

**DETECTION OF ADULTERATED NON-HALAL FAT (LARD) IN ANIMAL
FATS USING GC-FID, HPLC, DSC AND FTIR SPECTROSCOPY
EVALUATED WITH CHEMOMETRIC TECHNIQUES**

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(Matric No. 3090105)

Thesis submitted in fulfillment for the degree of
MASTER OF SCIENCE
(FOOD BIOTECHNOLOGY)

Faculty of Science and Technology
UNIVERSITI SAINS ISLAM MALAYSIA
NILAI

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AUTHOR'S 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 Sains Islam Malaysia or at any other institution.

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SIGNATURE PAGE

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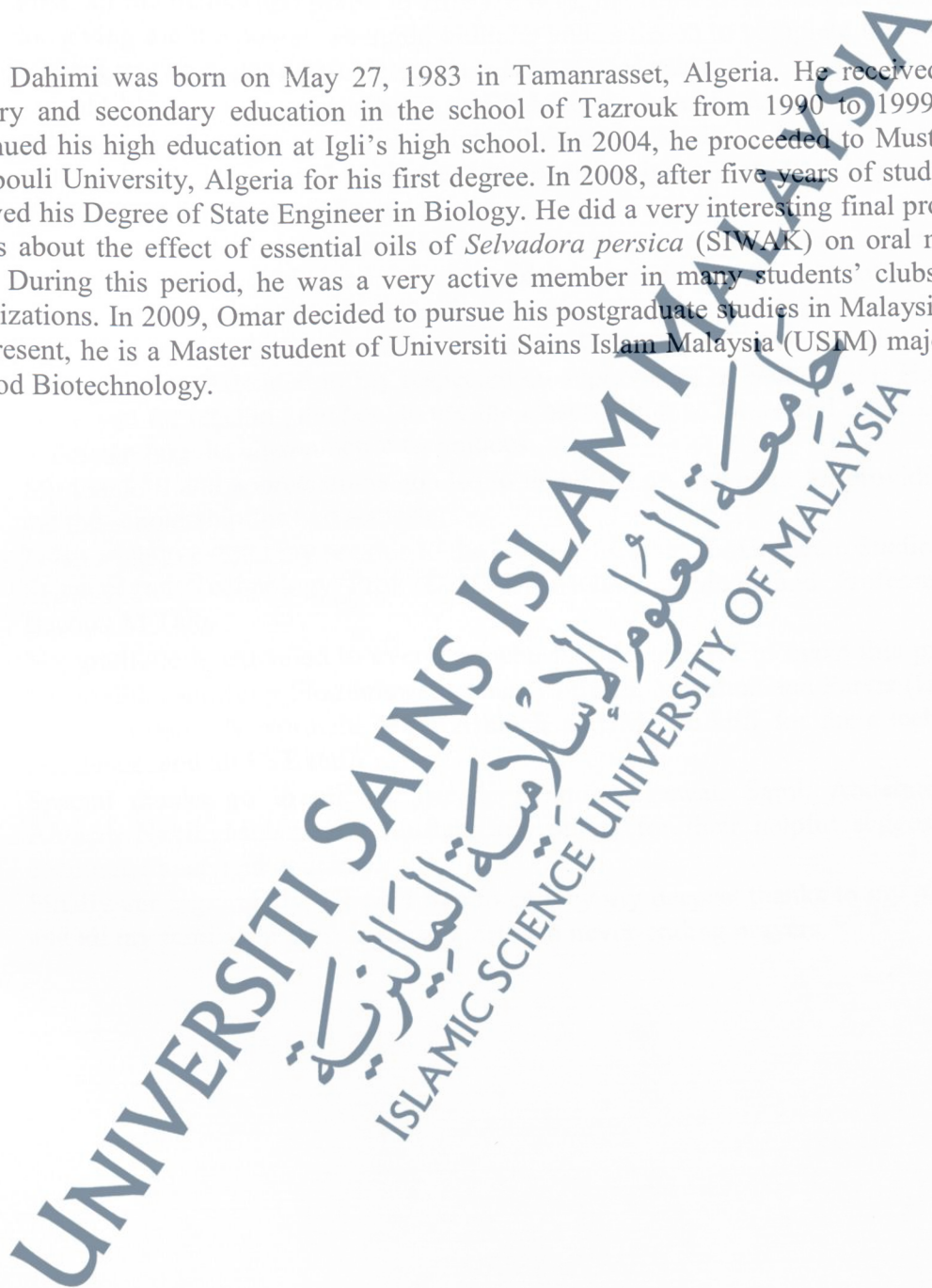
DEDICATION

This thesis is dedicated to my beloved parents, brothers, and sisters; to all members of my big family. It is also dedicated to teachers, scientists, researchers, and to everyone that is interested in the Halal food knowledge.



BIODATA OF AUTHOR

Omar Dahimi was born on May 27, 1983 in Tamanrasset, Algeria. He received his primary and secondary education in the school of Tazrouk from 1990 to 1999. He continued his high education at Igli's high school. In 2004, he proceeded to Mustapha Stambouli University, Algeria for his first degree. In 2008, after five years of study, he received his Degree of State Engineer in Biology. He did a very interesting final project. It was about the effect of essential oils of *Selvadora persica* (SIWAK) on oral micro flora. During this period, he was a very active member in many students' clubs and organizations. In 2009, Omar decided to pursue his postgraduate studies in Malaysia. At the present, he is a Master student of Universiti Sains Islam Malaysia (USIM) majoring in Food Biotechnology.



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ABSTRAK

Kehadiran lemak babi atau terbitannya dalam sebarang produk makanan adalah isu agama yang serius di kalangan golongan Muslim dan Yahudi. Keadaan sifat fizikokimia lemak babi, membuatnya sukar dibezakan dengan lemak haiwan lain seperti lemak lembu dan lemak ayam. Objektif utama kajian ini ialah: (1) untuk memantau asid lemak (FA), dan triasilgliserol (TAG) profil lemak babi, lemak lembu, dan lemak ayam menggunakan empat alat yang berbeza (GC, HPLC, DSC, dan FTIR), dan (2) untuk mengesan dan mencirikan kepekatan tertentu lemak babi yang dicampurkan dengan lemak lembu dan lemak ayam menggunakan peralatan yang telah ditetapkan dengan penggunaan kimometriks. Pengukuran telah dibuat dari lemak babi tulen yang diekstrak, lemak lembu, lemak ayam dan campuran lemak lembu (BT), lemak ayam (CF) dan lemak babi dengan campuran kandungan yang berbeza lemak haiwan BT atau CF: lemak babi LD laitu (99.5 % + 0.5% LD, 99 % + 1 % LD, 98 % + 2 % LD, 97 % + 3 % LD, 96 % + 4 % LD, dan 95 % + 5 % LD). Kemudian sampel telah dianalisis menggunakan GC-FID, HPLC, DSC, dan FTIR. Data semua sampel telah diskalakan ke dalam piawai, normalisasi, dan purata terpusat sebelum PCA dilakukan untuk setiap data berskala menggunakan perisian Unscrambler. Markah dan bebanan plot setiap data berskala dikaji dan dibandingkan. Keputusan GC-FID menunjukkan bahawa lemak babi mengandungi lebih tinggi asid lemak C18:2 (FA) dan rendah asid lemak C16:0 (FA), tetapi nilai berlawanan diperhatikan untuk lemak lembu dan lemak ayam dengan jumlah 0.60 % dan 0.14 % untuk C18 : 2 sis dan 50 % serta 71 % untuk C16 : 0. Jumlah C4: 0, C14: 0, dan C18: 0 adalah lebih kurang sama untuk semua lemak. Lain-lain asid lemak (FA) berada dalam jumlah yang kecil dan hampir sama untuk kedua-duanya. Di samping itu, dari hasil HPLC, ia telah mendapati bahawa triasilgliserol lemak babi mengandungi lebih tinggi PPL, triasilgliserol PPO dan rendah PLO tetapi berlawanan bagi lemak lembu manakala lemak ayam memberikan profil yang mirip seperti lemak babi. Sementara itu, jumlah OOP dan PPP adalah lebih kurang sama untuk semua lemak. Keputusan DSC menunjukkan bahawa DSC boleh digunakan untuk membezakan lemak babi dari lemak lembu dan lemak ayam berdasarkan suhu permulaan 'onset', entalpi, dan suhu akhir 'endset' penyejukan dan termogram leburan. Dengan menggunakan termogram lebur, ia adalah mungkin untuk mengesan kehadiran lemak khinzir dalam lemak lembu dan lemak ayam walaupun kurang daripada 1%, manakala, sukar menggunakan termogram penyejukan untuk menyukak kehadiran lemak babi yang dicampurkan dalam lemak ayam. Melalui teknik kemometriks, keputusan semua data analisis komponen utama (PCA) digambarkan bahawa PCA dapat mengenal pasti dengan ketara lemak babi, lemak lembu, lemak ayam dan campuran lemak bersama lemak babi lembu, lemak babi dan lemak ayam walaupun pada tahap kepekatan yang lebih rendah (0.5% lemak 99.5% lemak daging lembu / ayam lemak (b/b) Di sebaliknya, K-min kelompok hanya mampu untuk mengelaskan lemak khinzir tulen, lemak ayam tulen (CF) dan lemak lembu tulen (BT), tetapi ia tidak dapat membezakan lemak lembu dari campuran lemak lembu bersama lemak babi (≤ 5 % lemak babi). dan lemak ayam dari campuran lemak ayam bersama lemak babi (≤ 5 % lemak babi).

ملخص البحث

ان تواجد دهن الخنزير او مشتقاته في اي مركب يعتبر مشكلة حساسة في الدين الاسلامي واليهودي. اضافة الى الخصائص الفيزيوكيميائية لدهن الخنزير التي جعلته صعب التمييز بينه وبين الدهون الحيوانية الاخرى مثلا دهن البقر ودهن الدجاج. لهذا فان الاهداف الرئيسية لهذا البحث هي: كشف الاحماض الدهنية و ثلاثي الغريسدات لدهن الخنزير, دهن البقر, ودهن الدجاج باستعمال تقنية الغاز الغروماتوغرافي, السائل الغروماتوغرافي, وتقنية التأثير الحراري (الدي اس, سي او مطياف الاشعة فوق الحمراء). اما الهدف الثاني هو كشف و تشخيص اقل تركيز مضاف من دهن الخنزير الى دهن البقر ودهن الدجاج باستعمال التقنيات السابقة مدعمة بتقنية الكيموميترك الاحصائية. القياسات اجريت على الدهون الحيوانية المستخلصة (دهن الخنزير, دهن البقر, ودهن الدجاج) و دهن البقر مضاف اليه دهن الخنزير وكذلك دهن الدجاج مضاف اليه دهن الخنزير بتركيزات تتراوح بين (0.5 و 5%). بعد ذلك كل العينات تم تحليلها باستعمال التقنيات المشار اليها آنفا. بيانات كل العينات تم تحليلها باستعمال تقنية (ال بي سي, اي) من خلال برنامج انسكلابلر. نتائج تقنية الغاز كروماتوغرافي اظهرت ان دهن الخنزير يحتوي على نسبة عالية من (الحمض الدهني رقم 18 سيس. وكمية الحمضيين الدهنيين رقم 14 ورقم 4 ورقم 18 تقريبا متساوية عند كل العينات المدروسة. اما باقي الاحماض الدهنية نسبتها قليلة نسبيا و تقريبا متساوية القيمة في كل العينات. زيادة على هذا نتائج تقنية السائل الكروماتوغرافي اظهرت ان دهن الخنزير يحوي نسبة عالية من ثلاثي الغريسدات من نوع (بي بي ال و بي بي او) وكمية قليلة من (بي ال او) لكن عكس هذه النتائج ظهرت عند دهن البقر بينما دهن الدجاج فكانت نتائجها قريبة من نتائج دهن الخنزير. في حين ان باقي ثلاثي الغريسدات نتائجها كانت تقريبا متقاربة. من جهة اخرى نتائج تقنية التأثير الحراري (دي اس سي) بينت انه من خلال استعمال هذه التقنية يمكن تمييز دهن الخنزير عن باقي الدهون الاخرى اعتمادا على درجات

الحرارة البدئية والانتالي ودرجات الحرارة الانتهاية لمخطط الذوبانية و مخطط التجميد الخاص بالعينات. ووجد ايضا انه باستعمال المخطط الحراري للذوبانية فانه بالامكان جدا الكشف عن دهن الخنزير المضاف الى دهن البقر ودهن الدجاج حتى اقل من 1 % بينما صعب ان تتحص على ذلك باستعمال المخطط الحراري للتجميد خاصة عند دهن الدجاج مضاف اليه دهن الخنزير. علاوة على ذلك، من خلال تقنية الكيموميترك، نتائج البي سي اي لكل البيانات اطهرت انه بالامكان تحديد وكشف دهن الخنزير، دهن البقر ودهن الدجاج ودهن البقر ودهن الدجاج مضاف اليه دهن الخنزير حتى بنسبة 0.5% من دهن الخنزير . من جانب اخر تقنية كي مين كلاستر كانت قاردة فقط على تمييز دهن الخنزير عن دهن البقر ودهن الدجاج التقنية اما اذا كانت مضافة اليها دهن الخنزير فان هذه التقنية اي كي مين كلاستر) قادرة على تمييز دهن الخنزير عن باقي العينات.

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LIST OF ABBREVIATIONS

BF	Beef Fat
BT	Beef Tallow
CA	Cluster Analysis
DSC	Differential Scanning Calorimetry
ECN	Equivalent Carbon Number
FAO	Food and Agriculture Organization
FA	Fatty Acid
FAME	Fatty Acid Methyl Esters
FID	Flame Ionisation Detector
FTIR	Fourier Transform Infrared
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
IV	Iodine Value
MMM	Trimyristin
MPL	1-myristoyl-2-palmitoyl-3-linoleoyl-sn-glycerol
MPP	1-myristoyl-2-dipalmitoyl-sn-glycerol
MUFA	Monounsaturated Fatty Acids
OOL	1,2-dioleoyl-3-linoleoyl-sn-glycerol
OOO	Triolein
OPO	1,3-dioleoyl-2-palmitoyl-sn-glycerol
PCA	Principal Component Analysis
PLS	Partial Least Square
PPP	Tripalmitic acid
PSO	1-palmitoyl-2-stearoyl-3-oleoyl-sn-glycerol
PPS	1,2-dipalmitoyl-3-stearoyl-sn-glycerol
POO	1-palmitoyl-2-dioleoyl-sn-glycerol
PPL	2-dipalmitoyl-3-linoleoyl-sn-glycerol
PPO	1,2-dipalmitoyl-3-oleoyl-sn-glycerol
PUFA	Polyunsaturated Fatty Acids
SFA	Saturated Fatty Acids
SSO	1,2-distearoyl-3-oleoyl-sn-glycerol
SSS	Tristearic acid
TAG	Triacylglycerols
USTAG	Unsaturated triacylglycerols