REFERENCES

Abraham, T. E. 1995. Immobilized cyclodextrin glycosyltransferase for the continuous production of cyclodextrins. *Biocatal. Biotransform.* 12: 137-146.

Aloson, N., López-Gallego, F., Betancor, L., Hidalgo, A., Mateo, C., Guisan, J. M., and Fernandez-Lafuente, R. 2005. Immobilization and stabilization of glutaryl acylase on aminated sepabeads supports by the glutaraldehyde crosslinking method, *J. Mol. Catal. B: Enzym.* 35: 57-61.

Anderson, M. M., and Hatti-Kual, R. 1999. Protein stabilizing effect of poly ethyleneimine. Biotechnol. 72: 21-31.

Arica, M. Y, Bayramoğlu, G., and Bicak, N. 2003. Charaterisation of tyrosinase immobilized onto spacer-arm attached glycidyl methylate-based reactive microbeads. *Process Biochem.*

Asano, Y., and Nakazawa, A. 1985. Crystallization of phenylalanine dehydrogenase from *Sporosarcina ureae*. *Agric. Biol. Chem.* 49: 3631-3632.

Asano, Y., and Nakazawa, A. 1987. High yield synthesis of L-amino acids by phenylalanine dehydrogenase from *Sporosarcina ureae*. *Agric. Biol. Chem.* 51: 2035-2037.

Asano, Y., Nakazawa, A., and Endo, K. 1987a. Novel phenylalanine dehydrogenase from *Sporosarcina ureae* and *Bacillus sphaericus*: Purification and characterization. *J. Biol. Chem.* 262: 10346-10354.

Asano, Y., Nakazawa, A., Endo, K., Hibino, Y., Ohmori, M., Numuo, N., and Kondo, K. 1987b. Phenylalanine dehydrogenase of *Bacillus badius*: Purification, characterization and gene cloning. *Eur. J. Biochem.* 168: 153-159.

Asano, Y., Endo, K., Nakazawa, A., Hibino, Y., Okazaki, N., Ohmori, M., Numao, N., and Kondo, K. 1987c. *Bacillus* phenylalanine dehydrogenase produced in *Escherichia coli*: Its purification and application to L-phenylalanine synthesis. *Agric. Biol. Chem.* 51: 2621-2623.

Asano, Y., and Tanetani, M. 1998. Thermostable phenylalanine dehydrogenase from a mesophilic *Microbacterium* sp. strain DM 86-1. *Arch. Microbiol.* 169: 220-224.

Atherton, N. D., and Green, A. 1988. HPLC measurement of phenylalanine in plasma. *Clin. Chem.* 34: 2241-2244.

Atia, K. S. 2005. Co-immobilization of cyclohexanone monooxygenase and glucose-6-phosphate dehydrogenase onto polytehyleneimine-porous agarose polymeric composite using γ irradiation to use in biotechnological processes. *Radia. Phys. Chem.* 73: 91-99.

Baker, p. J., Waugh, M. L., Wang, X., Stillman, T. J., Turnbull, A. P., Engel, P. C., and Rice, D. w. 1998. Determinants of substrate specificity in super family of amino acid dehydrogenase. *Biochem.* 36: 16109-16115.

Bayramoğlu, G, Kaya, B., and Arica, M.Y. 2005. Immobilization of *Candida rugosa* lipase onto spacer-arm attached poly (GMA-HEMA-EGDMA) microspheres. *Food chem.* 92: 261-268.

Bayramoğlu, G, Kiralp, S., Yilmaz, M., Toppare, L., and Arica, M. Y. 2008. Covalent immobilization of chloroperoxidaes onto magnetic bead: Catalytic properties and stability. *J. Biochem. Eng.* 36: 180-188.

Blasi, L., Longo, L., Vasapollo, G., Cingolani, R., Ranaldi, R., Rizzello, T., Acierno, R., and Maffia, M. 2005. Characterization of glutamate dehydrogenase immobilization on silica surface by atomic force microscopy and kinetic analyses. *Enzyme. Microb. Technol.* 36: 818-823.

Boer, L. D., Rijssel, M. V., Euverink, G. J., and Dijkhuizen, L. 1989. Purification, characterization, and regulation of a monomeric L-phenylalanine dehydrogenase from the facultative methyltroph *Nocardia* sp. 239. *Arch. Microbiol.* 153: 12-18.

Bollag, D. M., Rozycki, M. D., and Edelstein, S. J. 1996. *Protein methods.* 2nd ed. New York: Wiley-Liss. pp. 72-77.

Bongaerts, J., Kramer, M., Muller, U., Raeven, L., Wubbolts, M. 2001. Metabolic engineering for microbial production of aromatic amino acids and derived compounds. *Metab. Eng.* 3: 289-300.

Brunhuber, N. M. W., and Blanchard, J. S. 1994. The biochemistry and enzymology of amino acid dehydrogenases. *Critical reviews in biochemistry and molecular biology* 29: 415-467.

Brunhuber, N. M. W., Thoden, J. B., Blanchard, J. S., and Vanhooke, J. L. 2000. *Rhodococcus* L-phenylalanine dehydrogenase: Kinetics, mechanism, and structural basis for catalytic specificity. *Biochem.* 39: 9174-9187.

Budzinski, A. 2000. Aminosauren, Peptide und die Chemie dazu. Chemische Rundschau. 6: 10, ISSN 0009-2983.

Chao, Y. P., Lo, T. E., and Luo, N. S. 2000. Selective production of L-aspartic acid and L-phenylalanine by coupling reaction of aspartase and aminotransferase in *Escherichia coli*. *Enzyme. Microb. Technol.* 27: 19-25.

Chaplin, M. F., and Bucke, C. 1990. *Enzyme technology*, 80-89. Great Britain: Cambridge University Press.

Chase, H. A., and Yang, Y. 1998. Immobilization of enzymes on poly (vinyl alcohol)-coated perfluorocarbon supports: comparison of techniques for the immobilization of trypsin and α-amylase on poly (vinyl alcohol)-coated solid and liquid perfluorocarbons. *Biotechnol. Appl. Biochem.* 27: 205-216.

Chibata, I. Immobilization enzyme, research and development. 1987. Kodansha science book.

Chiou, SH., and Wu, WT. 2004. Immobilization of *Candida rugosa* lipase on chitosan with activatation of the hydroxyl groups. *Biomaterials*. 25: 197-204.

Chumphukam, O. 2004. Immobilization of phenylalanine dehydrogenase: A comparative study of various support, optimal conditions and characterization. Master's Thesis. Department of Biochemistry, Science, Chulalongkorn University.

Creighton, T. E. 1993. *Proteins: Structures and molecular properties.* 2nd ed. New York: W. H. Freeman and Company. pp. 262-264.

Fernandez-Lafuente, R., Rosell, C. M., Rodriguez, V., Santana, C., Soler, G., Bastida, A., and Guisan, J. M. 1993. Preparation of activated supports containing low pK amino groups. A new tool for protein immobilization via the carboxyl coupling method. *Enzyme. Microb. Technol.* 15: 546-550.

Field, R. 1972. The rapid determination of amino acid with TNBS. *Methods Enzymol.* 25: 464-467.

Galkin, A., Kulakova, L., Yoshimura, T., Soda, K., and Esaki, N. 1997. Synthesis of optically active amino acids from α-keto acids with *Escherichia coli* cells expressing heterologous genes. *Appl. Environ. Microbiol.* 63(12): 4651-4656.

Gao, B., Wang, X., and Shen, Y. 2006. Studies on characters of immobilizing penicillin G acylase on a novel composite support PEI/Sio_2. *J. Biochem. Eng.* 28: 140-147.

Gennari, F., Miertus, S., Stredansky, M., and Pizzio, F. 1998. Used of biocatalysts for industrial application. *Gen. Eng. Biotechnol.* Vol. 3

Girotti, S., Ferri, E., Ghini, S., and Budini, R. 1993. Bioluminescent flow sensor for L-phenylalanine determination in serum. *Talanta.* 40(3): 425-430.

Glazer, A. N., DeLang, R. J., and Sigma, D. S. 1976. Chemical modification of proteins, In T. S. Work and E. Work (eds.), *Laboratory techniques in biochemistry and molecular biology*, vol.4, pp. 69-134. Oxford: North-Holland publishing Co., Inc.

Groot, M. N. N., and de Bont, J. A. M. 1998. Conversion of phenylalanine to benzaldehyde initiated by an aminotransferase in *Lactobacillus plantarum. Appl. Environ. Microbiol.* 64: 3009-3013.

Guthrie, R., and Susi, A. 1963. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. *Pediatrics.* 32(3): 338-343.

Hanson, R. L., Howell, J. M., LaPorte, T. L., Donovan, M. J., Cazzulino, D. L., Zannella, V., Montana, M. A., Nanduri, V. B., Schwarz, S. R., Eiring, R. F., Durand, S. C., Wasylyk, J. M., Parker, W. L., Liu, M. S., Okuniewicz, F. J., Chen, B., Harris, J. C., Natalie, K. J., Ramig, K., Swaminathan, S., Rosso, V. W., Pack, S. K., Lotz, B. T., Bernot, P. J., Rusowicz, A., Lust, D. A., Tse, K. S., Venit, J. J., Szarka, L. J., and Patel, R. N. 2000. Synthesis of allysine ethylene acetal using phenylalanine dehydrogenase from *Thermoactinomyces intermedius. Enzyme. Microb. Technol.* 1: 348-358.

Harris, E. L. V., and Angal, S., eds. 1989. *Protein purification methods: A practical approach.* New York: IRL press. 179 pp.

Hoare D. G., and Koshland, Jr. 1967. A method for the quantitative modification and estimation of carboxylic acid groups in proteins. *J. Biol. Chem.* 242: 2247-2253.

Hong, J., Xu, D., Gong, P., Yu, J., Ma, H., and Yao, S. 2007. Covalent-bonded immobilization of enzyme on hydrophilic polymer covering magnetic nanogels. *Micropor. Mesopor. Mater.*

Hung, T-C., Giridhar, R., Chiou, S-H., and Wu, W-T. 2003. Binary immobilization of Candida rugosa lipase on chitosan. *J. Mol. Catal. B: Enzym.* 26: 69-78.

Huang, T., Warsinke, A., Kuwana, T., and Scheller, F. W. 1998. Determination of L-phenylalanine based on an NADH-detecting biosensor. *Anal. Chem.* 70: 991-997.

Hummel, W., Weiss, N., and Kula, M. R. 1984. Isolation and characterization of a bacterium possessing L-phenylalanine dehydrogenase activity. *Arch. Microbiol.* 137: 47-52.

Hummel, W., Schutte, H., Schmidt, E., Wandrey, C., and Kula, M. R. 1987. Isolation of L-phenylalanine dehydrogenase from *Rhodococcus* sp. M4 and its application for the production of L-phenylalanine. *Appl. Microbiol. Biotechnol.* 26: 409-416.

Hummel, W., and Kula, M. R. 1989. Dehydrogenases for the synthesis of chiral compounds. *Eur. J. Biochem.* 184: 1-13.

Inkure, S. 2005. Purification and characterization of phenylalanine dehydrogenase from *Bacillus lentus.* Master's Thesis. Biotechnology Program, Science, Chulalongkorn University.

Janson, J. C., and Ryden, L., eds. 1998. *Protein purification: Principles, high-resolution methods and applications.* New York: Wiley-Liss, Inc. pp. 283-309.

Kang, K., Kan, C., Yeung, A., and Liu, D. 2006. The immobilization of trypsin on soap-free P(MMA-EA-AA) latex particles. *Mater. Sci. Eng.* 26: 664-669.

Kataoka, K., Takada, H., Tanizawa, K., Yoshimura, T., Esaki, N., Ohshima, T., and Soda, K. 1994. Construction and characterization of chimeric enzyme consisting of an amino-terminal domain of phenylalanine dehydrogenase and a carboxy-terminal domain of leucine dehydrogenase. *J. Biochem.* 116: 931-936.

Kenedy, J. F., and Cabral, J. M. 1987. *Enzyme immobilization.* In Biotechnol, Vol. 9, Chapter 7a, 349-350. VCH, Weinheim.

Keradjipoulos, D., and Holldorf, A. W. 1979. Purification and properties of alanine dehydrogenase from *Halobacterium salinarium. Biochem. Biophys. Acta.* 570: 1-10.

Kiba, N., Kato, A., and Furusawa, M. 1995. Determination of branched-chain L-amino acids by flow-injection analysis with co-immobilized leucine dehydrogenase/ NADH oxidase and chemiluminescence detection. *Anal. Chim. Acta.* 311: 71-76.

Kiba, N., Itagaki, A., and Furusawa, M. 1997. Determination of L-phenylalanine in serum by flow-injection analysis using immobilized phenylalanine dehydrogenase and fluorometric detection. *Talanta.* 44: 131-134.

Kiba, N., Shimura, G., Hayashi, H., Tachibana, M., Tani, K., Koizumi, H., and Hibino, Y. 2000. Silmutaneous determination of L-phenylalanine and branched-chain L-amino acids in plasma by liquid chromatographic system with a co-immobilized enzyme reactor and fluorescence detection. *Anal. Sciences*. 16: 981-984.

Koeller, KM., and Wong, CH., 2001. Enzymes for chemical synthesis. *Nature*. 409: 232-240.

Leksakorn, A. 2001. Purification and characterization of phenylalanine dehydrogenase from thermotolerant *Bacillus badius* BC1. Master's Thesis. Department of Biochemistry, Science, Chulalongkorn University.

Lopez-Gallego, F., Betancor, L., Hidalgo, A., Alonso, N., Fernandez-Lorente, G., Guisan, J. M., and Fernandez-Lorente, R.2005. Preparation of a robust biocatalyst of amino acid oxidase on sepabeads supports using the glutaraldehyde crosslinking method. *Enzyme. Microb. Technol.*

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* 193: 265-275.

Martin, M. T., Alcalde, M., Plou, F., and Ballesteros, A. 2002. Covalent immobilization of cyclodextrin glucosyltransferase (CGTase) in activated silica and sepharose. *J. Biochem. Biophy.* 39: 229-234.

Mateo, C., Abian, O., Fernandez-Lafuente, R., and Guisan, J. M. 1999. Reversible enzyme immobilization via a very strong and nondistorting ionic adsorption on support polyethyleneimine composites. John Wiley & Sons. pp. 98-105.

Mateo, C., Palomo, J. M., Fernandez-Lorente, G., Guisan, J. M., and Fernandez-Lafuente, R. 2007. Improvement of enzyme activity, stability and selectivity via immobilization techniques. 40: 1451-1463.

McCaman, M., W., and Robins, E. 1962. Fluorometric method for the determination of phenylalanine in serum. *J. Clin. Med.* 59: 885-890.

Mean, G. E., and Feeney, R. E. 1971. Chemical modification of proteins. California: Holden-Day, Inc.

Mile, A. M., and Smith, R. L. 1993. Functional methionines in the collagen/gelatin binding domain of plasma fibronectin: effects of chemical modification by chloramine T. *J. Biochem.* 32: 8168-8178.

Misono, H., Yonezawa, J., Nagata, S., and Nagasaki, S. 1989. Purification and characterization of a dimeric phenylalanine dehydrogenase from *Rhodococcus maris* K-18. *J. Bacteriol.* 171: 30-36.

Mohy Eldin, M. S., Schroen, C. G. P. H., Janssen, A. E. M. and Mita, D.G. 2000. Immobilization of penicillin G acylase onto chemically grafted nylon particles. *J. Mol. Catal. B: Enzym.* 10: 445-451.

Műller, A. 2001. Hartes Gerangel um den Futtertring. Chemische Rundschau. 6: 1

National Institues of Health Consensus Development. 2001. Phenylketonuria: screening and management. *Pediatrics.* 108: 972-982.

Nelson, D. L., and Cox, M. M. 2000. *Lehninger Principles of Biochemistry.* 3rd. New York: Worth Publishers. p 243.

Neckers, L. M., Delisi, L. E. and Wyatt, R. J. 1980. Liquid-chromatography quantification of plasma phenylalanine, tyrosine, and tryptophan. *Clin. Chem.* 27(1): 146-148.

Ohshima, T., Nagata, S.,and Soda, K. 1985. Purification and characterization of thermostable leucine dehydrogenase from *Bacillus stearothermophilus. Arch. Microbiol.* 141: 407-411.

Ohshima, T., and Soda, K. 1990. Biochemistry and biothecnology of amino acid dehydrogenase. Adv. Biochem. Eng. Biotechnol. 42: 187-209.

Ohshima, T., Takada, H., Yoshimura, T., Esaki, N., and Soda, K. 1991. Distribution, purification, and characterization of thermostable phenylalanine dehydrogenase from thermophilic actinomycetes. *J. Bacteriol.* 173: 3943-3948.

Ohshima, T., and Soda, K. 2000. Stereoselective biocatalyst: Amino acid dehydrogenases and their applications. In: Ramesh N. Patel (ed), *Stereoselective Biocatalysis*, New York: Marcel Dekker. pp. 877-902.

Onprasert, K. 2000. Investigation of pyruvcate binding site of alanine dehydrogenase from *Aeromonase hydrophila* by chemical modification. Master's Thesis. Department of Biochemistry, Science, Chulalongkorn University.

Park, D., Haam, S., Jang, K., Ahn, I-S., and Kim, W-S. 2005. Immobilization of starch-converting enzymes on surface-modified carriers using single and co-immobilized system: properties and application to starch hydrolysis. *Process Biochem.* 40: 53-61.

Patel, R. N. 2001. Enzymatic synthesis of chiral intermediates for Omapatrilat, an antihypertensive drug. *Biomolecular Engineering*. 17: 167-182.

Prousoontorn, H. M., and Pantatan, S. 2007. Production of 2-O-α-glucopyranosyl L-ascorbic acid from ascorbic acid β-cycrodextrin using immobilized cycrodextrin glycosyltransferase. *J. Incl. Phenom. Macrocycl. Chem.* 57: 39-46.

Ragnitz, K., Syldatk, C., Pietzsch, M. 2001. Optimization of the immobilization parameters and operational stability of immobilized hydantoinase and L-*N*-carbamoylase from *Arthrobactor aurescens* for the production of optically pure L-amino acids. *Enzyme Microb. Technol.* 20: 713-720.

Robin, S. P., and Reeds, P. J. 1984. Measurement of α-keto acids in plasma using amino acid analyzer. *Anal. Biochem.* 142: 24-27.

Rudy, J. L., Rutledge, J. C. and Lewis, S. L. 1987. Phenylalanien and tyrosine in serum and eluates from dried blood spots as determinaed by reversed-phase liquid chromatography. *Clin. Chem.* 33(7): 1152-1154.

Sitthai, P. 2004. Cloning and expression of phenylalanine dehydrogenase gene from *Acinetobacter lwoffii* and the possibility for amino acid production. Master's Thesis. Department of Biochemistry, Science, Chulalongkorn University.

Subramanian, A., Kennel, S. J., Oden, P. I., Jacobson, K. B., Woodward, J., and Doktyez., M. J. 1999. Comparision of techniques for enzyme immobilization on silica support. *Enzyme. Mricrob. Technol.* 24: 26-34.

Tachibana, S., Suzuki, M., and Asano,Y. 2006. Application of enzyme chip to the microquantification of L-phenylalanine. *Anal. Biochem.* 359: 72-78.

Takada, H., Yoshimura, T., Ohshima, T., Esaki, N., and Soda, K. 1991. Thermostable phenylalanine dehydrogenase of *Thermoactinomyces intermedius*: Cloning, expression, and sequencing of its gene. *J. Biochem. (Tokyo)* 109: 371-376.

Tynan, J., Forde, J., McMahon, M., and Mulcahy, P. 2000. Synthesis of a highly substitured N^6-linked immobilized NAD^+ derivative using a rapid solid-phase modular approach: suitabikity for use with the kinetic locking-on tactic for bioaffinity purification of NAD^+-dependent dehydrogenase. *Protein Expression and Purification*. 20: 421-434.

Vanhooke, J. L., Thoden, J. B., Norbert, M., Brunhuber, W., Blanchard, J. S., and Holden, H. M. 1999. Phenylalanine dehydrogenase from *Rhodococcus sp.* M4:

High-resolution X-ray analyses of inhibitory ternary complexes reveal key features in the oxidative deamination mechanism. *Biochemistry.* 38: 2326-2339.

Villalonga, R., Tachibana, S., Perez, Y., and Asano, Y. 2005. Increased conformational and thermal stability properties for phenylalanine dehydrogenase by chemical glycosidation with end-group activated dextran. *Biotechnol. Lett.* 27: 1311-1317.

Villalonga, R., Fujii, V., Shinohara, H., Tachibana, S., and Asano, Y. 2008. Covalent immobilization of phenylalanine dehydrogenase on cellulose membrane for biosensor construction. *Sensor.Actuators B.* 129: 195-199.

Voet, D., and Voet, J. G. 2004. *Biochemistry.* 3rd ed. USA: Von Hoffmann Corporation. pp. 76-78, 985.

Wakayama, M., Tsutsumi, T., Yada, H., Sakai, K., and Moriguchi, M. 1996. Chemical modification of histidine residue of *N*-acetyl-D-glutamate amidohydrolase from Pseudomonas sp. 5f-1. *Biosci. Biotech . Biochem.* 60(4): 650-653.

Wang, Z-H., and Jin, G. 2004. Covalent immobilization of preteins for the biosensor based on imaging ellipsometry. J. Immunal. Methods. 285: 237-243.

Weetall, H. H.1993. Preparation of immobilized proteins covalent coupled through silane coupling agents to inorganic supports. *App. Biochem. Biotech.* Vol. 41.

Wendel, U., Koppelkamm, M., and Hummel, W. 1991. Emzymatic phenylalanine estimation for the management of patients with phenylketonuria. *Clinica. Chimica. Acta.* 201: 95-98.

Wong, CH., and Whitesides, GM. 1994. Enzyme in synthetic organic chemistry. New York: John Wiley & Sons. pp. 273-279.

APPENDICES

APPENDIX A

Preparation for protein determination

Reagent for determination of protein concentration (modified from Lowry *et al.*, 1951)

Solution A (0.5% copper sulfate and 1% potassium tartate, pH 7.0)

Potassium tartate	1	g
Copper sulfate	0.5	g

Adjusted pH to 7.0 and adjust the solution volume to 100 ml.

Solution B (2% sodium carbonate and 1 N sodium hydroxide)

Sodium carbonate	20	g
Sodium hydroxide	4	g

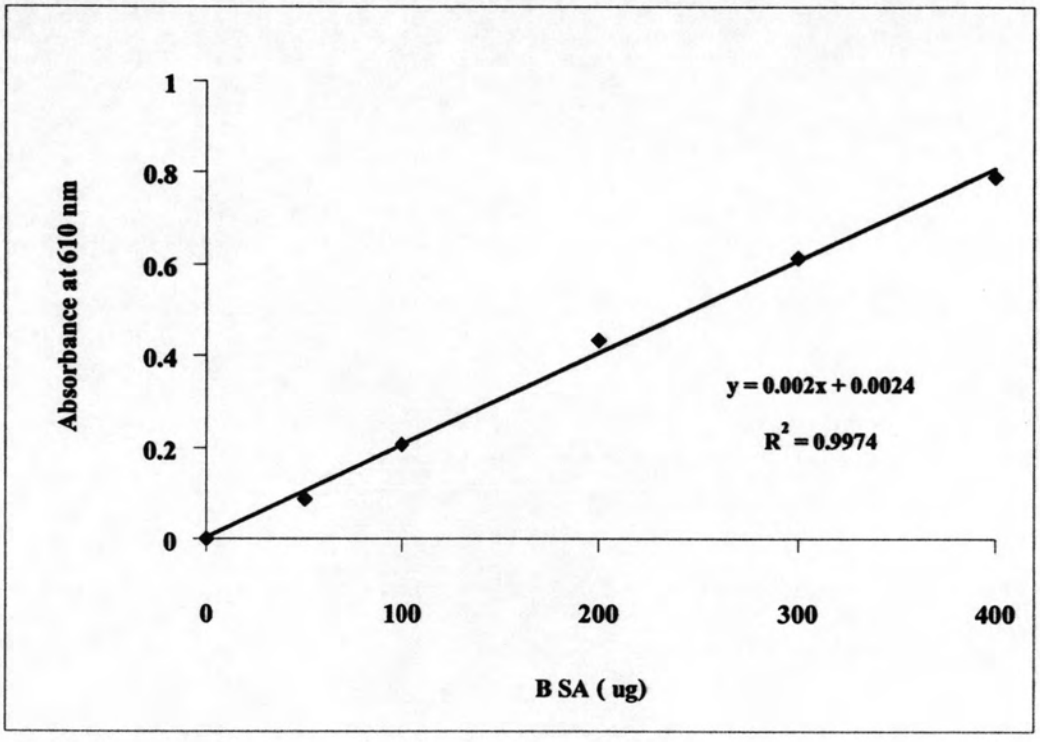
Dissolved in distilled water to 1 liter.

Solution C (phenol reagent)

Folin-Ciocalteu phenol reagent used in this work was reagent grade from Carlo Erba, Italy.

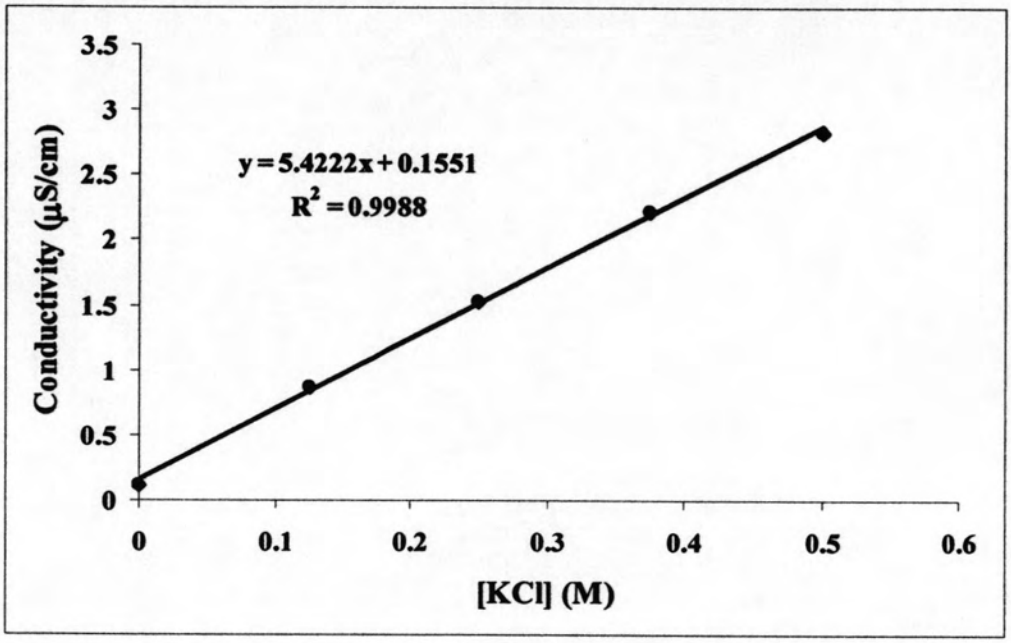
APPENDIX B

Standard curve for protein determination by Lowry' s method



APPENDIX C

Calibration curve for conductivity of potassium chloride



APPENDIX D

Preparation for non-denaturing polyacrylamide gel electrophoresis (Native-PAGE)

1. Stock solutions

2 M Tris-HCl (pH 8.8)

Tris (hydroxymethyl)-aminomethane	24.2	g

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

1 M Tris-HCl (pH 6.8)

Tris (hydroxymethyl)-aminomethane	12.1	g

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

1% (w/v) Bromophenol blue

Bromophenol blue	100	mg

Brought to 10 ml with distilled water and stirred until dissolved.

The aggregated dye was removed by filtration.

2. Working solutions

Solution A (30% (w/v) acrylamide, 0.8% (w/v) bis-acrylamide)

Acrylamide	29.2	g
N, N'-methylene-bis-acrylamide	0.8	g

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 C (0.5 M Tris-HCl, pH 6.8)

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

APPENDIX D (continued)

10% (w/v) Ammonium persulfate

Ammonium persulfate	0.5	g
Distilled water	5.0	ml

Electrophoresis buffer (25 mM Tris, 192 mM glycine)

Tris (hydroxymethyl)-aminomethane	3.0	g
Glycine	14.4	g

Dissolved and adjusted to total volunm 1 liter with distilled water (final pH should be approximately 8.3)

5x Sample buffer (312.5 mM Tris-HCl pH 6.8, 50% (v/v) glycerol, 1% (v/v) bromophenol blue)

1 M Tris-HCl (pH 6.8)	0.6	ml
Glycerol	5.0	ml
1% Bromophenol blue	0.5	ml
Distilled water	1.4	ml

3. Native-PAGE

7.7% Separating gel

Solution A	2.6	ml
Solution B	2.5	ml
Distilled water	4.9	ml
10% (w/v) Ammonium persulfate	50	µl
TEMED	5.0	µl

5.0% Stacking gel

Solution A	0.67	ml
Solution C	1.0	ml
Distilled water	2.3	ml
10% (w/v) Ammonium persulfate	30	µl
TEMED	5.0	µl

APPENDIX D (continued)

4. Protein staining solution

Staining solution, 1 liter

Coomassie brilliant blue R-250	1.0	g
Glacial acetic acid	100	ml
Methanol	450	ml
Distilled water	450	ml

Destaining solution, 1 liter

Methanol	100	ml
Glacial acetic acid	100	ml
Distilled water	800	ml

5. Enzyme activity staining solution

1 M Tris-HCl, pH 8.5

Tris (hydroxymethyl)-aminomethane 6.06 g

Adjusted to pH 8.5 with 1 N HCl and made up volume to 100 ml with distilled water

40 mM L-phenylalanine

L-phenylalanine 0.066 g

Dissolved with 10 ml distilled water

50 mM NAD^+

NAD^+ 0.359 g

Dissolved with 10 ml distilled water

0.25 mg/ml phenazine methosulfate

Phenazine methosulfate 0.0025 g

Dissolved with 10 ml distilled water

2.5 mg/ml nitroblue tetrazolium

Nitroblue tetrazolium 0.025 g

Dissolved with 10 ml distilled water

Activity staining solution (4.25 mM Tris-HCl, pH 8.5, 40 μM L-phenylalanine 50 μM NAD^+, 250 μg phenazine methosulfate and 2.5 mg nitroblue tetrazolium)

1 M Tris-HCl, pH 8.5	4.25	ml
40 mM L-phenylalanine	1.0	ml
50 mM NAD^+	1.0	ml
0.25 mg/ml phenazine methosulfate	1.0	ml
2.5 mg/ml nitroblue tetrazolium	1.0	ml
Distilled water	1.75	ml

APPENDIX E

Preparation for denaturing polyacrylamide gel electrophoresis

1. Stock solution

2 M Tris-HCl (pH 8.8)

Tris (hydroxymethyl)-aminomethane	24.2	g

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

1 M Tris-HCl (pH 6.8)

Tris (hydroxymethyl)-aminomethane	12.1	g

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

10% (w/v) SDS

Sodium dodecyl sulfate (SDS)	10	g

Added distilled water to a total volume of 100 ml.

50% (w/v) Glycerol

100% Glycerol	50	ml

Added distilled water to a total volume of 100 ml.

1% (w/v) Bromophenol blue

Bromophenol blue	100	mg

Brought to 10 ml with distilled water and stirred until dissolved.

The aggregated dye was removed by filtration.

2. Working solutions

Solution A (30% (w/v) acrylamide, 0.8% (w/v) bis-acrylamide)

Acrylamide	29.2	g
N, N'-methylene-bis-acrylamide	0.8	g

Adjusted volume to 100 ml with distilled water.

Filtered and stored in dark (brown bottle) at 4°C

APPENDIX E (continued)

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

2 M Tris-HCl (pH 8.8)	75	ml
10% (w/v) SDS	4	ml
Distilled water	21	ml

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

1 M Tris-HCl (pH 6.8)	50	ml
10% (w/v) SDS	4	ml
Distilled water	46	ml

10% (w/v) Ammonium persulfate

Ammonium persulfate	0.5	g
Distilled water	5.0	ml

Electrophoresis buffer (25 mM Tris, 192 mM glycine and 0.1% (w/v) SDS)

Tris (hydroxymethyl)-aminomethane	3.0	g
Glycine	14.4	g
SDS	1	g

Dissolved and adjusted to total volume to 1 liter with distilled water (final pH should be approximately 8.3)

5x Sample buffer (312.5 mM Tris-HCl pH 6.8, 50% (v/v) glycerol, 1% (w/v) bromophenol blue)

1 M Tris-HCl (pH 6.8)	0.6	ml
50% (v/v) Glycerol	5.0	ml
10% (w/v) SDS	2	ml
1% (w/v) Bromophenol blue	1	ml
β-Mercaptoethanol	0.5	ml
Distilled water	1.4	ml

APPENDIX E (continued)

3. SDS-PAGE

10% Separating gel

Solution A	3.3	ml
Solution B	2.5	ml
Distilled water	4.2	ml
10% (w/v) Ammonium persulfate	50	µl
TEMED	5	µl

5.0% Stacking gel

Solution A	0.67	ml
Solution C	1.0	ml
Distilled water	2.3	ml
10% (w/v) Ammonium persulfate	30	µl
TEMED	5	µl

4. Protein staining solution

Staining solution, 1 liter

Coomassie brilliant blue R-250	1.0	ml
Methanol	450	ml
Distilled water	450	ml

Destaining solution, 1 liter

Methanol	100	ml
Glacial acetic acid	100	ml
Distilled water	800	ml

APPENDIX F

Abbreviation for amino acid residues

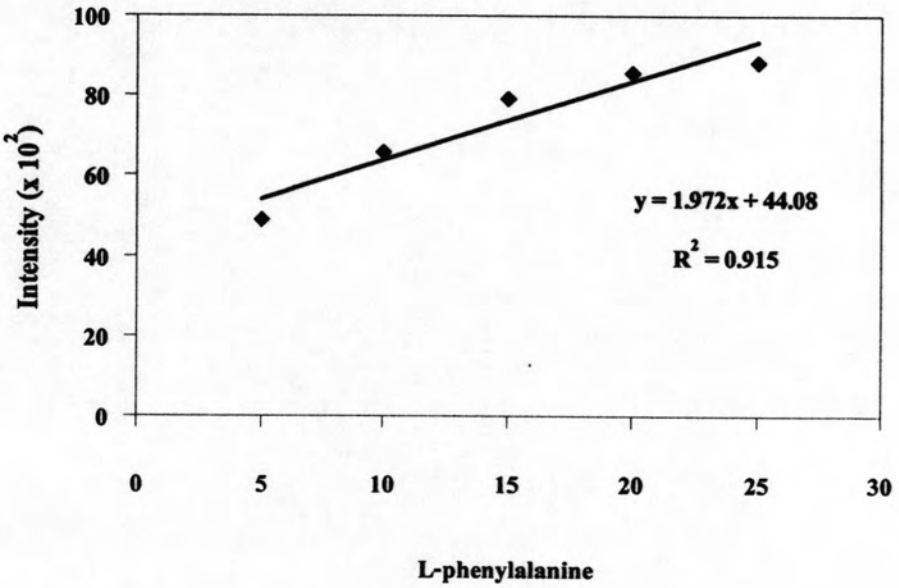
Amino acid	3 Letters-Abbreviation	1-Letter-Abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Source: Voet, 2004

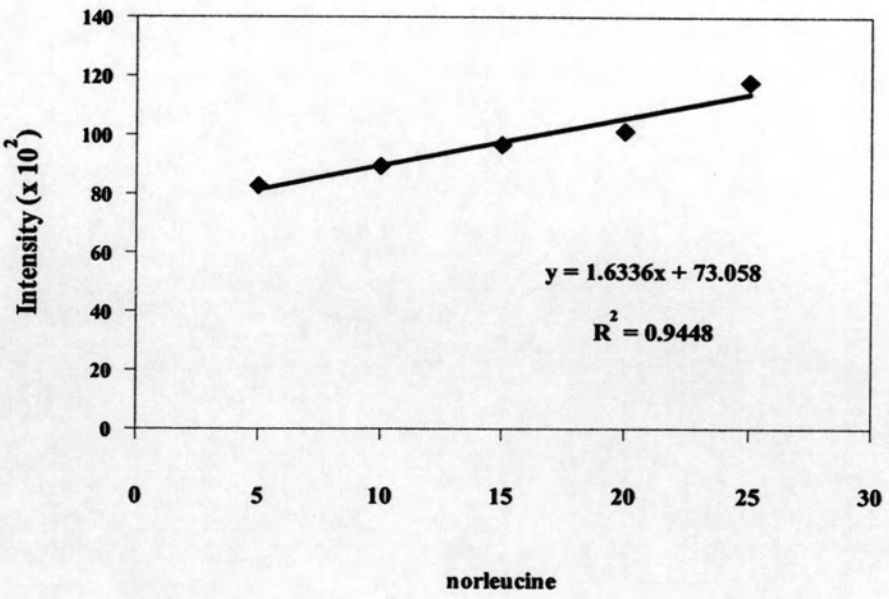
APPENDIX G

Calibration curve for the determination of amino acids by measuring the intensity of standard amino acids on TLC plate

(1)

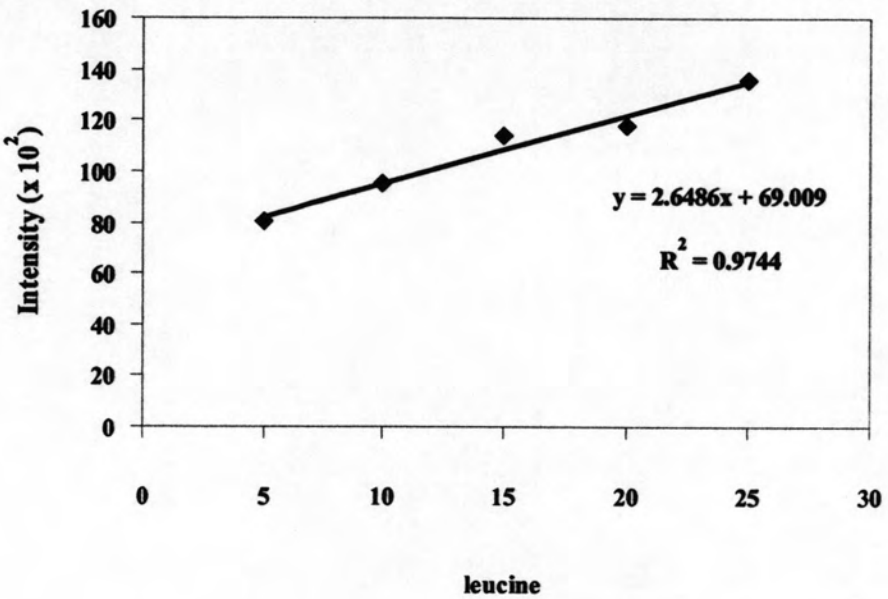


(2)

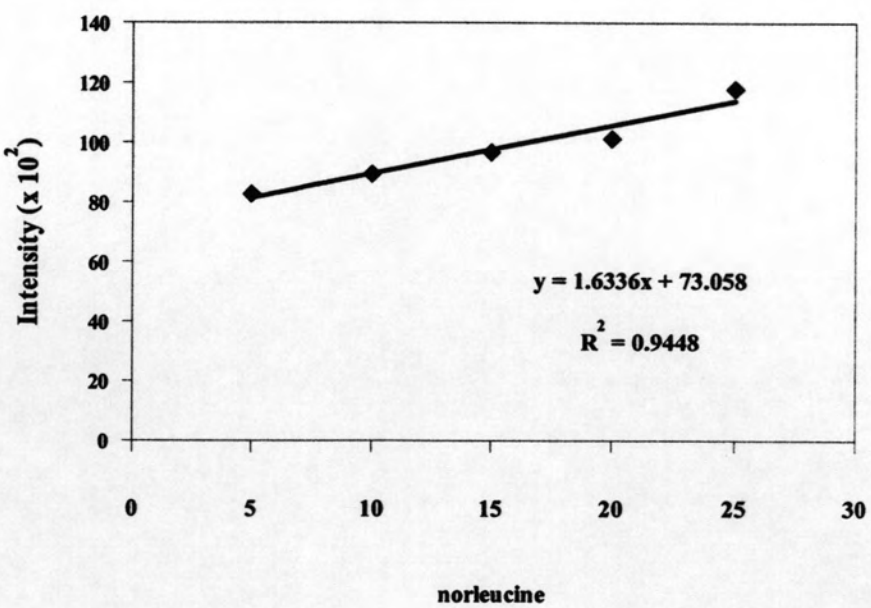


APPENDIX G (continued)

(3)

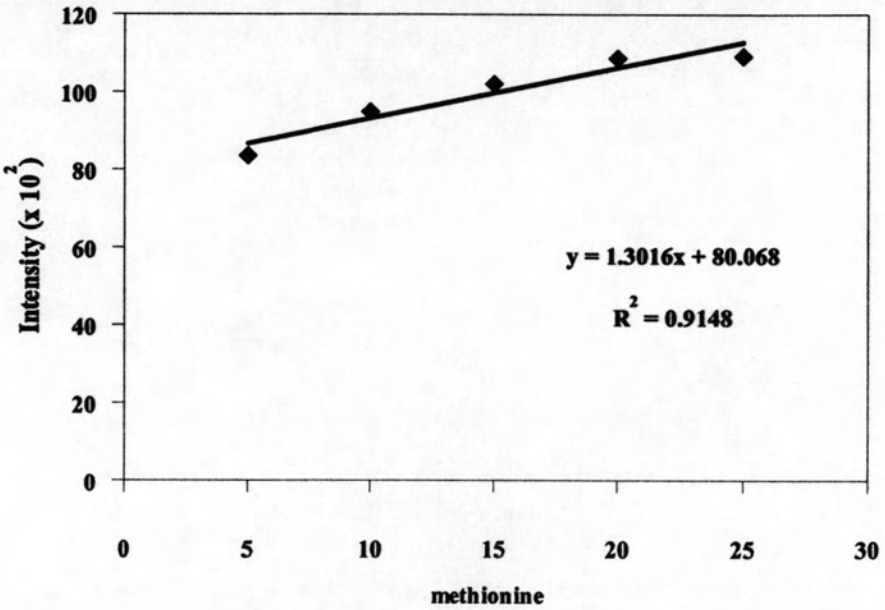


(4)



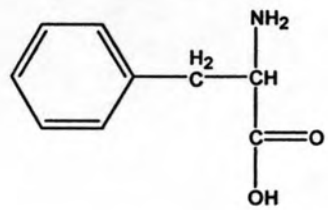
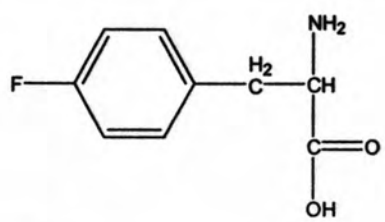
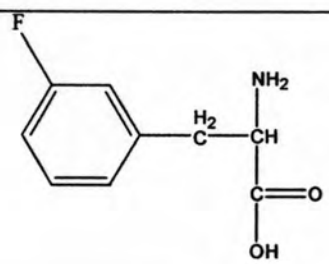
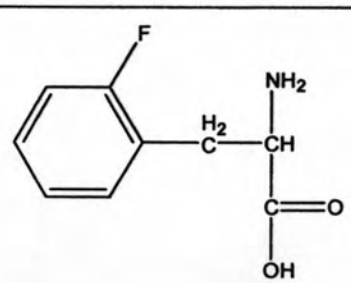
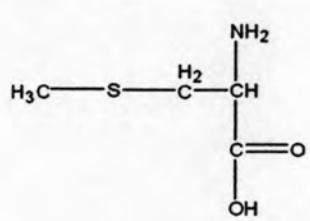
APPENDIX G (continued)

(5)

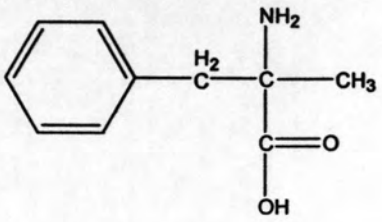
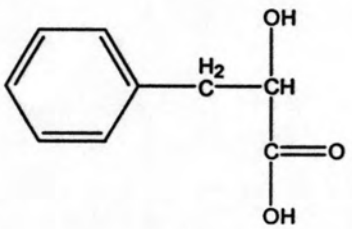
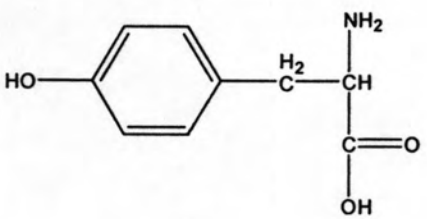
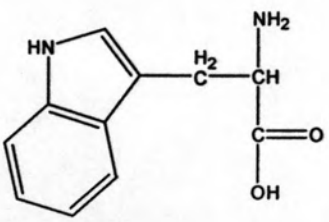
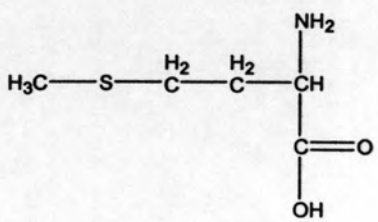


APPENDIX H

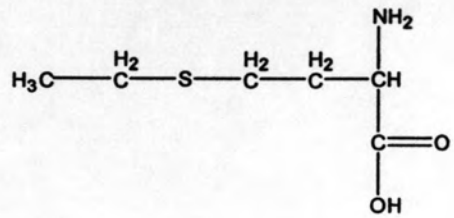
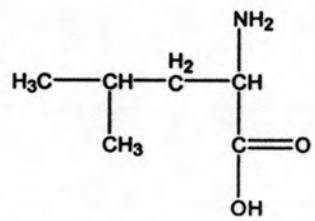
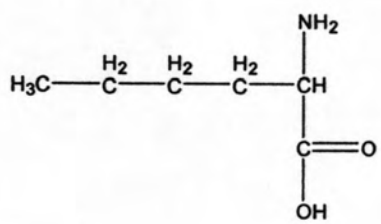
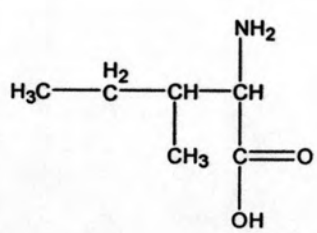
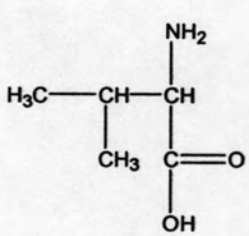
Structure of amino acids and their analogs

Amino acids and analogs	Structure
L-phenylalanine	
p-fluoro-DL-phenylalanine	
m-fluoro-DL-phenylalanine	
o-fluoro-DL-phenylalanine	
S-methyl-L-cysteine	

APPENDIX H (continued)

Amino acids and analogs	Structure
α-methyl-DL-phenylalanine	
L-α-phenyllactate	
L-tyrosine	
L-tryptophan	
L-methionine	

APPENDIX H (continued)

Amino acids and analogs	Structure
L-ethionine	
L-leucine	
L-norleucine	
L-isoleucine	
L-valine	

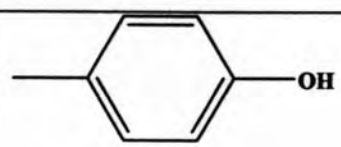
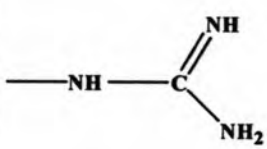
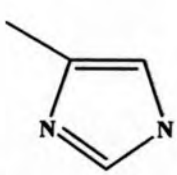
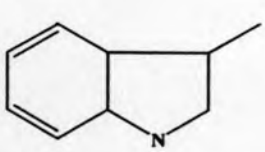
APPENDIX I

Amino acids and their corresponding keto acids

Amino acid	Keto acid
L-phenylalanine	β-phenylpyruvate
L-norleucine	α-ketocaproate
L-leucine	α-ketoisocaproate
L-norvaline	α-ketovalerate
L-methionine	α-keto-γ-methiol-n-butyrate

APPENDIX J

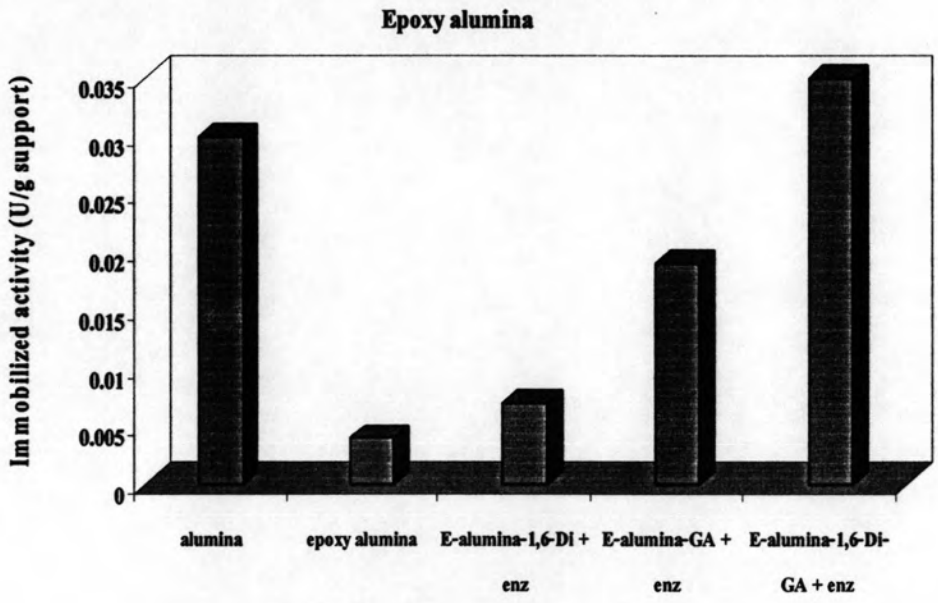
Reactive functional groups of protein

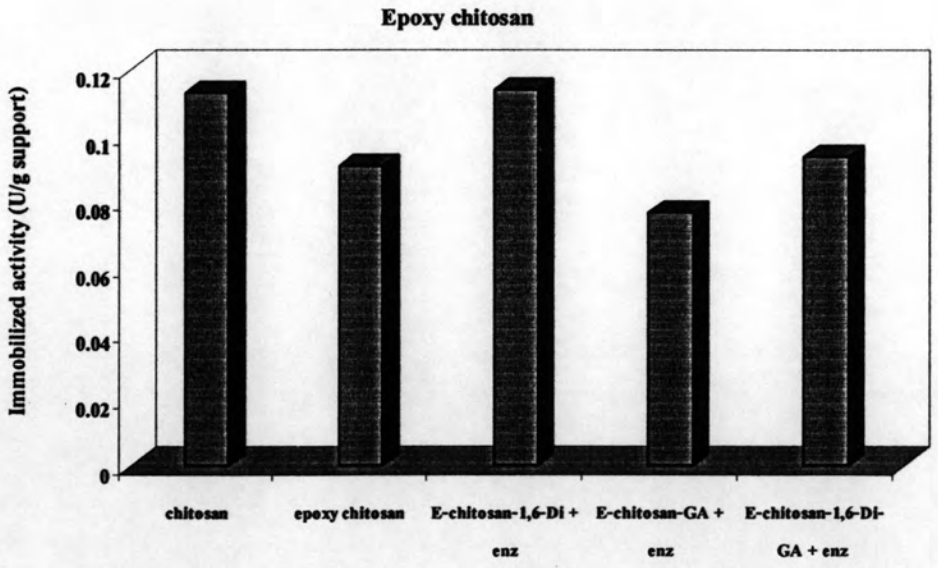
Reactive functional group		pKa
Formula	**Originating from amino acid**	
$-NH_2$	Lysine (Lys, ε-NH_2), N-terminal amino groups (α-NH_2)	10.53; 9.0-9.9
-SH	Sulfhydryl of cysteine (Cys)	8.27
-COOH	Carboxyl of aspartate (Asp), glutamate (Glu), C-terminal carboxyl groups	3.86; 4.07; 1.8-2.4
	Phenolic of tyrosine (Tyr)	10.07
	Guanidino of arginine (Arg)	12.48
	Imidazole of histidine (His)	6.10
-S-S-	Disulfide of cystine	-
	Indole of tryptophan (Trp)	-
CH_3-S-	Thioester of methionine (Met)	-
$-CH_2$-OH	Hydroxyl of serine (Ser) and threonine (Thr)	-

(Sources: Gemeiner, 1992)

APPENDIX K

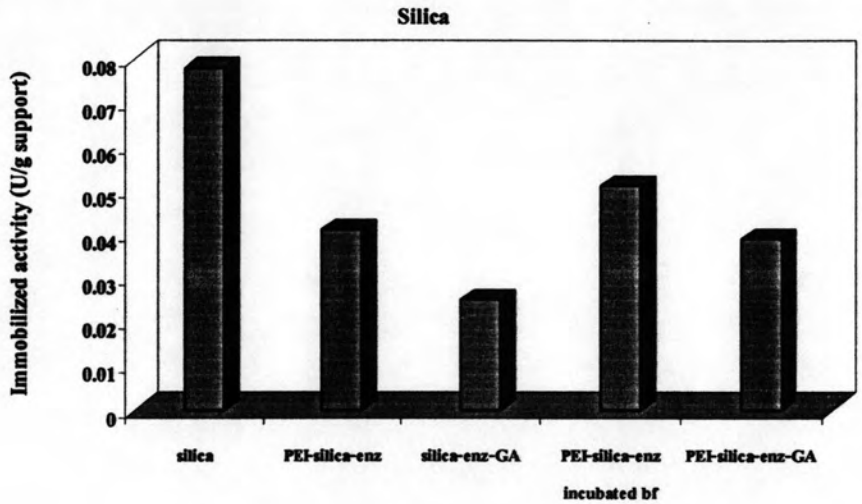
PheDH immobilization via its amino groups: Amination with 1, 6-diaminohexane

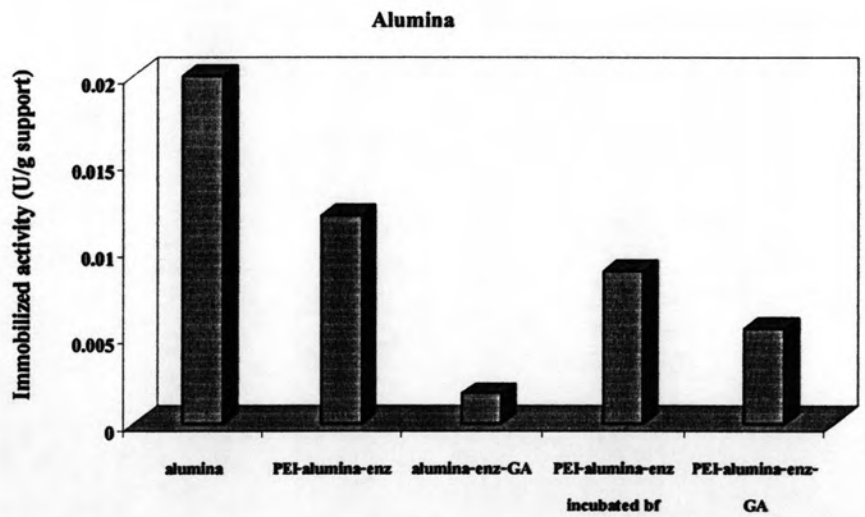




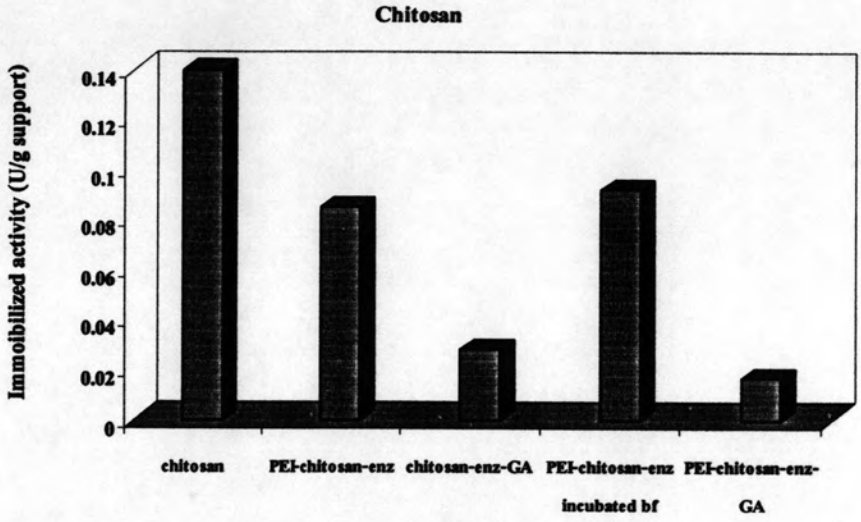
APPENDIX L

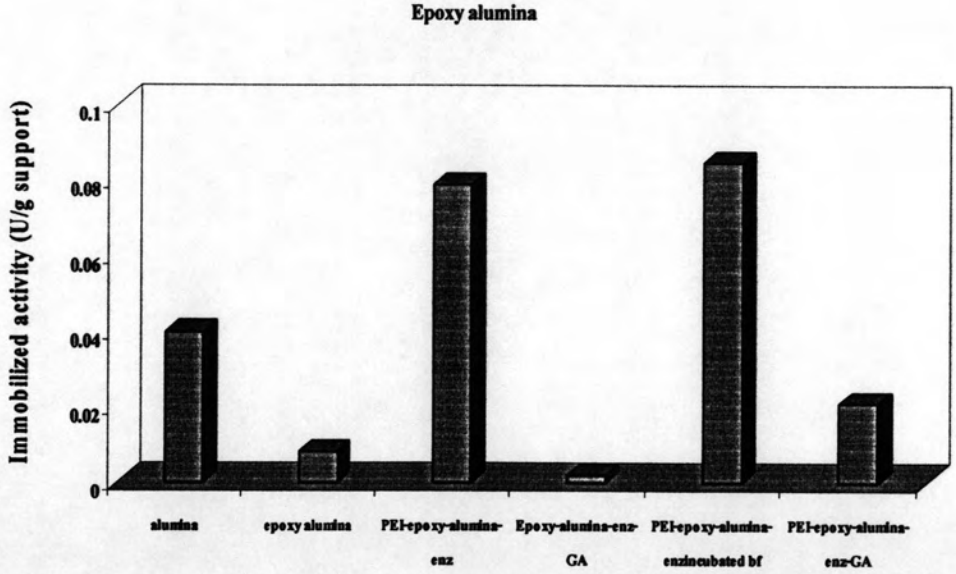
PheDH immobilization via ionic interaction





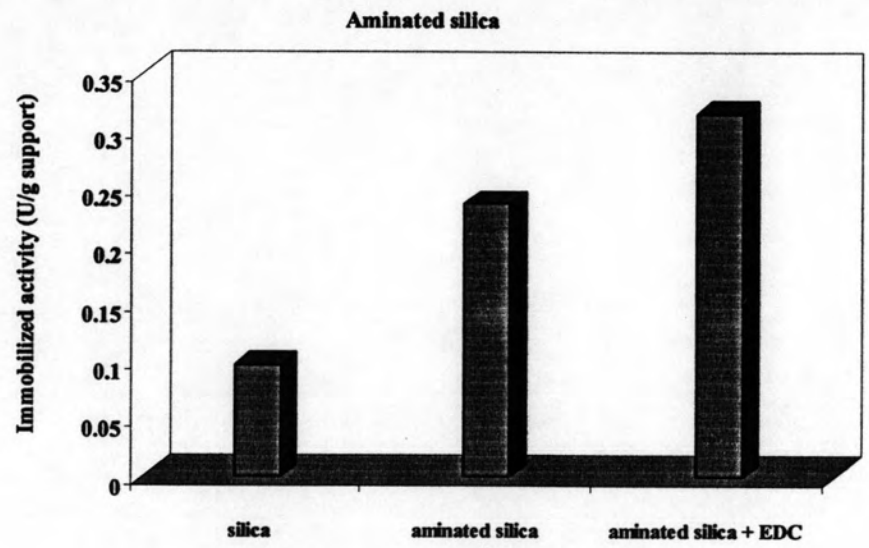
APPENDIX L (continued)

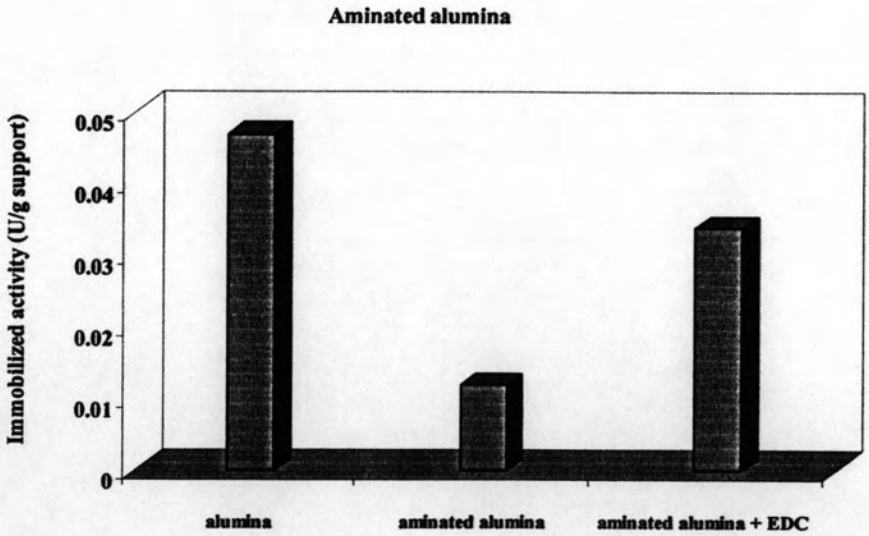




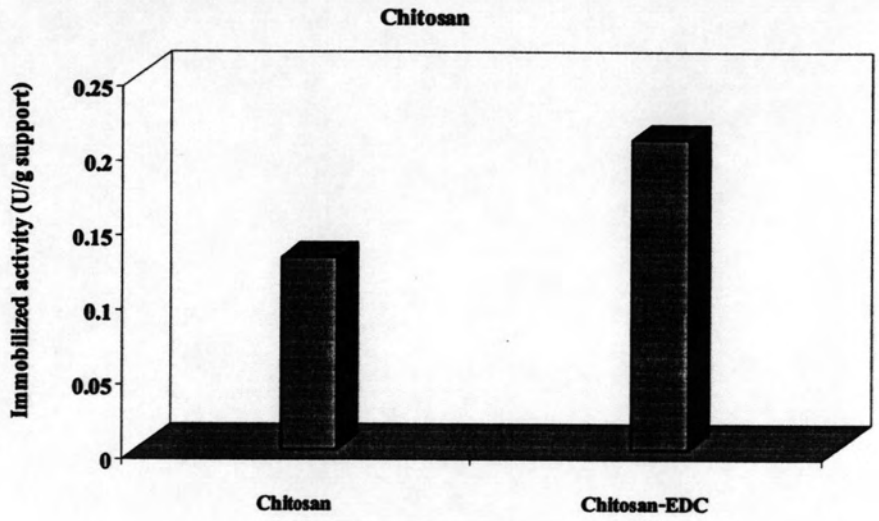
APPENDIX M

PheDH immobilization via its carboxylic group





APPENDIX M (continued)



APPENDIX N

Effect of pH on the activity of free PheDH

pH	A_{438}	Unit	Relative Activity (%)
8.5	1.716	9.30	67.39
9	2.087	11.29	81.81
9.5	2.568	13.80	100.00
10	1.988	10.75	77.90
10.5	0.523	2.83	20.50
11	0.052	0.23	1.67
11.5	0.052	0.23	1.67
12	0.055	0.30	2.16
12.5	0.052	0.23	1.67
13	0.054	0.29	2.12

Effect of pH on the activity of immobilized PheDH

pH	A_{438}	Immobilized activity (U/g)	Relative activity (%)
8.5	0.515	0.557	46.42
9	0.862	0.932	77.67
9.5	1.107	1.200	100.00
10	0.829	0.896	74.67
10.5	0.522	0.564	47.00
11	0.103	0.111	9.25
11.5	0.064	0.069	5.75
12	0.064	0.063	5.75
12.5	0.057	0.062	5.17
13	0.051	0.055	4.58

APPENDIX O

Effect of temperature on the activity of free PheDH

Temperature	A_{438}	Unit	Relative activity (%)
25	1.275	6.89	62.75
30	1.455	7.86	71.85
35	1.780	9.60	87.43
40	2.031	10.98	100.00
45	1.809	9.78	89.07
50	0.775	4.30	39.16
55	0.483	2.61	23.77
60	0.405	2.41	21.45

Effect of temperature on the activity of immobilized PheDH

Temperature	A_{438}	Immobilized activity (U/g)	Relative activity (%)
25	0.999	1.08	52.94
30	1.037	1.12	54.90
35	1.301	1.41	69.12
40	1.889	2.04	100.00
45	1.775	1.92	94.12
50	1.203	1.30	63.73
55	0.873	0.94	46.08
60	0.563	0.61	29.90

APPENDIX P

Effect of pH on the stability of free PheDH

Buffer	A_{438}	Immobilized activity (U/g)	Relative activity (%)
Citrate pH 4.0	0.050	0.27	72.97
5.0	0.065	0.35	94.59
6.0	0.068	0.37	100.00
Tris-HCl pH 7.0	0.952	5.15	95.90
7.5	0.970	5.24	97.58
8.0	0.991	5.37	100.00
8.5	0.944	5.10	94.97
9.0	0.866	4.68	87.15
KPB pH 6.0	0.947	5.12	92.85
7.0	0.982	5.20	94.37
7.5	1.019	5.51	100.00
8.0	0.963	5.21	94.56
8.5	0.913	4.94	89.66
Glycine-KCl-KOH pH 8.5	0.721	0.389	100.00
9.0	0.563	0.304	78.15
9.5	0.565	0.305	78.40
10.0	0.353	1.190	49.10
10.5	0.316	1.710	43.96
11.0	0.301	1.630	41.90
11.5	0.186	1.010	25.96
12.0	0.164	0.890	22.88
12.5	0.164	0.890	22.88
13.0	0.119	0.640	16.45

APPENDIX P (continued)

Effect of pH on the stability of immobilized PheDH

Buffer	A_{438}	Immobilized activity (U/g)	Relative activity (%)
Citrate pH 4.0	0.401	0.43	63.24
5.0	0.616	0.67	98.53
6.0	0.631	0.68	100
Tris-HCl pH 7.0	0.62	0.67	97.1
7.5	0.64	0.69	100
8.0	0.614	0.66	95.65
8.5	0.629	0.68	98.55
9.0	0.608	0.66	95.65
KPB pH 6.0	0.619	0.67	100
7.0	0.608	0.66	98.50
7.5	0.596	0.64	95.52
8.0	0.507	0.55	82.09
8.5	0.557	0.6	89.55
Glycine-KCl-KOH pH 8.5	0.555	0.6	96.39
9.0	0.567	0.61	98.39
9.5	0.575	0.62	100
10.0	0.565	0.61	91.91
10.5	0.526	0.57	91.91
11.0	0.527	0.57	91.91
11.5	0.526	0.57	91.91
12.0	0.252	0.57	91.91
12.5	0.313	0.34	54.84
13	0.115	0.12	19.35

APPENDIX Q

Effect of temperature on the stability of free PheDH

Temperature	A_{438}	Unit	Relative activity (%)
25	1.03	5.57	100
30	1.042	5.63	101.1
35	1.025	5.54	99.46
40	0.922	4.98	89.41
45	0.222	1.2	21.54
50	0.153	0.83	14.9
55	0.108	0.58	10.41
60	0.086	0.46	8.26
65	0.058	0.31	5.57
70	0.026	0.14	2.51

Effect of temperature on the stability of immobilized PheDH

Temperature	A_{438}	Immobilized activity (U/g)	Relative activity (%)
25	0.528	0.57	100
30	0.554	0.6	105.26
35	0.527	0.57	100
40	0.481	0.52	91.23
45	0.288	0.31	54.39
50	0.142	0.15	26.32
55	0.113	0.12	21.05
60	0.042	0.045	7.89
65	0.026	0.028	4.91
70	0.015	0.015	2.63

APPENDIX R

Storage stability at room temperature of free PheDH

Day	A_{438}	Immobilized activity (U/g)	Relative activity (%)
1	0.923	4.99	100.00
5	0.816	4.40	88.31
10	0.707	3.82	76.55
15	0.553	2.99	57.77
20	0.209	1.13	22.07
25	0.059	0.32	6.47
30	0	0	0
35	0	0	0
40	0	0	0

Storage stability at 4°C of free PheDH

Day	A_{438}	Immobilized activity (U/g)	Relative activity (%)
1	1.030	5.57	100.00
5	1.002	5.42	97.32
10	0.987	5.34	95.90
15	0.758	4.10	73.54
20	0.715	3.87	69.52
25	0.532	2.88	51.78
30	0.482	2.61	46.93
35	0.475	2.52	45.25
40	0.408	2.21	39.71

APPENDIX R (continued)

Storage stability at room temperature of immobilized PheDH

Day	A_{438}	Immobilized activity (U/g)	Relative activity (%)
1	1.463	1.58	100
5	1.453	1.57	99.37
10	1.222	1.21	76.58
15	0.932	1.00	63.69
20	0.762	0.82	52.23
25	0.333	0.36	22.78
30	0.244	0.26	16.56
35	0.217	0.23	14.65
40	0.049	0.05	3.31

Storage stability at 4°C of immobilized PheDH

Day	A_{438}	Immobilized activity (U/g)	Relative activity (%)
1	1.345	1.45	100
5	1.288	1.39	95.86
10	1.203	1.30	89.68
15	1.16	1.25	80.00
20	0.999	1.08	74.48
25	0.771	0.834	57.49
30	0.62	0.67	46.21
35	0.578	0.62	42.76
40	0.448	0.48	33.10

BIOGRAPHY

Miss Nipawan Tanchai was born on March 6th, 1982 in Chiangrai. After graduating with degree of Bachelor of Science from the Department of Biotechnology at Ramkhamhaeng University in 2004, she keeps on studying for Master of Science at the Biotechnology Program, Faculty of Science at Chulalongkorn University in that year.