DIFFERENTIATION OF LARD FROM OTHER ANIMAL FATS AND VEGETABLE OILS BASED ON N-ALKANE PROFILES AND CHEMOMETRICS ANALYSIS

By

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Chair : Helmi Bin Wasoh @ Mohamad Isa, PhD
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Food authenticity is an emerging topic in food industry and has been a significant concern to consumers and food authorities. One of the hot issues is the misused or adulteration of lard in food products due to production cost reduction since lard is the cheapest form of fat that is readily available and could be effectively blended with other vegetable oils. However, there are some limitations on the use of lard in the food industry from the perspective of Muslim religion, in addition to the risk of health and biological complications. In fact, it is very essential to distinguish lard from other common animal fat and vegetable oils so that misleading conclusions could be avoided. Therefore, this research attempted to differentiate lard from other animal fats and vegetable oils based on n-alkane profiles and chemometrics analysis. In this study, unsaponifiable fraction of animal fats (lard, beef, mutton, and chicken) and vegetable oils (coconut oil, peanut oil, crude palm oil and soybean oil) were isolated and analysed by gas chromatography-mass spectrometry (GC–MS) to establish n-alkane profiles of each fat. The n-alkane profiles were further subjected to chemometrics analysis to cluster and classify lard and other fats and oils. The differentiation pattern of the samples was observed using Principal Component Analysis (PCA), Hierarchical Clustering Analysis (HCA), Partial Least Squares - Discriminant Analysis (PLS-DA) and Random Forest (RF). Significant differences ($p < 0.05$) were found between the 17 n-alkanes found in all fats and oils (C_{10} to C_{27}). C_{24} was strongly correlated to lard, C_{13} was unique to palm oil, and the C_{15}, C_{10}, C_{12}, and C_{14} were correlated to vegetable oils. Tetracosane (C_{24}) with 15.72% of abundance was proposed as potential biomarker for differentiation of lard from other samples based on PLS-DA and random forest feature selection. Based on this finding, a more promising study may be achieved to protect the interest of various fats and oils in vast applications especially using chemometrics analysis and machine learning.

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

PEMBEZAAN LEMAK BABI DARIPADA LEMAK HAIWAN LAIN DAN MINYAK SAYUR BERDASARKAN PROFIL N-ALKANA DAN ANALISIS KEMOMETRI

Oleh

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Ketulenan makanan adalah isu penting dalam industri makanan dan telah menjadi kebimbangan kepada pengguna dan pihak berkuasa yang terlibat. Salah satu isu hangat ialah salahguna atau pencampuran lemak babi dalam produk makanan bagi mengurangkan kos pengeluaran kerana lemak babi adalah antara lemak termurah yang mudah didapati dan boleh diadun secara berkesan dengan minyak sayuran lain. Walau bagaimanapun, terdapat beberapa batasan penggunaan lemak babi dalam industri makanan dari perspektif agama Islam, di samping risiko komplikasi kesihatan dan biologi. Sebenarnya, adalah sangat penting untuk membezakan lemak babi daripada lemak haiwan dan minyak sayuran biasa yang lain supaya kesimpulan yang mengelirukan dapat dielakkan. Oleh itu, penyelidikan ini cuba membezakan lemak babi daripada lemak haiwan dan minyak sayuran lain berdasarkan profil n-alkana dan analisis kemometrik. Dalam kajian ini, pecahan yang tidak boleh ditapis dari lemak haiwan (lemak babi, daging lembu, daging kambing dan ayam) dan minyak sayuran (minyak kelapa, minyak kacang tanah, minyak sawit mentah dan minyak kacang soya) telah diasingkan dan dianalisis dengan kromatografi gas-spektrometri jisim (GC-MS) untuk menghasilkan profil n-alkana bagi setiap lemak. Seterusnya, profil n-alkana digunakan dalam analisis kemometrik untuk mengelompok dan mengklasifikasikan lemak babi dan lemak dan minyak lain. Corak pembezaan sampel diperhatikan menggunakan Analisis Komponen Utama (PCA), Analisis Pengelompokan Hierarki (HCA), Kuasa Dua Terkecil Separa - Analisis Diskriminasi (PLS-DA) dan Hutan Rawak (RF). Beza keertian antara 17 n-alkana yang terdapat dalam semua lemak dan minyak (C_{10} hingga C_{27}) adalah ($p < 0.05$). C_{24} berkorelasi kuat dengan lemak babi, C_{13} unik kepada minyak sawit, dan C_{15}, C_{10}, C_{12}, dan C_{14} dikaitkan dengan minyak sayuran. Tetracosane (C_{24}) dengan 15.72% kadar banyak telah dicadangkan sebagai penanda yang berpotensi untuk pembezaan lemak babi daripada sampel lain berdasarkan pemilihan ciri Kuasa Dua Terkecil Separa - Analisis Diskriminasi (PLS-DA) dan hutan rawak (RF). Berdasarkan penemuan

ini, kajian yang lebih berpotensi boleh dicapai untuk melindungi kepentingan pelbagai lemak dan minyak dalam aplikasi yang luas terutamanya dengan menggunakan analisis kemometrik dan pembelajaran mesin.

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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:

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
µL	Microliter
µm	Micrometre
Cm	Centimetre
G	Gram
M	Meter
Mg	Milligram
Min	Minute
mL	Millilitre
Mm	Millimetre
CA	Cluster analysis
COBAC	Computer-Based Analytical Chemistry
CAC	Chemometrics in Analytical Chemistry
COMPANA	Computer Application in Analytics
FFA	Free fatty acid
GC-MS	Gas Chromatography-Mass Spectrometry
HCA	Hierarchical Clustering Analysis
MS	Mass spectrometry
NIST	National Institute Standard and Technology
OOB	Out-of-bag
PCA	Principal Component Analysis
PLS-DA	Partial Least Squares - Discriminant Analysis
RF	Random Forest
SFA	Saturated fatty acid
TAG	Triglyceride
USFA	Unsaturated fatty acid
UPM	Universiti Putra Malaysia
VIP	Variable significance in projection

CHAPTER 1

INTRODUCTION

Food authentication has been a major concern, not just for fraud prevention, but also to verify the safety of foodstuffs that may be dangerous to consumers. Among foodstuffs, the authentication of edible fats and oils has received special attention in order to determine whether or not goods are adulterated. The use of lard in food production was the most common source of adulteration in edible fats and oils. Pig fat, often known as lard, is derived from the rendering process of pig adipose tissue. In some countries, lard is used instead of oil because it is cheaper and easier to get. (Azir et al., 2017).

However, from a religious perspective, certain religions like Islam, Hinduism, and Judaism prohibit their followers from consuming lard in any form (Al-Kahtani et al., 2017). In addition, diets rich in lard and pork are associated with certain health risks, including hypercholesterolemia and coronary health disease (Rohman & Che Man, 2008). Zoonotic infections including tularemia, anthrax, and trichinosis may spread to pork consumers since food products containing pork are susceptible to bacterial deterioration and pigs are also considered as a reservoir of infection (Yardimci, 2020). For these reasons, researchers developed various analytical approaches, including physical and chemical-based methods to distinguish lard (Rohman et al., 2012).

All fats (including animal fats and vegetable oils) are mainly composed of triglycerides (TAG) and other minor components, including mono- and diglycerides, free fatty acids (FFA), hydrocarbons (n-alkane), phosphatides, sterols, fatty alcohols, fat-soluble vitamins, tocopherols, and waxes (Gunstone, 2002). Most scientists used major components of fats and oils composition to detect lard adulteration. Rohman et al. (2012) have differentiated lard from other animal fats based on triacylglycerol composition combined with principal component analysis (PCA). Azizan et al. (2021) used the composition of fatty acids to detect lard adulteration in wheat biscuits combined with chemometrics.

In this study, animal fats (lard, beef, chicken, and mutton fats) and vegetable oils (coconut, crude palm, peanut, and soybean oils) have been chosen for n-alkane profiling as they are the common edible fats and oils consumed by Malaysians and readily available. n-Alkanes are linear saturated hydrocarbons which occur naturally in plant and animal tissues (Zakaria et al., 2018). Several analytical approaches have been developed to analyse n-alkane quantitatively. One of the methods was using gas chromatography accompanied by mass spectrometry (GC-MS) (Troya et al., 2015). It is undeniable that this parameter might be used to distinguish different oils in order to evaluate their authenticity and highlight the presence of adulterants. (Srbinovska et al., 2020).

Chemometrics is a chemical science that employs mathematical, statistical, and other approaches based on formal logic to develop or select optimal measuring processes and experiments, as well as to give the most relevant chemical information by evaluating multivariate chemical data (Heberger, 2018). Chemometric tools have been explored and used in various scientific and technological fields to fix issues. Different analytical and statistical approaches can be used to address a variety of issues in food chemistry, including adulteration, geographic or production origin analyses, process effects, and unit operations on food quality parameters (Granato et al., 2018).

However, based on our knowledge and intensive literature search, there are relatively few publications devoted to the analytical analysis of n-alkanes especially with combination chemometrics approach which could aid in the development of a potent instrument for the quality control of edible oils. Therefore, this study aimed to differentiate lard from other animal fats (beef, chicken, and mutton fats) and vegetable oils (coconut, crude palm, peanut, and soybean oils) based on n-alkane profiles established by GC-MS in combination with chemometrics analysis and machine learning.

1.1 Problem statements

i. Published reports on the use of chemometrics and machine learning analysis (that use n-alkane profiles data as matrix variables) for lard differentiation are still lacking due to attention was given more toward to the major components and DNA analysis.
ii. Whether it is possible to differentiate lard from other animal fats and vegetable oils based on minor component of oils like n-alkanes.

1.2 Hypothesis

It may be possible to differentiate lard from other animal fats and vegetable oils based on n-alkanes profiling by GC-MS in combination with chemometrics analysis and machine learning.

1.3 Objectives

This study embarks on the following specific objectives:
i. To extract and analyse n-alkanes from lard, other animal fats and vegetable oils using GC-MS.
ii. To perform clustering and classification analysis of lard, other animal fats and vegetable oils using the chemometrics approach.
iii. To investigate the use of GC-MS analysis coupled with multivariate analysis in identification of potential chemical markers.

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