UNIVERSITI PUTRA MALAYSIA

DETERMINATION OF BOVINE AND PORCINE GELATIN POLYPEPTIDES USING SODIUM DODECYL SULPHATE-POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

NUR AZIRA BINTI TUKIRAN

IPPH 2012 3

DETERMINATION OF BOVINE AND PORCINE GELATIN POLYPEPTIDES USING SODIUM DODECYL SULPHATE-POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

By

NUR AZIRA BINTI TUKIRAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Masters of Science

April 2012

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

DETERMINATION OF BOVINE AND PORCINE GELATIN POLYPEPTIDES USING SODIUM DODECYL SULPHATE-POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

By

NUR AZIRA BINTI TUKIRAN

April 2012

Chairman : Professor Amin bin Ismail, PhD

Institute : Halal Products Research Institute

Gelatin is used widely in food and pharmaceutical industries because of its unique physicochemical characteristics. However, the gelatin utilisation becomes an issue among Muslim, Jews and vegetarians due to its animal origin. As such, methods of gelatin detection and differentiation as well as gelatin stability in the processed foods have emerged as an important research area to be studied. Therefore, the aim of this study was to detect and differentiate the bovine and porcine gelatin polypeptides using a combination method of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and Principal Component Analysis (PCA).

Utilising optimal conditions of SDS-PAGE with 6% of resolving gel, 8 µg amount of protein, 8 M Urea-SDS sample buffer, together with sensitive silver staining had exhibited molecular weights of gelatin polypeptides ranged from 53 to 220 kDa. They can be used to differentiate between bovine and porcine gelatins. The gelatin of porcine exhibited wider molecular weight distribution as compared to bovine that consisted of 11 and 2 prominent bands, respectively. In addition, these prominent bands were stable under heat treatment and consistent even in different Bloom number offer suitability for the identification of gelatin sources. In order to determine the detection limit of SDS-PAGE in detecting the percentage of adulterated samples, PCA was applied to classify the percentage of adulteration in the samples. Results showed that SDS-PAGE combined with PCA was unable to detect the presence of less than 5% porcine gelatin adulterated in the bovine gelatin. To detect the presence of gelatin in the samples, both extracting solutions (cold acetone and deionised water) are suitable; however, for commercial processed products with mixed ingredients cold acetone was more efficient. In the study, the qualitative comparison using SDS-PAGE between samples could be differentiated by PCA, and the combination may provide robust information for gelatin species identification. This study proposed that this new approach employing SDS-PAGE and PCA together with a simple gelatin extraction method may provide a useful tool for food authenticity issues concerning gelatin.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PENENTUAN POLIPEPTIDA GELATIN "BOVINE" DAN "PORCINE" MENGGUNAKAN NATRIUM DODESIL SULFAT -ELEKTROFORESIS GEL POLIAKRILAMIDA (SDS-PAGE)

Oleh

NUR AZIRA BINTI TUKIRAN

April 2012

Pengerusi : Profesor Amin bin Ismail, PhD

Institut : Institut Penyelidikan Produk Halal

Gelatin digunakan dengan meluas dalam makanan dan industri farmaseutikal disebabkan oleh ciri-ciri fizikokimia uniknya. Bagaimanapun, penggunaan gelatin menjadi isu di kalangan komuniti Islam, Yahudi dan "vegetarian" disebabkan oleh sumber haiwannya. Oleh itu, kajian berkenaan pengesanan dan pembezaan gelatin serta kestabilannya di dalam matrik makanan yang telah diproses menjadi satu perkara penting untuk dikaji. Lantaran itu, tujuan kajian ini ialah untuk mengesan dan membezakan gelatin "bovine" dan "porcine" dengan menggunakan gabungan kaedah natrium dodesil sulfat-elektroforesis gel poliakrilamida (SDS-PAGE) dengan analisis komponen utama (PCA).

Menggunakan keadaan optimum SDS-PAGE; 6% gel “resolving”, 8 µg jumlah protein, 8 M Urea-SDS penimbal bersama dengan “silver staining” yang sensitif, gelatin “bovine” dan gelatin “porcine” telah mempamirkan corak polipeptida yang berbeza pada berat molekul diantara 53 hingga 220 kDa. Jalur-jalur polipeptida ini digunakan untuk membezakan di antara gelatin “bovine” dan “porcine”. Gelatin “porcine” menunjukkan taburan berat molekul yang lebih luas berbanding dengan gelatin “bovine” yang mana masing-masing mengandungi 11 dan 2 jalur polipeptida. Tambahan pula, jalur-jalur polipeptida ini adalah stabil di bawah perlakuan haba yang berlainan dan konsisten walau dalam nilai Bloom yang berbeza, dan ini menawarkan kesesuaian untuk pengenalpastian sumber gelatin. Untuk menentukan had pengesanan kaedah SDS-PAGE dalam mengesan peratusan pencemaran di dalam sampel, PCA digunakan untuk mengelaskan peratusan pencemaran. Hasil kajian mendapati SDS-PAGE dengan gabungan PCA tidak mampu mengesan kehadiran kurang daripada 5% gelatin “porcine” yang mana telah dicemarkan dengan gelatin “bovine”. Untuk mengesan kehadiran gelatin di dalam sampel, kedua-dua larutan (aseton yang telah disejukan dan air suling) adalah sesuai; walaubagaimanapun, untuk produk komersial yang telah diproses dengan bahan-bahan yang kompleks larutan aseton yang telah disejukan adalah lebih sesuai. Dalam kajian ini, perbandingan kualitatif di antara sampel-sampel dengan menggunakan SDS-PAGE boleh dibezakan oleh PCA, dan seterusnya gabungan kedua-dua kaedah ini boleh memberi maklumat yang lebih tepat dalam pengecaman spesis gelatin. Kajian ini mencadangkan bahawa, pendekatan baru dengan menggunakan SDS-PAGE and PCA bersama dengan kaedah pengekstrakan gelatin yang mudah boleh memberi satu kaedah yang berharga bagi menentukan ketulenan makanan yang berkaitan dengan gelatin.

ACKNOWLEDGEMENTS

In the name of Allah, The Most Merciful and The Most compassionate. Alhamdullillah, with His blessing I have completed my preparation of this master thesis.

Firstly I would like to express my appreciations and thanks to my supervisory committee chairman, Professor Dr. Amin bin Ismail, whose encouragement, guidance and support from the initial to the final level enabled me to develop an understanding of the subject. I wish also to express my appreciations to my co-supervisor, Professor Dato' Dr. Yaakob bin Che Man for his support and assistance.

Appreciation is extended to my colleagues, Aina, Raja, Akasya, Nadiha, Nashrah, Aimi, Alia, Hazirah, Nadia, Wan, Irmey, Salwani, Marina, Nora, Ifa, Ummi, Illiyin, Afiq, Hazim, Yanti, Azilah, Emy and to all of those who supported me in one way or another during the completion of the project.

My heartfelt gratitude goes to beloved family, mama, abah, Anis, Irsyad, Iffah, Irfan and Wafiq who have consistently motivated me, giving me spiritual encouragement and support.

Lastly, I would like to thank Universiti Putra Malaysia for providing funding for the study.

I certify that a Thesis Examination Committee has met on 6 April 2012 to conduct the final examination of Nur Azira binti Tukiran on her thesis entitled **"Determination of bovine and porcine gelatin polypeptides using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)"** in accordance with the Universities and University Collages Act 1971 and the Constitution of the University Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Shuhaimi bin Mustafa, PhD
Associate Professor
Halal Products Research Institute
Universiti Putra Malaysia
(Chairman)

Suraini bt Abd Aziz, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Chong Pei Pei, PhD
Associate Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Internal Examiner)

Abd. Karim Alias, PhD
Professor
School of Technology Industry
Universiti Sains Malaysia
Pulau Pinang, Malaysia
(External Examiner)

SEOW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 23 July 2012

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Amin bin Ismail, PhD
Professor
Halal Products Research Institute
Universiti Putra Malaysia
(Chairman)

Yaakob bin Che Man, PhD
Professor
Halal Products Research Institute
Universiti Putra Malaysia
(Member)

_____

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

_____

NUR AZIRA BINTI TUKIRAN

Date: 6 April 2012

TABLE OF CONTENTS