

**OPTIMIZATION ON ACIDOLYSIS REACTION OF PALM
STEARIN AND OLEIC ACID BY A DUAL LIPASE SYSTEM AND
ITS PHYSICOCHEMICAL CHARACTERISTICS**

NurAtikahbinti Muhammad
(Matric No. 3110196)

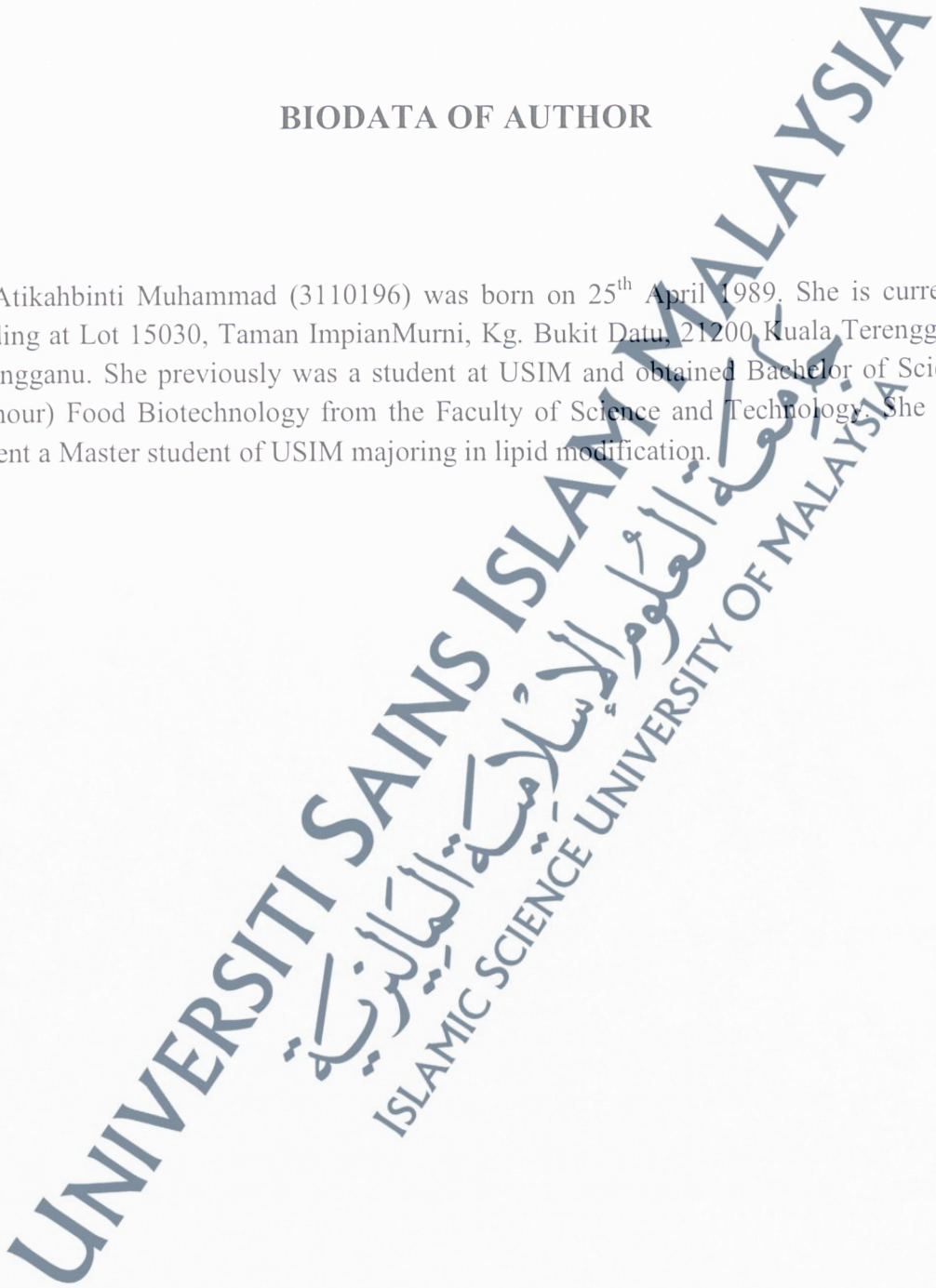
Thesis submitted in fulfillment for the degree of
MASTER OF SCIENCE
FOOD BIOTECHNOLOGY

Faculty of Science and Technology
UNIVERSITI SAINS ISLAM MALAYSIA
Nilai

May 2016

BIODATA OF AUTHOR

NurAtikahbinti Muhammad (3110196) was born on 25th April 1989. She is currently residing at Lot 15030, Taman ImpianMurni, Kg. Bukit Datu, 21200 Kuala Terengganu, Terengganu. She previously was a student at USIM and obtained Bachelor of Science (Honour) Food Biotechnology from the Faculty of Science and Technology. She is at present a Master student of USIM majoring in lipid modification.



ACKNOWLEDGEMENTS

Alhamdulillah, praise be to Allah, for His bless and mercy, all these pieces of work have been put in together as a thesis research. I would like to express sincere gratitude to my supervisor, Dr. NorlelawatibtArifin, Head Program of Food Biotechnology for her patience, motivation and immense knowledge in completing my research laboratory work and thesis. Her expertise and encouragement were such a big blessing for me. I would like to thanks to my co-supervisor as well, Prof Em Dr.Jalani bin Sukaimi for his assistance and help mainly at the beginning of the research progression and along the way until my laboratory work finished.

Besides both of them, I have owed so much Prof Dr.Lai Oi Ming from Bioscience Institute, UPM, for allow me conducting a part of the research in her laboratory. Besides, warm thanks also go to her graduate students whom willingly assisted me during my research work over there. All supports, assistants, and knowledge will be much appreciated.

A big thanks as well goes to all laboratory staff at Faculty Science and Technology for their helping hand and guide in the laboratory work. Warm gratitude and thanks to all postgraduate members and lab mates. Their companies along this tough journey with love and laugh have made it possible to walk through.

Special thanks to Ministry of High Education for their assistance in MyMaster15 program that provide funding for the study to be completed.

Finally, last but not least, to my beloved parents, husband and siblings for their love, concern and support in all ups and downs. There might be more to mention however, to make it compress, for those not mentioned here are forever part of the work as well.

All those people above help the perseverance accompany me through this long journey and have been the main part on this research to be completed. Thank you, everyone.

ABSTRACT

Acidolysis reaction makes the acyl group specifically to be incorporated into the triacylglycerol of palm stearin. The acidolysis reaction was optimized to measure its optimum condition of reaction and the effect of dual lipase system used in this reaction through its interesterification degree (ID). The final product is expected to have healthier fatty acid composition and characteristics compare to palm stearin. In this study, acidolysis reaction was catalyzed by lipase Amano AK and immobilized lipase *Thermomyces lanuginosus* (TL IM). All four parameters affecting the reaction-enzyme ratio (w/w), enzyme load (%), reaction time (h) and substrate molar ratio (w/w), were optimized via Response Surface Methodology (RSM) using Central Composite Rotatable Design (CCRD). The responses measured were percentage of free fatty acid (FFA) and fatty acid composition (FAC) of oleic acid. The target was to have the minimum FFA percentage and maximum FAC of oleic acid in the final product. The physicochemical analysis of triacylglycerol (TAG) composition, FAC percentage and its slip melting point (SMP) profile were conducted to the finished product to compare with the palm stearin. From the RSM, the optimum condition obtained were 3 hours of time reaction, 5:5 (w/w) of enzyme ratio, 1:3 (w/w) of substrate molar ratio and 10% (w/w) enzyme load. Among these four factors, the substrate molar ratio was found to have a significant effect ($p < 0.0001$) towards the percentage of FFA while in contrast towards percentage of FAC of oleic acid, the enzyme ratio, enzyme load, and reaction time had a significant effect ($p < 0.0001$). The synergistic effect between the two lipases with ratio 5:5 gives average interesterification degree (ID) of 0.73. Slip melting point for purified modified palm stearin (PMPS) was 46.13°C. TAG composition of oleic-oleic-oleic (OOO) and palmitic-oleic-oleic (POO) as well as percentage of FAC of oleic acid increased in their percentage after the reaction. These conclude that the model given by RSM can be implied to predict the responses as well as interactions between the parameters and the finished product. PMPS have a lower melting point and healthier fatty acid composition as compared to palm stearin.

Keywords: RSM, dual lipase, physicochemical, palm stearin, acidolysis

ABSTRAK

Tindak balas acidolisis boleh membuatkan asid lemak dimasukkan ke dalam triasilgliserol minyak stearin sawit secara spesifik. Tindak balas acidolisis telah dioptimumkan dan kesan sinergi dua lipase yang digunakan dalam tindak balas ini ditentukan melalui darjah interesterifikasi (ID). Hasil produk dijangka mempunyai struktur asid lemak dan ciri-ciri yang lebih sihat berbanding minyak stearin sawit. Dalam kajian ini, tindak balas acidolisis dimangkin oleh dua enzim iaitu lipase *Amano AK* dan lipozim *Thermomyces lanuginosus* (TL IM). Empat parameter yang mempengaruhi tindak balas-nisbah enzim (w/w), jumlah enzim (%), masa tindak balas (h) dan nisbah molar substrat (w/w), telah dioptimumkan melalui Kaedah Permukaan Tindak Balas (RSM) menggunakan Rekaan Komposit Berpusat (CCRD). Tindak balas yang diukur adalah peratusan asid lemak bebas (FFA) dan asid oleik di dalam komponen asid lemak (FAC). Sasarannya adalah untuk mempunyai peratusan FFA minimum dan maksimum FAC asid oleik dalam produk akhir. Analisis fizikokimia melalui komposisi triasilgliserol (TAG), peratus FAC dan titik takat lebur (SMP) Profil telah dijalankan untuk produk siap untuk membandingkan dengan stearin sawit. Dari RSM, keadaan optimum diperolehi adalah 3 jam masa tindak balas, 5:5 nisbah enzim, 1:3 dengan nisbah molar substrat dan 10% (w/w) jumlah enzim. Di antara empat faktor tersebut, nisbah molar substrat didapati mempunyai kesan yang signifikan ($p < 0.0001$) terhadap peratusan FFA manakala berbeza terhadap peratusan FAC asid oleik, nisbah enzim, jumlah enzim, dan masa tindak balas mempunyai kesanyang signifikan ($p < 0.0001$). Kesan sinergi antara kedua-dua enzim dengan nisbah 5:5 memberikan purata darjah interesterifikasi (ID) daripada 0.73. Titik takat lebur untuk minyak stearin sawit yang telah diubah suai (PMPS) adalah 46.13°C . Komposisi TAG, oleik-oleik-oleik (OOO) dan palmitik-oleik-oleik (POO) serta peratusan FAC asid oleik meningkat selepas tindak balas. Ini dapat menunjukkan bahawa model yang diberikan oleh RSM boleh meramalkan interaksi antara faktor dan tindak balas yang diukur serta produk hasil acidolisis (PMPS) mempunyai takat lebur yang lebih rendah dan komposisi asid lemak yang lebih sihat berbanding stearin sawit.

Kata kunci: RSM, dual lipase, fizikokimia, stearin sawit, acidolysis

ملخص

تفاعل التحليل الحمضي يجعل تنظيم مجموعة أسيلية أكثر تحديدا لإندماج في أسيل الغليسرول الثلاثي في ستيرين النخيل. هذا التفاعل محفز عن طريق نظام ثناء الليياز حيث يتكون من الليياز أمانوAK والليياز المثبت (TL IM) *Thermomyces lanuginosus* لمعرفة تأثير التآزر بين الليازين. العوامل الأربعة التي تؤثر على التفاعل تُستخدم جميعها على الاستجابة السطحية (RSM) (أ.إس.إم) وهي نسبة الإنزيم، كمية الإنزيم، وقت التفاعل، ونسبة المولار الركيزة. ومن (أ.إس.إم)، وكانت أفضل الظروف التي تم الحصول عليها هي 3 ساعات لوقت التفاعل، 5: 5 نسبة إنزيم الليياز أمانوAK نحو الليياز المثبت TL IM، ثم 3: 1 نسبة لستيرين النخيل نحو حمض الأوليك و% 10 (وزن / وزن) كمية الإنزيم. ومن بين العوامل الأربعة نسبة ركيزة المولار ثري ذا دلالة إحصائية عند مستوى ($P < 0.0001$) نحو (إف.إف.إي) مما تجعل التصنيفات أن تميل إلى نسبة ركيزة المولار من أجل تفعيل التفاعل الكيميائي. على الرغم من ذلك، نسبة الإنزيم، وكية الإنزيم ووقت التفاعل ذو دلالة إحصائية عند مستوى ($P < 0.0001$) نحو (إف.إف.إي.سي). وهذا يدل على أن أقل وقت التفاعل، يتسلى أن يكمل التفاعل، كمية الثابتة % 10، والنسبة المعدلة بين اللياسين للوصول إلى أقصى نطاق الدرجات ل (إف.إف.إي.سي) وأدنى نطاق الدرجات ل (إف.إف.إي.سي). ومن هنا، يلخص أن النموذج الذي يُعطى لأن إيس إم يمكن أن يُستخدم لتوقع إف.إف.إي.سي ووفاء إي.سي تحت جميع الحالات في القيمة التجريبية. والآثار التآزرية بين اللياسين بنسبة 5:5 يساهم إلى معدلة المستوى الإترستريفي ($ID = 0.73$). وهذا (ID) يتضح أن تفاعل اللياس يرتفع كفاءة نشاط لياس AK. معدل سترين النخيل الخالص PMPS يحصل على $46.13^{\circ}C$ لنقطة الانصهار. وبجانب ذلك، بناء على عناصر أسيل الغليسرول الثلاثي، نسبة 000 وPOO ترتفع بعد عملية التفاعل، بينما قيمة PPP وPOO تنخفض. ولذلك، المتقاج المعدل PMPS له نقطة الانصهار الأدنى والعناصر الأصح من ستيرين النخيل.

كلمات مفتاحية: RSM، ثناء الليياز، فيزيائي كيميائي، ستارين النخيل، التحليل بالحمض

CONTENT PAGE

Title page	
Thesis declaration	i
Biodata of author	ii
Acknowledgements	iii
Abstract	iv
Abstrak	v
Mulkhas	vi
Content page	vii
List of figures	xi
List of tables	xii
List of appendices	xiii
Abbreviation	xiv
Chapter I: Introduction	1
Chapter II: Literature review	6
2.1 Palm Stearin	6
2.2 Oleic Acid	8
2.3 Lipase application in food industry	9
2.3.1 Free lipase AK Amano	12
2.3.2 Immobilize lipase <i>Thermomyceslanuginoses</i> (TL IM)	13
2.4 Modifications of fats and oils	14

2.4.1 Enzymatic interesterification and acidolysis reaction	19
2.4.2 Dual lipase system	20
2.5 Optimization via Response Surface Methodology (RSM)	22
Chapter III: Enzymatic Acidolysis of Palm Stearin and Oleic Acid by	
Dual Lipase System via Response Surface Methodology (RSM)	24
3.1 Introduction	24
3.2 Materials	26
3.3 Method	27
3.3.1 Acidolysis of Palm Stearin and Oleic Acid	27
3.3.2 Experimental Design via RSM	24
3.3.3 Optimization and verification of modified palm stearin using RSM	29
3.3.4 Free Fatty Acid (FFA) Percentage	30
3.3.5 Fatty Acids Composition (FAC) using Gas Chromatography	30
3.4 Result and Discussion	31
3.4.1 Model Fitting and ANOVA	31
3.4.2 Effect of parameters on FAC	36
3.4.3 Effect of parameters on FFA	43
3.4.4 Optimization and Verification	49
3.5 Conclusion	51

Chapter IV: Interesterification Degree of Dual Lipase System in Acidolysis of

Palm Stearin and Oleic Acid

	53
4.1 Introduction	53
4.2 Materials	55
4.3 Method	56
4.3.1 Production and Purification of sample by using Short Path Distillation (SPD)	56
4.3.2 Enzyme reusability	57
4.3.3 Determination of TAG composition for Interesterification Degree	58
4.3.4 Interesterification Degree of Acidolysis of Palm Stearin and Oleic Acid	58
4.4 Result and Discussion	58
4.4.1 TAG composition	58
4.4.2 Interesterification Degree of lipase AK Amano and immobilized lipase TL IM in acidolysis of palm stearin and oleic acid	61
4.5 Conclusion	68
Chapter V: Physicochemical properties of purified modified palm stearin	69
5.1 Introduction	69
5.2 Materials	71
5.3 Method	71

5.3.1 Production and Purification of Modified Palm Stearin using Short Path Distillation	71
5.3.2 Determination of triacylglyceride (TAG) composition	71
5.3.3 Fatty acid composition (FAC) analysis	71
5.3.4 Analysis of slip melting point (SMP)	72
5.4 Result and discussion	72
5.4.1 TAG composition	72
5.4.2 Fatty acid composition (FAC)	73
5.4.3 Slip Melting Point (SMP)	75
5.5 Conclusion	77
Chapter VI: Summary and Recommendations	78
6.1 Summary	78
6.2 Recommendations	80
Bibliography	82
Appendices	89

LIST OF FIGURES

FIGURE 1: Oleic acid structure	8
FIGURE 2: Cutting down fatty acids at <i>sn</i> -1,3 position and replacement of new fatty acids	11
FIGURE 3: Effects of enzyme ratio with enzyme load towards FAC.....	36
FIGURE 4: Effects of enzyme ratio with substrate molar ratio towards FAC.....	37
FIGURE 5: Effects of enzyme ratio with reaction time towards FAC.....	38
FIGURE 6: Effects of enzyme load with substrate molar ratio towards FAC.....	40
FIGURE 7: Effects of enzyme load with reaction time towards FAC	41
FIGURE 8: Effect of substrate molar ratio with reaction time towards FAC	42
FIGURE 9: Effects of substrate molar ratio with enzyme ratio towards FFA.....	44
FIGURE 10: Effects of substrate molar ratio with enzyme load towards FFA	45
FIGURE 11: Effects of substrate molar ratio with enzyme load towards FFA	45
FIGURE 12: Effects of reaction time with enzyme load towards FFA	47
FIGURE 13: Effects of reaction time with enzyme ratio towards FFA	48
FIGURE 14: Effects of interactions between enzyme load and enzyme ratio towards FFA	48
FIGURE 15: Amount of OOO, POO, PPO and PPP between PS and PMPS	59
FIGURE 16: Comparisons of Interesterification degree (ID) of certain sample	62
FIGURE 17: Slip melting point among reusable enzymes batch samples	76

LIST OF TABLES

TABLE 1: Characteristics of palm stearin	7
TABLE 2: Fatty acid composition of palm stearin	8
TABLE 3: Oleic acid characteristics	9
TABLE 4: The previous studies on modifications of fat and oil and their parameters.....	15
TABLE 5: The parameter range of all four factors and responses.....	28
TABLE 6: Series of experiments generated by RSM.....	29
TABLE 7: FAC and FFA obtained for RSM.....	33
TABLE 8: ANOVA and statistical analysis for FAC and FFA.....	34
TABLE 9: Determining the value of each corresponding factors for optimization and verification	49
TABLE 10: The predicted and actual value from both response under optimum condition	51
TABLE 11: The interesterification degree (ID) of all samples and its parameters.....	65
TABLE 12: Reused enzyme batch samples' ID	67
TABLE 13: percentage of TAG composition and its values among the samples.....	72
TABLE 14: FAC of palm stearin and purified modified palm stearin.....	74
TABLE 15: Slip melting point of palm stearin and purified modified palm stearin	75

LIST OF APPENDICES

Appendix A: Amount of observed TAG for all samples.....	89
Appendix B: Analysis of variance (ANOVA) for FAC, SMP and TAG (OOO, POO, PPO and PPP).....	90



ABBREVIATION

ANOVA	Analysis of variance	LO	Linseed oil
ARA	Arachidonic acid	MCFA	Medium chain fatty acid
CCRD	Central composite rotatable design	MPOB	Malaysian Palm Oil Board
		MPOC	Malaysian Palm Oil Council
DHA	docosahexanoic acid	MUFA	Monounsaturated fatty acids
DOE	Design of experts	NaOH	Sodium hydroxide
ECN	Equivalent carbon number	OOO	Oleic-Oleic-Oleic
ELSD	Evaporating light scattering detector	PMPS	Purified modified palm stearin
		POO	Palmitic-Oleic-Oleic
FAC	Fatty acid composition	PPO	Palmitic-Palmitic-Oleic
FAME	Fatty acid methyl esters	PPP	Palmitic-Palmitic-Palmitic
FFA	Free fatty acid	PS	Palm stearin
FID	Flame ionization detector	PUFA	Polyunsaturated fatty acids
GC	Gas chromatography	RBD	Refined, bleached, deodorized
HDL	High density lipoprotein	RSM	Response surface methodology
HMFS	Human milk fat substitute	SL	Structured lipid
HPLC	High-performance liquid chromatography	SMP	Slip melting point
		TAG	triacylglycerols
ID	Interesterification degree	TL IM	<i>Thermomyces lanuginosus</i>
LDL	Low density lipoprotein		