UNIVERSITI PUTRA MALAYSIA

APPLICATION OF GAS CHROMATOGRAPHY-MASS SPECTROMETRY AND ELEMENTAL ANALYZER-ISOTOPE RATIO MASS SPECTROMETRY TECHNIQUES TO DISTINGUISH LARD FROM SELECTED ANIMAL FATS BEFORE AND AFTER CHEMICAL GLYCEROLYSIS

NINA NAQUIAH BINTI AHMAD NIZAR

IPPH 2013 3

APPLICATION OF GAS CHROMATOGRAPHY-MASS SPECTROMETRY AND ELEMENTAL ANALYZER-ISOTOPE RATIO MASS SPECTROMETRY TECHNIQUES TO DISTINGUISH LARD FROM SELECTED ANIMAL FATS BEFORE AND AFTER CHEMICAL GLYCEROLYSIS

By

NINA NAQUIAH BINTI AHMAD NIZAR

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

July 2013

Appendix B3

COPYRIGHT

DEDICATION

In the Name of Allah

Especially for:

Abah and Ibu,

Shahid, Hanis, Syahirah, Sharaf,

All my friends and relatives,

For the never ending love and support…

I love you Lillahitaala!

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

APPLICATION OF GAS CHROMATOGRAPHY MASS SPECTROMETRY AND ELEMENTAL ANALYZER-ISOTOPE RATIO MASS SPECTROMETRY TECHNIQUES TO DISTINGUISH LARD FROM SELECTED ANIMAL FATS BEFORE AND AFTER CHEMICAL GLYCEROLYSIS

By

NINA NAQUIAH BINTI AHMAD NIZAR

July 2013

Chair: Ir. Dzulkifly Bin Mat Hashim, MSc

Institute: Halal Products Research Institute

A study was conducted to differentiate lard from selected animal fats namely chicken fat, beef fat and mutton fat, before and after chemical glycerolysis. It was carried out using Gas Chromatography Mass Spectrometry (GCMS) and Elemental Analyzer–Isotope Ratio Mass Spectrometry (EA-IRMS) techniques. The comparison of overall fatty acid data obtained by Gas Chromatography analysis before and after chemical glycerolysis showed that lard and chicken fats shared common characteristics by having palmitic, oleic and linoleic acids as major fatty acids. On the other hand, beef and mutton fats shared common characteristics by possessing palmitic, stearic and oleic acid as major fatty

acids. Direct comparisons among the fatty acid data therefore may not be suitable for differentiation of animal fats. When the fatty acid distributional data of the animal fats was subjected to Principle Component Analysis (PCA), it was demonstrated that stearic, oleic and linoleic acids were the most discriminating parameters in the clustering of animal fats to four subclasses. The stable isotope analysis of lard and selected animal fats before chemical glycerolysis using EA-IRMS showed significant difference in the carbon isotope ratios ($\delta^{13}C$). The same finding was observed after chemical glycerolysis. This would be a good indicator in discrimination of lard, chicken, beef and mutton fats. The current finding leads to a more efficient method, to screen and ascertain the source of origin of fats used in food products.

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

APLIKASI TEKNIK KROMATOGRAFI GAS SPEKTROMETRI JISIM DAN ANALISIS ELEMEN NISBAH ISOTOP SPEKTROMETRI JISIM BAGI PEMBEZAAN LEMAK BABI DARIPADA LELEMAK HAIWAN LAIN SEBELUM DAN SELEPAS GLISEROLISIS KIMIA

Oleh

NINA NAQUIAH BINTI AHMAD NIZAR

Julai 2013

Pengerusi: Ir. Dzulkifly Bin Mat Hashim, MSc

Institut: Institut Penyelidikan Produk Halal

Satu kajian telah dijalankan untuk membezakan lelemak babi daripada lelemak haiwan lain iaitu lelemak ayam, lelemak lembu dan lelemak kambing, sebelum dan selepas gliserolisis kimia. Kajian ini telah dijalankan dengan menggunakan Teknik Kromatografi Gas Spektrometri Jisim (GCMS) dan Analisis Elemen Nisbah Isotop Spektrometri Jisim (EA-IRMS). Perbandingan keseluruhan data asid lemak yang diperolehi daripada analisis Kromatografi Gas sebelum dan selepas gliserolisis kimia menunjukkan lelemak babi dan lelemak ayam mempunyai ciri-ciri yang sama iaitu mempunyai asid palmitik, asid oleic dan asid linoleik sebagai komponen asid lemak yang utama. Sementara itu, lelemak lembu dan kambing pula berkongsi ciri-ciri yang sama dengan memiliki asid palmitik, asid stearik dan asid oleik sebagai asid lemak utama. Walau

bagaimanapun, perbandingan taburan data asid lemak sahaja tidak dapat membezakan lelemak haiwan. Oleh itu, taburan data asid lemak tersebut diproses menggunakan Analisis Prinsip Komponen (PCA). Analisis tersebut telah menunjukkan bahawa asid stearik, oleic dan linoleik adalah parameter yang paling utama dalam membezakan lelemak haiwan kepada empat kumpulan berasingan. Analisis isotop stabil lelemak babi dan lelemak haiwan sebelum gliserolisis kimia menggunakan EA-IRMS menunjukkan perbezaan yang signifikan dalam nilai isotop karbon ($\delta^{13}C$). Pemerhatian yang sama turut didapati selepas proses gliserolisis kimia dijalankan. Ini dapat dijadikan asas dalam pembezaan lelemak babi, ayam, lembu dan kambing. Penemuan ini akan membawa kepada kaedah yang lebih cekap untuk tujuan saringan (*screening*) makanan, selain dapat memastikan sumber lelemak yang digunakan dalam produk makanan.

ACKNOWLEDGEMENTS

Thank you Allah the Most Gracious; for His mercy granted me wisdom, faith and courage to initiate and successfully complete this endeavor. Alhamdulillah!

Thank you to my supervisors, Ir. Dzulkifly Mat Hashim and Dr. Nazrim Marikkar, for their sincere and knowledgeable guidance.

My family, especially Abah and Ibu for their love and encouragement. You saw me through.

To all my friends, near and far, your support means the world to me. Thank you, for being there.

To all staffs and colleagues of Halal Products Research Institute, UPM, your contributions, are deeply appreciated. Last but not least, I want to thank UPM for providing the funds and opportunity to study here.

Thank you so much!

APPROVAL SHEET 1

I certify that a Thesis Examination Committee has met on 11th July 2013 to conduct the final examination of Nina Naquiah binti Ahmad Nizar on her thesis entitled "**Application Of Gas Chromatography Mass Spectrometry and Elemental Analyzer-Isotope Ratio Mass Spectrometry Techniques to Distinguish Lard from Selected Animal Fats Before and After Chemical Glycerolysis**" 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 Masters of Science degree.
Members of the Thesis Examination Committee were as follows:

Hasanah, PhD
Prof. Dr.
Faculty of Food Technology
Universiti Putra Malaysia
(Chairman)

Badlishah, PhD
Prof. MadyaDr.
Faculty of Food technology
Universiti Putra Malaysia
(Internal Examiner)

Amin Ismail, PhD
Prof. Dr.
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

_____
Bujang Kim Huat
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Dzulkifly bin Mat Hashim, MSc, Ir.
Lecturer
Halal Products Research Institute
Universiti Putra Malaysia
(Chairman)

Mohammed NazrimMarikkar, PhD
Senior Lecturer
Faculty of Biotechnology & Biomolecular Sciences
Universiti Putra Malaysia
(Member)

_____

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

_____

NINA NAQUIAH BINTI AHMAD NIZAR

Date: 11th July 2013

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AOAC	Association of Analytical Chemist
AOCS	American oil Chemist Society
BF	Beef fat
ANOVA	Analysis of Variance
$\delta^{13}C$	Carbon Isotope Value
DSC	Differential Scanning Calorimetry
FA	Fatty Acid
FAME	Fatty Acid Methyl Esters
GC	Gas Chromatography
GCxGC-TOF-MS	Gas Chromatography x Gas Chromatography- Time of Flight-Mass Spectrometer
MF	Mutton fat
IUPAC	International Union of Pure and Applied Chemistry
LD	Lard
MAG	Monocylglycerol
DAG	Diacylglycerol
EA-IRMS	Elemental Analyzer-Isotope Ratio Mass Spectrometer
PCA	Principal Component Analysis
TAG	Triacylglycerol

CHAPTER 1

GENERAL INTRODUCTION

Lard is one of the important products traded worldwide. In 2010, the annual production of lard is 109.9 million tonnes (Food Outlook, 2012), wherein the highest production was from China (51.7 mt), followed by European Union (22.5 mt) and USA (9.9 mt) (FAO, 2010). For certain segments of the society, consumption of lard is not desirable due to religious restriction (Al-Taher, 2004) and various health reasons (Wang and Lin, 1995). According to past studies, lard is reported to be mixed with other fat species such as beef, mutton, and chicken in different food products (Anna, 2006; Saeed *et al*., 1989; Saeed *et al*., 1986). Hence, various efforts have been made to develop analytical approaches to differentiate lard from other animal and plant species. A considerable amount of literature on lipid-based methodologies for such purpose have been published (Sawaya *et al*., 1990; Marikkar *et al*., 2005a; Marikkar *et al*., 2005b; Rohman and Che Man, 2010; Rohman *et al*., 2011; Che Man *et al*., 2011; Nurjuliana *et al*., 2010).

In the last 40 years, extensive works on modification of fat, cholesterol content and fatty acid composition of animal products were done, in requirement of producing high quality food products, that meet the optimum dietary recommendations for human diet as well as enhancing the versatility of fats and

oils in different industrial applications (Jakobsen, 1999). The two most common methods used to modify the physico-chemical properties of the original fats and oil arechemical glycerolysis and fractionation. The products of the former are partial acylglycerols (MAG and DAG). In this research, fractionation of lardthat yields lard stearin and lard olein would be investigated. Since modifications affect the composition and physical-chemical properties of the original oil, therefore, a proper basis for differentiation of lard and selected animal fats in its modified forms is timely.

Another new and interesting field of research that could be explored for differentiation or detectionof this kind is the carbon isotope ratio analysis by using Isotope Ratio Mass Spectrometry (IRMS). Already there are some reports to illustrate the use of stable isotope ratio analysis of light elements such as carbon, hydrogen, nitrogen, and oxygen to verify authenticity and geographical origin of some food samples (Jochmann, 2009). Most of the past researches, however, were mainly focused on honey (Chesson *et al*., 2011; Simsek *et al*., 2012), fish oil (Aursand *et al*., 2000), vegetable oils such as olive oil, sunflower oil, groundnut oil, palm oil, rapeseed oil and corn oil (Angerosa *et al*., 1999; Kelly *et al*., 1997; Bianchi et al., 1993), and essential oils (Schipilliti *et al*., 2010). As of date, very few studies have been reported on the use of IRMS to investigate animal fats. It was reported by Hamilton, (1998) that non-maize oils (animal fats included) are clearly differentiated from maize oils. To the best of our

knowledge, there is hardly any studies to show the potential application of IRMS in Halal authentication purposes, thus establishing their isotope ratios for detection purposes has become important. The information of this kind would be greatly helpful as a basis for food control authorities who are required to carry out routine tests on commercial products that are suspected to contain lard (Yanty*et al*., 2011) other than to ensure food safety and to protect the consumers from fraud and deception.

The application of chromatographic analysis in the earlier studies on characterization and comparison of edible oils have shown to give accurate and consistent results (Aparicio and Aparicio-Ruíz, 2000). Animal oils from different species, brands and grades can be discriminated conveniently using Gas Chromatography (GC) (Araujo *et al*., 2010).However, there may be difficulties in sorting or differentiating the animal fats solely based on fatty acid distributional pattern. Thus, Principle Component Analysis (PCA) may be effectively applied in chromatography for the purpose of measuring similarity and dissimilarity among calculated data. Owing to the high occurrence of components in oils, the evaluation and comparison of the chromatographic profiles by visual methods may be enhanced by the usage of PCA (Cserháti, 2010). Therefore, in this work, a study to evaluate the potential of GC combined with PCA for the differentiation of lard and selected animal fats and its derivatives is important. As the fatty acid distributional data from gas chromatography techniques are

well established, it would be interesting to see its correlation with carbon isotope values acquired from IRMS.

Hence, the objectives of the present study are:

1. To distinguish lard from selected animal fats namely chicken, beef and mutton fats in terms of fatty acid components using Elemental Analyzer-Isotope Ratio Mass Spectrometry (EA-IRMS) andGas Chromatography Mass Spectrometry (GCMS)
2. To determine whether chemical glycerolysis of lard and the selected animal fats namely chicken, beef and mutton fatswould affect the ability to distinguish them using Elemental Analyzer-Isotope Ratio Mass Spectrometry (EA-IRMS) and Gas Chromatography Mass Spectrometry (GCMS)

REFERENCES

Al-Rashood, K. A. and Abou-Shaaban, R. R. A. (1996). Compositional and Thermal Characterization of Genuine and Randomized Lard : A Comparative Study. *JAOCS* 73(3): 303–309.

Al-Taher, F. (2004). Halal food production: Mian N. Riaz and Muhammad M. Chaudry (Eds.); CRC Press, Boca Raton, FL, 2004, 379pp, Hardcover. *Food Microbiology* 21(4): 490-490.

Alves, M.R., Casal, S., Oliveira, M.B.P.P and Ferreira, M.A. (2003). Contribution of FA profile obtained by high-resolution GC/chemometric techniques to the authenticity of green and roasted coffee varieties. AOCS Press, 80: 511-517.

Angerosa, F.B.O., Contento, S., Guillou, C., Reniero, F. and Sada, E. (1999). Application of stable isotope ratio analysis to the characterization of the geographical origin of olive oils. *Journal of agricultural and food chemistry* 47(3): 1013–1017.

Anna, J. (2006). Species specific detection of meat by polymerase chain reaction techniques.PhD Thesis, Corvinus University of Budapest.

AOAC (2007).Official methods of analysis of AOAC International.18th ed. Association of Official Analytical Chemists, Washington, DC.

AOCS (1993). Official method and recommended practices of the American Oil Chemists' Society, 6th ed, IL.

Aparicio, R. and Aparicio-Ruíz, R. (2000). Authentication of vegetable oils by chromatographic techniques. *Journal of chromatography A* 881(1-2): 93–104.

Araujo, P., Zeng, Y., Du, Z.-Y., Nguyen, T.-T., Froyland, L. and Grung, B. (2010). Discrimination of n-3 Rich Oils by Gas Chromatography. *Lipids* 45: 1147–1158.

Aursand, M., Mabon, F. and Martin, G. J. (2000). Characterization of Farmed and Wild Salmon (Salmo salar) by a Combined Use of Compositional and Isotopic Analyses. *JAOCS* 77(6): 659-666.

Baskaran, M. (2011).Handbook of Environmental Isotope Geochemistry, Advances in Isotope Geochemistry, Berlin Heidelberg: Springer-Verlag. Page 707.

Bianchi, G., Angerosa, F., Camera, L., Reneiro, F. and Anglani, C. (1993). Stable Carbon Isotope Ratios (13C / 12C) of Olive Oil Components, *J Agri. Food Chem*. 41:1936–1940.

Bojlul, B., Monahan, F. J., Moloney, A. P., Kiely, P. O., Scrimgeour, C. M. and Schmidt, O. (2007).Alteration of the carbon and nitrogen stable isotope composition of beef by substitution of grass silage with maize silage.*Rapid Commun.Mass Spectrom*. 19: 1937-1942.

Brodnjak-Voncina D., Kodba, Z.C. and Novic, M. (2005).Multivariate data analysis in classification of vegetable oils characterized by the content of fatty acids.*Chemometric and Intelligence Laboratory Systems*.75: 31- 43.

Carrasco-Pancorbo, A., Navas-Iglesias, N. and Cuadros-Rodríguez, L. (2009). From lipid analysis towards lipidomics, a new challenge for the analytical chemistry of the 21st century. Part I: Modern lipid analysis. *Trends in Analytical Chemistry*, 28(3): 263–278.

Carter, J. F. and Barwick, V. J. (2011). *Good Practice Guide for Isotope Ratio Mass Spectrometry*. FIRMS.ISBN 978-0-948926-31-0.

Che Man, Rohman, A. and Mansor, T. S. T. (2011). Differentiation of Lard From Other Edible Fats and Oils by Means of Fourier Transform Infrared Spectroscopy and Chemometrics. *JAOCS* 88: 187–192.

Che Man, Y. B. and Mirghani, M. E. S. (2001). Detection of Lard Mixed with Body Fats of Chicken , Lamb , and Cow by Fourier Transform Infrared Spectroscopy. *JAOCS 78*(7): 753-761.

Che Man, Y. B., Sazili, A. Q. and Regenstein, J. M. (2003). Chapter 3 Food Production From The Halal Perspective. pp: 1–59.

Cheng, S.F., Choo, Y.M., Ma, A.N. and Chuah, C.H. (2005). Rapid synthesis of palm-based monoacylglycerols. *Journal of the American Oil Chemists' Society* 82(11): 791–795.

Cheong, L.Z., Hong, Z., Lise, N., Kirsten J., Haagensen, J.A.J. and Xuebing, X. (2010). Physical and sensory characteristics of pork sausages from enzymatically modified blends of lard and rapeseed oil during storage. *Meat science* 85(4): 691–9.

Chesson, L., Tipple, B.J., Erkkila, B.R., Cerling, T.E. and Ehleringer, J. R. (2011). B-HIVE: Beeswax hydrogen isotopes as validation of environment. Part I: Bulk honey and honeycomb stable isotope analysis. *Food Chemistry* 125(2): 576–581.

Chin, S.T., Yaakob, C.M, Tan, C.P. and Dzulkifly, M.H. (2009) Rapid Profiling of Animal-Derived Fatty Acids Using Fast GCxGC Coupled to Time-of-Flight Mass Spectrometry. *J Am Oil ChemSoc* 86:949–958.

Christy, A.A. and Egeberg, P.K. (2006).Quantitative determination of saturated and unsaturated fatty acids in edible oils by infrared spectroscopy and chemometrics.*Chemometric Intelligence Laboratory Systems* 82: 130 – 136.

Codex Alimentarius Commission.(2001). Codex Standard for Named Animal Fats, Codex-Stan 211-1999.Volume 8. Rome: Food and Agriculture Organization of the United Nations, World Health organization (WHO).

Cordella, C., Moussa, I., Martel, A.C., Sbirrazzuoli, N. and Lizzani-Cuvelier, L. (2002). Recent Developments in Food Characterization and Adulteration Detection : Technique-Oriented Perspectives. *Journal of Agricultural and Food Chemistry* 50: 1751-1764.

Cordella, C.B.Y, Militão, J.S.L.T, Clément, M.C. and Cabrol-Bass, D. (2003). Honey characterization and adulteration detection by pattern recognition applied on HPAEC-PAD profiles. 1. Honey floral species characterization. *Journal of agricultural and food chemistry* 51(11): 3234–42.

Cserháti, T. (2010). Data evaluation in chromatography by principal component analysis. *Biomedical chromatography : BMC* 24(1): 20–28.

David, F., Sandra, P. and Wylie, P.L. (2003) Improving the Analysis of fatty Acid Methyl Esters Using Retention Time Locked Methods and Retention Time Databases. Agilent Technologies, Inc.

Damstrup, M.L., Jensen, T., Sparsø*c*, F.V., Kiil, S.Z., Jensen, A.D. and Xu X.(2005). Solvent Optimization for Efficient Enzymatic MonoacylglycerolProduction Based on a Glycerolysis Reaction *JAOCS* 82(8): 559-564.

Damstrup, M. L. (2008). Process Development of Enzymatic Glycerolysis for Industrial Monoacylglycerol Production. PhD Theses. Technical University of Denmark.

Deffense, E. (1993). Milk fat fractionation today: A review. *Journal of the American Oil Chemists' Society* 70(12): 1193–1201.

De Smet, S., Balcaen, A., Claeys, E., Boeckx, P. and Van Cleemput, O. (2004).Stable carbon isotope analysis of different tissues of beef animals in relation to their diet.*Rapid Commun.Mass Spectrom*. 18: 1227–1232.

Ghidini, S., Ianieri, A., Zanardi, E., Conter, M., Boschetti, T., Iacumin, P. andBracchi, P.G.(2006). Stable isotopes determination in food authentication: A review. *Ann. Fac. Medic. Vet. di Parma* 27: 193-204

Indrasti, D., Yaakob, C.M., Shuhaimi, M. and Dzulkifly, M.H. (2010).Lard detection based on fatty acids profile using comprehensive gas chromatography hyphenated with time-of-flight mass spectrometry. *Food Chemistry* 122: 1273–1277.

Fadzlillah, N. A., Che Man, Y. B., Jamaludin, M. A., Rahman, S. A., and Al-Kahtani, H. A. (2011). Halal Food Issues from Islamic and Modern Science Perspectives. *2nd International Conference on Humanities, Historical and Social Sciences IPEDR* 17: 159–163.

Food Agriculture Organization.(2010). Top Production- Indigenous Pigmeat 2010. Retrieved May 2012 from http://faostat.fao.org/site/339/default.aspx.

Food Outlook.(2012). Meat and Meat Products. Retrieved May 2012 from http://www.fao.org/docrep/012/ak341e/ak341e09.htm

Gan, H.L., Tan, C.P., Che Man, Y.B., NorAini, I. and Nazimah, S.A.H. (2005). Monitoring the storage stability of RBD palm olein using the electronic nose. *Food Chemistry* 89: 271–282.

Glaser K.R., Wenk C. and Scheeder, M. R. (2004). Evaluation of pork backfat firmness and lard consistency. *J Sci Food Agric* 84: 853-862.

Gonzalez, I., Gonzalez, C., Hernandez, J., Marques, E. and Sanz, P.F. (1999).Use of isotope analysis to characterize meat from Iberian-breed swine. *Meat Science* 52: 437–441.

Grompone, M. A. (1990). Characteristics of Uruguayan Mutton Tallow. *Journal of American Oil and Chemist Society*. 67(12): 1990.

Hamilton, R.J. (1998). Purity Criteria for Edible Vegetable Oils in Lipid Analysis of Oils and Fats.pp.284. Blackie Academic and Professional.

Hamm, W. (1995). Trends in edible oil fractionation. *Trends in Food Science and Technology* 6: 121–126.

Jafari, M., Kadivar, M. and Keramat, J. (2009). Detection of Adulteration in Iranian Olive Oils Using Instrumental (GC, NMR, DSC) Methods. *J Am Oil Chem Soc* 86:103–110.

Jakobsen, K. (1999). Dietary modifications of animal fats: status and future perspectives. *Lipid – Fett.* 101(12): 475–483.

Jaswir, I., Mirghani, M.E.S., Hassan, T.H. and Said, M.Z.M. (2003). Determination of Lard in Mixture of Body Fats of Mutton and Cow Using Fourier Transforms Infrared Spectroscopy. *Journal of Oleo Science* 52(12): 633–638.

Jochmann, M., Steinmann, D., Stephan, M. and Schmidt, T. C. (2009). Flow injection analysis-isotope ratio mass spectrometry for bulk carbon stable isotope analysis of alcoholic beverages. *Journal of agricultural and food chemistry* 57(22): 10489–10496.

Kallio, H., Yli-Jokipii, K., Kurvinen, J.P., Sjo¨vall, O and Tahvonen, R. (2001).Regioisomerism of Triacylglycerols in Lard, Tallow, Yolk, Chicken Skin, Palm Oil, Palm Olein, Palm Stearin, and a Transesterified Blend of Palm Stearin and Coconut Oil Analyzed by Tandem Mass Spectrometry. *J. Agric. Food Chem.* 49: 3363−3369.

Kamm, W., Dionisi, F. and Hischenhuber, C. (2007). Authenticity Assessment of Fats and Oils. *Food Reviews International* 17(3): 37–41.

Kelly, S. and Heaton, K. (2005).Tracing the geographical origin of food: The application of multi-element and analysis.*Trends in Food Science & Technology* 16: 555–567.

Lee, D., Noh, B., Bae, S. and Kim, K. (1998). Characterization of fatty acids composition in vegetable oils by gas chromatography and chemometrics. *Analytica Chemica Acta* 358:163-175.

Lee, K. and Foglia, T. A. (2000). Synthesis, Purification and Characterization of Structured Lipids Produced from Chicken Fat. *JAOCS* 77: 1027–1034.

Lipidlibrary (2012). A racemic mixture of *sn*-1,2- and 2,3-diacylglycerols Retrieved May 2012 from http://lipidlibrary.aocs.org/lipids/mg/index.htm

Lipidlibrary (2012).Stereo isomeric *sn*-1- and *sn*-2- and *sn*-3-monoacylglycerols. Retrieved May 2012 from http://lipidlibrary.aocs.org/lipids/mg/index.htm

Liu, X., Xu, S.P., Wang, J.H., Yuan, J.P., Guo, L.X., Xin, L. and Huang, X.N. (2007). Characterization of ganoderma spore lipid by stable carbon isotope analysis: implications for authentication. *Analytical and bioanalytical chemistry* 388(3): 723–31.

Liu, Y., Meng, Z., Shan, L., Jin, Q. and Wang, X. (2009). Preparation of specialty fats from beef tallow and canola oil by chemical interesterification: physico-chemical properties and bread applications of the products. *European Food Research and Technology* 230(3): 457-466.

Marikkar, J.M.N., Ghazali, H. M., Che Man, Y. B., Peiris, T. S. G. and Lai, O. M. (2005a). Distinguishing lard from other animal fats in admixtures of some vegetable oils using liquid chromatographic data coupled with multivariate data analysis. *Food Chemistry* 91(1): 5–14.

Marikkar, J.M.N., Ghazali, H. M., Che Man, Y. B., Peiris, T. S. G. and Lai, O. M. (2005b). Use of gas liquid chromatography in combination with pancreatic lipolysis and multivariate data analysis techniques for identification of lard contamination in some vegetable oils. *Food Chemistry90*(1-2): 23–30.

Marikkar, J.M.N., Jayasundera, J.M.M.A., Kumari, A.G.O. and Waidyarathana, K.P. (2008). A Predictive Model for Determination of the Iodine Value of Coconut Oil by GLC Analysis of the Component Fatty Acids.*Cord* 24(2): 21.

Marikkar, J.M.N., Lai, O. M., Ghazali, H. M., and Man, Y. B. C. (2001). Detection of Lard and Randomized Lard as Adulterants in Refined-Bleached-Deodorized Palm Oil by Differential Scanning Calorimetry. *JAOCS* 78(11): 1113–1119.

Marikkar, J.M.N., Lai, O.M., Ghazali, H.M. and Che Man, Y.B. (2002). Compositional and thermal analysis of RBD palm oil adulterated with lipase-catalyzed interesterified lard. *Food Chemistry* 76(2): 249–258.

Marikkar, J.M.N., Ghazali, H.M., Long, K. and Lai O.M. (2003). Lard uptake and its detection in selected food products deep-fried in lard. *Food Research International* 36: 1047–1060.

Michael J. Haas (2005). Animal Fats in Bailey's Industrial Oil and Fat Products, 6th. Ed. Edited by FereidoonShahidi. pp: 161-212. New York: John Wiley and Sons, Inc.

Mottram, H. R., Woodbury, S. E., Rossell, J. B. and Evershed, R. P. (2003). High-resolution detection of adulteration of maize oil using multi-component compound-specific $\delta^{13}C$ values of major and minor components and discriminant analysis. *Rapid communications in mass spectrometry : RCM*, *17*(7): 706–12.

Muccio, Z. and Jackson, G. P. (2009). Isotope Ratio Mass Spectrometry. *The Analyst* 134(2): 213–22.

Nasyrah, A. R., Marikkar, J. M. N. and Dzulkifly, M. H. (2012). Discrimination of plant and animal derived MAG and DAG by principal component analysis of fatty acid composition and thermal profile data. *IFRJ* 19(4): 1497–1501.

Negi, D. S. (2006). Base Catalyzed Glycerolysis of Fatty Acid Methyl Esters: Investigations towards the Development of a Continuous Process. PhD Theses. Technical University of Berlin

Nurjuliana, M., Che Man, Y.B. and Dzulkifly, M.H. (2011).Analysis of lard's aroma by an electronic nose for rapid halal authentication.*Journal of the American Oil Chemists' Society* 88(1): 75–82.

O' Brien, R. 2009.Fats and Oils. Formulating and Processing for Applications. pp. 54-55, 265-275, 15-57 3rd Ed. Boca Raton: CRC Press Taylor & Francis Group.

Osorio, M. T., Moloney, A. P., Schmidt, O., and Monahan, F. J. (2011).Multielement Isotope Analysis of Bovine Muscle for Determination of International Geographical Origin of Meat, *Journal of Agricultural and Food Chemistry* 59: 3285-3294.

Perini, M., Camin, F., Bontempo, L., Rossmann, A., and Piasentier, E. (2009). Multielement (H, C, N, O, S) stable isotope characteristics of lamb meat from different Italian regions *Rapid Commun. Mass Spectrom.* 23: 2573–2585

Piasentier, E.R.,Valusso, F.,Camin, and Versini,G. (2003). Stable isotope ratio analysis for authentication of lamb meat.*Meat science* 64(3): 239–47.

Rand R. Wilcox. (2003). Chapter 6 - Least squares regression and Pearson's correlation.Applying Contemporary Statistical Techniques.pp. 173-206. California: Elsevier Science (USA) Academic Press.

Rhodes, C. N., Lofthouse, J. H., Hird, S., Rose, P., Reece, P., Christy, J. and Macarthur, R. (2010).The use of stable carbon isotopes to authenticate claims that poultry have been corn-fed.*Food Chemistry*, *118*(4), 927-932.

Riaz, M.N. and Chaudary, M.M. (2004).*Halal Food Production*, pp. 1-379. Florida: CRC press.

Richter, E.K., Spangenberg, J.E., Michael, K. and Florian, L. (2010).Characterization of Rapeseed (*Brassica napus*) Oils by Bulk C, O, H, and Fatty Acid C Stable Isotope Analyses *J. Agric. Food Chem* 58: 8048–8055.

Rohman, A. and Che Man, Y.B. (2010). FTIR spectroscopy combined with chemometrics for analysis of lard in the mixtures with body fats of lamb, cow, and chicken. *International Food Research Journal* 17: 519–526.

Rohman, A., Che Man, Y.B. and Noviana, E. (2012).Analysis of Emulsifier in Food Using Chromatographic Techniques.In Press.

Rohman, A., Sismindari, Erwanto, Y. and Che Man, Y.B. (2011).Analysis of pork adulteration in beef meatball using Fourier transform infrared (FTIR) spectroscopy. *Meat Science* 88: 91–95.

Ruiz-Rodrigueza ,A., Reglero, G. and Iba˜nez, E. (2010). Recent trends in the advanced analysis of bioactive fatty acids. *Journal of Pharmaceutical and Biomedical Analysis* 51: 305–326.

Saeed T, Ali S.G., Rahman H.A.A and Sawaya WN. (1989). Detection of Pork and Lard as adulterants in Processed Meat: Liquid Chromatography analysis of derivatized triglycerides. *J Ass of Off Anal Chem* 72:921-925.

Saeed, T., Abu-Dagga, F. and Rahman, H.A. (1986).Detection of Pork and lard as Adulterants in Beef and mixtures.*J Ass of Off Anal Chem* 69: 999-1002.

Sawaya, W.N., Saeed T., Mameesh, M., El-Rayes, E., Husain, A., Ali, A. and Rahman, H.A. (1990). Detection of Pork in Processed Meat: Experimental comparison of methodology. *Food Chem* 37:201-219.

Schipilliti, L., Tranchida, P. Q., Sciarrone, D., Russo, M., Dugo, P., Dugo, G. and Mondello, L. (2010). Genuineness assessment of mandarin essential oils employing gas chromatography-combustion-isotope ratio MS (GC-C-IRMS). *Journal of separation science* 33: 617–25.

Seo, H.Y., Ha, J., Shin, D.B., Shim, S.L., No, K.M., Kim, K.S. and Lee, K.B. (2010). Detection of Corn Oil in Adulterated Sesame Oil by Chromatography and Carbon Isotope Analysis. *Journal of the American Oil Chemists' Society* 87(6): 621–626.

Shin, E.C., Craft, B.D., Pegg, R.B., Phillips, R.D. and Eitenmiller, R.R. (2011) Chemometric approach to fatty acid profiles in Runner-type peanut cultivars by principal component analysis (PCA).*Food Chemistry*119(3): 1262-1270.

Simsek, A., Bilsel, M. and Goren, A. C. (2012). 13C/12C pattern of honey from Turkey and determination of adulteration in commercially available honey samples using EA-IRMS. *Food Chemistry* 130(4): 1115–1121.

Ulfert Focken (2004).Feeding fish with diets of different ratios of C3- and C4-plant-derived ingredients: a laboratory analysis with implications for the back-calculation of diet from stable isotope data.*Rapid Commun. Mass Spectrom*18: 2087–2092.

Wang, F.S. and Lin, C.W. (1995).Turbidimetry for Crystalline Fractionation of Lard.*JAOCS* 72(5): 585-589.

Wikipedia 2013.[GCMS].Retrieved from http://en.wikipedia.org/wiki/GCMS.

Wikipedia 2012.[Schematic diagram for a GC system].Retrieved from http://en.wikipedia.org/wiki/GC system.

Wikipedia. 2012. Adipose tissue. en.wikipedia.org/wiki/Adipose_tissue. Accessed on December 2012.

Wood, J.D., Enser, M., Fisher, A.V., Nute, G.R., Sheard, P.R., Richardson, R.R., Hughes, S.I. and Whittington F.M. (2008).Fat deposition, fatty acid composition and meat quality: A review. *Meat Science* 78: 343–358.

Woodbury, S.E., Evershed, R.P. and Rossell, J.B. (1998).Purity assessments of major vegetable oils based on $\delta^{13}C$ values of individual fatty acids. *JAOCS* 75(3): 371–379.

Yang, T., Zhang, H., Mu, H., Sinclair, A. J., and Xu, X. (2004). Diacylglycerols from butterfat: Production by glycerolysis and short-path distillation and analysis of physical properties. *Journal of the American Oil Chemists' Society*, 81(10): 979–987.

Yanty, N. A. M., Marikkar, J. M. N. and Che Man, Y.B. (2011b). Effect of fractional crystallization on composition and thermal characteristics of avocado (Perseaamericana) butter. *J Therm Anal Calorim* 1-7.

Yanty, N. A. M., Marikkar, J. M. N., Che Man, Y. B. and Long, K. (2011). Composition and thermal analysis of lard stearin and lard olein. *Journal of oleo science* 60(7): 333–338.

Yılmaz, M.T. and Karakaya, M. (2009). Differential Scanning Calorimetry Analysis of Goat Fats: Comparison of Chemical Composition and Thermal Properties. *Journal of the American Oil Chemists' Society* 86(9): 877–883.

Yılmaz, M.T., Mustafa, K. and Aktas, N. (2010).Composition and thermal properties of cattle fats.*European Journal of Lipid Science and Technology* 112(3): 410–416.