
UNIVERSITI PUTRA MALAYSIA

# DETECTION OF GELATIN ORIGIN USING FOURIER TRANSFORM INFRARED SPECTROSCOPY AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

NORAKASHA BINTI RUSLI

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# DETECTION OF GELATIN ORIGIN USING FOURIER TRANSFORM INFRARED SPECTROSCOPY AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

**By**

**NORAKASHA BINTI RUSLI**

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

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Dedicate to those who wish to know and those who are devoted to the progress of society

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

**DETECTION OF GELATIN ORIGIN USING FOURIER TRANSFORM INFRARED SPECTROSCOPY AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY**

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**NORAKASHA BINTI RUSLI**

**February 2011**

**Chairman : Dzulkifly bin Mat Hashim, M.Sc**

**Institute : Halal Products Research Institute**

A study on detection of gelatin origin was conducted. Rapid method was developed using Fourier transform infrared (FTIR) spectroscopy to distinguish between bovine and porcine gelatin. The results showed that FTIR spectroscopy was capable of distinguishing bovine and porcine gelatin by analyzing the region between 3290- 3280 $cm^{-1}$ and 1660-1200 $cm^{-1}$ using discriminant analysis (DA). The Cooman's plot clearly showed that both gelatins were classified according to their respective group.

The subsequent study explains the application of high performance liquid chromatography (HPLC) in ascertaining the source of gelatin using amino acid analysis. A partial least square (PLS) calibration demonstrated good linear regression ($R^2$) of 0.991 and 0.983 for Sigma and Merck gelatin standards, correlation between actual values against predicted data obtained from the

cross-validation of gelatin mixture. Three major amino acids namely glycine (Gly), proline (Pro) and hydroxyproline (Hyp) were applied in principal component analysis (PCA) and the score plots obtained show good separation between pure bovine, pure porcine or the mixture of bovine and porcine gelatin for both Sigma and Merck standards.

Finally, the third study was conducted to know the capabilities of these detection methods to be applied in real food samples. Market samples were analyzed by both FTIR spectroscopy and amino acid analysis. Results presented by Cooman's plot and PCA proved that the samples were distinctly divided in two groups accordingly depending on their source without any confusion or mistake. However, the analysis cannot be done for the samples containing added ingredients such as flavouring and colouring.

In conclusion, this study provides methods for determining the source of gelatin focused on bovine and porcine. These methods are suitable to be use in the analysis of halal food authentication.

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

**PENGESANAN SUMBER GELATIN MENGGUNAKAN SPEKTROSKOPI FOURIER TRANSFORM INFRA MERAH DAN KROMATOGRAFI CECAIR PRESTASI TINGGI**

Oleh

**NORAKASHA BINTI RUSLI**

**Februari 2011**

**Pengerusi : Dzulkifly bin Mat Hashim, MSc.**

**Institut : Institut Penyelidikan Produk Halal**

Pengesanan sumber gelatin telah dikaji. Kaedah pantas telah dibangunkan menggunakan spektroskopi Fourier transform infra merah (FTIR) untuk membezakan antara sumber gelatin lembu dan khinzir. Hasil kajian menunjukkan bahawa spektroskopi FTIR dapat membezakan antara gelatin lembu dan khinzir dengan menganalisis julat di antara 3290- 3280 $cm^{-1}$ dan 1660- 1200 $cm^{-1}$ menggunakan analisis diskriminasi (DA). Plot Cooman's jelas menunjukkan bahawa kedua-dua gelatin tersebut dapat diklasifikasikan mengikut kumpulan masing-masing.

Kajian seterusnya menerangkan aplikasi kromatografi cecair prestasi tinggi (HPLC) dalam menentukan sumber gelatin menggunakan analisis asid amino. Kalibrasi 'partial least square' menunjukkan persamaan linear ($R^2$) yang baik

masing-masing bersamaan 0.991 dan 0.983 bagi gelatin standard Sigma dan Merck, iaitu korelasi anatara nilai sebenar dengan data ramalan yang diperolehi daripada model pengesahan campuran gelatin. Tiga asid amino utama iaitu glycine (Gly), proline (Pro) dan hydroxyproline (Hyp) telah digunakan dalam analisis komponen utama (PCA) and plot skor yang diperolehi menunjukkan pemisahan yang baik antara gelatin lembu asli, gelatin khinzir asli dan campuran antara gelatin lembu dan khinzir bagi kedua-dua standard Sigma dan Merck.

Akhir sekali, kajian ketiga dijalankan untuk mengetahui keupayaan kaedah pengesanan ini untuk digunapakai dalam sampel makanan sebenar. Sampel pasaran dianalisis menggunakan kedua-dua kaedah iaitu spektroskopi FTIR dan analisis amino asid. Hasil kajian yang ditunjukkan melalui plot Cooman's dan PCA membuktikan bahawa sampel terbahagi dengan jelas dalam dua kumpulan mengikut sumber masing-masing tanpa apa-apa kekeliruan dan kesilapan. Walau bagaimanapun, analisis ini tidak dapat dilakukan bagi sampel yang mengandungi bahan tambah seperti perasa dan pewarna.

Kesimpulannya, kajian ini menyediakan kaedah untuk menentukan sumber gelatin dengan menumpukan hanya kepada gelatin lembu dan khinzir. Kaedah ini sesuai untuk digunakan dalam analisis pengesahan makanan halal.

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## APPROVAL

I certify that an Examination Committee has meet on 23 February 2011 to conduct the final examination of Norakasha binti Rusli on her master thesis entitled Detection of Gelatin Origin using Fourier Transform Infrared Spectroscopy and High Performance Liquid Chromatography in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the master.

Members of the Examination Committee were as follows:

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Date: 22November 2011

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

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## 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.

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**NORAKASHA RUSLI**

Date: 23 February 2011

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