UNIVERSITI PUTRA MALAYSIA

DIFFERENTIATION OF LARD FROM OTHER FAT IN PASTRY PRODUCTS USING FOURIER TRANSFORM INFRARED SPECTROSCOPY COMBINED MULTIVARIATE ANALYSIS

WAN SITI FARIZAN BINTI MOHAMED RANI

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By

WAN SITI FARIZAN BINTI MOHAMED RANI

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December 2014

ESPECIALLY DEDICATED TO MY BELOVED FAMILY

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the Degree of Master of Science

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Chairman : Professor Amin Bin Ismail, PhD
Institute : Halal Products Research Institute

The determination of lard in pastry products is increasing important in the food industry. Hence, this study was used Fourier transform infrared (FTIR) spectroscopy combined with chemometric analysis for investigating the lard adulteration in the pastry products. The objectives of the study were to apply FTIR spectroscopy for detection and quantification of lard in locally manufactured pastry products and to use the combination of FTIR spectroscopy and chemometric in qualitative and quantitative analysis of lard in pastry.

The first objective employed 45 samples of extracted oil from pastries prepared in laboratory (SP samples). These SP samples (with known amount of adulterants) served as training set prior to classify the unknown edible oils extracted from 50 commercial pastries available in the market (CP samples). Since edible oils spectra seems similar through visual inspection, chemometrics analysis of partial least square (PLS)-second derivate and discriminant (DA) analysis have been proposed to quantify and classify the presence of lard. Subsequently, FTIR spectra were calculated at selected region of 1200-900cm^{-1} and also have treated with their normal, first and second derivatives. From the analysis, PLS-second derivative offers excellent relationship between FTIR-actual and predicted values with R^2 and RMSEC of 0.994 and 2.38, respectively. The highest R^2 and lowest values of RMSEC computed indicated minimum average error and goodness of the developed calibration set. The classification results showed DA and Coomans plot models were precisely grouped CP samples according to intensity of adulteration with 6 principal components (PCs) and 100% of variability described. In the second objective, qualitative and quantitative analysis of lard were performed by discriminant analysis (DA) and visualized as Coomans plot. DA and Coomans plot were successfully classified commercial baking fats and shortening to their respective axis of lard adulteration as lowest as 0.25ml (v/v) with high R^2 and low RMSECV in normal data pretreatment of 4.36 and 0.97, respectively.

The data sets were validated by normal, first and second data pre-treatment, baseline variation, calibration design and constrained mixtures of PLS. The data sets were split randomly into calibration (n = 141) and validation (n = 141) samples. Coefficient of determination (R^2) and standard error in cross validation (SECV) also were calculated. Prediction accuracy of FTIR calibration models were tested using validation set and evaluated by standard error of prediction (SEP), slope and bias. The study indicated that, FTIR spectroscopy combined with chemometric multivariate analysis provided as a rapid technique in determining lard adulteration in pastry products at regions of interest.

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

MEMBEZAKAN KANDUNGAN LEMAK BABI BERBANDING LEMAK LAIN DI DALAM PRODUK PASTRI MENGGUNAKAN FOURIER TRANSFORM INFRARED SPEKTROSKOPI DAN ANALISIS MULTIVARIASI

Oleh

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Penentuan lemak babi di dalam produk pastri semakin menjadi penting di dalam industri makanan sekarang. Oleh itu, kajian ini menggunakan Fourier transform infrared (FTIR) spektroskopi bersama dengan analisis kemometrik untuk mengkaji percampuran lemak babi di dalam produk pastri. Objektif kajian ini adalah menggunakan FTIR spektroskopi untuk mengesan dan mengenalpasti lemak babi di dalam produk pastri yang didapati di pasaran dan menggunakan kombinasi FTIR spektroskopi dan kemometrik di dalam analisis kualitatif dan kuantitatif lemak babi di dalam pastri.

Objektif pertama menggunakan 45 sampel minyak yang telah diekstrak daripada pastri yang dihasilkan di makmal (sampel SP). Sampel SP ini (mengandungi jumlah kontaminasi yang diketahui) dan bertindak sebagai set latihan untuk mengelaskan ekstrak minyak yang tidak diketahui daripada 50 pastri yang didapati daripada kedai (sampel CP). Oleh kerana spektra minyak hampir tidak dapat dikenalpasti dengan jelas menggunakan mata kasar, oleh itu analisis kemometrik seperti 'Partial Least Square' (PLS)-derivatif kedua dan analisa diskriminasi (DA) digunakan untuk membezakan dan mengelaskan kehadiran lemak babi. Spektrum FTIR dianalisis menggunakan derivatif biasa, pertama dan kedua di bahagian terpilih iaitu, 1200-900sm^{-1}. Daripada analysis ini, PLS-derivatif kedua didapati berjaya menghasilkan keputusan yang terbaik untuk bacaan sebenar dan ramalan oleh FTIR dengan nilai penentuan koefisi (R^2) dan 'root mean standard error calibration' (RMSEC) iaitu, 0.99 dan 2.38. Nilai tertinggi R^2 dan RMSEC menghasilkan purata kesalahan yang paling minima dan nilai kalibrasi yang terbaik. DA dan model Coomans plot didapati sangat tepat dalam mengelaskan 100% sampel CP menggunakan 6 prinsip komponen (PC). Sementara objektif kedua pula, mengaplikasikan DA ada

Coomans plot untuk analisa kualitatif dan kuantitatif lemak babi. Dalam bahagian ini, DA dan Cooman plot berjaya mengelaskan minyak dan lemak sayuran komersial dari percampuran lemak babi serendah nilai isipadu 0.25ml. Dan keputusan ini disokong oleh nilai R^2 yang tinggi dan RMSEV yang rendah menggunakan pembetulan data normal, iaitu 4.36 dan 0.97. Set data ini disahkan menggunakan PLS secara pembetulan biasa, pertama dan kedua derivatif; kepelbagaian garis dasar, rekabentuk kalibrasi dan memaksimumkan campuran. Data set ini juga diasingkan secara rawak kepada sampel kalibrasi dan validasi yang masing-masing berjumlah, 141. R^2 dan 'standard error cross validation' (SECV) juga dikenalpasti melalui pengiraan. Ramalan ketepatan kalibrasi FTIR diuji menggunakan set validasi dan dinilai dengan menggunakan 'standard error of prediction' (SEP), kecerunan dan kepincangan. Dari kajian ini, didapati bahawa gabungan FTIR spektroskopi bersama dengan analisa kemometrik menyediakan teknik yang terpantas untuk mengenalpasti percampuran lemak babi di dalam produk pastri.

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‘

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LIST OF ABBREVIATIONS

AMF	anhydrous milk fat
ATR	attenuated total reflectance
AV	anasidine value
CLA	conjugated linoleic acids
CLS	classical least square
CP	commercial puff pastry available at market
DA	Discriminant analysis
DAGs	diacylglycerides
DARA	discriminant analysis reflectance accessory
DATEMS	diacetyl tartaric acid esters of monoglycerides
DM	distance match
DPs	dropping points
DSC	differential scanning calorimetry
DTGS	deuterated triglycine sulphate
ELISA	enzyme-link immunosorbent assay
EM	electromagnetic
ENose	electronic nose
Fas	fatty acids
FFAs	free fatty acids
FTIR	Fourier transform infrared
GC	gas chromatography
GLC	gas liquid chromatography
HATR	horizontal attenuated liquid chromatography
HPLC	high-performance liquid chromatography
HRPI	Halal Products Research Institute
IR	infrared
IRDM	Infrared Data System
IV	iodine value
JAKIM	Department of Islamic Development Malaysia
KBr	potassium bromide
LOD	limit of detection
LOQ	limit of quantification
MB	multi-bounce
MGs	monoglycerides
MIR	mid-infrared
MLR	multiple linear regression
MS	mass spectrometer
NAICS	The North America Industry Classification System
Na2SO4	Sodium sulphate
NIR	near-infrared
NMR	nuclear magnetic resonance
OAV	odour activity values
OPS	oleoylpalmitoylstrearin
P	power
PC	principal component / number of factor
PCA	principle component analysis
PCR	principle component regression

PHO	partially hydrogenated vegetable oils
PLS	partial least square
PRESS	predicted residual error sum of squares
PUFA	polyunsaturated fatty acid
PV	peroxide value
QBB	name of brand of pure ghee
QC	quality control
R^2	coefficient of determination
RF	first derivative
RMS	root mean square
RMSEC	root mean standard error of calibration
RMSECV	root mean standard error of calibration and validation
RMSEP	root mean standard error of prediction
RN	normal derivative
RS	second derivative
SAP	saponification number
SECV	standard error in cross validation
SEP	standard error of prediction
SD	standard deviation
SFAs	saturated fatty acids
SFC	solid fat content
S/N	signal to noise
SP	pastry samples prepared in laboratory
SM	similarity match
SS	search standards
SSU	saturated-saturated-unsaturated
SUS	saturated-unsaturated-saturated
T	transmittance
TAGs	triglycerides
TFAs	trans fatty acids
TLC	thin-layer chromatography
UPS	unsaturated-palmitic-stearic
UV	ultraviolet
w/o	water-in-oil
ZnSe	Zinc Selenide

CHAPTER 1

GENERAL INTRODUCTION

Food products are analyzed for a variety of reason such as compliance with legal and labeling requirements, assessment of product quality and safety as well as detection of adulteration. The basic principle of analytical procedures and techniques commonly used to provide information about chemical composition, structure and physical properties of food materials. Pork and lard are serious matters in view of some religions such as Islam and Judaism (Regenstein *et al*., 2003; Al-Qaradawi, 2001). The practice of adulteration may occur to manufactured products and also to unmanufactured natural substances by claiming the product or ingredients is pure or genuine product. Adulteration of food products with cheap ingredients is not only economic fraud but it may cause severe health implication. Detection of adulteration is great importance for commercial and health issues.

One of the current issues related to fats adulteration is QBB Pure Ghee product that have been claimed to contain lard or any substance of porcine origin (Ann, 2010; Yusop, 2010). Therefore, rapid sensor techniques are increasingly important tools can be used within research and products assessment quality.

Methods based on near infrared (NIR), Fourier transform infrared (FTIR), Raman and fluorescence spectroscopy, as well as gas and liquid sensor are rapid and non-destructive to measure lipid oxidation, composition of fat and fat quality (Moya Moreno *et.al*., 1999) that are useful for industrial quality monitoring and/or for research purposes.

Compared to traditional methods, these techniques offer new and better insight into complex problems (Blanda *et al*., 2010). They can measure a great number of chemical compounds, enabling semi-fingerprinting of each sample. They also can be used for continuous and non-destructive measurements of products and processes over time, and they do in many cases allow imaging of chemical properties and chemical processes progress in various products. All these features make these methods versatile, flexible, effective and highly informative (Phillips *et al.,* 2010; Subramanian *et al.,* 2009).

These rapid and non-destructive measurement of FTIR instrumental methods have also been used efficiently in assessment of sensory attributes, composition and quality of fats (Lerma-Garcia, *et al.*, 2010; Allam & Hamed, 2007; Irwandi *et al*., 2003); determination of nutritional paramaters of yogurt (Moros *et al*., 2006); composition and flavour quality of Cheddar cheese

(Subramanian, *et al*., 2009); discrimination and classification of bacteria (Mariey *et al*., 2001; Kansiz *et al*., 1999); determination of health related compounds and properties in fruit and vegetables (Bureau *et al*., 2009; Sherazi *et al.,* 2009); monitoring of fermentation processes and protein denaturation and effective on-line solutions for complex products (Khanmohammadi *et al.,* 2009; Carbonaro *et al.,* 2008). The use of spectroscopic techniques as effective descriptive tools can be related to genomics and proteomics. All these spectroscopic techniques can be combined with images and spectral imaging to increase insight in complex problems.

In quantitative analysis of fats and oils, FTIR spectroscopy coupled with Smart-ARK accessory has received great attention for total analysis time of less than 2 min and sample volume of less than 1 ml. FT IR can be used to determine i.e; cis and trans content, adulterations, moisture content and specific application of parameters that correlate to lipid products for instance iodine value (IV), anisidine value (AV), saponification number, solid fat index, peroxide value (Yu *et al*., 2007; Ibrahim Nor *et al.,* 2005; Guillen and Cabo, 1997) and free fatty acid content (Al-Alawi, 2005).

The most common FTIR accessories used for fats and oils analysis are transmission cells and attenuated total reflectance (ATR). The ATR measurement technique has no limitations because liquid samples can be directly applied on the ATR crystal. Previous studies showed that lard content in foods can be determined using FTIR together with combination of statistical analysis such as partial least square (PLS), principle component analysis (PCA), classical least square (CLS) and multiple least regressions (MLR) (Marikkar *et al*., 2005).

1.1. Problem statements

Pastry is baked food made with one or mixture of edible fats and oils. Some bakers use a blend of lard and shortening, while others prefer to add some butter or margarine to the fat blend (Paul and Jenny, 2004). The main reason of such blending provide plasticity characteristic, so pastries can be folded without breaking. Furthermore, the edible fat content in pastry is significant as the amount needed is approximately 35-55% of the dough weight. Consequently, because of the costs of raw materials, some manufactures tend to mix non-halal fats such as lard in the pastry. In such condition, the lard would not be declared on the ingredient label of food packaging. In Malaysia, some consumers do not consume lard for variety of reasons such as concerning health and religion issues. The above issues oblige scientists to find easy, simple and rapid method available in the market to detect the presence of lard in pastry products. Previously, FTIR spectroscopy has been employed to determine foods adulteration (Rohman *et al.* 2011; Irwandi *et al*. 2003; Che Man and Mirghani, 2001). This fat-based method has the ability to provide information about the chemical structure of molecules and required only small amount of sample. In addition, no study

reported on the use of FTIR spectroscopy combined with chemometric analysis for differentiation of lard in pastry products.

Hence, this study was proposed to use FTIR spectroscopy combined with chemometrics data analysis to provide robust technique for detection and quantification of unknown extracted oils from commercial pastry sold by food vendors.

1.2. Significance of the study

The attempt of the study will be significant in determining lard adulteration in pastry products. The method that has been established in this present study will be useful and can be proposed as a screening method prior to detect the presence of lard in commercial pastry products available in the markets. This method can also be recommended to Department of Islamic Development Malaysia (JAKIM) in monitoring halal certification. Substantially, this study will be able to assist Muslims and non-pork consumer from consuming pastry products added with lard ingredient.

1.3. Objectives of the study

General objective

To evaluate the lard detection in pastry products sold in Klang Valley by employing combination of FTIR spectroscopy and multivariate chemometric analysis.

Specific objectives

i. To differentiate lard from butter, margarine and shortening in pastry products using combination of FTIR and chemometric analysis.
ii. To determine the limit of detection of lard from other fats in pastry products using FTIR.

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