UNIVERSITI PUTRA MALAYSIA

CORRELATION BETWEEN SPECTRAL PERMITTIVITY CHARACTERISTICS AND FATTY ACID METHYL ESTER COMPOSITION FOR LARD DETECTION IN EDIBLE OILS

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By

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

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Chairman: Fakhrul Zaman Rokhani, PhD
Faculty: Halal Products Research Institute

Lard adulteration in processed food has been a major public concern as it tightly connected to religion and believes, other than concern on the danger of heart coronary diseases. A number of works have been applied on discriminating lard from other animal fats and plant oils. Most of them are based on non-electrical properties sensing and require meticulous lab steps and huge lab-based equipment. Hence, the requirement to develop rapid, low cost, non-destructive and portable Halal authentication and verification system. This study aims to provide basic information that would serve as the foundation for studies on the development of adulteration monitoring systems and Halal sensors by means of spectral permittivity data.

In this research, we propose and experimentally demonstrate the use of spectral permittivity with correlation to fatty acid methyl ester composition as a technique to discriminate lard from other edible oils. The spectral permittivity profile for edible oil was investigated in a wide radio frequency range between 5 MHz to 30 MHz at different temperature of 45°C, 65°C and 85°C. Fatty acid composition of edible oil was investigated by fatty acid methyl ester (FAME) analysis using gas chromatography mass spectrometry (GCMS). From the spectral permittivity data and fatty acid methyl ester composition obtained, multiple data analysis techniques were implemented.

Relative standard deviation (RSD) is used as a mean to characterize measurement variability and validate the precision of the technique. One-way analysis of variance (ANOVA) was implemented for statistical analysis to investigate the significant difference between different edible oils samples. Tukey's range test was used to group the edible oils into different classes. Principal component analysis (PCA) was implemented to the data for statistical modeling to cluster different groups of edible oils, especially lard from other edible oils.

The spectral permittivity profile of edible oil shows slight decrease as the frequency is increased, which could be explained by the movement limitation of the dipoles at high frequency electric field. Increase in temperature shows that the spectral permittivity profile of edible oils were decreasing, which is caused by increased kinetic energy and mechanical amplitudes of motion of the molecules. Other than that, when the temperature increased, the viscosity of the material is reduced, causing reduction of relaxation time and higher dipole moment which also result in decreasing spectral permittivity. The study on fatty acid composition shows edible oils that have higher long chain fatty acid composition have lower spectral permittivity; and spectral permittivity of edible oils increases with increasing degree of fatty acid unsaturation. The technique was proved to have high precision and small variability. One-way ANOVA shows that there is significant difference between different edible oils. Tukey's range test and PCA shows good performance in separating different edible oils; especially in discriminating lard from other edible oils.

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

KORELASI PENCIRIAN SPEKTRUM KETELUSAN DAN KOMPOSISI METIL ESTER ASID LEMAK UNTUK PENGESANAN LEMAK KHINZIR DALAM MINYAK MASAK

Oleh

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Pencemaran minyak babi di dalam makan diproses telah menjadi kebimbangan umum kerana ianya berkait rapat dengan keagamaan dan kepercayaan, selain melibatkan bahaya penyakit jantung koronari. Beberapa kajian telah dijalankan di dalam usaha mendiskriminasi minyak babi daripada minyak haiwan yang lain dan minyak sayuran. Kebanyakan daripadanya adalah berasaskan sensor sifat bukan elektrik dan memerlukan langkah makmal yang teliti dan peralatan berasaskan makmal yang besar. Oleh itu, adalah satu keperluan untuk membangunkan sistem pengesahan Halal yang cepat, rendah kos, tahan dan mudah alih. Kajian ini bertujuan menyediakan maklumat asas bagi kajian mengenai pembangunan sistem pengawasan pencemaran minyak dan sensor Halal dengan menggunakan data spektrum dielektrik.

Di dalam kajian ini, penggunaan spektrum dielektrik bersama kolerasi kepada komposisi methyl ester lemak sebagai teknik untuk mendiskriminasi minyak babi dari minyak lain telah dicadangkan dan diuji. Profil spektrum dielektrik bagi minyak masak telah dikaji dalam julat frekuensi radio antara 5 MHz hingga 30 MHz pada suhu yang berbeza iaitu 45°C, 65°C dan 85°C. Komposisi asid lemak minyak masak telah dikaji melalui analisis metil ester asid lemak (FAME) menggunakan gas chromatography mass spectrometry (GCMS). Dari data spektrum dielektrik dan komposisi metil ester

asid lemak yang diperolehi, pelbagai teknik analisis data telah dijalankan. Sisihan piawai relatif (RSD) digunakan sebagai cara untuk mencirikan kepelbagaian ukuran dan mengesahkan ketepatan teknik. Analisis Varians Satu Hala (ANOVA) telah di implimentasi sebagai analisis statistik bagi mengkaji perbezaan ketara antara sampel minyak. Ujian rangkaian Tukey telah digunakan bagi mengklasifikasi minyak ke dalam kelas yang berbeza. Analisis Komponen Utama (PCA) telah di implimentasikan ke atas data untuk pemodelan statistik bagi membezakan kelompok minyak di dalam kelas yang berbeza, terutamanya lemak babi dari minyak yang lain.

Profil spektrum dielektrik minyak memperlihatkan sedikit penurunan apabila frekuensi meningkat. Ini dapat dijelaskan oleh pergerakan dipole yang terhad pada medan elektrik berfrekuensi tinggi. Peningkatan suhu menunjukkan bahawa profil spektrum dielektrik minyak berkurang, yang mana disebabkan oleh peningkatan tenaga kinetic dan amplitud mekanik gerakan molekul. Selain itu, apabila suhu meningkat, kelikatan bahan akan berkurang, menyebabkan pengurangan masa rehat dan masa dipole yang lebih tinggi, dimana ia juga menyebabkan penurunan pada spektrum dielektrik. Kajian ke atas komposisi asid lemak menunjukkan bahawa minyak masak yang mengandungi komposisi asid lemak berantai panjang yang lebih tinggi mempunyai spektrum dielektrik yang lebih rendah, dan spektrum dieletrik minyak meningkat dengan peningkatan kadar lemak tidak tepu.

Teknik ini telah dibuktikan mempunyai ketepatan yang tinggi dan variasi yang kecil. ANOVA Satu Hala membuktikan bahawa terdapat perbezaan yang signifikan antara minyak dari sumber yang berbeza. Ujian rangkaian Tukey dan PCA menunjukkan prestasi yang baik dalam memisahkan minyak dari sumber yang berbeza, terutamanya membezakan minyak babi daripada sumber haiwan lain dan sayuran.

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I certify that a Thesis Examination Committee has met on 18 December 2017 to conduct the final examination of Masyitah binti Amat Sairin on her thesis entitled "Correlation between Spectral Permittivity Characteristics and Fatty Acid Methyl Ester Composition for Lard Detection in Edible Oils" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
EMR	Electromagnetic radiation
DSC	Differential scanning calorimetry
E-Nose	Electronic nose
RBD	Refined, bleached and deodorized
GC-FID	Gas chromatography flame ionisation detector
GC-ToF	Gas chromatography hyphenated with time-of-flight
GCMS	Gas chromatography mass spectrometry
EA-IRMS	Element analyzer isotope ratio mass spectrometry
FTIR	Fourier transform infrared
FAME	Fatty acid methyl ester
PCA	Principle component analysis
FDA	Food and drug administration
USA	United States of America
IHS	Information handling services
HPLC	High performance liquid chromatography
PLS	Partial least square
CA	Cluster analysis
SMLR	Stepwise multiple linear regression
LSD	Least significant difference
ANOVA	Analysis of variance

MD	Mean difference
SD	Standard deviation
SE	Standard error
RMSECV	Root mean square root of error cross validation
SDD	Standard deviation difference
UHT	Ultra-high temperature
RSD	Relative standard deviation
SCFA	Short chain fatty acid
MCFA	Medium chain fatty acid
LCFA	Long chain fatty acid
VLCFA	Very long chain fatty acid
PC	Principal component

LIST OF NOMENCLATURES

R^2	Coefficient of determination
ε	Dielectric
ε_r	Complex permittivity
C_p	Parallel capacitance/Capacitance of material
C_o	Capacitance of free space
R_p	Parallel resistance
ω	Angular frequency
f	Frequency
ε'_r	Dielectric constant
ε''_r	Dielectric loss factor

CHAPTER 1

INTRODUCTION

1.1 Overview

Fats and oils have been prone to adulteration. In 2015, palm oil is recorded to be the most consumed oil around the world, while butter, lard, tallow and grease fall are placed 5th, 6th and 7th most consumed oil respectively (Manning, 2016). In the food industry, lard is mixed with plant oils to cut the production costs for cheaper shortenings, margarines and other oil-based foods (Aida et al., 2005). Lard adulteration is a delicate and serious concern because it involves religious believes. Both Islamic and Judaism religions prohibit their followers in consuming lard and pork or any of its derivatives (Siddiqui, 2012). In addition, vegetarian like Hindus and Buddhist would also have concern on the same problem.

A number of techniques have been implemented on Halal product (i.e. suitable to be consumed by Muslim and Jewish people) analysis in past studies. These techniques can be classified into two categories which are labeling-based and spectral-based. Labeling-based method includes polymerase chain reaction (PCR) techniques which observe the presence of DNA and is utilized to study pork, pig's fat and its derivatives in foods including sausages and pastries (Aida et al., 2005)(Che Man et al., 2007). Spectral-based methods includes techniques that obtaining chemical and physical information of material by interacting electromagnetic radiation (EMR) with molecules (Sneddon & Butcher, 2002) which includes electronic nose (E-Nose), Differential Scanning Calorimetry (DSC), liquid chromatography, gas chromatography, and spectroscopy. E-Nose technology was used by evaluating odor pattern to detect pork in food products and lard adulterated in palm oil (Che Man et al., 2005)(Nurjuliana, Che Man, & Mat Hashim, 2011). DSC used in the detection of the presence of lard/randomized lard as adulterants in refined-bleached-deodorized (RBD) palm oil (Sneddon & Butcher, 2002). There are several types of gas chromatography techniques used in the Halal product analysis which include, Gas Chromatography-Flame Ionisation Detector (GC-FID) that was used to differentiate lard from other edible fats (Dahimi et al., 2014), Gas Chromatography hyphenated with time-of-flight mass spectrometry (GC-ToF) was used to detect lard based on fatty acid profile (Indrasti et al., 2010) and Element Analyzer-Isotope Ratio Mass Spectrometry (EA-IRMS) and Gas Chromatography Mass Spectrometry (GCMS) was used to differentiate lard, chicken fat, beef fat and mutton fat (Nizar et al., 2013). Liquid chromatography technique has been used to distinguish lard from other animal fats in mixtures of vegetable oils (Marikkar et al., 2005). Fourier Transform Infrared (FTIR) spectroscopy is an example of a spectroscopy technique which has been vastly used in halal verification and detection such as in chocolate (Che Man et al., 2005), meatball (Rohman et al., 2011), cod-liver oil (Rohman & Che Man, 2009), cake formulation (Syahariza et al., 2005), fat mixtures

(Rohman & Che Man, 2011), ink for food packaging (Ramli et al., 2015) and many more.

In this study, spectral permittivity spectroscopy technique is explored as an approach to discriminate lard from other edible fats and oils.

1.2 Problem statement and motivation

In general, adulteration of edible oil causes two great concerns to the consumer. First, animal-based fats and oils adulteration in edible oil present great concern to followers of vegetarian diet as an example, Buddhist or Hindus (Davidson, 2003). In particular, lard adulteration prompt concern to followers of Islamic and Judaism religions as both religions prohibit the consumption of lard and pork (Al-Zamakhsyari, 1998)(Siddiqui, 2012). Several reported cases include shortening, butter, margarine and other specialty food oils made of vegetable oils blended with lard or tallow (Gillies, 1974)(Sonntag, 1982), goat and pig body fat detected in vegetable ghee and cow/buffalo ghee respectively (Lambelet, Singhal, & Ganguli, 1980)(Lambelet & Ganguli, 1983) and recently, sunflower oil contaminated with chicken fat (APK-Inform, 2015).

Second, health issues concern, which can be very vital to people who have allergies to a certain types of food as well as the relationship of dietary cholesterol and saturated fatty acid to coronary heart disease (Jakobsen, 1999). In 1997, European Commission reported that around 103,400 tons of olive oil adulterated with estimated 20,680 tons of hazelnut oil was to be placed on the market (Commission, 2001). The presence of nut oils could cause complication for people with food allergies to nuts (Johnson, 2014). In (Wang et al., 2016), non-optimal intakes of polyunsaturated fat, trans fat and saturated fat each contribute to significant coronary heart disease.

It is clear that there is high dependency of consumers throughout the world to oils and fats. With the vast consumption of oils and fats, greedy manufacturers attempted to reap profits by illicitly adulterating original oils with alternative substances leading to numbers of food fraud cases. While in certain cases oil adulteration is intended to enhance food flavor or give stability as ingredient, these may cause serious health related issues or unable to be consumed by certain group of people. Thus, concern in adulteration in oils and fat should not be taken lightly or ignored. It is crucial to develop a verification system that can distinguish adulterated edible oils to combat food fraud. This thus can induce a healthier and safer eating lifestyle among the citizen of all ages, races and religions.

1.3 Objectives

The overall goal of this study is to evaluate the spectral permittivity technique for discriminating lard from other edible fats and oils. In order to accomplish this goal, the following objectives were set;

i. To investigate the relation between spectral permittivity profile of fats and oils with their fatty acids composition shown by Fatty acid methyl ester (FAME) composition.
ii. To distinguish complex permittivity properties of lard from other edible fats and oils across the electromagnetic spectrum and the effect of frequency, temperature and composition.
iii. To develop analytical models to discriminate lard from other edible fats and oils based on their spectral permittivity profile.

1.4 Thesis organization

This thesis describes a study on the application of dielectric spectroscopy technique at wide to discriminate lard from other edible fats and oils. The research conducted in this thesis is expected to give a fundamental input for further development of sensing system in fats and oils production industry. A review of previous research and studies regarding the uses of spectral permittivity spectroscopy in monitoring adulteration in fats and oils are discussed in Chapter 2. Conventional techniques other than spectral permittivity spectroscopy to determine the adulteration of lard in edible fats and oils are first described. The concept of spectral permittivity spectroscopy is then explained. A brief introduction of statistical analysis techniques used in this study for characterization of edible oils in correlation to their fatty acid methyl ester (FAME) composition are also discussed. Chapter 3 provides the material, setup and experimental procedures in performing this research. Samples collection from oil extraction is described. The instrumentation setup and measurement procedure for spectral permittivity spectroscopy are described and illustrated by photos. The preparation of fatty acid methyl ester (FAME) from collected samples is explained. The instrumentation and setup procedure for gas chromatography mass spectrometry (GCMS) are described. The statistical analysis used is explained in the last section. Chapter 4 described the dielectric properties and fatty acid methyl ester (FAME) composition of edible fats and oils samples. The effects frequency and temperature on spectral permittivity properties of edible fats and oils samples are also described. The characterization models developed using Principal Component Analysis (PCA) is explained. Finally, Chapter 5 which is the final chapter in this thesis outlines the findings of this research and end with some suggestion and recommendation for future work in order to improve the result obtained in this study.

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