UNIVERSITI PUTRA MALAYSIA

APPLICATION OF DIFFERENT INSTRUMENTAL TECHNIQUES FOR ADULTERATION ANALYSIS OF BUTTER WITH LARD

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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February 2015

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Determination of food authenticity and detection of adulteration are major concerns not only to consumers, but also to industries and policy makers at all levels of the production process. The major authenticity issues from the food products are the alteration of food ingredients labeling whereby the high value materials are substituted with the cheaper ones without declaring it on the label. Butter is a fairly expensive raw material from an economic point of view, and thus the manufacturers are tempted to look for cheaper substitutes animal fats such as lard. Lard in food are serious matters in view of religious concerns, biological complication and health risk associated with daily intake. In the view of the risk associated with consumption of lard, it is essential to find the most effective, rapid, accurate and sensitive method to identifying the adulterated butter with lard. Therefore, the overall objective of this study is to apply different instrumental techniques for the analysis adulterated butter with lard.

In the first phase, butter is prepared through the conventional method, while lard was rendered from adipose tissue of pig using microwave oven. Subsequently, samples of butter and lard were mixed in different concentration (v/v). Each sample admixtures were subjected to analysis of Fourier Transform Infrared (FTIR) spectroscopy, Differential Scanning Calorimetry (DSC), Gas Chromatography Mass Spectrometry (GCMS), High Performance Liquid Chromatography (HPLC) and Nuclear Magnetic Resonance (NMR) spectroscopy.

Partial Least Square (PLS) from Fourier Transform Infrared Spectroscopy (FTIR) was successful used to predict lard adulteration in butter with the

equation y = 0.998x + 0.0617; was obtained with R^2 and RMSEC values were 0.998 and 1.42% (v/v), respectively using 2 PCs. Meanwhile, differential scanning calorimetry (DSC) analysis showed the cooling enthalpy and melting enthalpy for butter, which were -54.06±8.87 J/g and 35.97±4.18 J/g respectively, compared to the cooling and melting enthalpy of lard -10.72 ± 0.89 J/g and 13.95 ± 0.07 J/g, respectively. The cooling enthalpy supports their comparative FA and TAG saturation levels, as butter has a higher saturation FA and TAG, thus leading to a larger cooling enthalpy. In addition, fatty acid (FA) and triacylglycerol (TAG) analysis were also conducted using GCMS and HPLC. Furthermore, the ^{1}H-NMR peak signals at δ2.63 showed a specific characteristic of lard. Heteronuclear multiple bond correlation (HMBC), reconfirmed the assignments of the carbon atom of the *palmitoyloleoylinoleic*. The results from this study showed the complimentary of various instrumental techniques such as FTIR, DSC, HPLC, GCMS and NMR spectroscopy had adequate sensitivity and selectivity which would be an efficient tool for halal authentication.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGGUNAAN PELBAGAI INSTRUMEN UNTUK MENGESAN PENCAMPURAN LEMAK KHINZIR DALAM MENTEGA

Oleh

NURRULHIDAYAH BINTI AHMAD FADZLILLAH

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Penentuan ketulenan makanan dan pengesanan pencemaran merupakan kebimbangan utama bukan sahaja kepada pengguna, tetapi juga kepada industri dan penggubal dasar pada semua peringkat dalam proses pengeluaran makanan. Salah satu isu ketulenan utama daripada produk makanan adalah pemindaan pelabelan ramuan makanan, di mana bahan-bahan bernilai tinggi digantikan dengan bahan-bahan bernilai lebih rendah tanpa mengisytiharkan pada label. Dari sudut pandangan ekonomi, mentega merupakan bahan mentah yang mahal dan menyebabkan pengeluar makanan mencari pengganti yang lebih murah daripada lemak haiwan seperti lemak babi. Lemak babi dalam produk makanan merupakan perkara yang serius dari sudut agama, komplikasi biologi dan risiko. Berdasarkan risiko yang dikaitkan dengan penggunaan lemak babi, maka adalah penting untuk membangunkan kaedah yang paling berkesan, cepat, tepat dan sensitif untuk mengenal pasti mentega yang telah dicemari oleh lemak babi. Oleh itu, objektif keseluruhan dalam kajian ini adalah untuk menggunakan pelbagai kaedah instrumentasi untuk menganalisis mentega yang telah dicemari oleh lemak babi.

Dalam fasa pertama, mentega telah disediakan dengan menggunakan kaedah konvensional manakala lemak babi daripada adipos tisu di ekstrak dengan menggunakan ketuhar gelombang mikro. Kedua-dua sampel telah dicampurkan dengan kepekatan yang berbeza (v/v). Setiap campuran telah di analisis menggunakan Spektroskopi Pengubah Fourier Inframerah (FTIR), Pembezaan Pengimbasan Kalorimeter (DSC), Kromatografi Gas Spektrometri Jisim (GCMS), Kromatografi Cecair

Berprestatsi Tinggi (HPLC) dan Spektroskopi Magnetik Resonans Nuklear (NMR).

Kombinasi spektroskopi FTIR dan kalibrasi “partial least square” (PLS) telah dapat digunakan untuk mengesan dan menentukan lemak babi dalam mentega dengan regresi linear yang bagus, iaitu y = 0.998x + 0.0617, dan nilai R^2 dan nilai kesalahan standard rata-rata pada kalibrasi (RMSEC) masing-masing 0.998 dan 1.42% (v/v) diperoleh menggunakan 2 PCs. Manakala, Pembezaan Pengimbsan Kalorimeter (DSC) menunjukkan profil entalpi penyejukan dan pemanasan mentega, -54.06 ± 8.87 J / g dan 35.97 ± 4.18 J / g, berbanding profil entalpi penyejukan dan pemanasan lemak babi, - 10.72 ± 0.89 J / g dan 13.95 ± 0.07 J / g, masing-masing. Entalpi penyejukan telah menyokong perbezaan ketepuan di antara asid lemak (FA) dan triasilgliserol (TAG), seperti mentega yang mempunyai tahap ketepuan FA dan TAG yang lebih tinggi membawa kepada entalpi penyejukan yang lebih besar. Di samping itu, asid lemak (FA) dan triasigliserol (TAG) analisis juga telah dijalankan dengan menggunakan GCMS dan HPLC. Tambahan pula, Nuklear magnetik resonans spektroskopi memberi puncak pada isyarat δ2.63 ppm menunjukkan ciri-ciri tertentu lemak babi. Ikatan heteronukleus berganda (HMBC), ini mengesahkan semula pemberian atom karbon bagi *palmitoyloleoylinoleic.* Dapatan daripada kajian ini menunjukkan pelbagai teknik instrumental yang saling melengkapi seperti FTIR, DSC, HPLC, GCMS dan NMR spektroskopi mempunyai kepekaan dan kepilihan yang mencukupi, dan boleh menjadi alat yang efisien bagi pengesahan halal yang pantas dan boleh dipercayai

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I certify that a Thesis Examination Committee has met on 24 February 2015 to conduct the final examination of Nurrulhidayah bt Ahmad Fadzlillah on her thesis entitled "Application of Different Instrumental Techniques for Adulteration Analysis of Butter with Lard" 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 Doctor of Philosophy.

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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 **Doctor of Philosophy**. The members of the Supervisory Committee were as follows:

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This is to confirm that:

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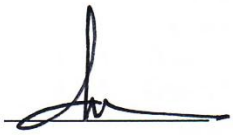
Signature: 
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LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists' Society
ATR	Attenuated total reflectance
BF	Beef fat
CF	Chicken fat
cm^{-1}	Reciprocal centimetre
DA	Discriminant analysis
FAME	Fatty acid methyl ester
FTIR	Fourier transform infrared spectroscopy
FID	Flame ionization detector
GC	Gas chromatography
HPLC	High performance liquid chromatography
NMR	Nuclear Magnetic Resonance Spectroscopy
IR	Infrared
MF	Mutton fat
MUFA	Monounsaturated fatty acid
MIR	Mid infrared
NIR	Near infrared
PC	Principal component
PCA	Principal component analysis
PLS	Partial least square
PRESS	Predicted residual error sum of square
PUFA	Polyunsaturated fatty acid
R^2	Coefficient of determination
RMSEC	Root mean standard error of calibration
RMSECV	Root mean standard error of cross validation
RMSEP	Root mean standard error of prediction
rpm	Revolution per minute
TG	Triglyceride
v/v	Volume/volume

CHAPTER 1

GENERAL INTRODUCTION

Butter, a type of edible fat, is one of the emerging dairy products in the market because of its nutritional benefits to human. Butter contains protein, calcium and phosphorus. The main vitamins in butter include A, D, E and vitamin K (Van Ruth *et al.,* 2008). Meanwhile, additional nutrients in butter include fatty acids, lactones, methyl ketones as well as dimethyl and diacetyl sulfide. Butter is rich in the most easily absorbable form of Vitamin A necessary for thyroid and adrenal health (Ecologist, 2015; Icattoreb, 2013; Tokach, 2013)

The denomination “butter” is reserved, for law, to the product got only and exclusively from the cream drawn from the milk of cow. It is one of the most expensive fats that have standard of quality. In this connection, the European Community has developed special regulation, strict standards for identifying butter and has established that butter must be obtained exclusively from cow's milk or cream (Commission of the European Communities, 1990; Coni *et al.,* 1994). According to (*Commission of the European Communities*, 1990; Coni*, et al.*, 1994), it has been stated under essential composition and quality factors section that commercial butter should consist of 80% milk fat, 16% water content and 2% milk solids other than fat, including salt.

Food Safety Information System Malaysia under Food Act 1983 and Food Regulation 1985 law has stated that butter is the solid product derived exclusively from milk or cream, shall contain not less than 80% of milk fat, 16% of water and may contain salt. Confirmation of the authenticity of a food or food ingredient is an increasing challenge for food scientist. This is especially the case when a substitute value claim, such as one relating to geographic origin or a particular processing history, is made on food label (Commission, 2011). Criteria and procedures for assessing the authenticity of these high-quality products need to be developed.

1.1 Problem statements

Adulteration is a serious problem for both producers and consumers in the food manufacturing industry. The driving force behind food adulteration is when the producer uses partially or totally low-cost ingredients rather than

more expensive ones to increase the revenue (Downey and Kelly, 2006). The growing interest in the authenticity of food products requires reliable verification methods because the properties of foods from different origins can be also differentiated.

Butter is a common raw material for many food products. It contains partially saturated animal milk fat. Butter is sometimes illegally mixed with cheaper animal fats such as lard for use in adulteration. This occurrence of adulteration is to gain economic benefit due to more expensive price of butter than lard and other animal fats (Koca *et al.*, 2010). However, in the case of adulteration using animal fats, this would affect in terms of consumer beliefs and practice such as for Hindus, Jews and Muslims (Didar *et al.*, 2013). For Muslims especially in Malaysia, adulteration using lard or improperly slaughtered animal fats is a big concern.

From the above considerations, the detection of other animal fats in butter is emphasized today more than ever, and a number of investigations have been carried out by several research groups to develop analytical methods for this purpose (Kamm *et al.*, 2001). When the added fat is of vegetable origin, it is usually easy for the analyst to detect its presence (Burros, 1986; Coni*, et al.*, 1994; Grob *et al.*, 1992; Hammond *et al.*, 1988; Plattner and J, 1981). In contrast, if the added fat is of animal origin, few analytical methods are available, and most of the methods are either difficult to be performed or time consuming ((Coni*, et al.*, 1994; Grob *et al.*, 1989).

Lard is one of the cheapest fats that can be found in some countries. Consequently, some manufacturers prefer to mix lard with other oils to reduce the production cost (Geeraert and Sandra, 1985; Luf *et al.*, 1987; Precht, 1991a; 1991b; Precht, 1992a; 1992b; Shukla *et al.*, 1983; Timms, 1980). It is widely applied as dough shortening and frying medium (Marikkar *et al.*, 2005). Medical reports on the effect of lard consumption in promoting carcinogenesis have alerted people to be more vigilant about pork and lard in food products (Marikkar*, et al.*, 2005; Matz, 1992). It is important to detect adulteration of foods with porcine-based products especially in monitoring and implementation of Halal regulations.

There is an increasing trend in some countries to mix pork and lard in their food products for the purposes of gaining extra economic profit. Methods have been developed for detection of lard in food product formulations, namely cake (Al-Rashood *et al.*, 1996), chocolate (Syahariza *et al.*, 2005) and biscuits (Che Man *et al.*, 2005), meat (Che Man *et al.*, 2011) ghee

and butter (Al-Jowder *et al.*, 1997), and vegetable oils (Farag *et al.*, 1983; Kowalski, 1989).

In last several years, there have been some issues associated with the adulteration of butter in Malaysia. Among the issues are pig DNA in Golden Churn Butter and in Kluang coffee. These issues have given a great impact to the producer and consumers since the majority of the population in Malaysia are Muslim. These are some examples of how important it is to develop a reliable technique to ensure the Halal status of food products, which is crucial for Muslims and Halal authentication (Marikkar*, et al.*, 2005). Some published articles related to food authentication are mainly on lard or pork. Such reports are as described in (Yahya Ishmael, 2005) for detecting lard in some vegetable oils and (Rohman and Man, 2010a) for analysis of pork in pork meatball. However, from an extensive literature search, there is no report regarding rapid detection of butter adulterated with lard using Nuclear Magnetic Resonance (NMR) spectroscopy. The application of NMR in this study is to identify selected TAG and FA composition as potential chemical marker for lard as an adulterant and its adulteration in butter Therefore, this study was carried out, and the general objective was to apply different instrumental techniques for adulteration analysis of butter with lard.

1.2 Significance of the study

Testing of food products for the purpose of labeling and authentication is necessary to avoid unfair competition and assure consumer's protection against fraudulent practices in the food industry. The major issues concerning authenticity are where, the potential financial rewards for substitution of cheaper ingredients are relatively high (Rohman and Sismindari, 2011).

The advance in food technology has resulted in the issues getting more complicated where ingredients used in foods are more difficult to understand by consumers unless they are directly involved in the related field. In Halal authentication, one cannot rely solely on physical inspection and documentation, but will need complementary evidence of the latest, high technology of analytical instrumentation. The analytical method used for the analysis of lard is based on the differences in the nature and the composition of minor and major components present or absent in lard and those in foods. These methods typically rely on physical-chemical constants or based on chemical and biological measurements (Lai *et al.*, 1994). Ideally, the methods developed should be rapid, accurate, reliable, easy to operate and low cost. As a result, high-resolution NMR spectroscopy and physicochemical methods have been developed for

analysis of lard in butter. The developed method can be used in food industries to authenticate butter from lard. This finding will also help the Department of Islamic Development Malaysia (JAKIM) as the authorized body responsible for the standardization of Halal certifications.

Global Halal industry has consistently been on the rise in many parts of the world at least for a decade already. The market for Halal food around the globe is getting bigger by years. There is an emerging interest among Muslim consumers everywhere to consume Halal products, and this becomes a good sign for global Halal market. The research conducted by the World Halal Secretariat estimated that the global Halal products market is estimated at an enormous USD 2.3 trillion (not including banking), which USD1.4 trillion (67 per cent) of this market represents food and beverages. Pharmaceuticals on the other hand make up USD506 billion (22 per cent), with cosmetics and personal care amounting to USD230 billion (Kowalski, 1989). Therefore, detection of lard in Halal food becomes more demanding.

The advancement of sophisticated technology in Halal industry will lead to the innovation outcomes that could give alternatives way to authenticate butter from which types of animal fat sources and would overcome the 'Halal' issues regarding animal fats. More complex techniques such as NMR have led to greater accuracy of the analytical results. There have been important efforts made to develop new applications of existing analytical techniques for detection and quantification of lard as an adulterant and its adulteration with butter.

1.3 Objectives

The objectives of the study were as follows:

1.3.1 General objective

The general objective of this research was to apply different instrumental techniques for adulteration analysis of butter with lard.

1.3.2 Specific objectives

i. To identify and quantify the functional groups in lard and adulterated butter using FTIR and PLS method.

ii. To identify the pure butter from the adulterated butter using thermal properties and triacylglycerol profile analysis

iii. To examine the fatty acid composition of butter adulterated with lard using gas chromatography mass spectrometry and multivariate data analysis.

iv. To determine the triacylglycerol marker profile of lard and butter adulterated with lard using high-resolution NMR spectroscopy.

v. To investigate the potential of NMR spectroscopy and principal component analysis (PCA) to detect the adulteration of butter with lard.

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LIST OF PUBLICATIONS

Journals:-

1. **Nurrulhidayah A.F**., Y.B Che Man, Amin I., Shuhaimi M., Alfi Khatib."FTIR-ATR spectroscopy based metabolite fingerprinting as a direct determination of butter adulterated with lard. International Journal of Food Properties. DOI: 10.1080/10942912.2012.692224.

2. **Nurrulhidayah A.F**., A. Rohman, Y.B Che Man, Amin I., Shuhaimi M., Alfi Khatib.2012 " Authentication analysis of butter from beef fat using FTIR spectroscopy coupled with chemometrics. International Food Research Journal 20(3): 1383-1388.

3. **Nurrulhidayah A.F**., A. Rohman, Y.B Che Man, Amin I., Shuhaimi M., Alfi Khatib. 2013 "Application of FTIR-ATR spectroscopy coupled with multivariate analysis of rapid estimation of butter adulteration. Journal of Oleo Science 62 (8): 555-562.

4. **Nurrulhidayah A.F**., A. Rohman, Y.B Che Man, Amin I., Shuhaimi M., Alfi Khatib. 2013 "Analysis of chicken fat as adulterant in butter using Fourier Transform Infrared Spectroscopy and chemometrics. Grasias Y Aceitas 64 (4): 349-355.

Awards:-

1. **Nurrulhidayah A.F.,**Y.B Che Man, Amin I., Shuhaimi M., Alfi Khatib. FTIR-ATR spectroscopy based metabolite fingerprinting as a direct determination of butter adulterated with lard. **Pameran Rekacipta, Penyelidikan dan Inovasi 2012, Universiti Putra Malaysia (Gold medal).**

2. **Nurrulhidayah A.F.,**Y.B Che Man, Amin I., Shuhaimi M., Alfi Khatib, A.Rohman. Analysis butter adulterated with lard using FTIR-^{1}H-NMR spectroscopy. **World Halal Research 2012 (TOP 5 Winner-Gold).**

3. **Nurrulhidayah A.F**.,Y.B Che Man, Amin I., Shuhaimi M., Alfi Khatib, A. 2012. Analysis butter adulterated with lard using FTIR and ^{1}H-NMR spectroscopy for Halal verification. **Research Innovation, Invention Design 2012. (Gold medal).**

4. **Nurrulhidayah A.F**.,Y.B Che Man, Amin I., Shuhaimi M., Alfi Khatib. 2013. Application of FTIR and proton NMR spectroscopy for analysis butter adulterated with lard for Halal verification. **Malaysia Technology Expo 2013. (Silver Medal)**

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