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

NORAKASHA BINTI RUSLI

IPPH 2011 1
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

DECEMBER 2011
Dedicate to those who wish to know and those who are devoted to the progress of society
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 (R²) 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 pengesahan 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.
ACKNOWLEDGEMENTS

In the name of Allah, Most Gracious, Most Merciful. Alhamdulillah, after a long journey, undergoing ups and downs in this research, finally the study on “Detection and verification of gelatin sources using FTIR and HPLC technique” have been completed with Allah’s grace. I would like to thank Allah S.W.T for giving me the strength to fulfill this requirement in order to complete my research.

I would also like to express my appreciation to my project supervisor, Mr. Dzulkifly bin Mat Hashim for all his valuable advice, encouragement, supervision and willingness to share his expertise throughout this project. I would also like to thank my committee members, Prof. Dr. Yaakob bin Che man and Assoc. Prof. Dr. Shuhaimi bin Mustafa for their wisdom and guidance in helping me completing my project.

Thank you to my husband, Rozzamri bin Ashari for his inspiration, motivation, moral support, financial support and time. For my late parents, Mariah binti Salim and Rusli bin Abu Hassan, this is the best gift that I can give to you. Thank you for all the love and advices that you instill within me, educating me to grow into this wonderful human being today with great moral values. Your memories and words have always been my inspiration and
motivation to achieve greatness in life. To my family and friends, this is a proof that no victory can be achieved without effort and patience.

My thanks also go to all staff in Halal Products Research Institute, Cik Noorfaizan, Cik Mafuza, Cik Wan Siti Farizan, En. Abdul Rohman, and all my lab mates. I would like to express my deepest gratitude to the Ministry of Science, Technology and Innovation Malaysia (MOSTI) for funding this study (Science Fund No. 05-01-04-SF0625 awarded to Prof. Dr. Yaakob B. Che Man).
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:

**Shahida Ahmad, PhD**
Faculty of Biotecnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

**Amin Ismail, PhD**
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal examiner)

**Tan Chin Ping, PhD**
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Internal examiner)

**Abdul Karim Alias, PhD**
Professor
School of Industrial Technology
Universiti Sains Malaysia
(External examiner)

---

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

Date: 22 November 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:

**Dzulkifly Mat Hashim**  
Lecturer  
Halal Products Research Institute  
Universiti Putra Malaysia  
(Chairman)

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

**Shuhaimi Mustafa, PhD**  
Associate 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.

________________________
NORAKASHA RUSLI

Date: 23 February 2011
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>v</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>vii</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>ix</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>xi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xvi</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xvii</td>
</tr>
</tbody>
</table>

## CHAPTER

### 1 GENERAL INTRODUCTION

### 2 LITERATURE REVIEW

#### 2.1 Gelatin

- 2.1.1 Introduction
- 2.1.2 Types and extraction of gelatins
- 2.1.3 Chemical composition of gelatin
- 2.1.4 Physical and chemical properties of gelatin
- 2.1.5 Functional properties of gelatin
  - 2.1.5.1 Gelling properties
  - 2.1.5.2 Foaming properties
  - 2.1.5.3 Emulsifier
  - 2.1.5.4 Stabilizer
  - 2.1.5.5 Microencapsulation
  - 2.1.5.6 Film forming
  - 2.1.5.7 Fruit and juice clarifying agent
  - 2.1.5.8 Nutritional properties
  - 2.1.5.9 Multiple applications of gelatin
- 2.1.6 Current analysis of gelatin
- 2.1.7 Market demand of gelatin

#### 2.2 Fourier Transform Infrared Spectroscopy

- 2.2.1 Mid infrared
2.2.2 Principles of Attenuated Total Reflectance
2.2.3 FTIR applications
2.3 Investigation of protein using HPLC
  2.3.1 Amino acid analysis
  2.4 Method development
  2.5 Method validation

3 POTENTIAL USE OF FOURIER TRANSFORM INFRARED SPECTROSCOPY FOR DIFFERENTIATION OF BOVINE AND PORCINE GELATINS
  3.1 Introduction
  3.2 Materials and methods
   3.2.1 Sample preparation
   3.2.2 FTIR measurement
   3.2.3 Acquisition of infrared spectra and data
   3.3.4 Discriminant analysis
  3.3 Results and discussion
   3.3.1 Spectral for gelatins
   3.3.2 Discriminant analysis and characterization of FTIR spectrum of standard gelatin
  3.4 Conclusion

4 AMINO ACIDS ANALYSIS FOR DISTINCTION OF BOVINE AND PORCINE GELATINS
  4.1 Introduction
  4.2 Materials and methods
   4.2.1 Materials
   4.2.2 Standard solutions
   4.2.3 Sample preparation
   4.2.4 Hydrolysis
   4.2.5 Derivatisation
   4.2.6 Chromatographic conditions
   4.2.7 Linearity
   4.2.8 Accuracy/ Recovery
   4.2.9 Precision
   4.2.10 Statistical analysis
4.3 Results and discussion
   4.3.1 Characteristics of validation method
   4.3.2 Analyzing amino acids in gelatins
   4.3.3 Partial least square and principal component analysis

4.4 Conclusion

5 APPLICATION OF FTIR AND HPLC IN DETERMINING SOURCE OF GELATIN IN MARKET SAMPLES
   5.1 Introduction
   5.2 Materials and methods
      5.2.1 Materials
      5.2.2 FTIR spectroscopic analysis
      5.2.3 Amino acid analysis
      5.2.4 Statistical analysis
   5.3 Results and discussion
      5.3.1 FTIR spectroscopic analysis
      5.3.2 Amino acid analysis
      5.3.3 Principal Component analysis
   5.4 Conclusion

6 GENERAL CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH
   6.1 Conclusion
   6.2 Recommendations

REFERENCES
BIODATA OF STUDENT
LIST OF PUBLICATIONS