CHEMOMETRIC EVALUATION ON PROFILES OF LARD AND SELECTED EDIBLE FATS AFTER HEATING-PROCESS USING SPECTROSCOPY AND CHROMATOGRAPHY TECHNIQUES

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Thesis submitted in partial fulfilment for the degree of
DOCTOR OF PHILOSOPHY IN
SCIENCE AND TECHNOLOGY

UNIVERSITI SAINS ISLAM MALAYSIA

February 2023

AUTHOR DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries, which have been duly acknowledged.

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ACKNOWLEDGEMENTS

I am grateful to Allah SWT, and His Messenger Muhammad SAW, the only inner strength to finish this research, especially during the Covid-19 pandemic constraints.

The most appreciation is to Associate Prof. ChM. Dr. Mohd Sukri Hassan, the main supervisor of this research project, for his continued support and guidance. The second is thanks to co-supervisor, Associate Prof. ChM. Dr. Juliana Jumal, for advice on research and thesis writing.

The following thanks to the Deans of the Faculty of Science and Technology (FST), Heads of the Centre Graduates Studies (CGS) Department, International Fatwa and Centre (iFFAH), and FST staff for cooperation in the lab work. I would like to thank the postgraduate students (especially Nurul Elma and Khalilah) for their bitter and sweet memories.

Special thanks to my beloved husband (Sharil Nizam), children (Aryana & Adam), and sister (Nurulhuda) for their endless support.

Finally, highly appreciative is to the Ministry of Higher Education Malaysia (MOHE) for financial research support of the FRGS-50715 and USIM grant under Centre of Excellence (COE), iFFAH (PPPI /KHAS_IHRAM /04/061007/13818).

ABSTRAK

Tujuan penyelidikan ini adalah menilai profil lemak babi menggunakan kaedah kemometriks untuk mendiskriminasikan lemak babi daripada jenis lemak lain setelah proses pemanasan dan penentuan biomarker dalam lemak babi. Dalam penyelidikan ini, dua jenis persampelan dilakukan. Pertama, sejumlah 270 sampel lemak babi daripada lemak perut (BL), lemak belakang (BK) dan lemak bahu (SF) dikumpulkan dari wilayah utara, selatan dan tengah, Semenanjung Malaysia dan diuji menggunakan teknik FTIR. Penilaian secara kemometriks ke atas profil lemak babi dilakukan menggunakan PCA dan dilanjutkan dengan menggunakan teknik Hotelling T^2. Persamaan vektor skor ditemui pada 3PC pertama (99.5% selang keyakinan) dan berjaya disahkan oleh projeksi PCA. Ini menunjukkan profil FTIR bagi lemak babi adalah serupa bagi semua wilayah dan bahagian badan. Kedua, untuk tujuan diskriminasi, sebanyak 60 sampel-sampel lemak dari babi, ayam, lembu, kambing dan tumbuhan telah disediakan menggunakan dua belas protokol proses pemanasan (pada suhu 120 ° C, 180 ° C, 240 ° C selama 0.5, 1, 2 & 3 jam). Lemak-lemak haiwan dan tumbuhan tanpa pemanasan juga termasuk dalam tambahan kajian ini. Selepas proses pemanasan, sampel-sampel lemak dianalisis menggunakan FTIR, ^{1}H-NMR, ^{13}C-NMR, GC-FID dan LC-MS/MS. Diskriminasi lemak-lemak dilakukan menggunakan teknik-teknik pengkelasan multivariasi (LDA, MDA, QDA & SVMDA) dan regresi multivariasi (OSC-PLSR, PCR & PLSR) pada FTIR dan ^{1}H-NMR data. Pengkelasan multivariasi mendapati MDA, QDA, dan SVMDA pada ^{1}H-NMR memberikan keputusan yang terbaik untuk pengelasan lemak babi daripada lemak-lemak lain. Bagi regresi multivariasi, keputusan OSC-PLSR pada FTIR (R^2, *adj.* R^2, RMSEC, RMSEV & MSEP; 0.985, 0.984 , 0.049, 0.051 & 0.056) mengatasi prestasi keputusan OSC-PLSR pada ^{1}H-NMR. Akhir sekali, pemprofilan lemak babi oleh ^{13}C-NMR, GC-FID dan LC-MS/MS untuk menentukan biomarker lemak babi telah dilakukan menggunakan teknik PCA. Data ^{13}C-NMR-PCA menunjukkan lemak babi dapat dibezakan dengan lemak ayam pada C-2 oleh isomer TAG yang dikaitkan dengan resonan δ 34.21 dan δ 62.10. Manakala, data GC-FID-PCA mendapati bahawa lemak haiwan dan lemak tumbuhan boleh dibezakan selepas degradasi isomer asid lemak (FAs) *cis* kepada *trans*. Lemak babi yang dipanaskan pada 180 °C (pada 0.5, 1 & 2 jam) didapati berbeza lebih ketara oleh LC-MS/MS-PCA yang disumbangkan oleh asid lemak tepu (SFA) daripada kumpulan lipid diasilgliserol (DAG) dan asid fosfatidik (PA). Aplikasi kemometrik didapati berjaya mendiskriminasikan lemak babi dari lemak-lemak lain setelah proses pemanasan dan biomarker FAs yang dikenalpasti secara tentatif.

ABSTRACT

This research aims to evaluate the profile of lard using the chemometric method to discriminate lard from other types of fat after the heating-process and determination of biomarkers in lard. In this research, two types of samples were conducted. First, a total of 270 lard were collected from belly fats (BL), back fats (BK), and shoulder fats (SF) from northern, southern, and central regions of Peninsular Malaysia, which were measured using FTIR. PCA utilised the chemometrics evaluation on lard profiles using PCA and extended Hotelling T^2. The scores vectors were found inside Hotelling T^2 eclipses at first 3 PCs (99.5% confidence interval) and successfully validated by PCA projection. This indicates that the FTIR profile for lard is undifferentiated for all regions and body parts. Second, for discrimination purposes, a total of 60 fats, lard, chicken, beef, mutton, and plant have undergone twelve heating-process protocols (at 120 °C, 180 °C, 240 °C for 0.5. 1, 2 &3 hrs). In addition, each species without any heating was included in this study. After the heating-process, fat samples were analysed using FTIR, ^{1}H-NMR, ^{13}C-NMR, GC-FID, and LC-MS/MS. The classification was performed using multivariate classification (LDA, MDA, QDA & SVMDA) and multivariate regression (OSC-PLSR, PCR & PLSR) on FTIR and ^{1}H-NMR. Multivariate classification found MDA, QDA, and SVMDA on ^{1}H-NMR provided the best results for classifying lard from other fats. In the multivariate regression, the result of OSC-PLSR on FTIR (R^2, *adj.* R^2, RMSEC, RMSEV & MSEP; 0.985, 0.984, 0.049, 0.051 & 0.056) was found to outperform than the OSC-PLSR on ^{1}H-NMR. Finally, profiling lard by ^{13}C-NMR, GC-FID, and LC-MS/MS combined with the PCA were conducted to determine the biomarker of lard. The ^{13}C-NMR-PCA data found that the lard differed against the chicken fats at C-2 of the TAG isomer, denoted by δ 34.21 and δ 62.10 resonances. Meanwhile, the GC-FID-PCA data found that animal fats and plant fats can differ after fatty acids (FAs) degradation of the *cis* into *trans* isomers. The lard heated at 180 °C (at 0.5, 1 & 2 hrs.) was found to differ significantly from others by LC-MS/MS-PCA, contributed by saturated fatty acids (SFA) of the diacylglycerol (DAG) and phosphatidic acids (PA) lipid classes. The application of chemometrics was found to discriminate lard against other fats after the heating-process successfully, and FAs were tentatively identified as biomarkers.

الملخص

يهدف هذا البحث إلى تقييم خصائص شحم الخنزير باستخدام طريقة القياس الكيميائي يميز شحم الخنزير عن الأنواع الأخرى من الدهون بعد عملية التسخين وتحديد المؤشرات الحيوية في شحم الخنزير. في هذا البحث تم إجراء نوعين من العينات. أولا، المجموع 270 شخم من ودهون الكتف (SF) ودهون الظهر (BK) دهون البطن (BL) تم جمع من المناطق الشمالية والجنوبية والوسطى لشبه جزيرة ماليزيا، والتي كانت تقييم القيا سات الكيميائية على ملفات PCA استخدمت. FTIR تم القياس با ستخدام تعريف شحم الخنزير باستخدام الممتد Hotelling T^2 و PCA. Hotelling T^2 تم العثور على ناقلات الدرجات داخل. يخسوف عند أول 3 PCA قطع (فاصل ثقة 99.5٪) وتم التحقق من صحته بنجاح بواسطة لشحم الخنزير غير متمايز لجميع المناطق FTIR تنبؤ. يشير هذا إلى أن ملف تعريف وأجزاء الجسم. ثانيًا، لأغراض التمييز، ما مجموعه 60 دهون شحم دجاج، خضعت لحوم البقر والضأن والنبات لاثني عشر بروتوكولًا لعملية التسخين (120 °C, 180 °C & 240 °C عند 0.5, 1, 2 & 3 hrs). بالإضافة إلى ذلك كان كل نوع دون أي تدفئة المدرجة في هذه الدراسة. بعد عملية التسخين، تم تحليل عينات الدهون باستخدام ^{13}C-NMR, ^{1}H-NMR, GC-FID, LC-MS/MS, FTIR. على FTIR و^{1}H-NMR تم التصنيف باستخدام تصنيف متعدد المتغيرات (LDA, MDA, QDA & SVMDA). تم العثور على تصنيف (OSC-PLSR, PCR & PLSR). أفضل النتائج لتصنيف (C-1-SVMDA, MDA & QDA) شحم الخنزير على ^{1}H-NMR قدم الخنزير من الدهون الأخرى. في الانحدار متعدد المتغيرات، تكون نتيجة على FTIR ($adj.\ R^2$ = 0.985) كان على ^{1}H (R^2 = 0.984, RMSEC = 0.049, RMSEV=0.051 & MSEP= 0.056) OSC-PLSR - NMR-. أخيرًا، تم إجراء تنميط شحم الخنزير تفوق أداء لتحديد PCA جنبًا إلى جنب مع ^{13}C-NMR, GC-FID, LC=MS/MS. أن شحم الخنزير العلامة الحيوية لدهن الخنزير. وجدت بيانا ^{13}C-NMR-PCA يختلف عن 34.21, 62.10 δ يُشار إليها بصدى TAG من أيزومر2-C دهون الدجاج في أن الدهون الحيوانية والدهون. وفي الوقت نفسه، وجدت بيانات GCFID-PCA, النباتية يمكن أن تختلف فيما بعد لرابطة الدول المستقلة إلى أيزومرات عابرة على FAs. يسخن شحم الخنزير من LC-MS/MS 180 °C (0.5, 1,& 2 hrs.) تم العثور عليه بشكل أكثر وضوحًا للاختلاف بساهم عن طريق الأحماض الدهنية المشبعة (SFA) من دياسيل جلسرين (DAG) وأحماض الفوسفاتيد(PA) فئات الدهون. تم العثور على تطبيق القياسات الكيميائية للتمييز بنجاح شحم ضد الدهون الأخرى بعد المرور بعملية التسخين، وكان FAs مؤقتًا تم تحديدها على أنها مؤشرات حيوية.

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LIST OF UNITS OF MEASUREMENTS

°C	degree Celsius
g	gram
Hertz	Hz
kg	kilogram
L	litre
mg	milligram
mL	millilitre
M	molar
mol	mole
ppm	parts per million
µL	microlitre

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LIST OF SYMBOLS

C	hyperplane SVM parameter
df	degree of freedom
hr	hour
hrs	hours
min	minutes
sec	second
vs	versus
α	significance level
δ	chemical shift
ε	epsilon
υ	*Nu*

LIST OF EQUATIONS

LIST OF ABBREVIATIONS

1D	One-Dimensional
2D	Two-Dimensional
3D	Three-Dimensional
ANN	Artificial Neural Network.
ANOVA	Analysis of Variance
ATR	Attenuated Total Reflectance
CI	Confidence Interval
COW	Correlation Optimized Warping
CRM	Certified Reference Material
DA	Discriminant Analysis
DAG	Diacylglycerol
DSG	Derivatives Savitzky-Golay
EA/IRMS	Elemental Analyzer/Isotope Ratio Mass Spectrometry
EVCO	Extra Virgin Coconut Oil
FAME	Fatty Acid Methyl Ester
FAMEs	Fatty Acid Methyl Esters (*plural*)
FA	Fatty Acid
FAs	Fatty Acids (*plural*)
FTIR	Fourier Transform Infrared
GC	Gas Chromatography
GC × GC-TOF-MS	Dimensional Gas Chromatography Coupled with Time-Of-Flight Mass Spectrometry
GC-FID	Gas Chromatography with Flame Ionization Detection
GC-MS	Gas Chromatography-Mass Spectrometry
GL	Glycerolipid
GLC	Gas Liquid Chromatography
GPPL	Glycerophospholipid
HCA	Hierarchical Cluster Analysis
HPLC	High-Performance Liquid Chromatography
IPA	Isopropyl Alcohol
IR	Infrared
k-NN	*k*-Nearest Neighbours
K-S	Kennard-Stone
LC-MS/MS	Liquid Chromatography with Tandem Mass Spectrometry
LDA	Linear Discriminant Analysis
MAG	Monoacylglycerol
MDA	Mahalanobis Discriminant Analysis
MSC	Multiplicative Scatter Correction
MSEP	Mean Standard Error Prediction
MUFA	Monounsaturated Fatty Acids

MVR	Multivariate Regression
NIR	Near-Infrared Spectroscopic
NMR	Nucleus Magnetic Resonance
OPLS	Orthogonal Partial Least Squares
OPLS-DA	Orthogonal Partial Least Squares Discriminant Analysis
OPLSR	Orthogonal Partial Least Squares Regression
OSC-PLSR	Orthogonal Signal Correction Partial Least Squares Regression
PA	Phosphatidic Acids
PC	Principal Component
PCs	Principal Components (*plural*)
PCA	Principal Component Analysis
PCh	Phosphatidylcholines
PCR	Principal Component Regression
PG	Phosphatidyl-Glycerol
PI	Phosphatidylinositol
PLS	Partial Least Squares
PLSR	Partial Least Squares Regression
PUFA	Polyunsaturated Fatty Acids
QDA	Quadratic Discriminant Analysis
R^2	Coefficient of Determination
RBF	Radial Basis Function
RF	Random Forest
RMSEC	Root Mean Square Error of Calibration
RMSEV	Root Mean Square Error of Validation
SFA	Saturated Fatty Acids
SVM	Support Vector Machines
SVMDA	Support Vector Machines Discriminant Analysis
SVMR	Support Vector Machines Regression
TAG	Triacylglycerol
TAGs	Triacylglycerols (*plural*)
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
VCO	Virgin Coconut Oil