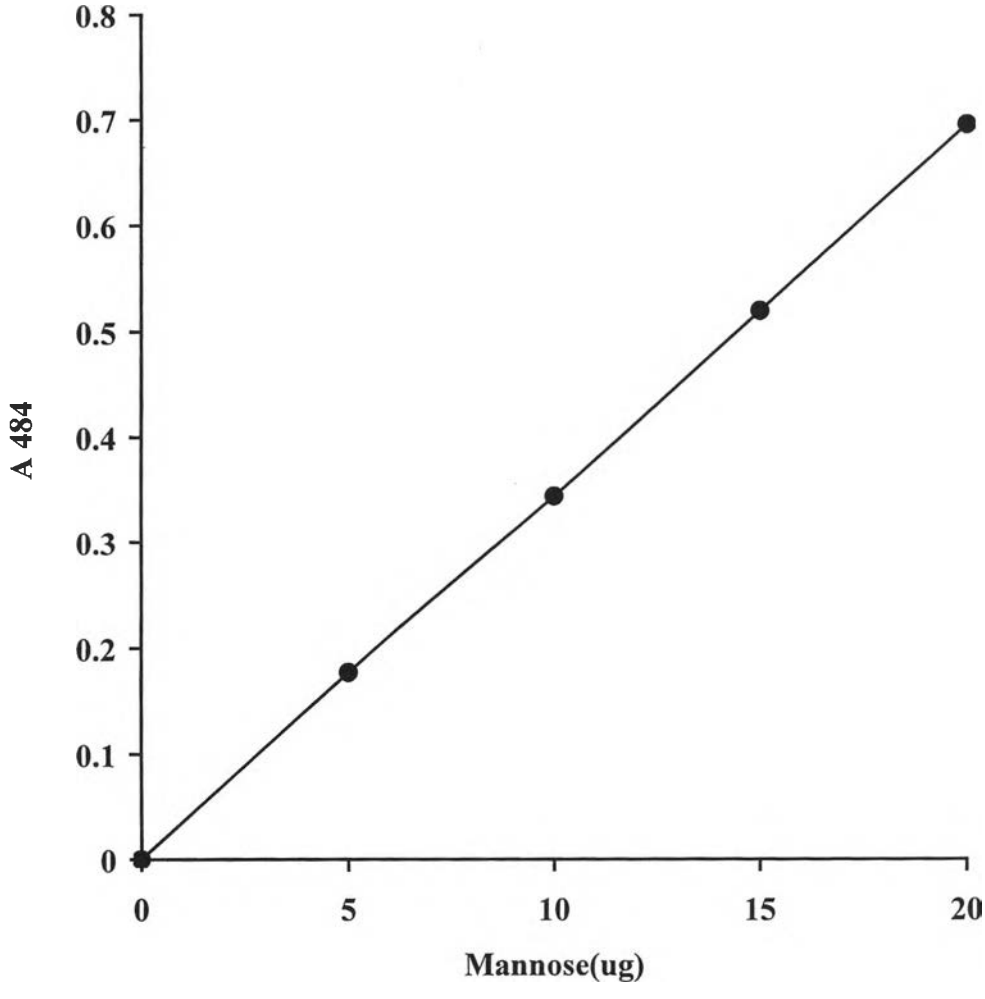
Periodic acid	1.4	g
5% Acetic acid	200	ml

0.2% Sodium metabisulfite

Sodium metabisulfite	0.4	g
5% Acetic acid	200	ml

APPENDIX : D

1. Calibration curve of sugar determination by phenol-sulfuric acid method.



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