A STUDY ON THE GENE EXPRESSION LEVEL IN HaCaT KERATINOCYTE CELLS TO RELATE WITH HALAL AND HARAM STATUS WHEN EXPOSED TO PLANT AND ANIMAL FATS USING cDNA MICROARRAY

BY

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A thesis submitted in fulfilment of the requirement for the degree of Master of Science (Halal Industry Science)

International Institute for Halal Research & Training (INHART)
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Halal and haram are ingrained in the daily life of a Muslim; guided by Al-Quran and As-Sunnah. This concerns of halal and haram has also opened a vast market operated by not only Muslims but non-Muslims all over the world. The rapid growth of halal market demands the use of technologies to ensure the quality and safety of halal products. These technologies range from compact and mobile test kits to the high-end techniques such as Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC) and Polymerase Chain Reaction (PCR). In line with the halal market growth, more scientific research related to halal by using the above techniques has also been reported. However, microarray has received very little attention in halal research. Therefore, this study explores the use of cDNA microarray to investigate the effects of fat from haram sources on HaCaT keratinocyte human skin cells in comparison to halal fat sources at gene expression level. The haram fat sources used in this study were lard and non-halal slaughtered lamb fat while Halal fat sources were virgin coconut oil (VCO) and halal slaughtered lamb fat. The RNAs extracted from treated cells were used in cDNA microarray (Agilent 8x60K SurePrint G3 Human GE). The data analyzed by GeneSpring GX 13.0, detected 50,739 genes from the four treatments and after further filtration; 53 genes were obtained with p-value of <0.05 and fold change of ≥2.0 (FC range between -2.457 to 6.813). The most regulated genes were NLRP5, FABP3, RPS21, PRKDC, ERCC4, ACTG1P4, and RACGAP1P. Selected genes (FABP3, PRKDC, GULP1, and XPOT) were then validated using real-time PCR. The gene expressions from real-time PCR were found to be consistent with microarray data. Finally, pathway analysis using Ingenuity Pathway Analysis (IPA® 2016) software gave some insights into the underlying molecular networks and pathways. Although the results were not entirely conclusive, some patterns were observed; the four fat emulsion treatments were involved in similar bio functionalities (cellular growth and proliferation, cell cycle and cellular movement) and associated diseases (developmental disorders involving the cell growth, connective tissue and hematological disease). In conclusion, the study showed that halal and haram fat sources caused differential gene expression in human cells. However, more work is warranted to further elucidate the pathways involved in order to understand the potential benefits and/or the perceived harmful effects of the fats.
خلاصة البحث

الحلال والحرم مثابرين في الحياة اليومية للمسلمين، منتقدة بالقرآن الكريم والسنة. وقد تحمل القلق من الحلال والحرم أيضاً سوقاً واسعاً ليس فقط من قبل المسلمين ولكن غير المسلمين في جميع أنحاء العالم. ويتطلب النمو السريع في سوق الحلال استخدام التكنولوجيا لضمان جودة وسلامة المنتجات الخالية. ويتراوح هذه التكنولوجيا بين مجموعات الاختبار الدقيقة والمتفلقة إلى التقنيات المتطورة مثل التحليل الطيفي للأذى تحت الحمراء (FTIR)، والمسح النفاثي للمحسس الكهرو (DSC)، وتفاعل البوليمير المتسلسل (PCR)، وتمكن مع نمو السوق الحلال، تم أيضاً القيام من أثر الدورة العلمية المتعلقة بالحلال باستخدام التقنيات المذكورة أعلاه. ومع ذلك، تلقى ميكرواري القليل جداً من الاهتمام في بحث الحلال. لذلك، تستكشف هذه الدراسة استخدام HAcaT

HAcaT للتحقق من تأثير الدهون من المصادر المحمصة على هاكات الخلايا الكبائية خلايا الجلد البشرية بالمقارنة مع مصادر الدهون الحلال في مستوى التعبير الجيني. وكانت مصادر الدهون المحمصة مستخدمة RNAs في هذه الدراسة هي دهن الخنزير ودهن الضأن المذبوخ بطرق غير شرعية، بينما كانت مصادر الدهون الحلال زيت

حمض الزيت الزيت (VCO) ودهن الضأن الحلال. تم استخدام الحمض النووي الربي المستخرج من الخلايا المختلفة (Agilent 8x60K SurePrint G3 Human GE cDNA الخلايا المعاقدة ميكرواري cDNA) التي تم تحليلها كشفت عن 50,739 من المعالجات الأربعة. بعد مزيد من الترشيح، تم الحصول على 53 FC تتراوح بين 2.457 و 6.813، وكانت الجينات الأكثر تنظيمًا هي Actg1p4 ، Er C c4 ، Pr KDC ، Rs P21 ، FAPB3 ، NLR P5 ، GULP1 ، Pr KDC ، FAPB3 ، RAC GAP1P. تم التحقق من صحة الجينات المحددة (RT-PCR) باستخدام الوقت الحقيقي PCR، Xpot. تم العثور على أن العوامل الجينية من

IPAF (2016) بعض الدراسات بدراسة مسح الدهون الخالية من لوحظت بعض الأفكار، حيث تشارح مستقبل الدهون الخالية ووظائف جسمية الملف الخلايا والاحتل، ودورها في الحركة الكلوية والامراض المرتبطة بها (الضياعات) التي ترتبط على نحو خاص والدهون السليمة وأمراض الدم. وعندما، أظهرت الدراسة أن مصاب الدهون الخالية والحرم تسبب في التعبير الجيني النافذ في الخلايا البشرية. ومع ذلك، فإنه لا يوجد من إجراء المزيد من البحوث لزيادة توضيح الممارسات التي يجري عليها في الفئران المحتملة أو الآثار الضارة المتصورة للدهون.
I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science (Halal Industry Science).

Yumi Zuhanis Has-Yun Hashim
Supervisor

Noriah Ramli
Co-Supervisor

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science (Halal Industry Science).

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This thesis was submitted to the International Institute for Halal Research & Training and is accepted as a fulfilment of the requirement for the degree of Master of Science (Halal Industry Science).

Irwandi Jaswir
Deputy Dean, International Institute for Halal Research & Training

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Hamzah Mohd Salleh
Dean, International Institute for Halal Research & Training
DECLARATION

I hereby declare that this dissertation 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.

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Signature .......................................................... Date ........................................
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# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Description</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Islamic principles regarding <em>halal</em> and <em>haram</em> according to Al-Qhardawi</td>
<td>09</td>
</tr>
<tr>
<td>2.2</td>
<td>Academic entities in <em>halal</em> research and development in South East Asia</td>
<td>11</td>
</tr>
<tr>
<td>2.3</td>
<td>List of some high-end technologies in <em>halal</em> researches</td>
<td>13</td>
</tr>
<tr>
<td>2.4</td>
<td>Internal parasites and bacteria of pigs</td>
<td>21</td>
</tr>
<tr>
<td>2.5</td>
<td>Fatty acids commonly found in fats and oils</td>
<td>23</td>
</tr>
<tr>
<td>2.6</td>
<td>A comparison between oligonucleotide and cDNA microarray</td>
<td>27</td>
</tr>
<tr>
<td>3.1</td>
<td>Real-Time PCR cycler program</td>
<td>51</td>
</tr>
<tr>
<td>4.1</td>
<td>The specific growth rate, doubling time and saturation density of both T-flasks</td>
<td>55</td>
</tr>
<tr>
<td>4.2</td>
<td>Measurement of purity and concentration of RNA and RIN from various treated HaCaT Cells</td>
<td>62</td>
</tr>
<tr>
<td>4.3</td>
<td>Measurement of purity and concentration of cDNA from control, lard and non-<em>halal</em> slaughtered lamb fat treated on HaCaT cells</td>
<td>64</td>
</tr>
<tr>
<td>4.4</td>
<td>List of 97 genes filtered with p-value &lt;0.05 by Benjamini Hochberg FDR and fold change in GeneSpring GX 13.0 analysis</td>
<td>68</td>
</tr>
<tr>
<td>4.5</td>
<td>List of 53 genes filtered by fold change cutoff $\geq 2.0$ in GeneSpring GX 13.0 analysis</td>
<td>72</td>
</tr>
<tr>
<td>4.6</td>
<td>List of 20 genes with highest fold change</td>
<td>74</td>
</tr>
<tr>
<td>4.7</td>
<td>Molecular networks of HaCaT cells treated with virgin coconut oil based on IPA software</td>
<td>78</td>
</tr>
<tr>
<td>4.8</td>
<td>Molecular networks of HaCaT cells treated with lard based on IPA software</td>
<td>85</td>
</tr>
<tr>
<td>4.9</td>
<td>Molecular networks of HaCaT cells treated with <em>halal</em> slaughtered lamb fat based on IPA software</td>
<td>92</td>
</tr>
<tr>
<td>4.10</td>
<td>Molecular networks of HaCaT cells treated with non-<em>halal</em> slaughtered lamb fat based on IPA software</td>
<td>96</td>
</tr>
</tbody>
</table>
4.11 The summary of top diseases and function (disease, cellular functions and physiological system development) of genes in HaCaT cells treated with VCO, lard, *halal* and non-*halal* slaughtered lamb fats respectively
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure No.</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>05</td>
</tr>
<tr>
<td>2.1</td>
<td>20</td>
</tr>
<tr>
<td>2.2</td>
<td>22</td>
</tr>
<tr>
<td>3.1</td>
<td>32</td>
</tr>
<tr>
<td>4.1</td>
<td>54</td>
</tr>
<tr>
<td>4.2a</td>
<td>56</td>
</tr>
<tr>
<td>4.2b</td>
<td>56</td>
</tr>
<tr>
<td>4.3</td>
<td>57</td>
</tr>
<tr>
<td>4.4</td>
<td>57</td>
</tr>
<tr>
<td>4.5</td>
<td>58</td>
</tr>
<tr>
<td>4.6</td>
<td>79</td>
</tr>
<tr>
<td>4.7</td>
<td>80</td>
</tr>
<tr>
<td>4.8</td>
<td>81</td>
</tr>
<tr>
<td>4.9</td>
<td>86</td>
</tr>
<tr>
<td>4.10</td>
<td>87</td>
</tr>
</tbody>
</table>
4.11 The third highest scored networks analyzed by IPA® 2016 showing differently expressed genes following treatment of lard on HaCaT cells

4.12 The highest scored networks analyzed by IPA® 2016 showing differently expressed genes following treatment of *halal* slaughtered lamb fat on HaCaT cells

4.13 The second highest scored networks analyzed by IPA® 2016 showing differently expressed genes following treatment of *halal* slaughtered lamb fat on HaCaT cells

4.14 The third highest scored networks analyzed by IPA® 2016 showing differently expressed genes following treatment of *halal* slaughtered lamb fat on HaCaT cells

4.15 The highest scored networks analyzed by IPA® 2016 showing differently expressed genes following treatment of non-*halal* slaughtered lamb fat on HaCaT cells

4.16 The second highest scored networks analyzed by IPA® 2016 showing differently expressed genes following treatment of non-*halal* slaughtered lamb fat on HaCaT cells

4.17 The third highest scored networks analyzed by IPA® 2016 showing differently expressed genes following treatment of non-*halal* slaughtered lamb fat on HaCaT cells

4.18 Fold change comparison graph for lard treatment

4.19 Fold change comparison graph for non-*halal* slaughtered lamb fat treatment
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTB</td>
<td>Beta-actin</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary deoxyribonucleic acid</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>cRNA</td>
<td>Complementary ribonucleic acid</td>
</tr>
<tr>
<td>DHA</td>
<td>Docohexanoic acid</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s Modified Eagle Medium</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl Sulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalogram</td>
</tr>
<tr>
<td>EPA</td>
<td>Eicosapentaenoic acid</td>
</tr>
<tr>
<td>EtBr</td>
<td>Ethidium bromide</td>
</tr>
<tr>
<td>EVOO</td>
<td>Extra virgin olive oil</td>
</tr>
<tr>
<td>FA</td>
<td>Fatty acid</td>
</tr>
<tr>
<td>FABP3</td>
<td>Fatty acid binding protein 3</td>
</tr>
<tr>
<td>FBS</td>
<td>Fetal bovine serum</td>
</tr>
<tr>
<td>FC</td>
<td>Fold change</td>
</tr>
<tr>
<td>FE</td>
<td>Feature extraction</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier Transform Infrared</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Glyceraldehyde 3-phosphate dehydrogenase</td>
</tr>
<tr>
<td>GULP1</td>
<td>GULP, Engulfment adaptor PTB domain containing 1</td>
</tr>
<tr>
<td>HaCaT</td>
<td>Human keratinocyte skin cell</td>
</tr>
<tr>
<td>IPA</td>
<td>Ingenuity Pathway Analysis</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffer saline</td>
</tr>
<tr>
<td>P.B.U.H</td>
<td>Peace be upon him</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PRKDC</td>
<td>Protein kinase, DNA-activated, catalytic polypeptide</td>
</tr>
<tr>
<td>qPCR</td>
<td>Quantitative polymerase chain reaction</td>
</tr>
<tr>
<td>R.A</td>
<td>Ṣallahu anhu (Peace be upon him)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>RIN</td>
<td>RNA integrity number</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Real-Time polymerase chain reaction</td>
</tr>
<tr>
<td>S.W.T</td>
<td>Subhanallahu wata’ala</td>
</tr>
<tr>
<td>VCO</td>
<td>Virgin coconut oil</td>
</tr>
<tr>
<td>XPOT</td>
<td>Exportin tRNA</td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS

Abstract ......................................................................................................................... i
Abstract in Arabic .......................................................................................................... ii
Approval Page ................................................................................................................ iii
Declaration ..................................................................................................................... iv
Copyright Page ............................................................................................................ v
Acknowledgements ....................................................................................................... vi
List of Tables ................................................................................................................... vii
List of Figures ............................................................................................................... ix
List of Abbreviations ................................................................................................... xi

CHAPTER ONE: INTRODUCTION .............................................................................. 1
1.1 Background of the Study ....................................................................................... 1
1.2 Problem Statement ............................................................................................... 3
1.3 Research Hypothesis ......................................................................................... 3
1.4 Research Objectives ......................................................................................... 4
1.5 Research Methodology ..................................................................................... 5
1.6 Scope of Study .................................................................................................... 5
1.7 Thesis Organization ........................................................................................... 6

CHAPTER TWO: LITERATURE REVIEW ................................................................. 7
2.1 *Halal* and *Haram* ........................................................................................ 7
2.1.1 Definition and Principles of *Halal* and *Haram* ....................................... 8
2.1.2 *Halal* Authorities and Entities ................................................................. 10
2.1.3 *Halal* Science and Technologies ............................................................... 12
   2.1.3.1 *Halal* Slaughtering ......................................................................... 15
   2.1.3.2 Alcoholic Drinks .............................................................................. 17
   2.1.3.3 The Prohibition of Pigs .................................................................... 18
      2.1.3.3.1 Chemical Evidence ................................................................... 18
      2.1.3.3.2 Microbial Evidence .................................................................. 20
      2.1.3.3.3 Gelatins ................................................................................... 21
2.2 Fats and Oils ...................................................................................................... 22
   2.2.1 Comparison of Animal Fats and Plant Oils ............................................ 23
   2.2.2 Application of Fats and Oils .................................................................. 24
2.3 Human Keratinocyte (HaCaT) Skin Cell ....................................................... 25
2.4 Gene Expression ................................................................................................. 25
   2.4.1 DNA Microarray and Its Applications ............................................... 26
      2.4.1.1 cDNA Microarray ....................................................................... 28
   2.4.2 Real-Time PCR and Its Applications .................................................. 28
      2.4.2.1 Comparison of PCR and Real-Time PCR .................................... 29
   2.3.2.2 Real-Time PCR as Validation to Microarray .................................... 29
2.5 Summary ........................................................................................................... 30
CHAPTER THREE: MATERIALS AND METHODS

3.1 Introduction
3.2 Materials
  3.2.1 Cell Line
  3.2.2 Oil and Fat Samples
  3.2.3 Culture Medium
  3.2.4 Consumable and Other Items
  3.2.5 Chemicals and Reagents
  3.2.6 Equipment and Instruments
3.3 Cell Maintenance in T-Flask
  3.3.1 Medium Preparation
  3.3.2 Routine Cell Maintenance
    3.3.2.1 Thawing Cells
    3.3.2.2 Freezing Cells
    3.3.2.3 Subculturing Monolayer Cells
    3.3.2.4 Cell Counting
    3.3.2.5 Changing Medium
3.4 Growth Profile of HaCaT Cells
  3.4.1 Media and Cell Volumes for Specific Seeding Concentration
  3.4.2 Growth Profile in Different T-Flasks
5.1 Conclusion
3.5 Treatment of HaCaT Cells with Fat Emulsions
  3.5.1 Formulation of Gum Arabic Solution
  3.5.2 Formulation of Fat Emulsion
    3.5.2.1 Preparation of Fats and Oil
    3.5.2.2 Preparation of Fat Emulsions
    3.5.2.3 Study on Effects of Different Concentration of Fat Emulsion on Cell Growth
  3.5.3 Treatment of Fat Emulsion on HaCaT Cells for Gene Expression Study
3.6 Gene Expression Study of Treated HaCaT Cells
  3.6.1 RNA Extraction
    3.6.1.1 Determination of RNA Concentration and Purity
    3.6.1.2 Determination of RNA Integrity Number (RIN)
  3.6.2 Microarray
    3.6.2.1 Sample Preparation
    3.6.2.2 Hybridization
    3.6.2.3 Slide Washing
    3.6.2.4 Scanning and Feature Extraction
  3.6.2.5 Genes and Pathway Analysis
3.7 Validation of Microarray Analysis by Real-Time PCR
  3.7.1 Sample Preparation
    3.7.1.1 Gene Selection
    3.7.1.2 Reverse Transcription of RNA
    3.7.1.3 Reconstitution of Primers
    3.7.1.4 SYBR Green Mastermix Preparation for Real-Time PCR
  3.7.2 Real-Time PCR
  3.7.3 Real-Time PCR Analysis
CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Halal is an important concern and continuously revolves around Muslims and increasingly among other population as well. Worldwide, halal labels or logo are synonym to the assurance for the safety and wholesomeness of the products. Halal is comprehensive and does not cover only food but everything in daily basis such as cosmetics, personal care, pharmaceuticals, clothes, logistics, finance and trades. Muslims need to be cautious in determining what to consume and what not, and this is guided by the verse in the Quran:

“O mankind, eat from whatever is on earth [that is] lawful and good and
do not follow the footsteps of Satan. Indeed, he is to you a clear enemy. “

(Surah Al-Baqarah; 2: 168)

In the present time, the increase of halal awareness helps to expand and flourish the halal market all around the world. To aid the understanding and muamalah of halal needs in human life, government and non-governmental organizations authorities are established. Interest in halal science also showed an increasing trend. For instance, there are studies on the effects of slaughtering on animals according to halal and shechita methods (Gregory et al., 2008) and the validation of food authentication by using high
end equipment such as Fourier transform infrared (FTIR) and polymerase chain reaction (PCR) (Rohman et al., 2011; Aida et al., 2005).

There are numerous experiments regarding halal issues can be elegantly performed and simplified throughout sophisticated technologies. To the best of our knowledge, the application of microarray technology in halal science related research is very limited. This high end technology has shown excellent performance in various fields of research such as cancer, toxicology, environmental safety, ecology, clinical genotyping and pathological testing (Brennan et al., 2005; Chin & Kong, 2002; Call et al., 2003; Huyghe et al., 2009). A study conducted by Liu and coworkers (2006) on gene expression of superior frontal cortex from alcoholic and non-alcoholic individuals reported that the alteration of genes from alcoholic person led to several neural diseases such as Alzheimer and other psychiatric diseases. This is the evidence to the prohibition of alcoholic beverages as Allah S.W.T mentioned in the Quran:

“They question you about strong drink (khamr) and games of chance. Say; in both is great abuse and usefulness for mankind; but the abusive side of them is greater than their usefulness. “

(Surah Al-Baqarah; 2: 219)

To this end, microarray technology especially cDNA microarray has a huge potential to be one of the useful tools of halal science research. In this present study, we used cDNA microarray to investigate the effect of halal and haram fats on human keratinocyte cells at the gene expression level.
1.2 PROBLEM STATEMENT

With the increasing of *halal* and *haram* awareness and market demands, a lot of work has been directed towards *halal* authentication of food and consumer products with major concerns on alcohol and components of pig origins. Various approaches and lab-based investigations such as enzymatic and proteomics have been applied in the *halal* science related research. However, less has been focused on the effect of *haram* substances at the gene expression level. In particular, little is known about the effects of swine components (the fats) on human at gene expression level despite the perceived harmful effects of pigs due to its *haram* status which is parallel to one of the Islamic principles on *halal* and *haram* stated by Al-Qhardawi (1994); prohibition of things due to their impurity and harmfulness. Components from swine are vastly being used in many areas such as baking, clothing and even constructions. It is therefore, the interest of this study to investigate the effects of fat from pig at the gene expression level using microarray analysis in comparison to other fat/lipid emulsions.

1.3 RESEARCH HYPOTHESIS

The different types of fat/lipid emulsions used to treat HaCaT human keratinocytes may result in different effects on cell behavior. The lipid emulsion from plant source which is from virgin coconut oil (VCO) may benefit the growth of cells as have been shown in nutraceuticals and cosmeceuticals. Meanwhile, the animal source of fat emulsion particularly lard, *halal* slaughtered lamb fat and non-*halal* slaughtered lamb fat may either encourage or inhibit cell growth.
The phenotypic characteristics of the cells, including the cell behavior upon treatment of the fat/lipid emulsions are the consequence of events occurring at the molecular level. Thus, investigating the effects at the gene expression may provide further depth of understanding of the underlying mechanisms. It is noteworthy that, this study is undertaken not to challenge the shariah prohibition of haram components in food and consumer products; rather it is to attest their harmful or negative effects. Microarray technology, which can simultaneously analyze thousands of genes, holds a great potential as a tool to decipher the effects of the different types of fats (halal and haram) on human cells and provide biomarker(s) that can be used to develop halal authentication protocols.

1.4 RESEARCH OBJECTIVES

The research objectives of this study are:

1. To investigate the effects of fat from haram sources on human skin keratinocyte (HaCaT) cells in comparison to halal fat sources at the gene expression level by microarray analysis.

2. To validate the result of microarray on halal and haram sources on HaCaT cells by using real-time PCR.

3. To identify the potential pathways related to the genes affected by the halal and haram fat emulsions on HaCaT cells.
1.5 RESEARCH METHODOLOGY

The major steps that were involved in this study are displayed in Figure 1.1. The detailed methodology is described in Chapter three of this thesis.

1.6 SCOPE OF STUDY

This study used HaCaT human keratinocyte cells as \textit{in vitro} model to investigate the effects of different types of \textit{halal} and \textit{haram} fat sources on human cells at gene expression level. The fats used in the study were from virgin coconut oil (VCO), \textit{shariah}...
slaughtered lamb, conventional slaughtered lamb, and lard. These fats were incorporated in gum arabic solution to produce emulsions and used in the treatment of HaCaT cells. The cDNA microarray platform was used to study the gene expression from the treated cells as compared to non-treated cells. Real-time PCR was used to validate a selection of selected genes obtained from microarray work. Pathway analysis was carried out to provide insights of potential underlying mechanism of how the different fats affected the cells.

1.7 THESIS ORGANIZATION

The outline of the chapters in this thesis is as follows:

i. Chapter One comprises of background, problem statement, research hypothesis, objectives of the research, research methodology and scope of study.

ii. Chapter Two reveals the literature review that is related to the study on halal and haram and the gene expression study.

iii. Chapter Three discusses the materials and details of methodologies used in this study.

iv. Chapter Four presents the results and discussion on the experiments conducted.

v. Chapter Five gives the conclusions of the research with recommendations for further related studies.
CHAPTER TWO

LITERATURE REVIEW

In this chapter, the halal and haram definitions are described with details on the halal authorities and entities, halal science research and technologies involved with halal authentication as well as slaughtering in Islam. Meanwhile, the gene expression section is further detailed with subtopics of DNA microarray and its applications; and end with real-time PCR as validation technique for microarray.

2.1 HALAL AND HARAM

Halal and haram are pertinent in Islam and comprehensively covers daily life items including food, beverages, clothes, personal care products and cosmetics as well as services such as logistics, tourism and hospitality. Allah S.W.T. said in the Quran:

“O ye who believe! Eat of the good things wherewith We have provided you, and render thanks to Allah of it is (indeed) He whom ye worship.”

(Surah Al-Baqarah 2: 172)

Allah has ordered Muslims to be concerned of what surrounds them. When halal sources are obtained, praises are due to Him the God Almighty. And when halal sources are very scarce, Muslims are urged to initiate and develop alternatives to the haram when