

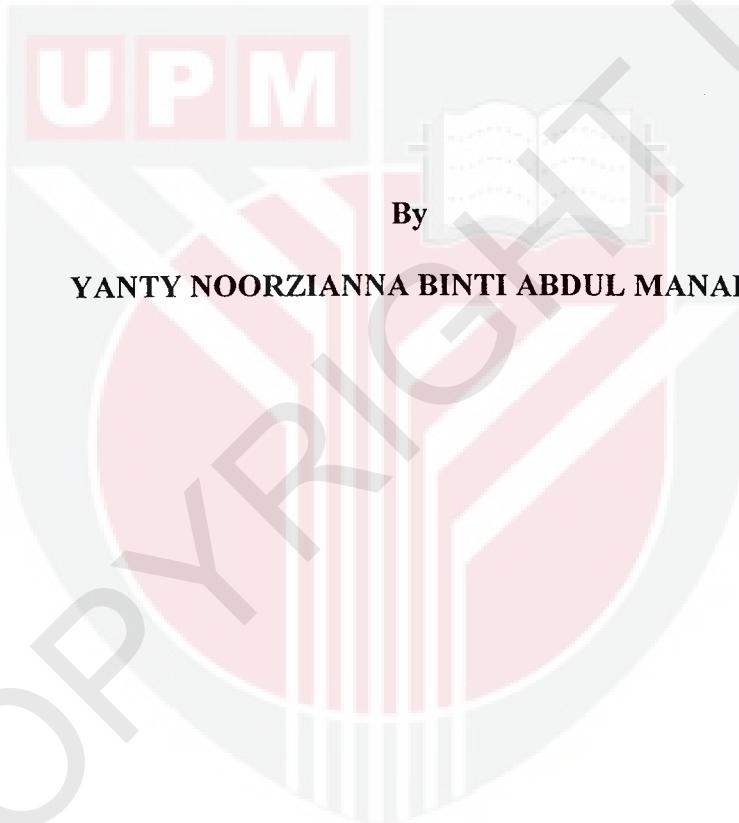


***CHARACTERISATION OF OILS AND FATS FROM SEEDS OF SEVERAL  
MALAYSIAN FRUITS AND THEIR ENZYMATIC INTERESTERIFICATION***

**YANTY NOORZIANNIA BINTI ABDUL MANAF**

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in  
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By

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**September 2009**

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The processing of many fruits results in the accumulation of large quantities of seeds and kernels. Proper utilization of these by-products could reduce waste disposal problems and serves as a potential new source of fats for use in food and non-food systems. To date, a large number of plant seeds have been analyzed and some of these have been cultivated as new oil crops.

In this study, the oils/fats from the seeds of honeydew melon (*Cucumis melo* var. *inodorus*), musk lime (*Citrus microcarpa*), papaya (*Carica papaya* L., variety Hong Kong variety) and rambutan (*Nephelium lappaceum* L.) were extracted and their physico-chemical characteristics determined. Honeydew melon seeds contained 25.0% of oil of which linoleic acid (69.0%) was the dominant fatty acid. It was found to be a rich source of unsaturated fatty acid (86.1%). Musk lime seeds contained 33.8% oil comprising 73.6% unsaturated fatty acids with linoleic (31.8%) and oleic (29.6%) acids

as the predominant fatty acids. The iodine and saponification values of musk lime seed oil (MLSO) were 118.1 g I<sub>2</sub>/100 g and 192.6 mg KOH/g, respectively. It contained POL (18.9%) as the most prominent TAG, followed by PLL (13.7%) and OLL (11.9%). The complete melting and crystallization transition temperatures of MLSO were 10.7°C and -4.2°C, respectively. The oil content of papaya seeds (Hong Kong variety) was 27.0% with oleic acid (73.47%) as the dominant fatty acid followed by palmitic acid (15.8%). It contained OOO (40.4%) as the most prominent TAG followed by POO (29.1%) and SOO (9.9%). Rambutan seeds contained 38.0% of oil. Compared to oils from the other seeds, oleic (42.0%) and arachidic (34.3%) acids were the dominant fatty acids. Most of TAG peaks have long retention times and could not be identified due to non-availability of required standards.

The individual seed oils (honeydew melon, musk lime, papaya and rambutan) and oil blends (rambutan:honeydew melon, rambutan:musk lime, rambutan:papaya, honeydew melon:musk lime, honeydew melon:papaya and musk lime:papaya) at 1:1 ratio were interesterified using three lipases namely Lipozyme IM, Lipozyme TL and Novozym 435 from Novozyme (Copenhagen, Denmark). The immobilized lipase was added at 0.01% (w/w) to 6.0 g of oil in 10 ml of *n*-hexane. The reaction mixture was then agitated in an orbital shaker at (200 rpm) kept at 40°C for 6, 12, 24 h, where each reaction was carried out in duplicate and a sample without enzyme was used as the control. HPLC analysis showed that several TAGs increase in concentration, while other TAG decreased in concentration as indicated by the increase and reduction in peak areas, respectively. Lipozyme IM and Lipozyme TL gave increments of similar TAG

peaks after interesterification except for musk lime seed oil. Interesterification of musk lime gave increments of similar TAG peaks when Lipozyme TL and Novozym 435 were used. The reduction of some TAG peaks, and formation of acylglycerol including free fatty acid content during interesterification indicate that hydrolysis also took place, a phenomenon confirming the reversibility of lipase reaction on its substrate before interesterification can take place for all samples. The amount of TAG of all seed oil samples were decreased after 24 h when all three enzymes were used. Novozym 435 gave the highest amount of TAG after interesterification followed by Lipozyme TL and Lipozyme IM for all oil samples. Lipozyme IM gave significantly ( $P<0.05$ ) higher percentage of partial acylglycerol and free fatty acid content than Lipozyme TL and Novozyme 435. Thus, Lipozyme IM showed that it had a highest hydrolytic activity.

The interesterification process led to some changes in the heating and crystallization profiles of the seed oils. Peak broadening was seen clearly in honeydew melon seed oil, indicating these changes could possibly be due to the formation of TAG molecular species with wider melting ranges. In heating and cooling profiles of papaya seed oil, the major peak became sharper compared to the original oil, indicating that homogeneity of TAG component was formed. It is most likely due to its high content of OOO. Most of the reactions involving all seed oils and oil blends increased the melting temperatures after 24 h of interesterification. It might be due to more formation of saturated TAG. In many cases, interesterification of seed oil blends changed the existing thermal transitions and produced additional peaks. This could be due to the changes in the TAG profile and formation of free fatty acid caused by the enzymatic interesterification.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai  
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**CIRI-CIRI MINYAK DAN LEMAK DARIPADA BIJI BUAH-BUAHAN  
MALAYSIA DAN INTERESTERIFIKASI BERENZIM**

Oleh

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Kebanyakan pemprosesan buah-buahan menyebabkan pengumpulan biji dan isirong dalam kuantiti yang banyak. Masalah pembuangan sisa-sisa dapat dikurangkan dengan memanfaatkan biji dan isirong ini dan seterusnya ianya berpotensi untuk penghasilan sumber bahan makanan dan bahan bukan makanan. Setakat ini, banyak biji tumbuhan telah dianalisis dan sesetengahnya telah ditanam sebagai tanaman sumber minyak baru.

Dalam kajian ini, ciri-ciri fisiko-kimia biji buah-buahan daripada tembikai susu (*Cucumis melo* var. *inodorus*), limau kasturi (*Citrus microcarpa*), betik (*Carica papaya* L., variati Hong Kong) dan rambutan (*Nephelium lappaceum* L.) telah dianalisis. Biji tembikai susu mengandungi 25% minyak di mana asid lemak dominan adalah asid linoleik (69%). Ianya kaya dengan sumber asid lemak tak tepu (86.1%). Biji limau kasturi mengandungi 33.8% minyak. Ia mengandungi 73.6% asid lemak tak tepu di mana asid linoleik (31.8%) dan asid oleik (29.6%) adalah asid lemak dominan. Biji betik daripada variati Hong Kong mengandungi 27.0% minyak di mana asid oleik

(73.47%) adalah asid lemak dominan diikuti oleh asid palmitik. Ia mengandungi OOO (40.4%) yang merupakan TAG dominan diikuti oleh POO (29.1%) dan SOO (9.9%). Biji rambutan mengandungi 38.0% lemak. Ia menagandungi 49.1% asid lemak tepu di mana asid oleik (42.0%) dan asid arakidik (34.3%) merupakan asid lemak dominan. Kebanyakan puncak TAG tidak dapat dikenalpasti disebabkan ketiadaan TAG piawai. Suhu peleburan dan suhu pengkristalan bagi minyak minyak rambutan adalah 44.2°C dan -44.5°C.

Setiap minyak biji (tembikai susu, limau kasturi, betik dan rambutan) dan campuran minyak biji dengan nisbah 1:1 (rambutan:tembikai susu, rambutan:limau kasturi, rambutan:betik, tembikai susu:limau kasturi, tembikai susu:betik dan limau kasturi:betik) telah diinteresterifikasi menggunakan tiga lipase iaitu Lipozyme IM, Lipozyme TL dan Novozym 435 dari Novozyme (Copenhagen, Denmark). Lipase pegun, 0.01% telah dimasukkan ke dalam 6.0 g minyak dalam 10 ml *n*-heksana. Pencampuran reaksi telah diaduk menggunakan penggoncang orbital (200 rpm) pada 40°C selama 6, 12, 24 jam, di mana setiap reaksi telah dilakukan duplikasi dan sampel tanpa enzim digunakan sebagai kawalan. Analisis HPLC menunjukkan kebanyakan puncak TAG meningkat, manakala sesetengahnya menurun dengan pengurangan luas puncak. Penggunaan Lipozyme IM dan Lipozyme TL menunjukkan peningkatan ketinggian puncak pada TAG yang sama pada setiap sampel minyak selepas interesterifikasi kecuali bagi minyak biji limau kasturi. Terdapat peningkatan ketinggian puncak pada TAG yang sama apabila minyak biji limau kasturi diinteresterifikasikan menggunakan Lipozyme TL and Novozym 435. Sesetengah penurunan ketinggian

puncak dan penghasilan asilglicerol termasuk kandungan asid lemak bebas adalah disebabkan hidrolisis di mana fenomena ini memastikan bahawa reaksi lipase secara berbalik pada substrak sebelum proses interesterifikasi. Amaun TAG bagi kesemua minyak biji dan campurannya semakin menurun selepas 24 jam apabila ketiga-tiga enzim digunakan. Lipozyme IM memberi peratus amaun TAG yang paling rendah diikuti oleh Lipozyme TL dan Novozym 435. Pada masa yang sama, selepas 24 j interesterifikasi, Lipozyme IM memberikan peratus asilglicerol dan asid lemak bebas yang lebih tinggi secara sikhnifikan ( $P<0.05$ ) berbanding Lipozyme TL and Novozym 435, bagi kesemua sampel minyak. Lipozyme IM memberikan penghasilan asid lemak bebas yang paling tinggi, menunjukkan enzim ini mempunyai aktiviti hidrolitik yang paling tinggi berbanding dua enzim yang lain.

Proses interesterifikasi menyebabkan perubahan profail peleburan dan pengkristalan bagi minyak biji dan campurannya. Pelebaran puncak dapat dilihat dengan jelas bagi minyak biji tembikai susu menunjukkan perubahan ini mungkin disebabkan penghasilan spesis molekular TAG dengan kelebaran julat peleburan. Bagi profail peleburan dan penyejukkan minyak biji betik, puncak major menjadi lebih tirus selepas diinteresterifikasi menunjukkan TAG yang homogen telah terhasil. Kemungkinan besar berlakunya keadaan ini adalah disebabkan minyak biji betik mengandungi OOO. Bagi kebanyakan kes, interesterifikasi minyak biji ini meningkatkan suhu peleburan manakala suhu penyejukkan telah menurun selepas 24 jam reaksi interesterifikasi. Ini mungkin disebabkan TAG tenu terhasil. Bagi kebanyakan kes, interesterifikasi campuran minyak biji telah mengubah transisi termal yang sedia ada dan puncak

tambahan telah terhasil. Ini mungkin disebabkan oleh perubahan pada profail TAG dan pembentukan asid lemak bebas disebabkan interesterifikasi berenzim.



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## TABLE OF CONTENTS

	Page
<b>ABSTRACT</b>	ii
<b>ABSTRAK</b>	v
<b>ACKNOWLEDGEMENTS</b>	ix
<b>APPROVAL</b>	x
<b>DECLARATION</b>	xii
<b>LIST OF TABLES</b>	xvii
<b>LIST OF FIGURES</b>	xix
<b>LIST OF ABBREVIATIONS</b>	xxvi
 <b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	<b>1</b>
<b>2 LITERATURE REVIEW</b>	<b>4</b>
Fats and oils	4
Fats and Oils from Tropical/Subtropical Fruit Seeds	5
Honeydew melon seed	7
Musk lime seed	12
Papaya seed	14
Rambutan seed	17
Modification of fats and oils	18
Blending	20
Fractionation	21
Hydrogenation	24
Interestesterification	25
Acidolysis	25
Alcoholysis	28
Transesterification	29
Enzymatic interestesterification	29
Lipase	31
Source of lipases	32
Specificity of lipases	34
Nonspecific lipases catalysts	35
$sn$ -3 specific lipases catalysts	36
$sn$ -2 specific lipases catalysts	37

<b>3</b>	<b>PHYSICO-CHEMICAL PROPERTIES OF HONEYDEW MELON, MUSK LIME, PAPAYA AND RAMBUTAN SEEDS AND SEED OILS</b>	<b>38</b>
	Introduction	38
	Materials and Methods	40
	Materials	40
	Methods	41
	Results and Discussion	45
	Honeydew melon seed and seed oil	45
	Proximate analysis of honeydew melon seed	45
	Iodine, saponification, unsaponifiable and free fatty acid values	46
	Fatty acid composition of honeydew melon seed oil	48
	Triacylglycerol profile of honeydew melon seed oil	50
	Physical nature and thermal behavior of honeydew melon seed oil	51
	Musk lime seed and seed oil	52
	Proximate analysis of musk lime seed	52
	Iodine, saponification, unsaponifiable and free fatty acid values	52
	Fatty acid composition of musk lime seed oil	54
	Triacylglycerol profile of musk lime seed oil	56
	Physical nature and thermal behavior of musk lime seed oil	57
	Papaya seed and seed oil	59
	Proximate analysis of papaya seed	59
	Iodine, saponification, unsaponifiable and free fatty acid values	60
	Fatty acid composition of papaya seed oil	61
	Triacylglycerol profile of papaya seed oil	61
	Physical nature and thermal behavior of papaya seed oil	63
	Rambutan seed and seed oil	65
	Proximate analysis of rambutan seed	65
	Iodine, saponification, unsaponifiable and free fatty acid values	66
	Fatty acid composition of rambutan seed oil	68
	Triacylglycerol profile of rambutan seed oil	70
	Physical nature and thermal behavior of rambutan seed oil	71
	Conclusion	73

<b>4</b>	<b>ENZYMATIC INTERESTERIFICATION OF SELECTED FRUIT SEED OILS AND THEIR BLENDS</b>	<b>75</b>
	Introduction	75
	Materials	79
	Methods	79
	Results and Discussion	81
	Triacylglycerol profile changes of honeydew melon seed oil after interesterification	81
	Thermal behavior changes of honeydew melon seed oil after interesterification	85
	Triacylglycerol profile changes of musk lime seed oil after interesterification	90
	Thermal behavior changes of musk lime seed oil after interesterification	94
	Triacylglycerol profile changes of papaya seed oil after interesterification	99
	Thermal behavior changes of papaya seed oil after interesterification	103
	Triacylglycerol profile changes of rambutan seed oil after interesterification	108
	Thermal behavior changes of rambutan seed oil after interesterification	112
	Triacylglycerol profile changes of rambutan:honeydew melon (1:1) seed oil blend after interesterification	117
	Thermal behavior changes of rambutan:honeydew melon (1:1) seed oil blend after interesterification	121
	Triacylglycerol profile changes of rambutan:musk lime (1:1) seed oil blend after interesterification	126
	Thermal behavior changes of rambutan:musk lime (1:1) seed oil blend after interesterification	130
	Triacylglycerol profile changes of rambutan:papaya (1:1) seed oil blend after interesterification	134
	Thermal behavior changes of rambutan:papaya (1:1) seed oil blend after interesterification	139
	Triacylglycerol profile changes of honeydew melon:musk lime (1:1) seed oil blend after interesterification	143
	Thermal behavior changes of honeydew melon:musk lime (1:1) seed oil blend after interesterification	148
	Triacylglycerol profile changes of honeydew melon:papaya (1:1) seed oil blend after interesterification	152
	Thermal behavior changes of honeydew melon:papaya (1:1) seed oil blend after interesterification	156
	Triacylglycerol profile changes of musk lime:papaya (1:1) seed oil blend after interesterification	161
	Thermal behavior changes of musk lime:papaya (1:1) seed oil blend after interesterification	165

Conclusion

170

**5 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS**

171

**REFERENCES**

176

**APPENDICES**

193

**BIODATA OF STUDENT**

194

**LIST OF PUBLICATIONS**

195



## LIST OF TABLES

Table	Page
1 Physico-chemical characteristics of fruit seed oils	8
2 Previous report on physical modification of fats and oils	19
3 Previous researches on chemical modification of fats and oils	26
4 Previous study on enzymatic interesterification of fats and oils	27
5 Proximate composition of honeydew melon and other melon seeds	46
6 Iodine, saponification, unsaponifiable and free fatty acid contents of honeydew melon and other melon seed oils	47
7 Fatty acid composition of honeydew melon and other seed oil	49
8 Proximate analysis of musk lime and other Citrus seeds	53
9 Iodine, saponification, unsaponifiable and free fatty acid contents of musk lime and other Citrus seed oils	54
10 Fatty acid composition of musk lime and other Citrus seed oils	55
11 Proximate analysis of papaya seeds	59
12 Iodine, saponification, unsaponifiable and free fatty acid contents of papaya seed oils	60
13 Fatty acid composition of papaya seed oils	62
14 Iodine, saponification, unsaponifiable and free fatty acid contents of rambutan seed oil and other vegetable fats	67
15 Fatty acid composition of rambutan seed oils	69
16 Solid fat content of rambutan seed oil and other vegetable fat	73
17 Composition of honeydew melon seed oil before and after interesterification	85
18 Composition of musk lime seed oil before and after interesterification	94

19	Composition of papaya seed oil before and after interesterification	103
20	Composition of rambutan seed oil before and after interesterification	112
21	Composition of rambutan:honeydew melon seed oil before and after interesterification	121
22	Composition of rambutan:musk lime seed oil before and after interesterification	130
23	Composition of rambutan:papaya seed oil before and after interesterification	138
24	Composition of honeydew melon:musk lime seed oil before and after interesterification	147
25	Composition of honeydew melon:papaya seed oil before and after interesterification	156
26	Composition of musk lime:papaya seed oil before and after interesterification	165
27	TAG composition of honeydew melon and musk lime seed oils	193
28	TAG composition of papaya seed oil	193

## LIST OF FIGURES

<b>Figure</b>		<b>Page</b>
1	Triacylglycerol profile of honeydew melon seed oil	50
2	Melting profile of honeydew melon seed oil	51
3	Cooling profile of honeydew melon seed oil	51
4	Triacylglycerol profile of musk lime seed oil	57
5	(A) Cooling and (B) heating thermograms of musk lime seed oil	58
6	Solid fat content of musk lime seed oil	58
7	Triacylglycerol profile of papaya seed oil	63
8	Solid fat content of papaya seed oil	64
9	Cooling profile of papaya seed oil	65
10	Heating profile of papaya seed oil	65
11	Triacylglycerol profile of rambutan seed oil	71
12	(A) Heating and (B) Cooling thermograms of rambutan seed oil	72
13	HPLC chromatogram of interesterified honeydew melon seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	82
14	HPLC chromatogram of interesterified honeydew melon seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	83
15	HPLC chromatogram of interesterified honeydew melon seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	84
16a	Melting profile of interesterified honeydew melon seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	87
16b	Crystallisation of interesterified honeydew melon seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	87
17a	Melting profile of interesterified honeydew melon seed oil using	88

	Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	
17b	Crystallisation of interesterified honeydew melon seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	88
18a	Melting profile of interesterified honeydew melon seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	89
18b	Crystallisation of interesterified honeydew melon seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	89
19	HPLC chromatogram of interesterified honeydew lime seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	91
20	HPLC chromatogram of interesterified lime seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	92
21	HPLC chromatogram of interesterified lime seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	93
22a	Melting profile of interesterified lime seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	96
22b	Crystallisation of interesterified lime seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	96
23a	Melting profile of interesterified lime seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	97
23b	Crystallisation of interesterified lime seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	97
24a	Melting profile of interesterified lime seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	98
24b	Melting profile of interesterified lime seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	98
25	HPLC chromatogram of interesterified papaya seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	100
26	HPLC chromatogram of interesterified papaya seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	101
27	HPLC chromatogram of interesterified papaya seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	102

28a	Melting profile of interesterified papaya seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	105
28b	Crystallisation of interesterified papaya seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	105
29a	Melting profile of interesterified papaya seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	106
29b	Crystallisation of interesterified papaya seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	106
30a	Melting profile of interesterified papaya seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	107
30b	Crystallisation profile of interesterified papaya seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	107
31	HPLC chromatogram of interesterified rambutan seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	109
32	HPLC chromatogram of interesterified rambutan seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	110
33	HPLC chromatogram of interesterified rambutan seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	111
34a	Melting profile of interesterified rambutan seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	114
34b	Crystallisation of interesterified rambutan seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	114
35a	Melting profile of interesterified rambutan seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	115
35b	Crystallisation of interesterified rambutan seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	115
36a	Melting profile of interesterified rambutan seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	116
36b	Crystallisation of interesterified rambutan seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	116
37	HPLC chromatogram of interesterified rambutan:honeydew melon	118

	seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	
38	HPLC chromatogram of interesterified rambutan:honeydew melon seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	119
39	HPLC chromatogram of interesterified rambutan:honeydew melon seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	120
40a	Melting profile of interesterified rambutan:honeydew melon seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	123
40b	Crystallisation of interesterified rambutan:honeydew melon seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	123
41a	Melting profile of interesterified rambutan:honeydew melon seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	124
41b	Crystallisation of interesterified rambutan:honeydew melon seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	124
42a	Melting profile of interesterified rambutan:honeydew melon seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	125
42b	Crystallisation of interesterified rambutan:honeydew melon seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	125
43	HPLC chromatogram of interesterified rambutan:musk lime seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	127
44	HPLC chromatogram of interesterified rambutan:musk lime seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	128
45	HPLC chromatogram of interesterified rambutan:musk lime seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	129
46a	Melting profile of interesterified rambutan:musk lime seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	131
46b	Crystallisation of interesterified rambutan:musk lime seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	132

47a	Melting profile of interesterified rambutan:musk lime seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	132
47b	Crystallisation of interesterified rambutan:musk lime seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	133
48a	Melting profile of interesterified rambutan:musk lime seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	133
48b	Crystallisation of interesterified rambutan:musk lime seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	134
49	HPLC chromatogram of interesterified rambutan:papaya seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	135
50	HPLC chromatogram of interesterified rambutan:papaya seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	136
51	HPLC chromatogram of interesterified rambutan:papaya seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	137
52a	Melting profile of interesterified rambutan:papaya seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	140
52b	Crystallisation of interesterified rambutan:papaya seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	141
53a	Melting profile of interesterified rambutan:papaya seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	141
53b	Crystallisation of interesterified rambutan:papaya seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	142
54a	Melting profile of interesterified rambutan:papaya seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	142
54b	Crystallisation of interesterified rambutan:papaya seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	143
55	HPLC chromatogram of interesterified honeydew melon:musk lime seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	144
56	HPLC chromatogram of interesterified honeydew melon:musk lime seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of	145

	incubation	
57	HPLC chromatogram of interesterified honeydew melon:musk lime seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	146
58a	Melting profile of interesterified honeydew melon:musk lime seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	149
58b	Crystallisation of interesterified honeydew melon:musk lime seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	149
59a	Melting profile of interesterified honeydew melon:musk lime seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	150
59b	Crystallisation of interesterified honeydew melon:musk lime seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	150
60a	Melting profile of interesterified honeydew melon:musk lime seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	151
60b	Crystallisation of interesterified honeydew melon:musk lime seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	151
61	HPLC chromatogram of interesterified honeydew melon:papaya seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	153
62	HPLC chromatogram of interesterified honeydew melon:papaya seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	154
63	HPLC chromatogram of interesterified honeydew melon:papaya seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	155
64a	Melting profile of interesterified honeydew melon:papaya seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	158
64b	Crystallisation of interesterified honeydew melon:papaya seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	158

65a	Melting profile of interesterified honeydew melon:papaya seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	159
65b	Crystallisation of interesterified honeydew melon:papaya seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	159
66a	Melting profile of interesterified honeydew melon:papaya seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	160
66b	Crystallisation of interesterified honeydew melon:papaya seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	160
67	HPLC chromatogram of interesterified musk lime:papaya seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	162
68	HPLC chromatogram of interesterified musk lime:papaya seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	163
69	HPLC chromatogram of interesterified musk lime:papaya seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	164
70a	Melting profile of interesterified musk lime:papaya seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	167
70b	Crystallisation of interesterified musk lime:papaya seed oil using Lipozyme IM (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	167
71a	Melting profile of interesterified musk lime:papaya seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	168
71b	Crystallisation of interesterified musk lime:papaya seed oil using Lipozyme TL (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	168
72a	Melting profile of interesterified musk lime:papaya seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	169
72b	Crystallisation of interesterified musk lime:papaya seed oil using Novozym 435 (a) control (b) 6 h (c) 12 h (d) 24 h of incubation	169

## LIST OF ABBREVIATIONS

<b>ANOVA</b>	:	analysis of variance
<b>AOCS</b>	:	American Oil Chemists' Society
<b>AOS</b>	:	arachidyl-oleoyl-stearyl-glycerol
<b>ASO</b>	:	arachidyl-stearoyl-oleoyl-glycerol
<b>AOA</b>	:	arachidyl-oleoyl-stearoyl-glycerol
<b>B</b>	:	Blue
<b>C6:0</b>	:	capric/hexanoic acid
<b>C7:0</b>	:	enanthic acid
<b>C8:0</b>	:	caprylic acid
<b>C9:0</b>	:	pelargonic acid
<b>C10:0</b>	:	capric/decanoic acid
<b>C12:0</b>	:	lauric acid
<b>C13:0</b>	:	tridecanoic acid
<b>C14:0</b>	:	myristic acid
<b>C14:1</b>	:	myristoleic acid
<b>C15:0</b>	:	pentaenoic acid
<b>C15:1</b>	:	10-pentadecenoic acid
<b>C16:0</b>	:	palmitic acid
<b>C16:1</b>	:	palmitoleic acid
<b>C18:0</b>	:	stearic acid
<b>C18:1</b>	:	oleic acid
<b>C18:2</b>	:	linoleic acid

<b>C18:3</b>	:	linolenic acid
<b>C20:0</b>	:	arachidic acid
<b>C20:1</b>	:	eicosanoic (gadoleic) acid
<b>C20:3</b>	:	eicosatrienoic acid
<b>C20:4</b>	:	arachidonic acid
<b>C22:0</b>	:	behenic acid
<b>C22:1</b>	:	erucic acid
<b>C24:0</b>	:	lignoceric acid
<b>DAG</b>	:	Diacylglycerol
<b>MAG</b>	:	Monoacylglycerol
<b>mL</b>	:	Milliliter
<b>NMR</b>	:	nuclear magnetic resonance
<b>OOA</b>	:	dioleoyl-3-arachidyl glycerol
<b>AOA</b>	:	oleoyl-arachidyl-oleoyl-glycerol
<b>OLL</b>	:	1-oleoyl-dilinoleoyl glycerol
<b>OOL</b>	:	dioleoyl-3-linoleoyl glycerol
<b>OOO</b>	:	trioleoyl glycerol
<b>POL</b>	:	palmitoyl-oleoyl-linoleoyl glycerol
<b>POO</b>	:	1-palmitoyl-dioleoyl glycerol
<b>PPL</b>	:	dipalmitoyl-3-linoleoyl glycerol
<b>PPO</b>	:	dipalmitoyl-3-oleoyl glycerol
<b>PORIM</b>	:	palm oil research institute of Malaysia
<b>PUFA</b>	:	polyunsaturated fatty acid

<b>rpm</b>	:	revolution per minute
<b>SAS</b>	:	statistical analysis system
<b>SFC</b>	:	solid fat content
<b>R</b>	:	Red
<b>SOO</b>	:	l-stearoyl-dioleoyl glycerol
<b>SPSS</b>	:	statistical package for the social sciences
<b>TAG</b>	:	Triacylglycerol
<b>Y</b>	:	Yellow

# CHAPTER I

## INTRODUCTION

Fats and oils are mainly derived from plant and animal sources. About 71% of edible oils or fats are derived from plant sources (Salunkhe *et al.*, 1992). Some oil crops such as groundnut can be used directly as a food, but others are exclusively processed to obtain fat or oil and cake or meal (Hatje, 1989).

The potential supply of lipid from fruit and fruit by-product may be enormous and should be investigated. Millions of pounds of fruit seeds are discarded yearly resulting in disposal problems, while proper utilisation of these waste products could lead to new sources of oil and meal (Kamel and Kakuda, 1992). However, the level of lipids in fruit seeds may vary with respect to cultivar and degree of ripeness (Ramadan and Morsel, 2003). In order to decide the method of lipid extraction from a fruit seed, its oil/fat content has to be pre-determined. With that, a knowledge on oil composition and physico-chemical characteristics would help to one think about its one potential uses as food or industrial raw material.

Fats and oils modification for better nutrition and improved physical properties is one of the major areas of research. Although seed oils can be used in food directly, there is plenty of scope for value addition. Value addition can be done through modification techniques. Among the modification techniques, interesterification is one of the most versatile as by rearranging the acyl groups within components of a mixed triacylglycerol (TAG), the physical properties of fats and oils may be

improved (Gunstone, 2000). Interesterification can be effected by using either lipase (Aguedo *et al.*, 2008; Lee *et al.*, 2008) as catalysts or chemicals such as metal salts (Farmani *et al.*, 2008; Azadmard-Damirchi and Dutta, 2008).

Lipases are found widely in animals, plants and microorganisms in nature (Wong, 1995). The important advantage of using lipases in oil and fat modification is that the oil quality and characteristics can be protected at the low temperatures. This is because by using lipases, the reaction can be done at lower temperature range (25-30°C) (Graile *et al.*, 1991). Furthermore, lipases are position-specific, and capable to react selectively with *sn*-1, *sn*-2, *sn*-3 positions of the TAG (Forsell *et al.*, 1993). The other reason for considering lipases applications in lipid processing is because of its mildness, both in the equipment setup and reaction conditions, hence reducing the financial constraints encountered in the process construction (Che Omar, 1996). Besides this, lipases are natural, biodegradable and renewable.

Most lipase-catalysed interesterification of fats and oils are carried out to obtain more liquid oil by exchanging the fatty acid (FA) residues to increase unsaturation and/or shorter chain TAG (Gunstone, 2000). In this study, Lipozyme IM and Lipozyme TL were selected as the enzymes because they are a highly specific lipase that has been shown to retain their 1,3 specificity for longer periods of time (Lee and Akoh, 1996; Li and Ward, 1993) and maintains a higher level of activity under dry conditions (Valivety *et al.*, 1992; Bloomer *et al.*, 1990). This feature is particularly important in this reaction as the addition of water has been found to promote hydrolysis (Gitlesen *et al.*, 1995). Immobilised Novozym 435 also was chosen as a nonspecific lipase in this study. However, the application of these lipases into the

modification of the Malaysian fruit seed oils is limited.

Therefore, the objectives of this study are:

1. To investigate the physico-chemical characteristics of oils extracted from honeydew melon (*Cucumis melo* var. *indorus*), musk lime (*Citrus microcarpa*), papaya (*Carica papaya* L.) and rambutan (*Nephelium lappaceum* L.) seed.
2. To study the effect of enzymatic interesterification reaction using lipases from Lipozyme IM, Lipozyme TL and Novozym 435 on the physico-chemical properties individual seed oil and their blend (1:1) that have been extracted.

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