ANALYSIS OF PROTEINS IN RICE USING CAPILLARY ELECTROPHORESIS

LINNGA RAJAN S/O PERANJISI

DISSERTATION SUBMITTED AS PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE WITH HONOURS

INDUSTRIAL CHEMISTRY PROGRAMME
SCHOOL OF SCIENCE AND TECHNOLOGY
UNIVERSITI MALAYSIA SABAH

OCTOBER 2008

PUMS99:1

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS@

JUDUL: Analysis of protein in rice using capillary electrophoresis

IJAZAH: Sarjana Muda Sains (Kimia Industri)

SAYA LINNGA RAJAN A/L PERANJISI SESI PENGAJIAN: 2005 - 2009
(HURUF BESAR)

mengaku membenarkan tesis (LPSM/Sarjana/Doktor Falsafah) ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis adalah hakmilik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institutsi pengajian tinggi.
4. Sila tandakan (/)

☐	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau Kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)
☐	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
☑	TIDAK TERHAD	

Disahkan Oleh

(TANDATANGAN PENULIS)

(TANDATANGAN PUSTAKAWAN)

Alamat Tetap: 55, Jalan SG 1/17,
Taman Sen Gombak,
68100, Batu Caves, Selangor

Nama Penyelia

Tarikh: 20.2.2009

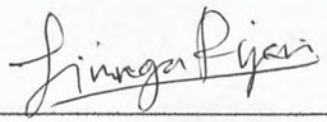
Tarikh: _____

CATATAN:- *Potong yang tidak berkenaan.
**Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa /organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.
@Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan atau disertai bagi pengajian secara kerja kursus dan Laporan Projek Sarjana Muda (LPSM).

DECLARATION

I declare that this dissertation is my original work except for quotations and summary that has been cited in reference

5th December 2008,



LINNGA RAJAN S/O PERANJISI
HS2005-2791

CERTIFIED BY

Name : Linnga Rajan s/o Peranjisi

Title : Analysis of Proteins in Rice Using Capillary Electrophoresis.

Dr Noumie Surugau (Supervisor)

Prof. Dr. Marcus Jopony (Examiner 1)

Dr Sazmal Effendi Arshad (Examiner 2)

Prof. Dr. Mohd Harun Abdullah (Dean)

ACKNOWLEDGEMENT

I would like to take this opportunity to thank all the people who have assisted me throughout the completion of this project. Firstly, I would like to express my sincere gratitude and appreciation to Dr. Noumie Surugau, my supervisor for her constant support and guidance throughout the long course of the entire study and also special thanks to all Industrial Chemistry lecturer.

I also offer thanks to Mr. Chong Tong Seng from Biochemistry/Plant Physiology Lab who has assisted me well along the completion of this project, his technical help and to all my fellow course mates for their support and encouragement. Special thanks are given to my parents, Mr. and Mrs. Peranjisi-Vasugi and both my sisters for their love, support, patience and encouragement. Without their support and help my degree's study would not have been possible. The support of all of my friends, Gomethy, Makesan, Indrani, Loges, Puneeta, Rathy, Sathya , Vijay and my beloved brothers in IP have made me strong enough to complete my studies. Their support and care is very much appreciated by me.

Overall I am overwhelmed by the constant help everyone have offered me in the completion of my project. My sincere thanks to all of them.

ABSTRACT

The analysis of protein in Sabah local rice varieties was a pioneer research in order to study the protein fractions or composition in it. The local rice varieties that had been used in this study were Huma rice, Bario rice, White Glutinous rice and Black Glutinous rice. Each of the rice samples was extracted into its protein fractions: albumin, globulin, glutelin and prolamin according to its solubility by using Osborne Fractionation method. The protein fractions were analyzed using Capillary Electrophoresis (CE). Each fraction was analyzed in a 30 cm uncoated fused silica capillary at 25°C with zero pressure, voltage of 5kV and detected by UV detector at wavelength 214nm. Capillary cleaning protocols were carried out before and after analysis. Each fraction was analyzed using two different buffers at two different concentrations and pH. 100mM Tris-HCl buffer at pH 7.00 gave the sharper peak and less stacking compared to 50mM phosphate buffer. Among the four types of local rice, Huma rice and Bario exhibited a high proportion of glutelin followed by White Glutinous rice and Black Glutinous rice whereas globulin can be said as second major protein component in the local rice varieties. The abundance of albumin and prolamine in the local rice varieties are relatively low. Huma rice exhibited most of the peaks for its protein fraction whereas White Glutinous rice exhibited two peaks for its protein fraction, globulin and glutelin. The most hydrophobic protein, prolamin, in which theoretically should be less in rice, exhibited a peak as one of the protein components in Black Glutinous rice despite albumin, globulin and glutelin.

ABSTRAK

Analisis protein dalam beras Sabah merupakan satu kajian utama untuk mengetahui atau mengkaji komponen protein di dalamnya. Empat variati beras tempatan yang digunakan dalam kajian ini ialah Beras Huma, Beras Bario, Beras Pulut Putih dan Bears Pulut Hitam, diekstrak kepada empat kandungan pecahan protein iaitu albumin, globulin, glutelin dan prolamin mengikut keterlarutan dan cara Pecahan Osborne. Pecahan protein ini kemudiannya dianalisis dengan menggunakan kapilari elektroforesis (CE). Setiap pecahan telah dianalisis dalam 30 cm kapilari silica tanpa penyaduran pada suhu 25°C tanpa tekanan, bervoltan 5kV, dikesan dengan menggunakan pengesan UV pada jarak gelombang 214nm. Protokol pembersihan kapilari telah dijalankan sebelum dan selepas analisis. Setiap pecahan dianalisis dengan meggunakan dua jenis larutan penimbal pada kepekatan dan pH yang berbeza. 100mM larutan penimbal Tris-HCl pH 7.00 melakarkan puncak yang lebih tajam berbanding dengan 50mM larutan penimbal fosfat. Beras Huma dan Beras Bario menunjukkan komponen protein glutelin yang tinggi diikuti dengan Beras Pulut Putih dan Beras Pulut Hitam manakala globulin boleh dikatakan sebagai komponen protein yang kedua tertinggi dalam beras-beras tersebut. Beras Huma melakarkan kebanyakan puncak bagi pecahan proteinnya manakala Beras Pulut Putih hanya melakarkan satu puncak bagi pecahan proteinnya. Protein yang paling hidrofobik iaitu, prolamin, berdasarkan pada teori yang sepatutnya menunjukkan komponen protein yang rendah sebaliknya menjadi salah satu komponen protein dominan dalam Beras Pulut Hitam.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

g	Gram
mg	Miligram
µl	Microlitre
mL	Mililitre
nm	Nanometer
%	Percentage
°C	Degree Celsius
rpm	Rotation per minute
π	Pi
maU	Mili-absorbance units
CE	Capillary Electrophoresis
EOF	Electroosmotic flow
ALB	Albumin
GLOB	Globulin
GLU	Glutelin
PRO	Prolamin
MES	2-(N-morpholino)ethanesulphonic acid
BES	N,N-Bis(2-hydroxyethyl)aminoethanesulphonic acid
MOPS	3-(N-morpholino)propanesulphonic acid
Tris	Tris(hydroxymethyl)aminomethane
Bicine	N,N-Bis(2-hydroxyethyl)glycine
CHES	3-(cyclohexylamino)ethanesulphonic acid
CAPS	3-(cyclohexylamino)propanesulphonic acid
HPMC	Hydroxypropyl methyl cellulose

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Proteins are an abundant component in all cells and almost all except storage proteins are important for biological functions and cell structure (Suzanne, 2003). Protein, as macro-molecules, perform important roles in functionality in foods and pharmaceuticals, as well as in biological systems (Vojdani, 1996). Proteins are key ingredients in many foods as they contribute to the nutritional value, flavor and other important functional properties of food systems (Hamada *et al.*,1998).Proteins are made up of successive carboxylic acid-amine residues, amino acids, that are covalently bonded together in a head-to-tail arrangement through substituted amide linkages (Rosenberg, 1996).

On a world wide scale, about 70% of the protein available for human consumption is derived from plant sources. The percentage varies in different parts of the world, ranging from slightly over 30% in the United States to about 90% in India. Cereal grains contribute more than 70% of the plant proteins and about 50% of the total proteins. The major cereal grains are wheat, rice and corn. 50 million metric tons which is contributed by wheat in total annual cereal protein production. Milled rice accounts for 18% of the cereal production because, in general, rice contains less protein than average of the other grains (Nakai & Modler, 2000).

Electrophoresis is defined as the migration of charged molecule in a solution through an electrical field (Suzanne, 2003). It is a separation method basically used in biochemical or analytical separation. By using this method, high separation efficiency can be achieved using a relatively limited amount of equipment. Electrophoresis has been applied to a variety of difficult separation method such as in biological and biochemical research, protein chemistry, pharmacology, forensic science, food control and molecular biology (Westermeier, 1993). It is important to choose and carry out the appropriate electrophoresis technique for specific separation.

The principle of electrophoresis separation is by injecting a small band of the sample into an aqueous buffer solution. A homogenous buffer solution is used over the whole separation time and range so as to ensure a constant pH value. A high dc potential is applied across the length of the buffer by a pair of electrodes located at the end of the buffer and this cause the ions of the sample to move toward one or other of the

electrodes. The rate of migration of a given species depends upon its charge and size. Separations are then based on differences in charge-to-size ratios for the various analytes in a sample (Skoog *et al.*, 2007).

One of the types of electrophoresis is the capillary electrophoresis which has been developed and used in last decade become an important separation tool used by chemist. Capillary electrophoresis (CE) is a powerful separation tool for the determination of charged particle, based on the difference in electrophoretic mobilities.It has become one of the most powerful techniques in protein separation in which it has features such as high frequency, small sample requirement and short analysis time (Lin *et al.*, 2007).

1.2 Objectives of the Study

The objectives of this study are:

a) To fractionate the storage proteins in four local rice varieties according to their solubility using Osborne Fractionation method and analyse each fraction using capillary electrophoresis.
b) To compare the abundance of protein in the local rice based on the electrophero-gram of each fraction.
c) To investigate the effect of buffers at two different concentration and pH in the analysis of rice protein.

1.3 Scope of study

This study will focus on the analysis of protein fractions in local rice varieties using capillary electrophoresis. The factors such as buffer type, concentration and pH that affects in the separation of rice protein using capillary electrophoresis will be investigated rice. The local rice varieties studied are Huma rice, Bario rice, White Glutinous rice and Black Glutinous rice.

CHAPTER 2

LITERATURE REVIEW

2.1 Proteins

Proteins are long chains of amino acids linked together by peptide (amide) bonds with positively charged nitrogen-containing amino group at one end and negatively charged carboxyl group at the another end (Campbell & Farrell, 2006). Proteins have 20 amino acids in which the residues in a protein are often chemically altered in post-translational modification either before the protein can function in the cell or as a part of control mechanisms. All amino acids possess common structural features, including an α carbon to which an amino group, a carboxyl group and a variable side chain are bonded. The end of the protein with a free carboxyl group is known as the C-terminus or carboxy terminus, whereas the end with a free amino group is known as the N-terminus or amino terminus. The structure of a basic amino acid is shown in Figure 2.1.

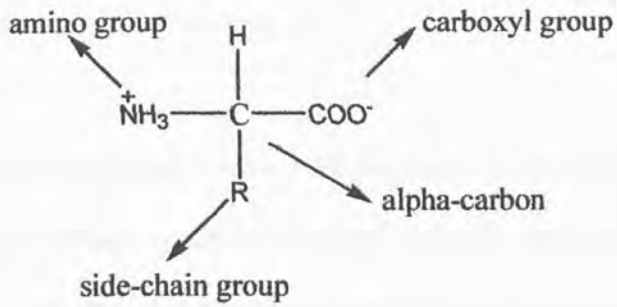


Figure 2.1 Basic structure of an amino acid.

Every amino acids has a carboxyl group and an amino group in which each group can exist in an acidic form or basic form, depending on the pH of the solution in which the amino dissolves (Bruice, 2004). Each protein molecule has a precise length composed of an exact sequence of amino acids which are arranged in a linear, unbranched fashion. The amino acids backbone may have many post-translational modifications contributing to the size, charge and function of the mature protein (Rosenberg, 1996).There are four levels of protein structure. The primary structure is the sequence of amino acids that make up a specific protein. Secondary structure is regular local structural arrays found in proteins which can be referred to as independent foldings units. In tertiary structure, the intermolecular arrangement of the secondary structure is independent foldings units with respect to each other. The quaternary structure shows the stoichiometry and spatial

arrangement of the subunits and is determined by the amino acid sequences of the subunits.

2.1.1 Chemical characteristics of proteins

Proteins have ionic and hydrophobic sites, both internally (within the folds of the tertiary structure) and on the surface where the primary structure comes in contact with the environment. The ionic sites of a protein are provided by the charged amino acids at physiological pH and by covalently attached modifying group. Proteins are polyelectrolyte containing positively and negatively charged groups. The net charge on a protein is contributed by the free α-amino group of the N-terminal residue, the free α-carboxyl group of the C-terminal residue, those R groups capable of ionization, and on the unique array of modifications attached to the protein. At physiological pH, the α-COO^- and α-NH_3^+ groups are ionized with the deprotonated carboxyl group bearing a negative charge and the protonated amino group, a positively charge. The difference in the charge of one terminal of respective amino acids enable in the analysis of proteins in rice due to its charge (Rosenberg, 1996).

2.2 Cereal Proteins

Cereal grains are important dietary source of the world's population, contributing 70% and 50% of the total calories and protein, respectively. Cereal grains are widely used for human foods and animal feeds around the world. As a major source of energy, cereals are often a primary nutrition protein provider and important functional properties to many foods. Five main cereals are wheat, maize, barley, oats and rice (Eskin, 1990). Plant proteins contain glutelins, prolamins, globulin and albumin. The study of cereal proteins has undergone many analytical techniques due to their relationship with quality and varietals identification (Nakai & Modler, 2000). The relative proportions of protein fractions in cereal seeds is shown in Table 2.1 .

Table 2.1 Relative proportions(%) of the Osborne protein fractions in cereal seeds

Cereal	Non-protein, N	Albumins	Globulins	Prolamins	Glutelins	Residues
Barley	11.6	-	15.6	45.2	18.0	5.0
Wheat	-	33.1	-	60.7	-	6.2
Maize	4.4	0.9	1.5	55.4	22.9	-
Rice	-	15.7	-	6.7	61.5	15.4
Oats	11	-	56.0	9.0	23	-

Source: From Bright and Shewry (1983).

One of the classical investigations of plant proteins was the study of Osborne (1907) on fractionation of cereal proteins according to their solubility. The original fractionation procedure divided cereal proteins into four major groups on the basis of solubility: water-

soluble albumins, salt-soluble globulins, alcohol-soluble prolamins and acid- or alkali-soluble glutelins.

2.3 Rice and Protein in Rice

Rice is a staple for a large part of the world's human population, making it the second most consumed cereal grain. Rice also provides more than one fifth of the calories consumed worldwide by humans.

Protein content of brown rice is about 8% and milled rice accounts for 6%-7%. As the second major constituent of milled rice, it is the major protein source in the diets of tropical Asians. The contents of milled rice or rice endosperm consist of 3.8%-8.8% albumin, 9.6%-10.8% globulin, 2.6%-3.3% prolamin and 66%-78% of glutelin (Borght *et al.*, 2006). 85%-90% of the rice proteins are storage proteins, mainly glutelins. Rice is extremely low in prolamins compared with other cereal grains (Nakai & Modler, 2000). Moreover, rice prolamins are heterogeneous and known for its complexity which is difficult in characterization (Dong *et al.*, 1999). The major protein components in rice is called glutelin, belong to the globulin group (Oszvald *et al.*, 2007).

The range of molecular weights (MWs) for rice albumin is wide but in majority the components have apparent MWs of 18-20k Da whereas rice globulins consist of α-, β-, γ- and δ- globulins with apparent MWs of 25.5, 15, 200k Da respectively. Rice prolamins consists of three polypeptide subunits with apparent MWs of 10, 13 and 16k Da. Rice glutelin is the major storage protein, composed of two major polypeptide

subunits classified as α, β or acidic, and β, or basic subunits with apparent MWs of 30-39 and 19-25k Da, respectively (Borght *et al.*, 2006).

2.4 Capillary Electrophoresis

The separation techniques using electrophoresis method was first developed by the Swedish chemist Arne Tiselius in the 1930s. He carried out this electrophoresis method by using the macromolecules which has electric charge to achieve the separations of blood plasma proteins in free solution. Tiselius developed a "moving boundary" method and he was able to separate albumin from α-, β-, and γ-globulin. From the idea and methods of Tiselius give rise to various and contemporary electrophoresis in analytical chemistry especially in separation chemistry using macromolecules. Due to his achievement and work in electrophoresis, Tiselius was awarded the Nobel Prize 1948 (Camilleri, 1998).

Now, capillary electrophoresis (CE) becomes a new analytical method and the analysis can be carried out in free solution without the use of supporting medium. It is also a powerful and increasingly important separation technique in analytical chemistry. CE a separation tool for determination of charged species, based on the differences in electrophoretic mobilities (Lin, 2007).

REFERENCES

Agboola, S., Ng, D., Mills, D. Characterisation and functional properties of Australian rice protein isolates. *Journal of Cereal Science* **41**, 283-290.

Baker, D.R. 1995. Capillary Electrophoresis. John Wiley & Sons.Inc, N.Y. pp. 94-95.

Bruice, P. Y .2004. *Organic Chemistry*. 4th Ed. Pearson Educational International, United State. pp. 965-968.

Bean, S. R., Tiley, M.2003. Separation of water soluble proteins from cereals by High Performance Capillary Electrophoresis. *Journal of Cereal Chemistry* **80**(5), 505-510.

Bean, S.R., Bietz, J.A., Lookhart, G.L. 1998. High-performance capillary electrophoresis of cereal proteins. *Journal of Chromatography* **814**, 25-41.

Bean, S.R., Lookhart, G.L.1996.Improvements in Cereal Protein Separations by Capillary Electrophoresis: Resolution and Reproducibility. *Journal Cereal Chemistry* **73**(1), 81-87

Borght, A.V.D., Vandeputte, G.E., Derycke, V., Brijs, K., Daenen, G., Delcour, J.A. 2006. Extractability and chromatographic separation of rice endosperm proteins. *Journal of Cereal Science* **44**, 68-74.

Baxter, G., Blanchard, C., Zhao, J. 2004. Effects of prolamin on the textural and pasting properties of rice flour and starch. *Journal of Cereal Science* **40**, 205-211.

Camilleri, P. C. 1998. *Capillary Electrophoresis: Theory and Practice*.2nd Ed.CRC Press, Florida. pp 26-27.

Campbell, M.K., Farrell, S.O.2006. *Biochemistry*.5th Ed. Thomson-Brooks/Cole, United States. pp 121.

Dong, Y.Y., Wang, J., Qiu, Y.Y. 1999. Varetial Identification of Rice Prolamins by Capillary Zone Electrophoresis. *Journal of Chromatography* **50**, 376-378.

Eskin, N.A.M., 1990. *Biochemistry of Foods*. 2nd Ed. Academic Press. United States. pp. 125-127

Hamada, J.S., Spanier, A.M., Bland, J.M., Diack, M. 1998. Preparative separation of value-added peptides from rice bran proteins by high performance liquid chromatography. *Journal of Chromatography A* **827**,319-327.

Kulka, S., Quintas, G., Lendl, B. 2006. On-line capillary electrophoresis FTIR detection for the separation and characterization of proteins. *Journal of Vibrational Spectroscopy* **42**, 392-396.

Lander,J.P. 1996. Handbook of Capillary Electrophoresis. 2nd Edition. CRC.Press LLC, Florida, pp.4-35

Lookhart, G., Bean, S. 1995. Separation and characterization of wheat protein fractions by high performance capillary electrophoresis. *Journal of Cereal Chemistry* **72** (6). 527-532

Lucy,A.C., Macdonald,M.A., Gulcev, D.M. 2007. Non covalent capillary coatings for protein separations in capillary electrophoresis. *Journal of Chromatography A* **1184**, 81-105

Oszvald, M., Tomoskozi, S., Larroque, O., Keresztenyl, E., Tamas, L., Bekes., F.2007. Characterisation of rice storage proteins by SE-HPLC and micro z-arm mixer. *Journal of Cereal Science*. 2-9.

Nakai, S., Modler, H.W. 2000. Food Proteins: Processing Application. Wiley-VCH. pp. 243-253.

Righetti, P.G. 1996. *Capillary Electrophoresis in Analytical Biotechnology*. CRC Press. Florida. pp.2-3, 37-43.

Royle, L., Radcliffe, M.C.1999. Analysis of Caramels by Capillary Electrophoresis and Ultrafiltration. *Journal of Food Science and Agricluture* **79,** 1709-1714.

Skoog, D.A., Holler, F.J., Crouch, S.R.2007. *Principle of Instrumental Analysis*.6th Ed. Thomson-Brooks/Cole. pp. 867-875.

Skoog, D.A., West, D.M., Holler, F.J., Crouch, S.R.2004. *Fundamentals of Analytical Chemistry*. 8th Ed.Thomson-Brooks/Cole.pp.1003-1005

Steenson, F.D., Sathe, S.K. 1995. Characterisation and Digestibility of Basmati Rice (Oryza Sative L.Var. Dehraduni) Storage Proteins. *Journal of Cereal Proteins* **72** (3), 273-280

Suzanne, N.S. 2003. *Food Analysis*. 3rd Ed. Kluwer Academic/Plenum Publishers. New York. pp. 133-134, 256-257.

Venn, R.F. 2000. *Principles and Practice of Bioanalysis*.1st Ed. Taylor and Francis Inc. New York. pp. 160-169.