



**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)**

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JUNE 2017

ABSTRACT

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 متأصلان في الحياة اليومية للمسلمين، منقادة بالقرآن الكريم والسنة. وقد فتح القلق من الحلال والحرم أيضاً سوقاً واسعة ليس فقط من قبل المسلمين ولكن غير المسلمين في جميع أنحاء العالم. ويتطلب النمو السريع في سوق الحلال استخدام التكنولوجيا لضمان جودة وسلامة المنتجات الحلال. وتتراوح هذه التكنولوجيا بين مجموعات الاختبار المدججة والمتنقلة إلى التقنيات المتطورة مثل التحليل الطيفي للأشعة تحت الحمراء (FTIR)، والمسح التفاضلي للمسح الكهربائي (DSC)، وتفاعل البوليميراز المتسلسل (PCR). وتماشياً مع نمو السوق الحلال، تم أيضاً القيام عن المزيد من البحوث العلمية المتعلقة بالحلال باستخدام التقنيات المذكورة أعلاه. ومع ذلك، تلقى ميكروأري القليل جدا من الاهتمام في بحوث الحلال. لذلك، تستكشف هذه الدراسة استخدام cDNA ميكروأري للتحقق من تأثير الدهون من المصادر المحرمة على هياكات الخلايا الكيراتينية خلايا الجلد البشرية HaCaT بالمقارنة مع مصادر الدهون الحلال في مستوى التعبير الجيني. وكانت مصادر الدهون المحرمة المستخدمة في هذه الدراسة هي دهن الخنزير ودهن الضأن المدبوح بطرق غير شرعية، بينما كانت مصادر الدهون الحلال زيت جوز الهند البكر (VCO) ودهن الضأن الحلال. تم استخدام الحمض النووي الريبوزي RNAs المستخرج من الخلايا المعالجة في cDNA ميكروأري (Agilent 8x60K SurePrint G3 Human GE). البيانات التي تم تحليلها كشفت عن 50,739 جيناً من العلاجات الأربعة وبعد مزيد من الترشيح. تم الحصول على 53 جيناً مع قيمة $p > 0.05$ ومضاعف تغيير $2.0 \leq FC$ (تتراوح بين -2.457 إلى 6.813). وكانت الجينات الأكثر تنظيمًا هي ACTG1P4، ERCC4، PRKDC، RPS21، FABP3، NLRP5، و RACGAP1P. تم التحقق من صحة الجينات المحددة (GULP1، PRKDC، FABP3)، XPOT باستخدام الوقت الحقيقي PCR. تم العثور على أن التعبيرات الجينية من RT-PCR متسقة مع بيانات ميكروأري. وأخيراً، أعطى تحليل المسار باستخدام تحليل مسار الإبداع (IPA® 2016) بعض الرؤى في الشبكات الجزيئية الكامنة والمسارات. على الرغم من أن النتائج لم تكن قاطعة تماماً، لوحظت بعض الأنماط؛ حيث تشارك مستحلب الدهون الأربعة في وظائف حيوية مماثلة (النمو الخلوي والانتشار، ودورة الخلية والحركة الخلوية) والأمراض المرتبطة بها (اضطرابات النمو التي تنطوي على نمو الخلايا والنسيج الضام وأمراض الدم). وكاستنتاج، أظهرت الدراسة أن مصادر الدهون الحلال والحرام تسببت في التعبير الجيني التفاضلي في الخلايا البشرية. ومع ذلك، فإنه لا بد من إجراء المزيد من الأبحاث لزيادة توضيح المسارات التي ينطوي عليها فهم الفوائد المحتملة أو الآثار الضارة المتصورة للدهون.

APPROVAL PAGE

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).

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DECLARATION

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ACKNOWLEDGEMENTS

First and foremost, all praises to Allah S.W.T and peace and blessing upon Prophet Muhammad S. A.W. The journey to completing this research project is not an easy one. I am very grateful to be supervised under Assoc. Prof. Dr. Yumi Zuhani Hashim and Assoc. Prof. Dr. Noriah Ramli as my co-supervisor and the rest of lecturers in INHART. They have taught and guided me in every step towards the completion of this research with patience and kindness. I would like to thank the Ministry of Higher Education (MOHE) for grant and scholarship that have been given to me along the period of conducting this research. Not forgetting Mr. Salleh Syed Ibrahim, representing the Division of Veterinary Service (DVS) who supplied important samples for this research. Big hearts I convey to my dearest parents, Mr. Salleh Jaafar, Mrs. Pauziah Mohd Said and the rest of my family, who taught me how to be strong and resilient in stress-prone environment and financing me on my way to completing this research. Special shout out to the coolest boss ever; Mr. Eidit Hashim for ever encouraging me to manage my stress wisely. Thank you, Boss.

For my study and work colleagues, lab mates, and my true companions that stayed by my side in low and high water during this particular time of my life, I would be stranded and lost if not for your support and morale you gave to me. I would like to thank Sister Irmanisha Ibrahim for hearing my troubles out, Sister Nurhusna Samsudin for thoughtful ideas, Sister Rahilah Ab. Rahman for years of fun car-pooling, Sister Nurasyikeen Abd. Mutalib for much interesting story-telling, Brother Phirdaous Abbas and Brother Mohd Azmir Ariffin for technical aides and fun moments. I would also like to thank my long distant best friends whom have stayed in my life, Sister Nik Adlin Bahrudin, Sister Noorhanan Mat Junoh, Sister Norafifah Abd. Ghapar, Sister Munirah Mohamad, Sister Norizzati Omar, Sister Nor Nabilah Sulaiman, and Sister Nur Hidayah Musthafa. Thank you for the best 9 years of laughter and sincere thoughts that we have during this precious friendship. It has been and will always be my favourite time in the world to me. I love you with all my heart.

These years of completing this research have been a blast for me and I am very grateful for it and will ever be in my life. Thank you for the best memories made here, International Islamic University Malaysia and many places I have been with all the people I adore much. Thank you from the bottom of my heart.

LIST OF TABLES

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

- 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

101

LIST OF FIGURES

<u>Figure No.</u>		<u>Page No.</u>
1.1	Application of cDNA microarray for detection of <i>haram</i> fat on HaCaT keratinocyte cells	05
2.1	Eight different forms of triglycerides. S represents saturated fatty acid and U, unsaturated fatty acid	20
2.2	Basic structure of fat and oil	22
3.1	Overview of experimental studies	32
4.1	Growth curve of HaCaT cells in the presence of DMEM and 10% FBS in 25 cm ² and 75 cm ² T-flasks.	54
4.2a	Gum arabic solution at different concentration (0.02 to 0.1 g/mL)	56
4.2b	Emulsion of fat is formed after adding 1 mL of liquid fat (VCO) into set of different concentrations of gum arabic solution	56
4.3	Graph of number of cells were treated with 0.5 % (v/v), 0.10 % (v/v) and 0.20 % (v/v) of fat emulsions harvested after 24 h	57
4.4	Graph of average for each fat emulsion treated on HaCaT cells	57
4.5	Graph of normalized percentage of treated cell number of fat samples to percentage of cell number by negative control	58
4.6	The highest scored networks analyzed by IPA® 2016 showing differently expressed genes following treatment of virgin coconut oil (VCO) on HaCaT cells	79
4.7	The second highest scored networks analyzed by IPA® 2016 showing differently expressed genes following treatment of virgin coconut oil (VCO) on HaCaT cells	80
4.8	The third highest scored networks analyzed by IPA® 2016 showing differently expressed genes following treatment of virgin coconut oil (VCO) on HaCaT cells	81
4.9	The highest scored networks analyzed by IPA® 2016 showing differently expressed genes following treatment of lard on HaCaT cells	86
4.10	The second highest scored networks analyzed by IPA® 2016 showing differently expressed genes following treatment of lard on HaCaT cells	87

4.11	The third highest scored networks analyzed by IPA® 2016 showing differently expressed genes following treatment of lard on HaCaT cells	88
4.12	The highest scored networks analyzed by IPA® 2016 showing differently expressed genes following treatment of <i>halal</i> slaughtered lamb fat on HaCaT cells	93
4.13	The second highest scored networks analyzed by IPA® 2016 showing differently expressed genes following treatment of <i>halal</i> slaughtered lamb fat on HaCaT cells	94
4.14	The third highest scored networks analyzed by IPA® 2016 showing differently expressed genes following treatment of <i>halal</i> slaughtered lamb fat on HaCaT cells	95
4.15	The highest scored networks analyzed by IPA® 2016 showing differently expressed genes following treatment of non- <i>halal</i> slaughtered lamb fat on HaCaT cells	97
4.16	The second highest scored networks analyzed by IPA® 2016 showing differently expressed genes following treatment of non- <i>halal</i> slaughtered lamb fat on HaCaT cells	98
4.17	The third highest scored networks analyzed by IPA® 2016 showing differently expressed genes following treatment of non- <i>halal</i> slaughtered lamb fat on HaCaT cells	99
4.18	Fold change comparison graph for lard treatment	102
4.19	Fold change comparison graph for non- <i>halal</i> slaughtered lamb fat treatment	103

LIST OF ABBREVIATIONS

ACTB	Beta-actin
ANOVA	Analysis of variance
cDNA	Complementary deoxyribonucleic acid
CO ₂	Carbon dioxide
cRNA	Complementary ribonucleic acid
DHA	Docohexanoic acid
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
EEG	Electroencephalogram
EPA	Eicosapentaenoic acid
EtBr	Ethidium bromide
EVOO	Extra virgin olive oil
FA	Fatty acid
FABP3	Fatty acid binding protein 3
FBS	Fetal bovine serum
FC	Fold change
FE	Feature extraction
FTIR	Fourier Transform Infrared
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GULP1	GULP, Engulfment adaptor PTB domain containing 1
HaCaT	Human keratinocyte skin cell
IPA	Ingenuity Pathway Analysis
mRNA	Messenger ribonucleic acid
PBS	Phosphate buffer saline
P.B.U.H	Peace be upon him
PCR	Polymerase chain reaction
PRKDC	Protein kinase, DNA-activated, catalytic polypeptide
qPCR	Quantitative polymerase chain reaction
R.A	<i>Radiallahu anhu</i>

RIN	RNA integrity number
RNA	Ribonucleic acid
RT-PCR	Real-Time polymerase chain reaction
S.W.T	<i>Subhanallahu wata'ala</i>
VCO	Virgin coconut oil
XPOT	Exportin tRNA

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 <i>Halal</i> and <i>Haram</i>	7
2.1.1 Definition and Principles of <i>Halal</i> and <i>Haram</i>	8
2.1.2 <i>Halal</i> Authorities and Entities.....	10
2.1.3 <i>Halal</i> Science and Technologies.....	12
2.1.3.1 <i>Halal</i> 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	31
3.1 Introduction.....	31
3.2 Materials	31
3.2.1 Cell Line.....	31
3.2.2 Oil and Fat Samples	33
3.2.3 Culture Medium.....	34
3.2.4 Consumable and Other Items.....	34
3.2.5 Chemicals and Reagents.....	35
3.2.6 Equipment and Instruments.....	35
3.3 Cell Maintenance in T-Flask	36
3.3.1 Medium Preparation	36
3.3.2 Routine Cell Maintenance.....	37
3.3.2.1 Thawing Cells.....	37
3.3.2.2 Freezing Cells.....	37
3.3.2.3 Subculturing Monolayer Cells.....	38
3.3.2.4 Cell Counting.....	38
3.3.2.5 Changing Medium.....	39
3.4 Growth Profile of HaCaT Cells	39
3.4.1 Media and Cell Volumes for Specific Seeding Concentration	39
3.4.2 Growth Profile in Different T-Flasks.....	40
3.4.3 Specific Growth Rate and Doubling Time.....	40
3.5 Treatment of HaCaT Cells with Fat Emulsions	41
3.5.1 Formulation of Gum Arabic Solution	41
3.5.2 Formulation of Fat Emulsion	41
3.5.2.1 Preparation of Fats and Oil	42
3.5.2.2 Preparation of Fat Emulsions	42
3.5.2.3 Study on Effects of Different Concentration of Fat Emulsion on Cell Growth.....	43
3.5.3 Treatment of Fat Emulsion on HaCaT Cells for Gene Expression Study	43
3.6 Gene Expression Study of Treated HaCaT Cells	44
3.6.1 RNA Extraction.....	44
3.6.1.1 Determination of RNA Concentration and Purity..	45
3.6.1.2 Determination of RNA Integrity Number (RIN)...	46
3.6.2 Microarray..	46
3.6.2.1 Sample Preparation	46
3.6.2.2 Hybridization.....	47
3.6.2.3 Slide Washing.....	47
3.6.2.4 Scanning and Feature Extraction...	48
3.6.2.5 Genes and Pathway Analysis.....	48
3.7 Validation of Microarray Analysis by Real-Time PCR.....	48
3.7.1 Sample Preparation	49
3.7.1.1 Gene Selection.....	49
3.7.1.2 Reverse Transcription of RNA.....	49
3.7.1.3 Reconstitution of Primers...	50
3.7.1.4 SYBR Green Mastermix Preparation for Real-Time PCR.....	50
3.7.2 Real-Time PCR.....	51
3.7.3 Real-Time PCR Analysis.....	51

3.8 Summary	52
CHAPTER FOUR: RESULTS AND DISCUSSION	53
4.1 Introduction.....	53
4.2 Growth Profile of HaCaT Keratinocyte Human Skin Cells	53
4.2.1 Growth Profile in Different T-flasks.....	53
4.2.1.1 Specific Growth Rate and Doubling Time of 25 cm ² and 75 cm ² T-flasks	54
4.3 Study of HaCaT Cells Treatment with Fat Emulsion.....	55
4.3.1 Gum Arabic Solution.....	55
4.3.2 Effect of Different Concentration of Fat Emulsions on HaCaT Cell Number	56
4.3.3 Treatment of HaCaT Cells with Fat Emulsions for Gene Expression Study.....	58
4.4 Microarray Based Gene Expression Study.....	61
4.4.1 RNA Sample Preparations.....	61
4.4.1.1 Purity, Concentration and RNA Integrity Number (RIN)..	61
4.4.1.2 Reverse Transcription.....	63
4.4.2 Microarray Analysis	64
4.4.2.1 Quality Control.....	64
4.4.2.2 GeneSpring Analysis.....	65
4.4.2.2.1 Selected Genes.....	66
4.4.2.3 Pathway Analysis.....	74
4.4.2.3.1 Top Three of High Scorer Networks by Virgin Coconut Oil Treatment.....	75
4.4.2.3.2 Top Three of High Scorer Networks by Lard Treatment	82
4.4.2.3.3 Top Three of High Scorer Networks by <i>Halal</i> and Non- <i>Halal</i> Slaughtered Lamb Fats Treatments	89
4.4.2.3.4 Top Diseases and Bio Functions of the Treatments.....	100
4.4.3 Validation of Microarray by Real-Time PCR.....	102
4.4.3.1 Fold Change Comparison... ..	102
4.5 Summary.....	103
CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS	104
5.1 Conclusions.....	104
5.2 Recommendations	106
REFERENCES.....	107
APPENDIX A:	115
APPENDIX B:	116
APPENDIX C:	118
APPENDIX D:	119
APPENDIX E:	120
APPENDIX F:	121
APPENDIX G:	122

APPENDIX H:	123
APPENDIX I:	130
APPENDIX J:	131
APPENDIX K:	134
APPENDIX L:	136
APPENDIX M:	156

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.

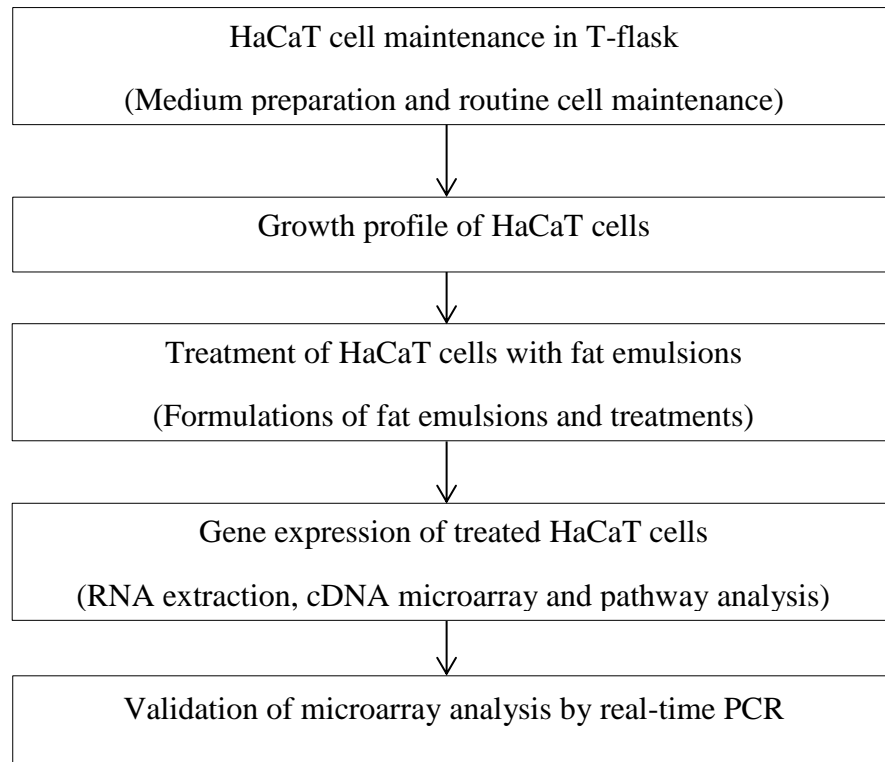


Figure 1.1: Application of cDNA microarray for *haram* detection of fat on HaCaT keratinocyte cells.

1.6 SCOPE OF STUDY

This study used HaCaT human keratinocyte cells as *in vitro* model to investigate the effects of different types of *halal* and *haram* fat sources on human cells at gene expression level. The fats used in the study were from virgin coconut oil (VCO), *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