INVESTIGATION OF BIOMARKING PATTERNS IN FATS FROM VARIOUS SOURCES FOR HALAL AUTHENTICATION USING INFRARED INSTRUMENTS

BY

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ABSTRACT

An important issue in food industry is the authenticity of food products. Tampering with the authenticity of food product can involve the alteration of correct labeling of food ingredients, whereby high value raw materials such as cow skin are substituted with cheaper materials such as pig skin.. The verification of labels in any food product is necessary in order to prevent adulteration practices. Authentication techniques for detection of adulteration in meat products is being developed using current technology which enables the food product to be analysed for authenticity. Applications include the effective determination of illegal substances in halal products. Scientists have introduced various halal authentication techniques. Infrared (IR) spectroscopy is a rapid and non-destructive technique, thus allowing the screening of a large number of samples that can be recovered after measurement and used later for further analyses. In this study,there are four types of identification using pig biomarker based on FTIR technique was carried out such as comparing the spectral pattern of fat in FTIR spectrum in pure meat from pig, lamb, cow, chicken, and palm oil, study effect of the cooking and mixing process on the spectral pattern of fat, authenticate food products using the biomarker pattern determined, study modelling experiment to determine significance of different fat using Minitab software. Five fat samples and sixteen wavelenghts were discerned for use as biomarkers. The sixteen wavelenghts identified included four wavelenghts in the functional group region and twelve wavelenghts in the fingerprint region. Prominent peaks in the functional group region were at wavelenghts 3007 nm, 2948.9 nm, 2918 nm and 2850 nm. Twelve prominent wavelengths in fingerprint group were identified: wavelengths 1743.1, 1466, 1416.5, 1377.7, 1236, 1216.3, 1178, 1141, 1116.6, 1098.4, 1082.7 and 965.1. The sixteen wavelengths in the spectrum can be plotted to distinguish pig fat against beef fat, lamb fat, and palm oil, but not pig fat against chicken fat becauses the biomarkers for pig and chicken fats were visually similar. At frequency 1236 and 3007 nm of the score plots. the biomarker wavelengths for pig and chicken fat as well as pig fat and beef fat, lamb fat and palm oil were located significantly far away. Using these two wavelengths for idenfication of all the fats in food samples would sufficiently distinguish between the fats and oil. The score plot for the animal fats processed differently (via oven, baked, fried, and boiled) remains grouped within the same type of animal fat. This suggest that processing did not cause structural changes in the fat derived from the four types of animal meat. Twenty-six fats at two frequencies along the graph (1236 and 3007 nm), indicating Meat Ball B (MBB) fat, Meat Ball A (MBA) fat, and Sausage B (SB) fat samples were able to identify each fat distinctly as there was clear distance between the biomarker points. Using values for points at these two frequencies for identification of the food samples was chosen as biomarkers as they were located distinctly apart from each other. The first two samples, MBB and SB that were located very close to PF indicated that MBB and SB samples contained pork fat; MBA was located close to CF, indicating the possibility that this sample contained chicken fat. Modeling experiment using interaction plot for group, showed a significant difference between CF-LF, BF-CF, BF-PF, LF-PF. The opposite looks very similar is between LF-PO, BF-PO, CF-PF.

خلاصة البحث

بصحة المنتج الغذائي إلى تغيير وضع العلامات الصحيحة للمكونات الغذائية ، حيث يتم استبدال المواد الخام ذات القيمة العالية مثل جلد البقر بمواد أرخص مثل جلد الخنزير .. يلزم التحقق من الملصقات في أي منتج غذائي لمنع ممارسات الغش. ويجري تطوير تقنيات التوثيق لكشف الغش في منتجات اللحوم باستخدام التكنولوجيا الحالية التي تمكن من تحليل المنتج الغذائي للتأكد من صحته. وتشمل التطبيقات التحديد الفعال للمواد غير المشروعة في المنتجات الحلال. أدخلت العلماء تقنيات التوثيق الحلال المختلفة. يعتبر التحليل الطيفي بالأشعة تحت الحمراء (IR) تقنية سريعة وغير مدمرة ، مما يسمح بفحص عدد كبير من العينات التي يمكن استعادتها بعد القياس واستخدامها لاحقًا لإجراء مزيد من التحليلات.
في هذه الدراسة ، هناك أربعة أنواع من تحديد الهوية باستخدام مرقم بيولوجي للخنازير استناداً إلى تقنية TIRF التي أجريت مثل مقارنة النمط الطيفي للدهون في طيف FTIR في اللحوم النقية من الخنازير ولحم الضأن والبقرة والدجاج وزيت النخيل ، ودراسة تأثير عملية الطهي والاختلاط على النمط الطيفي للدهون ، مصادقة المنتجات الغذائية باستخدام نموذج العلامات البيولوجية المحددة ، تجربة دراسة النمذجة لتحديد كمية الدهون المختلفة باستخدام برنامج Minitab.
تم التعرف على خمس عينات من الدهون وستة عشر من الموجات البارزة لاستخدامها كمؤشرات حيوية. تضمنت ستة عشر wavelenghts حددت أربعة wavelenghts في منطقة المجموعة الوظيفية واثني عشر wavelenghts في منطقة بصمات الأصابع. كانت القمم البارزة في منطقة المجموعة الوظيفية في موجات موجية 3007 نانومتر ، 2948.9 نانومتر ، 2918 نانومتر و 2850 نانومتر. تم تحديد اثني عشر طولًا موجيًا بارزًا في مجموعة بصمات الأصابع: الطول الموجي 1743.1 و 1466 و 1416.5 و 1377.7 و 1236 و 1216.3 و 1178 و 1141 و 1116.6 و 1098.4 و 1082.7 و 965.1. يمكن رسم الأطوال الموجية الستة عشر في الطيف لتمييز دهون الخنازير ضد دهن اللحم البقري ، ودهون الحمل ، وزيت النخيل ، ولكن ليس الدهون الخنازير ضد دهون الدجاج ، كما أن المؤشرات الحيوية للدهون والدجاج كانت متشابهة بصريًا. على تردد 1236 و 3007 نانومتر من المؤامرات النتيجة. موجات العلامات البيولوجية للدهون والخنزير والدهون وكذلك الدهون والخنزير والدهون. لحم الضأن. وكان زيت النخيل يقع بعيدا جدا. إن استخدام هذين الموجتين من أجل تعريف جميع الدهون في عينات الطعام سوف يميز بشكل كاف بين الدهون والزيوت. وتبقى قطعة النقاط للدهون الحيوانية المعالجة بشكل مختلف (عبر الفرن والمخبوز والمقلي والمسلوق) مجمعة ضمن نفس النوع من الدهون الحيوانية. هذا يشير إلى أن المعالجة لم تتسبب في تغييرات هيكلية في الدهون المستمدة من أربعة أنواع من اللحوم الحيوانية. ستة وعشرون من الدهون في اثنين من الترددات على طول الرسم البياني (1236 و 3007 نانومتر) ، مشيرا إلى الدهون الكرة MBB) B) الدهون ، والدهون اللحم A (ماجستير في إدارة الأعمال) الدهون ، وعينات من الدهون SB) Sausage B) كانت قادرة على التعرف على كل الدهون بوضوح كما كانت هناك مسافة واضحة بين نقاط المرقم الحيوي. اختيرت قيم استخدام النقاط في هذين الترددين لتحديد عينات الغذاء كمؤشرات حيوية لأنها كانت منفصلة بوضوح عن بعضها البعض. أشارت أول عيّنتان ، BBM و SB التي كانتا قريبة جدا من PF إلى أن عينات MBB و SB تحتوي على دهون الخنزير. كان موقع ماجستير إدارة الأعمال بالقرب من قوات التحالف ، مما يدل على إمكانية احتواء هذه العينة على دهون الدجاج. أظهرت تجربة النمذجة

باستخدام مؤامرة تفاعلية للمجموعة اختلافًا كبيرًا بين LF-CF و CF-BF و PF-BF و PF-LF. عكس
يبدو متشابهة جدا بين PO-LF ، PO -BF، PF-CF

APPROVAL PAGE

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Irwan Saputra

Signature …………………………………… Date ……………………..

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ELISA	Enzyme Linked Immunosorbent Assays
Enose	Electronic Nose
FTIR	Fourier Transform Infrared
GCMS	Gas Chromatography Mass Spectroscopy
IR	Infrared
MIR	Medium Infrared
NIR	Near Infrared
PC1	Principle Component 1
PC2	Principle Component 2
PCA	Principle Component Analysis
RIA	Radio Immunoassays
PCR	Polymerase Chain Reaction

LIST OF SYMBOLS

+	Addition
-	Subtraction
×	Multiplication
÷	Division
=	Equality
( )	Calculate expression inside first
[ ]	Calculate expression inside first
{}	A collection of elements
∑	Summation - sum of all values in range of series
ω	Omega
Δ	Change / Difference
&	And

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Food is an interesting topic of discussion and study among researchers due to its necessity for human well- being and survival. The production of food has evolved in line with modern advancements in science and technology. Several ingredient sources are being used in the production of food. These ingredients may be either permissible (halal) or prohibited (haram) (Fadzlillah et al, 2011).

Adulteration is defined as the addition of undeclared substances or materials to a product so as to increase bulk product or weight. Making the product appear more valuable than it actually is (Hargin, 1996). In the case of meat and meat, adulteration is not only refers to the replacement of ingredients but also to inappropriate information concerning the origin of raw materials (Montowska & Pospiech, 2014).

Some halal meat issues that have arisen are the mixing of meats from animals sources prohibited in Islam like pork. For example, the mixing beef and pork meats is often done by butchers solely for the benefit of gaining extra profit because pork is cheaper than beef. Visual inspection alone is impossible to differentiate between beef and pork meats. The development of current technology enables the food product to be analysed in terms of its contents accurately and therefore. the determination of illegal adulterants in halal products can be done effectively (Jamaludin & Radzi, 2009).

Scientists have introduced various halal authentication techniques. For example, Enzyme Linked Immunosorbent Assays (ELISA), Radio Immunoassays (RIA), HPLC, FTIR, Electronic Nose coupled with GC-MS, and PCR assays have been applied to analyse harmful meat. pathogens and chemicals in either processed and unprocessed food. The use of instruments such as Fourier Transform Infrared (FTIR) spectroscopy to detect pig derivatives in the meat products described earlier (Brangule et al., 2017).

1.2 STATEMENT OF THE PROBLEM

There are various approaches to detect and measure the level of adultery in food products. The first approach is to determine the ratio between chemical constituents. This approach assumes that this ratio is constant in certain food products. In this approach every addition of food products will modify or change the value of this ratio or will highlight anomalies in their chemical composition. Usually, this approach is associated with a number of analyzes and often uses chemometrics for analysis. The second approach looks for specific markers in food products, whether chemical or morphological components, which prove the presence of adulterants in food products. The third approach uses analytical methods derived from physical analysis by considering all samples to show the effect of counterfeiting on their physico-chemical properties (Cordella et al., 2002).

Analytical methods commonly used to detect adulteration of oils and fats is based on differences in the properties and contents of the components. These analytical methods usually depend on physical-chemical constants or on chemical and biological measurements (Reid et al., 2006).

Analytical methods can provide accurate results in determining the adulteration of a food product. Among the methods used in halal authentication are Fourier Transform Infrared (FTIR) Spectroscopy, a technique used to measure the vibration of the bonds in molecular functional groups (Che Man & Mirghani, 2001).

Infrared (IR) light is used to generate information on the molecular composition and structure of various types of materials including fats and oils. FTIR techniques and chemometric analysis are able to detect and measure pig fat levels in food samples (Yusof et al., 2007).

Infrared spectroscopy (IR) has been known as an analytical method suitable for authenticity studies (Xing et al., 2007). Analysis of food samples using the medium infrared spectrum (MIR) (4000-400 cm) provides relevant and valuable information about the existence of molecular bonds (Rohman and Che Man, 2009).

The infrared spectrum which is a modern analytical instrument produces a large amount of data that includes several thousand wavelengths of data (wave number). A computer that is used systematically can process large amounts of data with minimal information loss. A chemometric can process large data systematically and allows to get deeper information and more complete data interpretation. Chemometrics is the chemical discipline that uses mathematics and statistics to design or select optimal experimental procedures to provide maximum relevant chemical information by analyzing chemical data. and to obtain knowledge about chemical systems. The main objectives of multivariate methods are data reduction, classification and classification of observations and modeling of relationships that may exist between variables. Multivariate methods can also predict whether new observations are included in qualitative or quantitative groups (Downey, 1998). Principal component analysis (PCA) is a technique to reduce the difficulty of processing datasets, improve interpretation and at the same time minimize information loss. PCA also makes new uncorrelated variables which in turn maximize variance. In other words, PCA is an adaptive data analysis technique because the technical variants have been and can be developed according to different types of data and structures (Goodacre and Anklam, 2001), (Pavia et al, 2001).

Some FTIR spectroscopy studies have been conducted to pigs and / or analysis of lard, but from many studies, no research that aims to develop further research such as comparing patterns of spectral variety of fat to determine biomarkers, studied the effect of cooking on the pattern of spectral grease, products food authentication using biomarker patterns are determined, and experimental modeling studies to determine the significance of different fats.

1.3 PURPOSE OF THE STUDY

Biomarkers are expected to be highly accurate, efficient, and reliable for assessing disease risk and biological effect,simple to perform, and inexpensive.

Although several studies have been conducted to identify the biomarkers for non-halal meats like pig meat, these studies are still in their infancy stages, and as a result there is no universal biomarker which could be used for clear cut identification.

As the biomarker information is important, extensive investigation of biomarking pattern is required. For this reason, This study reports the results of investigation of biomarking pattern of fats from various sources based on Fourier Transform Infrared Spectroscopy (FTIR) and PCA techniques. The results such as comparing of spectral pattern of various fats to determine biomarker, study the effect of cooking process on the spectral pattern of fat, authenticate food product using the biomarker pattern determined, and study modelling experiment to determine significance of different fat.

1.4 RESEARCH OBJECTIVES

The study aimed to achieve the following objectives:

General objective: To identificate biomarkers in pure samples, to identificate biomarkers in mixture samples, and to authenticate food products.

Specific objectives:

1- To compare of spectral pattern of fats in FTIR spectrum in various animal meat and palm oil for determining the biomarker.

2- To study effect of cooking process on the spectral pattern of fats.

3- To authenticate of food products using determined biomarker pattern.

4- To study modelling of experiment for identificating a point of biomarker pattern.

1.5 RESEARCH QUESTIONS

1. What can be determined by comparing the biomarker patterns in the FTIR spectral fat in a variety of meat and palm oil?Is there study effect of cooking process on the spectral pattern of fat?
2. What food products can be authenticated by using biomarkers determined?
3. What is the different fat can be determined by modeling the experiment?

1.6 THEORETICAL FRAMEWORK

This study have been divided into three parts, which are identification of biomarkers in pure samples, identification of biomarkers in mixture samples, and food product authentication (Figure 3.1).

To compare the spectral patterns of fat in the FTIR spectrum of pure meat from pig, lamb, beef, chicken, and palm oil (Objective 1), there are three things to look for, which is an isolation that would determine that how to obtain the spectral patterns of fat. Obtanined specral pattern which will be purified and will be determinated later molecular structure, validated by some of the cooking process, compared with the FTIR spectrum.

For the effect of the cooking process on the spectral patterns of fat (Objective 2), to make sure, look at the effect of cooking on the spectral pattern of fat. The spectral pattern of fat will be determinated later molecular structure, compared with the FTIR spectrum and show the results of estimation using chemometric analysis.

To authenticate food products using specified biomarker patterns (Objective 3), it wanted to determine product authentication market. Samples will be collected from the market and then determined from the authentication kosher with chemometric analysis.

For modeling experiments to identificating point biomarker patterns (Objective 4), it is estimated that it would be difficult to identify samples containing lard from other fats in the spectral diagram. Modeling experiments will help to separate the distance between the fat.

1.7 RESEARCH HYPOTHESES

The hypotheses are as follows:

H1 The comparing of spectral pattern of fat in FTIR spectrum in various animal meats and palm oil can be used to determine biomarker

H2 The spectral biomarker of fat obtained from FTIR spectrum, software of spectrum reader, and software of statistic reder.

H3 The cooking process on the spectral pattern of fat has no effect on the spectral pattern of fat.

H4 The biomarker pattern determined can be used to authenticate food product.