The Role of standardized analytical method in supporting halal certification in Indonesia

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2019

INTRODUCTION TO HALAL ANALYSIS

- Halal authentication analysis is intended to confirm that the products (food, cosmetics, pharmaceuticals) are free-non halal-components
- The advanced technology in the industries has led to the use of non-halal components in the product.
  - *Pork*
  - *Lard*
  - *Porcine gelatines*
- Montowska and Pospiech (2010) reported that some food and pharmaceutical products available in the market may be labelled with incorrect or missing information related to ingredients sources.

Montowska, M., & Pospiech, E. (2010). Authenticity determination of meat and meat products on the protein and DNA basis. *Food Reviews International*, *27*(1), 84–100.

PRESIDENT
REPUBLIC OF INDONESIA

LAW OF REPUBLIC OF INDONESIA
NUMBER 33 YEAR 2014
CONCERNING
HALAL PRODUCT ASSURANCE BY

THE GRACE OF GOD ALMIGHTY

PRESIDENT OF REPUBLIC OF INDONESIA,

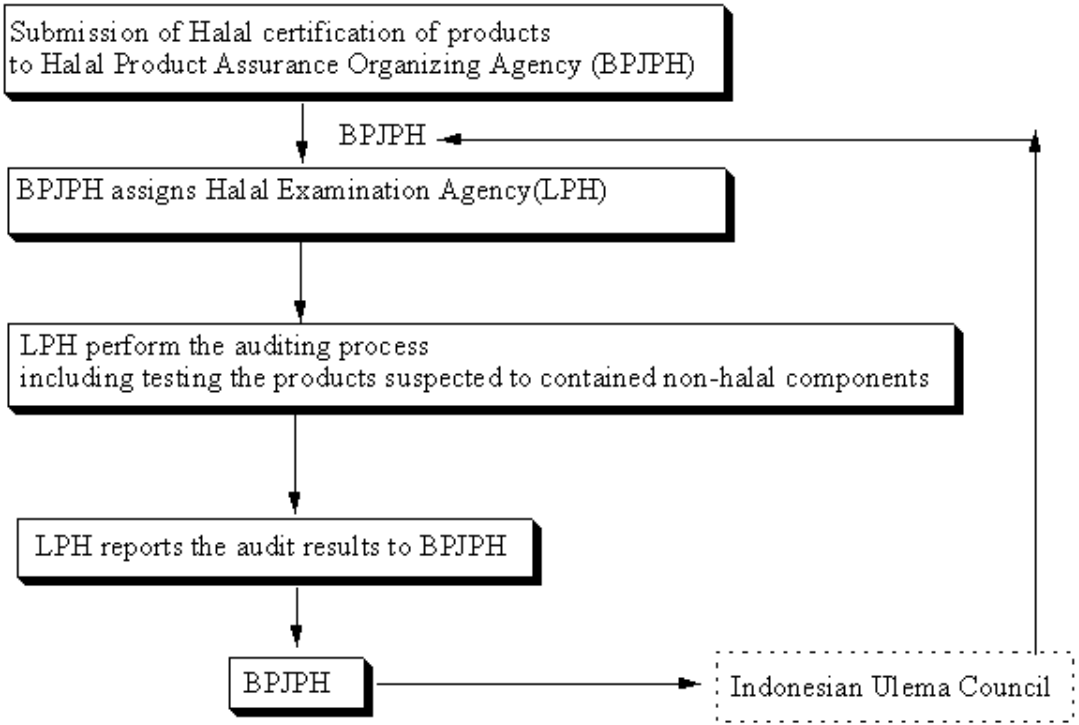
- Article 4
- Products that enter, circulate, and traded in the territory of Indonesia must be certified halal.

MANDATORY OF HALAL CERTIFICATION

HALAL CERTIFICATION in Indonesia

- Indonesia has IMPLEMENTED Indonesian Act No. 33, 2014 (*Undang-Undang Jaminan Produk Halal*, Act on Halal Products assurance)
- Halal certification is carried out by Halal Product Assurance Organizing Agency or *Badan Pelaksana Jaminan Produk Halal*
- The auditing process is carried out by Halal Examination Agency (*Lembaga Pemeriksa Halal*)

HALAL CERTIFICATION in Indonesia



Role the standardized analytical methods

Halal Examination Agency (LPH)

- Halal auditing is carried out by LPH
- To establish LPH, the following requirements must be fulfilled (Article 13):
  - having its own office and equipment;
  - having accreditation from BPJPH;
  - having a minimum of 3 (three) Halal Auditor; and
  - having a laboratory or cooperation agreement with other institutions which own a laboratory

The collaboration of BPJPH with LPH as intended is conducted for Product examination and/or testing of products (Article 9).

Need standardized methods for non-halal testing

ANALYSIS OF NON-HALAL COMPONENT

Food Reviews International
Publication details, including instructions for authors and subscription information:
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Analysis of Pig Derivatives for Halal Authentication Studies

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Non-halal components commonly found in food, cosmetics and pharmaceutical products are pig derivatives (lard, pork, porcine gelatin) and alcohol

- To comply with halal requirement, more stringent auditing/monitoring system is needed by Halal Authorities or Certification Bodies
- Reliable state-of-the-art scientific methods are required for analysis of non-halal components (e.g porcine origin, alcohol) in halal food
- Analytical techniques become major challenge for authentication of halal products

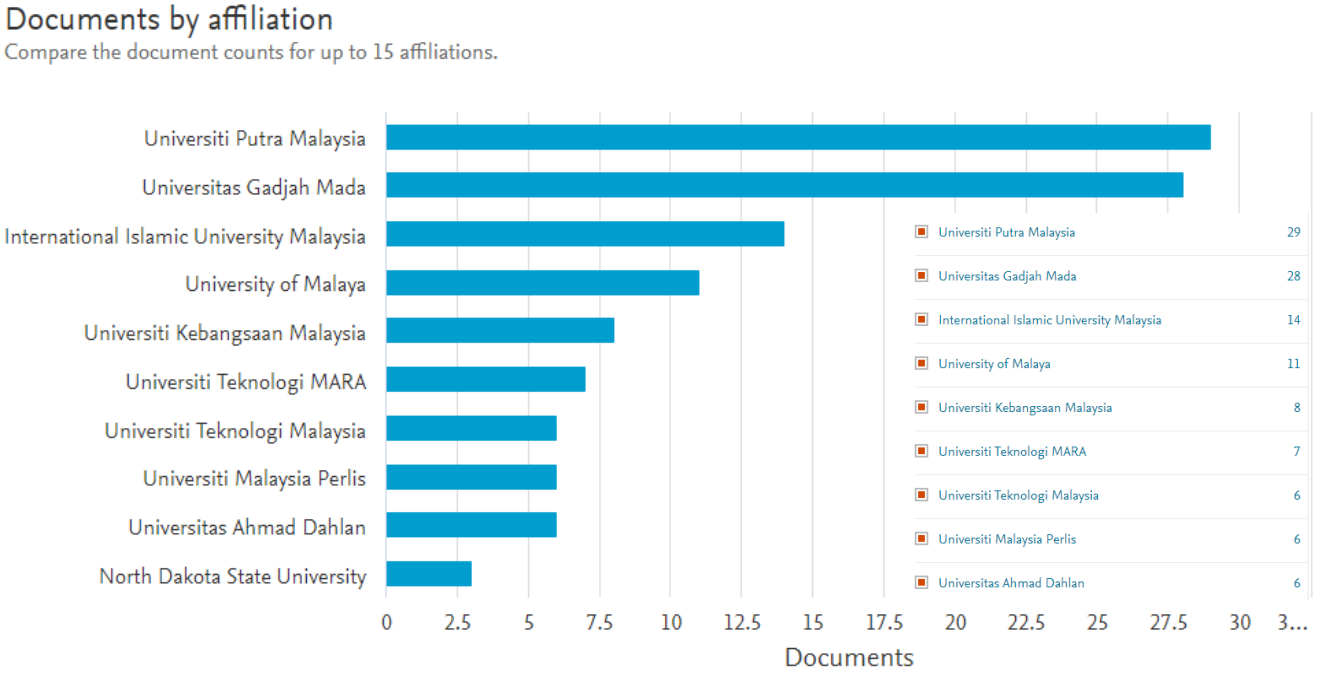
METHODS FOR HALAL AUTHENTICATION

- **Lard**
  - FTIR spectroscopy, especially combined with chemometrics (Lard, lipid based-products)
  - GC-MS (certain fatty acids in lard)
  - Differential scanning calorimetry (Lard, lipid based food)
  - Electronic nose or fast gas chromatography (analysis aroma profile)
- **Pork**
  - Real-time PCR
  - Enzyme immunosorbent assay
- **Porcine Gelatin**
  - RT-PCR (DNA-based methods for analysis of porcine DNA and non-allowed meat DNA)
  - LC-MS (peptide profile)

METHODS FOR HALAL AUTHENTICATION

- *Screening/exploratory*
  - FTIR spectroscopy
  - Differential scanning calorimetry (Lard, lipid based food)
  - Electronic nose or fast gas chromatography (analysis aroma profile)
- *Confirmatory*
  - RT-PCR (DNA-based methods for analysis of porcine DNA and non-allowed meat DNA)
  - LC-MS-MS (peptide profile)
  - GC-MS (certain fatty acids as markers in lard)

Number of Publication by Affiliation keywords: Halal Authentication



STANDARD METHODS

- Candidate of standard method
  - Lard: GC-MS
  - Pork: DNA based method
  - Gelatin: LC-MS/MS and PCR based methods
- To be standard methods, analytical techniques used must be:
  - Specific
  - Accurate
  - Precise
  - Sensitive
  - Robust

INFRARED SPECTROSCOPY

IR spectroscopy is based on the interaction between EMR and matters (samples) in IR regions

FTIR spectroscopy

Rapid and sensitive
Non destructive
Ease in sample presentation
used for qualitative
quantitative analyses

FINGER PRINT TECHNIQUE

AUTHENTICATION OF HALAL MEAT USING FTIR SPECTROSCOPY

JOURNAL OF ADVANCED VETERINARY AND ANIMAL RESEARCH
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MINI REVIEW

The employment of Fourier transform infrared spectroscopy coupled with chemometrics techniques for traceability and authentication of meat and meat products

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ABSTRACT

Meat-based food such as meatball and sausages are important sources of protein needed for the human body. Due to different prices, some unethical producers try to adulterate high-price meat such as beef with lower priced meat like pork and rat meat to gain economical profits, therefore, reliable and fast analytical techniques should be developed, validated, and applied for meat traceability and authenticity. Some instrumental techniques have been applied for the detection of meat adulteration, mainly relied on DNA and protein using polymerase chain reaction and chromatographic methods, respectively. But, this method is time-consuming, needs a sophisticated instrument, involves complex sample preparation which make the method is not suitable for routine analysis. As a consequence, a simpler method based on spectroscopic principles should be continuously developed. Food samples are sometimes complex which resulted in complex chemical responses. Fortunately,

ARTICLE HISTORY

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KEYWORDS

FTIR spectroscopy; authentication analysis; chemometrics; meat; meat products

GENERAL STEP FOR HALAL MEAT AUTHENTICATION IN FOOD PRODUCTS

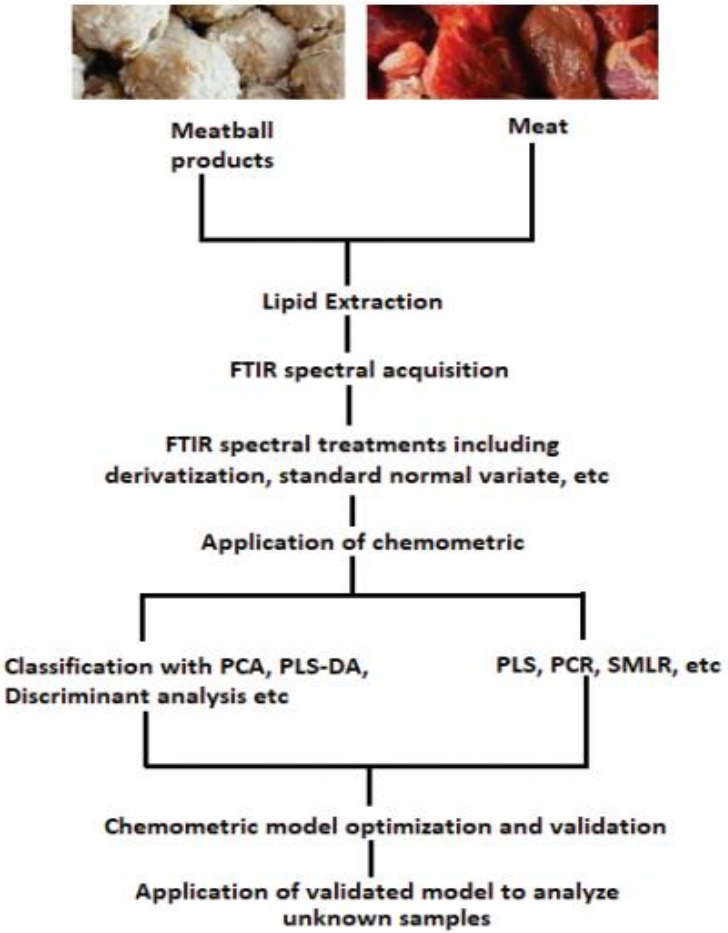


Figure 1. The sketch of application of FTIR spectroscopy in combination with chemomet-

Differentiation of Lard From Other Edible Fats and Oils by Means of Fourier Transform Infrared Spectroscopy and Chemometrics

Yaakob B. Che Man · A. Rohman · T. S. T. Mansor

Received: 3 May 2010 / Accepted: 20 July 2010 / Published online: 13 August 2010

Abstract Fourier transform infrared (FTIR) spectra at mid infrared regions (4,000–650 cm^{-1}) of lard and 16 edible fats and oils were compared and differentiated. The chemometrics of principal component analysis and cluster analysis (CA) was used for such differentiation using FTIR economical point of views. The food industry prefers to blend lard with some vegetable oils to minimize production costs because lard or industrially modified lard can be mixed efficiently with vegetable oils to produce cost-effective margarines, shortenings, and other oil-based

ANALYSIS OF LARD IN COSMETICS

J Am Oil Chem Soc (2012) 89:1537–1543
DOI 10.1007/s11746-012-2052-8

ORIGINAL PAPER

Quantitative Analysis of Lard in Cosmetic Lotion Formulation Using FTIR Spectroscopy and Partial Least Square Calibration

Endang Lukitaningsih · Miftahus Sa'adah · Purwanto · Abdul Rohman

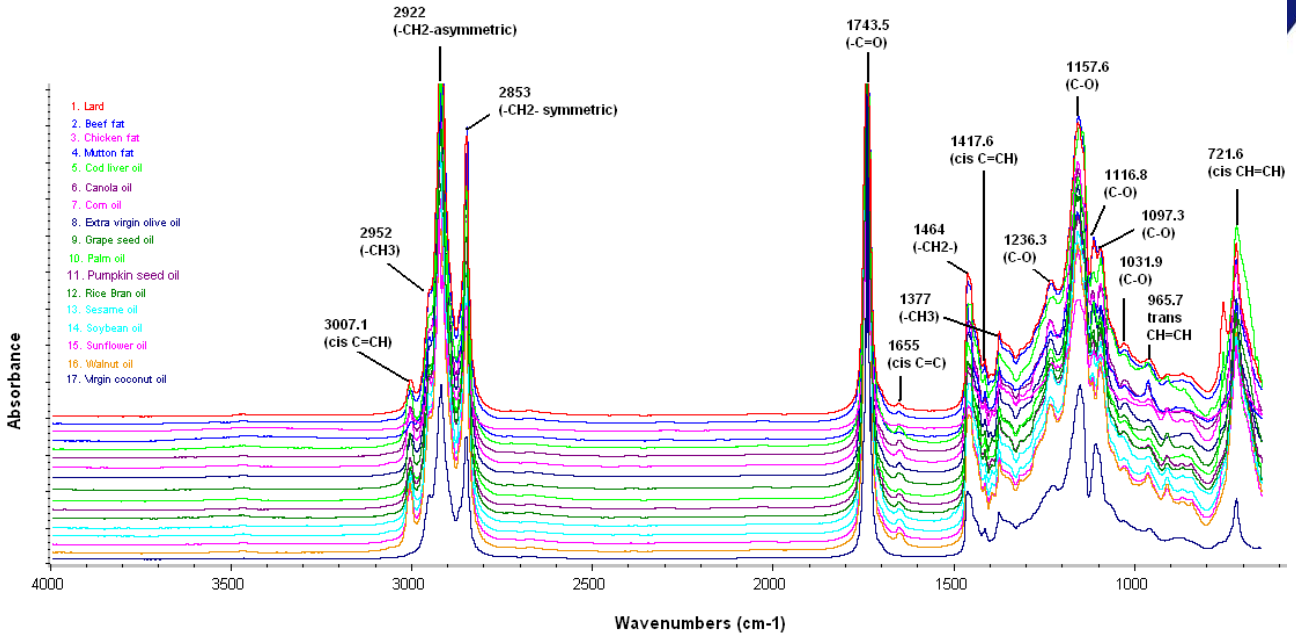
Received: 13 November 2011 / Revised: 26 February 2012 / Accepted: 6 March 2012 / Published online: 18 March 2012

Abstract Fourier transform infrared (FTIR) spectroscopy in combination with chemometrics of partial least squares (PLS) has been optimized for rapid determination of lard in a binary mixture with palm oil in a cosmetic lotion formulation. Lard, palm oil, and a binary mixture were

Introduction

In recent years, the use of personal care products in the form of cream and lotion cosmetic products has increased tremendously [1]. Human exposure to cosmetic formula-

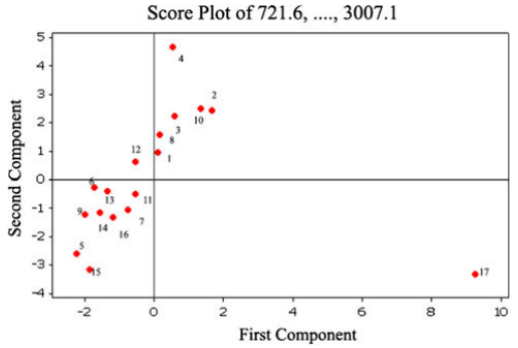
FTIR SPECTRA OF LARD AND OTHERS

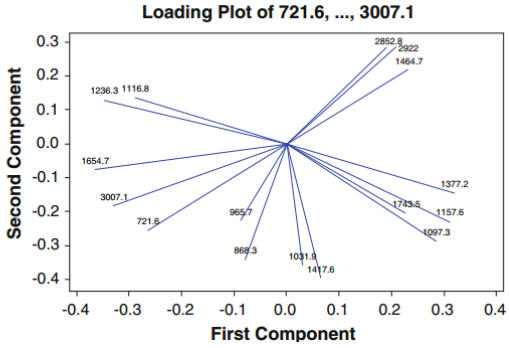


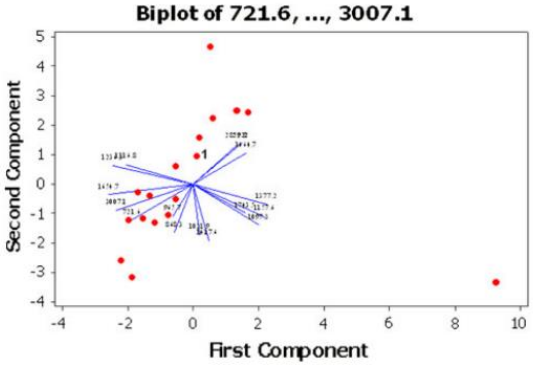
Rohman et al. (2011): JAOCS

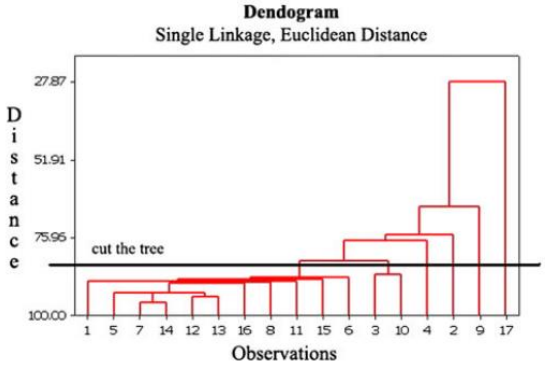
Jumlah Puncak (peak) /Bahu (shoulder)
Intensitas (absorbansi atau transmitans) puncak/bahu
Frekuensi eksak tiap puncak/bahu

CLASSIFICATION OF LARD AND OTHERS









1 Lard, 2 beef fat, 3 chicken fat, 4 mutton fat, 5 cod liver oil, 6 canola oil, 7 corn oil, 8 extra virgin olive oil, 9 grape seed oil, 10 palm oil, 11 pumpkin seed oil, 12 rice bran oil, 13 sesame oil, 14 soybean oil, 15 walnut oil, 16 sunflower oil, 17 virgin coconut oil

AUTHENTICATION OF HALAL MEAT USING FTIR SPECTROSCOPY

Meat adulterant	Meat adulterated	Meat-based products	Chemometrics	Wavenumbers (cm^{-1})	Results	References
Pork	Beef	Beef jerkys (dendeng)	LDA	Whole mid IR region (4,000–650)	LDA model could classify and predict the adulteration of Beef jerkys with pork, allowing 100% accuracy of the sample tested.	[39]
Pork offal (PO)	Beef offal (BO)	Fresh meat	SIMCA, LDA	1,002–1,240, 1,700–1,714, and 1,764–1,795 (BO) and 1,105–1,182 (PO).	SIMCA with mean-centered data could provide best model for the identification of BO, while LDA using non-scaled spectra offered best performance in classifying of PO	[40]
Pork	Beef	The mixture of beef-pork	PLS-Kernel calibration	Absorbance ratios of $A_{1,654\ cm^{-1}}/A_{1,745\ cm^{-1}}$, $A_{1,540\ cm^{-1}}/A_{1,745\ cm^{-1}}$, and $(A_{1,395\ cm^{-1}} + A_{1,450\ cm^{-1}})/ A_{1,175\ cm^{-1}}$	PLS-kernel calibration could predict the levels of pork in the mixture of pork-beef	[41]
Pork	Minced beef	Pork-beef fillet	PLSR	3,200–800 cm^{-1}	PLSR could predict the levels of pork with RMSEC of 4.88%, RMSEP of 9.45% and RMSECV of 10.30%	[42]
Pork	Beef	Ham sausages	PLSDA	Whole mid IR region (4,000–650)	PLSDA with standard normal variate treatment could classify halal (beef) sausage with sensitivity and specificity of 0.913 and 0.929.	[43]
Pork	Beef	Beef Meatballs	PLSR	1,200–1,000 cm^{-1},	PLSR could predict pork in beef meatballs with R^2 for	

Advantages and Disadvantages of FTIR

- Advantages
  - Simple and some cases without any sample preparation
  - Specific because FTIR spectra are fingerprint in nature

- Disadvantages
  - The developed method can only be used for formulations of samples consistent with those tested
  - If the composition of the sample to be analyzed is different, FTIR spectra of the analyte in the mixture will also be different.
  - the presence of non-halal components in the different food samples is quantified using different spectral regions.

ANALYSIS OF NON-HALAL MEAT

Meat Science 96 (2014) 94–98

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Analysis of lard in meatball broth using Fourier transform infrared spectroscopy and chemometrics

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Keywords:
Lard
Meatball broth
FTIR spectroscopy
Chemometrics

ABSTRACT

Meatball is one of the favorite foods in Indonesia. For the economic reason (due to the price difference), the substitution of beef meat with pork can occur. In this study, FTIR spectroscopy in combination with chemometrics of partial least square (PLS) and principal component analysis (PCA) was used for analysis of pork fat (lard) in meatball broth. Lard in meatball broth was quantitatively determined at wavenumber region of 1018–1284 cm^{-1}. The coefficient of determination (R^2) and root mean square error of calibration (RMSEC) values obtained were 0.9975 and 1.34% (v/v), respectively. Furthermore, the classification of lard and beef fat in meatball broth as well as in commercial samples was performed at wavenumber region of 1200–1000 cm^{-1}. The results showed that FTIR spectroscopy coupled with chemometrics can be used for quantitative analysis and classification of lard in meatball broth for Halal verification studies. The developed method is simple in operation, rapid and not involving extensive sample preparation.

Meat Science 88 (2011) 91–95

Contents lists available at ScienceDirect

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Analysis of pork adulteration in beef meatball using Fourier transform infrared (FTIR) spectroscopy

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Keywords:
Beef meatball
Pork
Adulteration
FTIR spectroscopy
Partial least square (PLS)

ABSTRACT

Meatball is one of the favorite foods in Indonesia. The adulteration of pork in beef meatball is frequently occurring. This study was aimed to develop a fast and non destructive technique for the detection and quantification of pork in beef meatball using Fourier transform infrared (FTIR) spectroscopy and partial least square (PLS) calibration. The spectral bands associated with pork fat (PF), beef fat (BF), and their mixtures in meatball formulation were scanned, interpreted, and identified by relating them to those spectroscopically representative to pure PF and BF. For quantitative analysis, PLS regression was used to develop a calibration model at the selected fingerprint regions of 1200–1000 cm^{-1}. The equation obtained for the relationship between actual PF value and FTIR predicted values in PLS calibration model was $y = 0.990x + 0.004$, with coefficient of determination (R^2) and root mean square error of calibration are 0.999 and 0.442, respectively. The PLS calibration model was subsequently used for the prediction of independent samples using laboratory made meatball samples containing the mixtures of BF and PF. Using 4 principal components, root mean square error of prediction is 0.742. The results showed that FTIR spectroscopy can be used for the detection and quantification of pork in beef meatball formulation for Halal verification purposes.

Meat Science 100 (2015) 301–305

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

The employment of FTIR spectroscopy in combination with chemometrics for analysis of rat meat in meatball formulation

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Keywords:
FTIR spectroscopy
Meatball
Partial least square
Principal component analysis
Rat meat

ABSTRACT

For Indonesian community, meatball is one of the favorite meat food products. In order to gain economical benefits, the substitution of beef meat with rat meat can happen due to the different prices between rat meat and beef. In this present research, the feasibility of FTIR spectroscopy in combination with multivariate calibration of partial least square (PLS) was used for the quantitative analysis of rat meat in the binary mixture of beef in meatball formulation. Meanwhile, the chemometrics of principal component analysis (PCA) was used for the classification between rat meat and beef meatballs. Some frequency regions in mid infrared region were optimized, and finally, the frequency region of 750–1000 cm^{-1} was selected during PLS and PCA modeling. For quantitative analysis, the relationship between actual values (x-axis) and FTIR predicted values (y-axis) of rat meat is described by the equation of $y = 0.9417x + 2.8410$ with coefficient of determination (R^2) of 0.993, and root mean square error of calibration (RMSEC) of 1.79%. Furthermore, PCA was successfully used for the classification of rat meat meatball and beef meatball.

http://doi.org/10.5455/javar.2018.c281
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Vol 5 No 3, Pages 307-314.

Original Article

The potential use of infrared spectroscopy and multivariate analysis for differentiation of beef meatball from dog meat for Halal authentication analysis

W.S. Rahayu[1,2], S. Martono[1], Sudjadi[1] and Abdul Rohman[1,3,#]

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ABSTRACT

Objective: The objective of this research was to assess the suitability of FTIR spectroscopy coupled with multivariate analysis of partial least square regression (PLSR) along with pattern recognition technique of principal component analysis (PCA) for rapid quantitative and qualitative (identification) analysis of dog meat in beef meatball formulation.

Materials and Methods: The lipid fraction of meatball was obtained by employing two different extraction techniques, namely Bligh-Dyer and Folch method. FTIR spectral bands correlated with beef fat, pork fat, chicken fat and rat fat were measured, interpreted, and qualitatively analyzed. The small variations among spectra were exploited as a basis tools to differentiate between dog fat and

The use of FTIR spectra for porcine gelatin

- Porcine gelatin is frequently used in capsule shell (pharmaceutical products) or candies (food products).
- FTIR spectra could be applied as screening method
- Need further confirmation using real-time PVR or LC-MS/MS

Food Chemistry 277 (2019) 373–381

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

A rapid ATR-FTIR spectroscopic method for classification of gelatin gummy candies in relation to the gelatin source

Nur Cebi[a,*], Canan Ekinci Dogan[b,*], Ayten Ekin Mese[c], Durmus Ozdemir[c], Muhammet Arıcı[a], Osman Sagdic[a]

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ARTICLE INFO

Keywords:
FTIR spectroscopy
Gelatin
Chemometrics
Source determination
Gummy candy
Halal food

ABSTRACT

Gelatin is widely used in gummy candies because of its unique functional properties. Generally, porcine and bovine gelatins are used in the food industry. FTIR-ATR combined with chemometrics analysis such as hierarchical cluster analysis (HCA) (OPUS Version 7.2 software), principal component analysis (PCA) (OPUS Version 7.2 software) and partial least squares-discriminant analysis (PLS-DA) (Matlab R2017b) were used for classification and discrimination of gelatin gummy candies related to their gelatin source. The spectral region between 1734 and 1528 cm^{-1} was selected for chemometric analysis. The potential of FTIR spectroscopy for determination of bovine and porcine source in gummy candies was examined and validated by a real-time polymerase chain reaction (PCR) method. Twenty commercial samples were tested by developed ATR-FTIR methodology and RT-PCR technique, mutually confirming and supporting results were obtained. Gummy candies were classified and discriminated in relation to the bovine or porcine source of gelatin with 100% success without any sample preparation using FTIR-ATR technique.

Food Chemistry 190 (2016) 1109–1115

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

An evaluation of Fourier transforms infrared spectroscopy method for the classification and discrimination of bovine, porcine and fish gelatins

Nur Cebi, M. Zeki Durak *, Omer Said Toker, Osman Sagdic, Muhammet Arici

Yildiz Technical University, Chemical and Metallurgical Engineering Faculty, Food Engineering Department, 34210 Istanbul, Turkey

ARTICLE INFO

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Keywords:
ATR–FTIR
Gelatin
Bovine
Porcine
Fish

ABSTRACT

The objective of this research was to develop a rapid spectroscopic technique as an alternative method for the differentiation and authentication of gelatin sources in food products by using attenuated total reflectance Fourier transform infrared (ATR–FTIR) spectra combined with chemometrics. Clear discrimination and classification of all the studied gelatin sources (bovine, porcine, and fish) were achieved by hierarchical cluster and principle component analysis (PCA). Amide-I (1700–1600 cm^{-1}) and Amide-II (1565–1520 cm^{-1}) spectral bands were used in a chemometric method. Moreover, ATR–FTIR spectral data successfully discriminated pure bovine gelatin from mixture of bovine and porcine gelatins, which is very important for the food industry. The method that we adopted could be beneficial for rapid, simple and economic determination of both gelatin presence and its origin from food products such as yogurt, ice cream, milk dessert or other gelatin containing products such as pharmaceuticals and cosmetics.

Differentiation of gelatin sources

- Suitable for analysis of gelatin sources
  - Porcine
  - Bovine
  - Porcine
- Need classification chemometrics
  - PCA
  - Cluster analysis

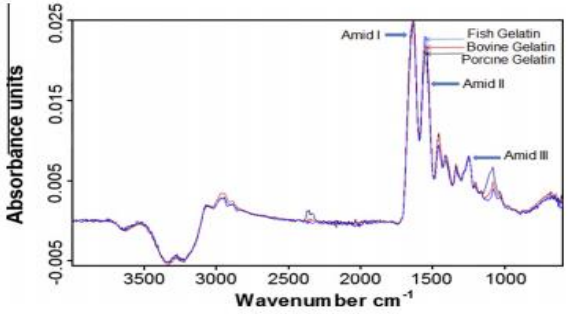

Fig. 1. FTIR spectrum of the fish gelatin, bovine gelatin and porcine gelatin.

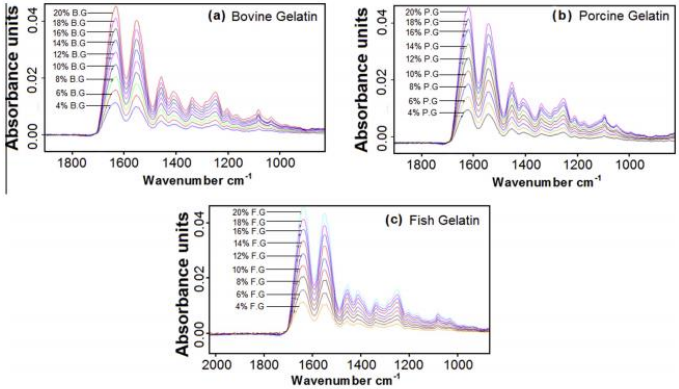

Fig. 2. Concentration-dependent FTIR spectra of bovine gelatin (B.G) (a), porcine gelatin (P.G) (b) and fish gelatin (F.G) (c).

Analysis of porcine in food products

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A rapid ATR-FTIR spectroscopic method for classification of gelatin gummy candies in relation to the gelatin source

Nur Cebi[a,*], Canan Ekinci Dogan[b,*], Ayten Ekin Mese[c], Durmus Ozdemir[c], Muhammet Arıcı[a], Osman Sagdic[a]

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ARTICLE INFO

Keywords:
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Gelatin
Chemometrics
Source determination
Gummy candy
Halal food

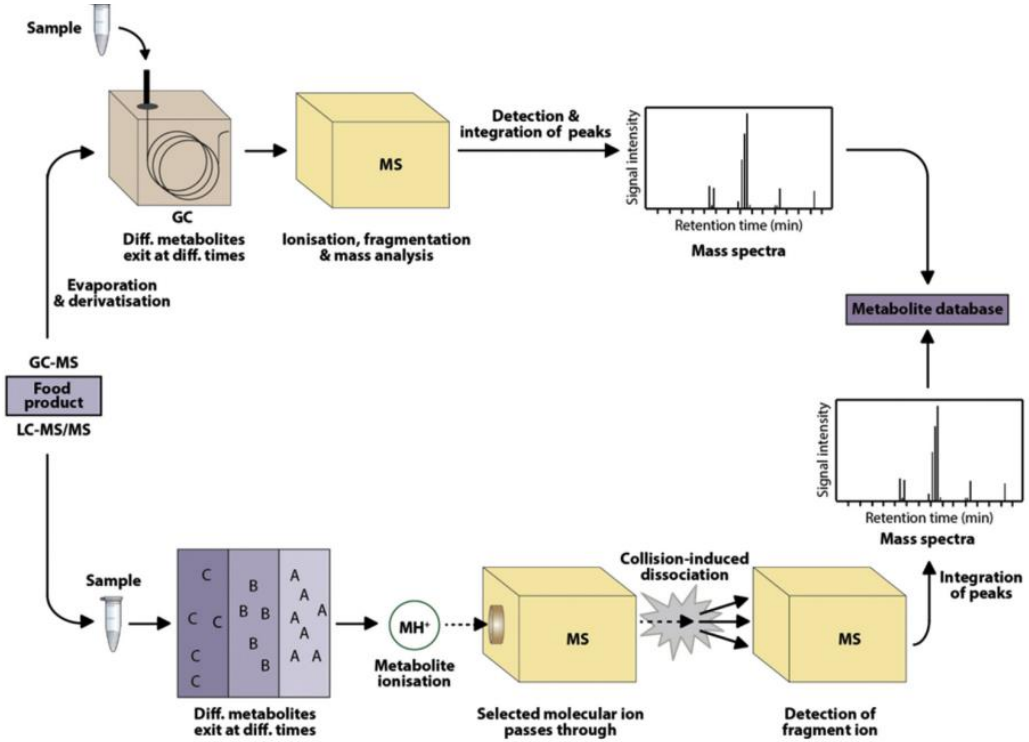
ABSTRACT

Gelatin is widely used in gummy candies because of its unique functional properties. Generally, porcine and bovine gelatins are used in the food industry. FTIR-ATR combined with chemometrics analysis such as hierarchical cluster analysis (HCA) (OPUS Version 7.2 software), principal component analysis (PCA) (OPUS Version 7.2 software) and partial least squares-discriminant analysis (PLS-DA) (Matlab R2017b) were used for classification and discrimination of gelatin gummy candies related to their gelatin source. The spectral region between 1734 and 1528 cm^{-1} was selected for chemometric analysis. The potential of FTIR spectroscopy for determination of bovine and porcine source in gummy candies was examined and validated by a real-time polymerase chain reaction (PCR) method. Twenty commercial samples were tested by developed ATR-FTIR methodology and RT-PCR technique, mutually confirming and supporting results were obtained. Gummy candies were classified and discriminated in relation to the bovine or porcine source of gelatin with 100% success without any sample preparation using FTIR-ATR technique.

Chromatographic-based methods: LC-MS and GC-MS

- In liquid chromatography (LC) and gas chromatography (GC), non-halal components are separated into its separate components via interaction between a liquid or gas mobile phase, respectively
- This methodology focuses on searching the specific markers present in porcine (fatty acids composition, triglyceride, peptide, proteins).
  - GC-MS: fatty acid markers in lard
  - LC-MS: specific peptide markers in porcine gelatines
- Oftentimes, GC and LC are combined with mass spectrometry (MS).
- Need the chemometrics techniques for making profiling between non-halal and halal components

Chromatographic-based methods: LC-MS and GC-MS



GC-MS coupled with chemometrics

- The analytes must be volatile and stable to high temperature
- Typically used for analysis
  - Fatty acid composition of lard and other animal fats
  - MAG and DAG
- GC-MS is used for searching the specific fatty acids markers in lard

FATTY ACID ANALYSIS USING 2D-GC

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Analytical Methods

Lard detection based on fatty acids profile using comprehensive gas chromatography hyphenated with time-of-flight mass spectrometry

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ABSTRACT

Comprehensive gas chromatography hyphenated with time-of-flight mass spectrometry was applied to detect the differences between lard (LA) and three other commonly animal-derived fats, namely cattle fat (CA), chicken fat (CF) and goat fat (GF). Combination of two different microbore columns (SLB-5ms and DB-wax) allowed the discrimination of lard from other animal fats by three fatty acid methyl esters (FAMEs) constituents involving methyl trans-9,12,15-octadecatrienoate (C18:3 n3t), methyl 11,14,17-eicosatrienoate (C20:3 n3t) and methyl 11,14-eicosadienoate (C20:2 n6). The FAME profiles could be used as a basis for discriminating lard from other animal fats in food authentication process.

Fatty acids composition of animal fats

Different carbon chain length and level of unsaturation of FAME components were grouped clearly on the GC GC

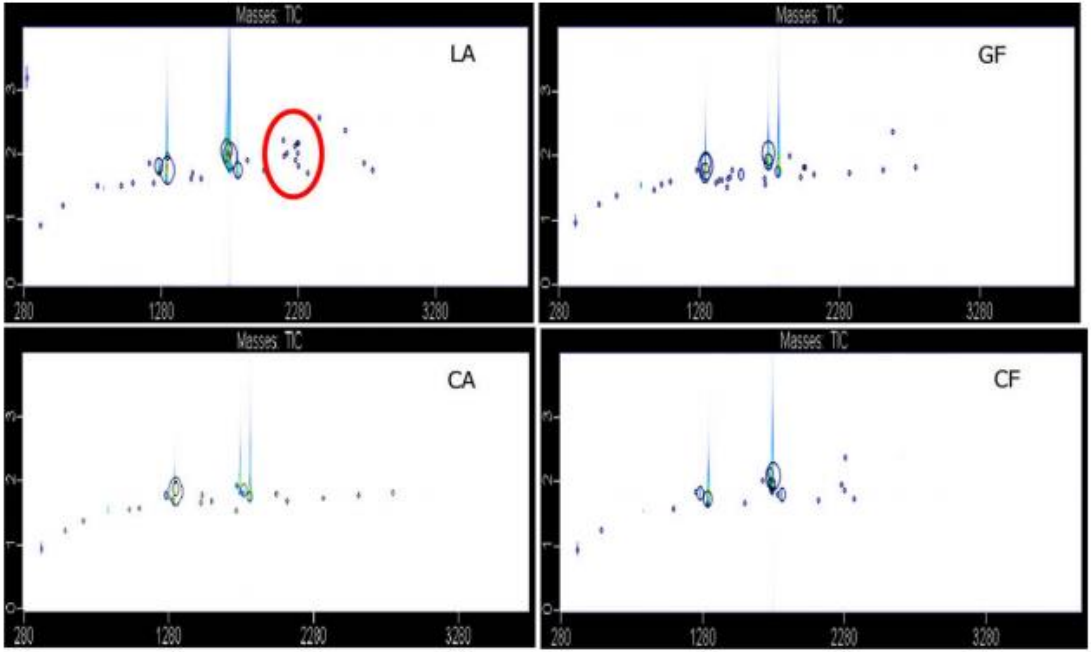


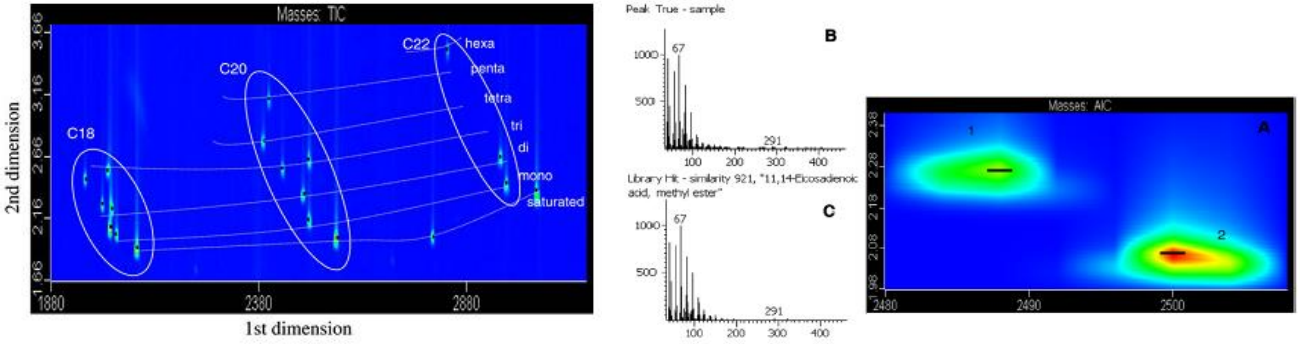
Fig. 2. 2D-contour plot chromatogram of animal-derived FAME; lard (LA), goat fat (GF), cattle fat (CA), and chicken fat (CF).

Composition of fatty acids in animal fats

Table 1
Lard FAME profiles and other animal fats by GC × GC-TOF-MS.

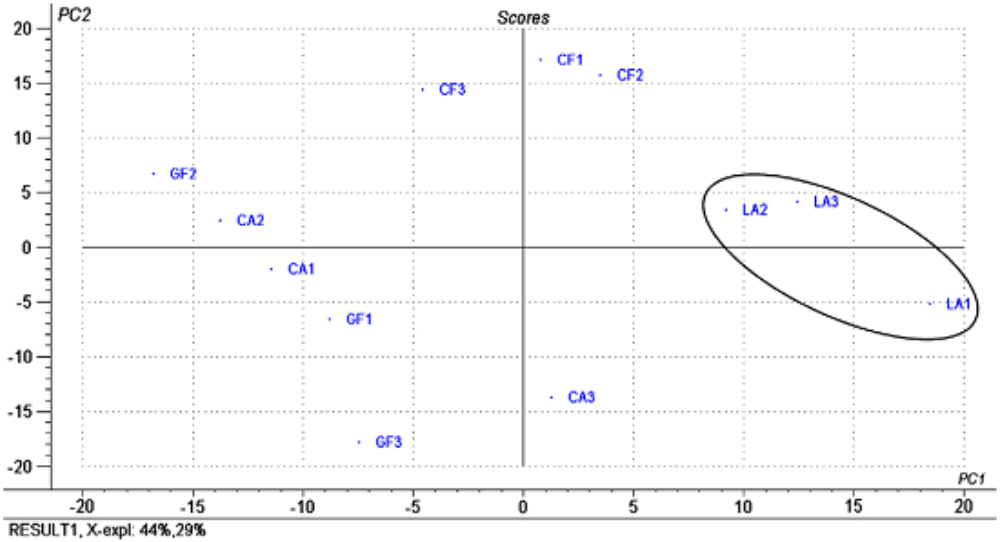
Formula	FAME compound	Composition (%)			
		LA	CA	CF	GF
C8:0	Methyl octanoate	0.006 ± 0.004^{a}	nd	nd	0.007 ± 0.012^{b}
C9:0	Methyl nonanoate	0.009 ± 0.016^{a}	nd	nd	0.116 ± 0.112^{b}
C10:0	Methyl decanoate	0.010 ± 0.005^{a}	0.024 ± 0.023^{b}	0.259 ± 0.440^{d}	0.102 ± 0.074^{c}
C12:0	Methyl dodecanoate	0.066 ± 0.014^{a}	0.915 ± 1.527^{c}	0.545 ± 0.211^{b}	0.138 ± 0.038^{d}
C13:0 ai	Methyl 10-methyl dodecanoate	nd	nd	nd	0.100 ± 0.127
C13:0	Methyl tridecanoate	nd	0.017 ± 0.016^{a}	nd	0.021 ± 0.004^{b}
C14:0 ai	Methyl 12-methyl tridecanoate	nd	0.113 ± 0.029^{a}	nd	0.157 ± 0.031^{b}
C14:1	Methyl 11-tetradecenoate	0.109 ± 0.095^{b}	2.202 ± 1.891^{d}	0.244 ± 0.160^{c}	0.031 ± 0.040^{a}
C14:0	Methyl tetradecanoate	1.058 ± 0.309^{a}	7.854 ± 2.179^{d}	1.609 ± 0.731^{b}	4.571 ± 1.169^{c}
4,8,12m-C15:0	Methyl 4,8,12-trimethyl tridecanoate	nd	nd	nd	0.273 ± 0.134
C15:0 i	Methyl 13-methyl tetradecanoate	nd	0.341 ± 0.057^{a}	nd	0.498 ± 0.050^{a}
C15:0 ai	Methyl 12-methyl tetradecanoate	nd	0.298 ± 0.258^{a}	nd	0.643 ± 0.168^{b}
C15:1	Methyl pentadecenoate	nd	nd	nd	0.017 ± 0.016
C15:0	Methyl pentadecanoate	0.082 ± 0.006^{a}	0.914 ± 0.311^{b}	0.1059 ± 0.043^{c}	1.107 ± 0.258^{d}
C16:0 i	Methyl 14-methyl pentadecanoate	nd	0.306 ± 0.062^{a}	nd	0.400 ± 0.074^{b}
C16:2 n6	Methyl 7,10-hexadecadienoate	0.059 ± 0.055^{a}	nd	0.107 ± 0.064^{b}	nd
C16:1 n7	Methyl 9-hexadecenoate	0.359 ± 0.033^{b}	0.128 ± 0.111^{a}	0.327 ± 0.337^{b}	0.474 ± 0.073^{c}
C16:1 n9t	Methyl 7-hexadecenoate	5.954 ± 3.300^{c}	4.768 ± 2.199^{b}	8.961 ± 2.986^{d}	1.500 ± 0.706^{a}
C16:1 n9c	Methyl 7-hexadecenoate	nd	0.952 ± 1.649^{b}	nd	0.026 ± 0.046^{a}
C16:0	Methyl hexadecanoate	15.979 ± 5.608^{a}	22.418 ± 7.679^{b}	24.128 ± 4.618^{c}	25.352 ± 12.099^{d}
2m-C16:0	Methyl 2-methyl hexadecanoate	nd	nd	nd	0.010 ± 0.017
7m-C16:1	Methyl 7-methyl hexadece-6-noate	nd	nd	nd	0.110 ± 0.167
2,6,10,14m-C15:0	Methyl 2,6,10,14-tetramethyl pentadecanoate	nd	nd	nd	0.210 ± 0.273
C17:1 n7	Methyl 10-heptadecenoate	nd	nd	nd	0.033 ± 0.030
C17:0 i	Methyl 15-methyl hexadecanoate	0.017 ± 0.008^{a}	0.476 ± 0.096^{b}	nd	0.708 ± 0.105^{c}
C17:0 ai	Methyl 14-methyl hexadecanoate	nd	1.164 ± 0.501^{a}	nd	1.492 ± 0.526^{a}
C17:1	Methyl heptadecenoate	0.225 ± 0.139^{a}	0.752 ± 0.321^{b}	nd	0.746 ± 0.354^{b}
C17:0	Methyl heptadecanoate	0.272 ± 0.207^{b}	1.839 ± 0.598^{c}	0.153 ± 0.048^{a}	2.777 ± 0.763^{d}
C18:0 i	Methyl 16-methyl heptadecanoate	nd	0.103 ± 0.091^{a}	nd	0.200 ± 0.179^{b}
3,7,11,15m-C16:0	Methyl 3,7,11,15-tetramethyl hexadecanoate	nd	0.002 ± 0.004^{a}	nd	0.10 ± 0.046^{b}
C18:3 n3t	Methyl trans-9,12,15-octadecatrienoate	6.754 ± 4.684	nd	nd	nd
C18:3 n3c	Methyl cis-9,12,15-octadecatrienoate	0.043 ± 0.043^{a}	nd	0.144 ± 0.087^{b}	nd
C18:2 n6t	Methyl trans-9,12-octadecadienoate	17.568 ± 1.473^{d}	2.006 ± 0.707^{b}	14.853 ± 7.622^{c}	0.394 ± 0.682^{a}
C18:1 n9t	Methyl trans-9-octadecenoate	32.000 ± 4.172^{b}	23.692 ± 10.317^{a}	25.142 ± 18.030^{ab}	23.400 ± 6.239^{a}
C18:1 n9c	Methyl cis-9-octadecenoate	2.437 ± 1.226^{a}	5.363 ± 2.065^{b}	nd	8.315 ± 4.059^{c}
C18:1	Methyl 7-octadecenoate	nd	0.579 ± 1.003	nd	nd
C18:1 n7	Methyl 11-octadecenoate	0.578 ± 0.944^{a}	0.851 ± 0.816^{a}	nd	0.724 ± 0.658^{a}
C18:0	Methyl octadecanoate	14.365 ± 2.097^{b}	21.836 ± 1.624^{c}	10.471 ± 4.147^{a}	24.053 ± 10.14^{d}
C19:0 i	Methyl 17-methyl octadecanoate	nd	nd	nd	0.351 ± 0.587

IDENTIFICATION OF FATTY ACID



- GC x GC-TOF/MS was used to look for specific fatty acids as chemical markers for identification of lard.
- Three fatty acids were successfully used as fatty acid markers in lard
  - trans-9,12,15-octadecatrienoate (C18:3 n3t)
  - 11,14,17-eicosatrienoate (C20:3 n3t)
  - 11,14-eicosadienoate (C20:2n6)

Classification of animal fats



GELATINE ANALYSIS

- Some reviews on gelatin analysis existed, mainly based on the physico-chemical properties and molecular biology-based methods
- Some of the methods are only suitable for analysis of pure compounds such as FTIR spectroscopy and HPLC
- LC-MS/MS and real-time PCR are potential to be developed as official methods for detection (confirmation) of gelatins due to its capability to find specific markers
  - Real time PCR → DNA
  - LC-MS/MS → specific peptides

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Mass spectrometric detection of marker peptides in tryptic digests of gelatin: A new method to differentiate between bovine and porcine gelatin

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ABSTRACT

Gelatin is a mixture of polypeptides obtained by hydrolysis of collagen primarily from bovine and porcine skin and bones. The similarity between different gelatins makes it difficult to trace their species origin. In this work, a new method for differentiation between bovine and porcine gelatin was developed based on detection and identification of marker peptides in digested gelatins. Sequence alignment analysis indicates that bovine and porcine Type I collagen contain differential sequences. The gelatins were digested by trypsin, and the resulting peptides were analyzed by high performance liquid chromatography/tandem mass spectrometry (HPLC–MS/MS). The marker peptides specific for bovine and porcine were successfully detected in the digested bovine and porcine gelatin, respectively. Comparative analysis indicated that more marker peptides could be detected in gelatin with a higher mean molecular weight. It was found that proline hydroxylation was a key factor affecting the peptide identification. For peptides such as GPPGSAGSPGK and GPPGSAGAPGK detected in digested bovine and porcine gelatin, respectively, the sequence should be verified manually since the mass shift caused by proline hydroxylation can be confused with the mass difference between Ser and Ala residues. The results indicate that detection of marker peptides in the digested gelatin sample using HPLC–MS/MS is an effective method to differentiate between bovine and porcine gelatin.

MASS SPECTRUM OF BOVINE GELATI

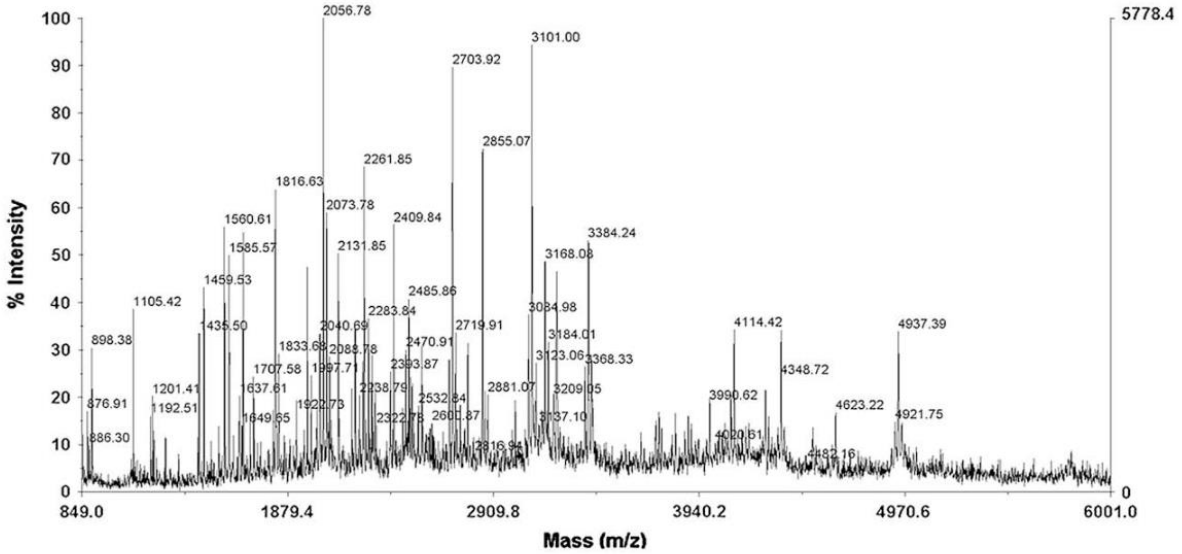

Fig. 2. MALDI-TOF mass spectra of bovine gelatin digested by trypsin at 37 °C for 10 h.

PEPTIDE MARKERS FOR DIFFERENTIATION BOVINE AND PORCINE GELATINES

Table 1
Marker peptides used for the differentiation of bovine and porcine gelatin.

No.	Position[a]	Bovine Type I collagen[b,e,f]	m/z[d]	Porcine Type I collagen[c,e,f]	m/z[d]
1.	α1 315–324	PGAPGPAGAR	850.5	PGPPGPAGAR	876.5
2.	α1 451–470	GEPGPTGIQGPPGPAGEEGK	1831.9	GEPGPTGVQGPPGPAGEEGK	1817.9
3.	α1 508–522	GPAGERGAPGPAGPK[g]	1318.7	GPAGERGSPGPAGPK[g]	1334.7
4.	α1 784–798	GEAGPSGPAGPTGAR	1281.6	GETGPSGPAGPTGAR	1311.6
5.	α1 921–936	PGEVGPPGPPGPAGEK	1442.7	PGEAGPPGPPGPAGEK	1414.7
6.	α1 937–960	GAPGADGPAGAPGTPGPQGIAGQR[g]	2057.0	GSPGADGPAGAPGTPGPQGIAGQR[g]	2073.0
7.	α1 987–996	QGPSGASGER	945.5	QGPSGPSGER	971.5
8.	α1 1029–1035	DGSPGAK	631.1	DGAPGPK	641.3
9.	α1 1039–1064	GETGPAGPPGAPGAPGAPGPVGPAGK	2121.1	GESGPAGPPGAPGAPGAPGPVGPAGK	2107.1
10.	α1 1069–1086	GETGPAGPAGPIGPVGAR	1560.7	GETGPAGPAGPVGPVGAR	1546.8
11.	α1 1144–1154	GPPGSAGSPGK[g]	911.4	GPPGSAGAPGK[g]	895.5
12.	α2 235–264	GSDGSVGPVGPAGPIGSAGPPGFPGAPGPK	2541.3	GNDGSVGPVGPAGPIGSAGPPGFPGAPGPK	2568.3
13.	α2 283–309	GEVGLPGLSGPVGPPGNPGANGLPGAK	2366.3	GEVGLPGVSGPVGPPGNPGANGLPGAK	2352.2
14.	α2 310–327	GAAGLPGVAGAPGLPGPR[g]	1514.9	GAAGLLGVAGAPGLPGPR[g]	1530.9
15.	α2 328–342	GIPGPVGAAGATGAR	1251.7	GIPGPAGAAGATGAR	1223.7
16.	α2 361–380	GEPGAVGQPGPPGPSGEEGK	1803.8	GEPGAAGPQGPPGPSGEEGK	1775.8
17.	α2 382–399	GSTGEIGPAGPPGPPGLR	1616.8	GPNGEVGSAGPPGPPGLR	1615.8
18.	α2 414–423	AGVMGPAGSR	902.5	AGVMGPPGSR	928.5
19.	α2 424–432	GATGPAGVR	785.4	GPTGPAGVR	811.4
20.	α2 451–464	GFPGSPGNIGPAGK	1255.6	GFPGSPGNVGPAGK	1241.6
21.	α2 465–476	EGPVGLPGIDGR	1166.6	EGPAGLPGIDGR	1138.6
22.	α2 499–506	GPSGDPGK	714.3	GPTGDPGK	728.4
23.	α2 520–543	GAPGPDGNNGAQGPPGLQGVQGGK[g]	2130.0	GAPGPDGNNGAQGPPGPQGVQGGK[g]	2114.0
24.	α2 544–569	GEQGPAGPPGFQGLPGPAGTAGEAGK	2305.1	GEQGPAGPPGFQGLPGPAGTAGEVGK	2333.2
25.	α2 574–588	GIPGEFGLPGPAGAR	1395.7	GIPGEFGLPGPAGPR	1421.7
26.	α2 592–609	GPPGESGAAGPTGPIGSR	1564.8	GPPGESGAAGPAGPIGSR	1534.8
27.	α2 622–645	GEPGVVGAPGTAGPSGPSGLPGER	2103.1	GEPGVLGAPGTAGPSGPSGLPGER	2117.1
28.	α2 664–671	GDIGSPGR	758.4	GDVGSPGR	744.4
29.	α2 676–693	GAPGAIGAPGPAGANGDR	1505.8	GAPGAVGAPGPAGANGDR	1491.7
30.	α2 748–777	GENGPVGPTGPVGAAGPSGPNGPPGPAGSR[g]	2565.3	GENGPVGPTGPVGAAGPAGPNGPPGPAGSR[g]	2549.3
31.	α2 795–815	TGPPGPSGISGPPGPPGPAGK	1781.9	IGPPGPSGISGPPGPPGPAGK	1794.0

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Short communication

Identification of five gelatins by ultra performance liquid chromatography/time-of-flight mass spectrometry (UPLC/Q-TOF-MS) using principal component analysis

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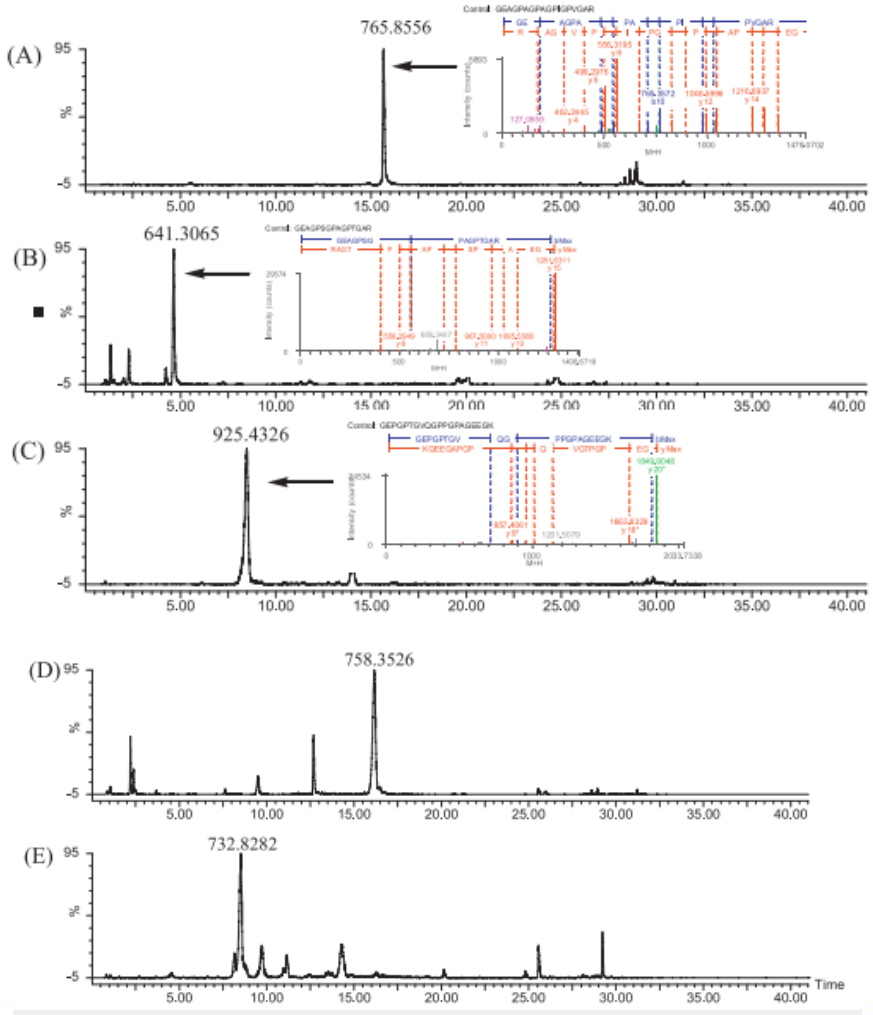
ABSTRACT

An ultra-performance liquid chromatography-quadrupole-time of flight mass spectrometry (UPLC/Q-TOF-MS) method coupled with a principal component analysis (PCA) was developed and applied toward identifying donkey-hide gelatin, bovine-hide gelatin, pig-hide gelatin, tortoise shell glue, and deerhorn glue. The UPLC-MS data of the trypsin digested samples were subjected to principal component analysis (PCA) in order to classify these five gelatins. Additionally, marker peptides given by the loadings plot of PCA were identified based on a comparison of recorded LC–MS data with a previously reported database of the corresponding gelatin variants. The results from this study indicate that the proposed method is reliable, and it has been successfully applied to the identification of variants of gelatins commonly used

Selected ion monitoring chromatograms of marker peptides in (A) donkey-hide gelatin, m/z 765.8556, doubly-charged ion of fragment GEAGPAGPAGPIGPVGAR. (B) bovine-hide gelatin, m/z 641.3065, doubly-charged ion of fragment GEAGPSGPGPTGAR. (C) pig-hide gelatin, m/z 925.4326, doubly-charged ion of fragment GEPGPTGVQGPPGPAGEEGK. (D) glue of tortoise shell m/z 758.3530, sequence unknown. (E) deerhorn glue m/z 732.8282, sequence unknown.



Classification of gelatines

multivariate statistical analysis of the UPLC/MS dataset was to convert the 3D LC/MS data into a 2D matrix expressed as an Exact Mass Retention Time (EMRT) pair using Markerlynx, which is an Application Manager for the MassLynx™ Software. The data set was visualized using unsupervised PCA to check for outliers and classification trends among the gelatins. Preliminary PCA was performed on all observations using 8556 pareto-scaled variables. The final PCA score plot demonstrated that the five different types of gelatins cluster together, and all were found to lie inside the Hotelling T2 (0.95) ellipse, as is shown in Fig. 2A. In the scores plot obtained by PCA, donkey-hide gelatin, bovine-hide gelatin, pig-hide gelatin, deer-horn glue are close to each other, and the tortoise shell

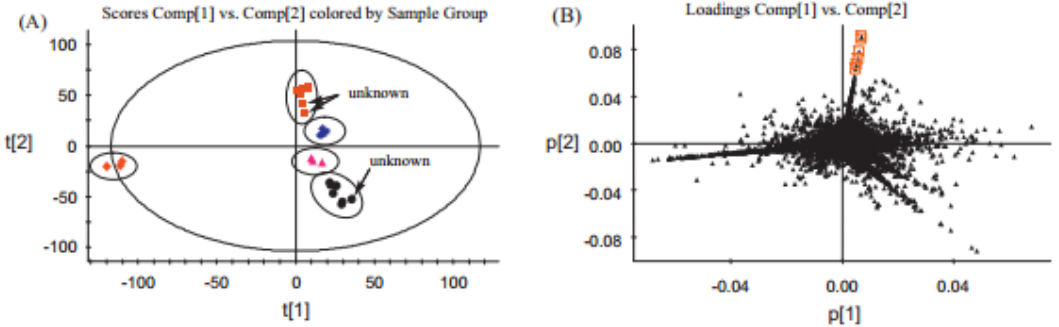


Fig. 2. PCA score plot (A) and loading plot (B) of (■) donkey-hide gelatin, (●) bovine-hide gelatin, (✱) pig-hide gelatin, (▲) deerhorn glue and (◆) glue of tortoise shell.

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SHORT COMMUNICATION

The employment of q-PCR using specific primer targeting on mitochondrial cytochrome-b gene for identification of wild boar meat in meatball samples

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ABSTRACT

Objective: The objective of this study was to employ real-time or quantitative polymerase chain reaction (q-PCR) using novel species specific primer (SSP) targeting on mitochondrial cytochrome-b of wild boar species (CYTBWB2-wb) gene for the identification of non-halal meat of wild boar meat (WBM) in meatball products.

Materials and Methods: The novel SSP of CYTBWB2-wb was designed by our group using PRIMERQUEST and NCBI software. DNA was extracted using propanol-chloroform-isoamyl alcohol method. The designed SSP was further subjected for validation protocols using DNA isolated from fresh meat and from meatball, which include specificity test, determination of efficiency, limit of

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