



UNIVERSITI PUTRA MALAYSIA

***FORMULATION OF FAT SUBSTITUTE USING PLANT-BASED FATS
SIMULATING THE PROPERTIES OF LARD***

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IPPH 2015 7



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By

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

October 2015

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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October 2015

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Lard (LD) is a solid fat used as shortening in the manufacture of bread, cakes, cookies and other products due to its flavor and physical properties. However, the limitation of lard applications were concerned as the Muslims, Jews and vegetarians are not permissible to consume both pork and lard in any products. In addition, consumption of lard and other animal fats is not recommended since there is a growing negative perception about the implication of animal fats on human health. Therefore, lard alternative products are highly demanded from many Muslim majority countries to regularize food formulation according to the syariah compliance. If an alternative halal product is made available, it may serve as an import substitute as well as to satisfy the demand for alternative halal products. However, past studies on lard alternative fat substitute is very limited. Hence, a study was carried out to formulate lard alternative fat substitute by mixing various plant fats such as mee fat [*Madhuca longifolia* (MF)], palm stearin (PS), avocado fat (Avo), cocoa butter (CB), palm oil (PO) and soybean oil (SBO). At first, the binary (MF:PS), ternary (Avo:PS:CB) and quaternary (PO:PS:SBO:CB) (w/w) mixtures were formulated using the above mentioned fats at different ratios and their physico-chemical properties were compared to that of LD such as slip melting point (SMP), iodine value (IV), fatty acid (FA) composition using GC, triacylglycerol (TAG) composition using HPLC, thermal behavior using DSC and solid fat content (SFC) NMR. None of the plant based mixtures had a SMP and IV that were similar to that of lard. Even though there were diversity in fatty acids and triacylglycerol molecules, however, some of plant based mixtures showed similarity with some fatty acids and triacylglycerol molecules of LD. Binary (MF:PS) mixtures had higher saturated fatty acids (44.25-45.77%) and UStSt triacylglycerol contents (38.21-44.76%) compared to that of lard (37.38 and 26.60%, respectively). Meanwhile, the saturated fatty acid and UStSt triacylglycerol contents of ternary (Avo:PS:CB) (36.65-38.01% and 24.89-33.61%, respectively) and quaternary (PO:PS:SBO:CB) (34.44-36.79% and 22.47-24.86%, respectively) mixtures were almost similar to lard (37.38 and 26.60%, respectively). The cooling and heating profiles of plant based mixtures were differed from lard. However, the major melting peak of MF:PS (99:1), Avo:PS:CB (84:7:9) and all quaternary mixtures was found to be closest to that of lard at -3.59 °C. SFC profile compatibility of mixture was used as the main criteria to choose the best mixture under each set (binary, ternary and quaternary) as compared to that of lard. According to this,

the SFC of binary mixture of MF:PS (99:1), ternary mixture of Avo:PS:CB (84:7:9) and quaternary mixture of PO:PS:SBO:CB (38:5:52:5) were almost similar and the least difference to that of lard. In the next stage, these selected plant based mixtures and lard were subjected to shortening production. The formulated shortenings were compared to that of lard shortening in term of their hardness using a texture analyzer (TA), consistency using a cone penetrator, polymorphism using XRD and microstructure behavior using PLM. The hardness (26.19-28.35 g) and adhesiveness (82.46-137 g/s) of formulated plant based shortening were not significantly different ($p>0.05$) compared to that of lard shortening (26.67 g and 123.88 g/s, respectively). The formulated plant based shortenings and lard shortening were categorized as plastic fats based on their consistency value (319.20-326.26 g/cm²) and displayed a mixture of β' and β -form polymorphs of which the β' -form was found to be predominant. However, the polymorphism characteristic was not affected by crystallization behavior where the number and size of crystals in the formulated plant based shortenings were dissimilar to those of lard shortening. In the final stage, the functional properties of formulated plant based shortenings and lard shortening were compared in the production of cookie dough and cookie. The dough made with formulated plant based shortenings and LD shortening had a better consistency with increase of mixing time. However, dough made with binary (337 BU) and quaternary (300 BU) mixture shortenings had a closer consistency value at 15 min of the mixing time and there were also no significant differences ($p>0.05$) with dough made with LD shortening (333 BU). The dough made with formulated plant based shortenings and lard shortening had a maximum cookie spread at 3 min while baking in the oven. In the meantime, there was no significant difference ($p>0.05$) in elasticity of dough made with binary mixture shortening (65 BU) with dough made with LD shortening (63 BU). However, there were no significant differences ($p>0.05$) among cookies of different types of shortenings with regard to cookie hardness (1008.12-1015.75 g), diameter (72.33-72.95 mm), thickness (9.32-9.52 mm) and cookie spread ratio (7.65-7.8 mm). With regard to color, ANOVA results showed that there were no significant differences ($p>0.05$) in *L*, *a*, and *b* values of cookies made with binary (70.43, 8.12 and 19.55, respectively) and quaternary (69.23, 7.27 and 20.96, respectively) mixture shortenings and lard shortening (69.27, 7.03 and 18.79, respectively).

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

FORMULASI LEMAK TUMBUHAN SEBAGAI PENGANTI BAGI MENYERUPAI CIRI-CIRI LEMAK BABI

Oleh

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Lemak babi merupakan salah satu lemak yang digunakan sebagai *shortening* bagi pembuatan roti, kek, biskut dan lain-lain lagi. Walau bagaimanapun, aplikasi lemak babi adalah terbatas yang mana orang-orang Islam, Yahudi dan pengamal vegetarian dilarang untuk menggunakan lemak haiwan tersebut. Tambahan pula, lemak babi tidak disyorkan memandangkan terdapat persepsi negatif mengenai implikasi lemak haiwan terhadap kesihatan manusia. Oleh itu, penganti lemak babi sangat diperlukan oleh negara-negara majoriti penduduknya Muslim di mana pengganti lemak babi ini dapat digunakan dalam pembuatan makanan mengikut hukum syarak. Sekiranya terdapat alternatif produk halal, maka permintaan terhadap produk tersebut dapat dipenuhi. Walau bagaimanapun, kajian mengenai alternatif lemak babi sangat terhad. Oleh itu, tujuan kajian ini adalah untuk memformulasikan alternatif kepada pengganti lemak babi dengan mencampurkan pelbagai lemak daripada sumber tumbuhan seperti lemak mee [*Madhuca longifolia* (MF)], stearin sawit (PS), lemak avokado (Avo), lemak koko (CB), minyak sawit (PO) and minyak soya (SBO). Pada permulaannya, lemak yang telah dinyatakan seperti di atas digunakan bagi menghasilkan formulasi campuran binari (MF:PS), ternari (Avo:PS:CB) dan kuaternari (PO:PS:SBO:CB) pada nisbah yang berbeza dan membandingkannya dengan lemak babi dari segi ciri-ciri kimia-fizikal seperti titik lebur (SMP), nilai iodin (IV), komposisi asid lemak menggunakan GC, komposisi triasilgliserol menggunakan HPLC, sifat terma menggunakan DSC dan kandungan lemak pepejal menggunakan NMR. Tiada campuran lemak tumbuhan yang mempunyai SMP dan IV yang sama seperti lemak babi. Walaupun terdapat kepelbagaian asid lemak dan triasilgliserol, sesetengah asid lemak dan molekul triasilgliserol yang terdapat dalam campuran lemak tumbuhan adalah sama seperti lemak babi. Campuran binari mengandungi asid lemak tepu (44.25-45.77%) dan triasilgliserol UStSt (38.21-44.76%) yang lebih tinggi berbanding dengan lemak babi. Sementara itu, campuran ternari (Avo:PS:CB) (36.65-38.01% dan 24.89-33.61%, masing-masing) dan kuaternari (34.44-36.79% dan 22.47-24.86%, masing-masing) mengandungi asid lemak tepu dan triasilgliserol UStSt yang hampir sama dengan lemak babi (37.38 dan 26.60%, masing-masing). Profil penyejukan dan pemanasan bagi campuran lemak tumbuhan adalah berbeza dengan lemak babi. Walau bagaimanapun, lemak babi dan campuran MF:PS (99:1), Avo:PS:CB (84:7:9) dan semua campuran kuaternari mempunyai puncak pemanasan utama pada -3.59 °C.

Persamaan profil SFC digunakan sebagai kriteria utama untuk memilih campuran yang paling baik untuk setiap set (binari, ternari dan kuaternari) dalam menentukan persamaannya dengan lemak babi. Berdasarkan ciri-ciri ini, SFC campuran binari MF:PS (99:1), campuran ternari Avo:PS:CB (84:7:9) dan campuran kuaternari PO:PS:SBO:CB (38:5:52:5) menunjukkan persamaan yang paling ketara dengan lemak babi. Pada tahap seterusnya, campuran lemak tumbuhan yang telah dipilih dan lemak babi digunakan dalam penghasilan *shortening*. *Shortening* yang telah diformulasi akan dibandingkan dengan *shortening* lemak babi dari segi kekerasan menggunakan penganalisa tekstur (TA), konsistensi menggunakan penetrasi kon, polimorfik menggunakan XRD and sifat struktur mikro menggunakan PLM. Kekerasan (26.19-28.35 g) dan kelekatan (82.46-137 g/s) *shortening* berasaskan lemak tumbuhan tidak menunjukkan perbezaan ($p>0.05$) berbanding *shortening* lemak babi (26.67 g dan 123.88 g/s, masing-masing). *Shortening* berasaskan lemak tumbuhan dan *shortening* lemak babi dikategorikan sebagai lemak palstik berdasarkan nilai konsistensi (319.20-326.26 g/cm²) dan terdiri daripada campuran β' and β - polimorfik di mana β' merupakan polimorfik utama. Walau bagaimanapun, ciri-ciri polimorfik tidak mempengaruhi sifat pengkristalan di mana bilangan dan saiz kristal adalah berbeza di antara *shortening* berasaskan lemak tumbuhan dan *shortening* lemak babi. Pada tahap terakhir, perbandingan antara *shortening* berasaskan lemak tumbuhan dan *shortening* lemak babi digunakan dalam penghasilan doh dan biskut. Konsistensi *shortening* berasaskan lemak tumbuhan dan *shortening* lemak babi adalah lebih stabil apabila diadun lebih lama. Walau bagaimanapun, tiada perbezaan secara signifikan ($p>0.05$) terhadap konsistensi doh yang dihasilkan daripada *shortening* campuran binari (337 BU), kuaternari (300 BU) dan lemak babi (333 BU) pada 15 minit pengadunan. Pengembangan biskut adalah maksimum pada 3 min sewaktu dibakar di dalam oven bagi doh yang dihasilkan daripada *shortening* berasaskan lemak tumbuhan dan *shortening* lemak babi. Pada masa yang sama, kekenyalan doh yang dihasilkan daripada *shortening* campuran binari (65 BU) dan *shortening* lemak babi (63 BU) tidak menunjukkan perbezaan secara signifikan ($p>0.05$). Walau bagaimanapun, biskut yang diperbuat daripada *shortening* yang berbeza tidak menunjukkan perbezaan secara signifikan ($p>0.05$) terhadap kekerasan (1008.12-1015.75 g), diameter (72.33-72.95 mm), ketebalan (9.32-9.52 mm) dan nisbah pengembangan biskut (7.65-7.8 mm). Dari segi warna, nilai ANOVA menunjukkan tiada perbezaan secara signifikan ($p>0.05$) bagi nilai L , a , dan b bagi biskut yang dihasilkan daripada *shortening* campuran binari (70.43, 8.12 and 19.55, masing-masing), kuaternari (69.23, 7.27 and 20.96, masing-masing) dan lemak babi (69.27, 7.03 and 18.79, masing-masing).

ACKNOWLEDGEMENTS

Alhamdulillah thank you to Almighty God Allah for His mercies and blessings. I would like to express my most profound and sincere appreciation to my supervisor, Dr. Mohammed Nazrim Marikkar from the Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences for his guidance, advice, encouragement and understanding. His cooperation and support are always highly appreciated. My appreciation also goes to my co-supervisors Dr. Miskandar bin Mat Sahri and Prof. Dr. Shuhaimi bin Mustafa for their encouragement, opinion, comments and valuable moral support.

A special thanks to Dr. Ir. Filip Van Bockstaele and Prof. Dr. Koen Dewettinck from Department of Food Quality and Food Safety, Ghent University, Belgium for giving me the opportunity to use their lab. I would also like to thank my colleagues and staffs from Halal Research Products Institute UPM, The Malaysian Agricultural Research and Development Institute (MARDI), Malaysian Palm Oil Board (MPOB) and University of Ghent, Belgium for their help throughout the course of this project.

I would like to take this opportunity to express my warmest gratitude to my parents, husband, son, sister, brother in law and niece for their encouragement, support and love.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

°C	Degree celcius
<i>a</i>	Redness
ANOVA	Analysis of variance
AOAC	Association of analytical communities
AOCS	American Oil Chemists' Society
Avo	Avocado fat
<i>b</i>	Yellowness
C12:0	Lauric acid
C14:0	Myristic acid
C16:0	Palmitic acid
C16:1	Palmitoleic acid
C18:0	Stearic acid
C18:1	Oleic acid
C18:2	Linoleic acid
C18:3	Linolenic acid
C20:0	Arachidic acid
CB	Cocoa butter
CBE	Cocoa butter equivalents
CBS	Cocoa butter substitute
CHD	Coronary heart disease
cm	Centimeter
DSC	Differential scanning calorimetry
DSC	Differential scanning calorimeter
FAME	Fatty acid methyl ester
FAO	Food and Agricultural Organization
g	Gram
GLC	Gas liquid chromatography
g/s	Gram per second
g/cm ²	Gram per centimeter square
HPLC	High performance liquid chromatography
IV	Iodine value
<i>L</i>	Lightness
LD	Lard
LLL	Trilinoleoyl glycerol
LLLn	Dilinoleoyl-3-linoleoyl glycerol
MF	Mee (<i>Madhuca longifolia</i>) fat
mL/min	Milliliter per minute
MLCT	Medium-and long-chain TAGs
mm	Milimeter
MPOB	Malaysian Palm Oil Board
MUFA	Monounsaturated fatty acid
OLL	1-oleoyl- dilinoleoyl glycerol
OOL	Dioleoyl-3-linoleoyl glycerol
OOO	Trioleoyl glycerol
PDAGS	Stearin fraction of palm-based diacylglycerol
PLL	1-palmitoyl-dilinoleoyl glycerol
PLM	Polarized light microscopy
PMF	Palm mid fraction

pNMR	Pulse nuclear magnetic resonance
PO	Palm oil
POL	Palmitoyl-oleoyl-linoleoyl glycerol
POO	1-palmitoyl-dioleoyl glycerol
PORAM	Palm Oil Refiners Association of Malaysia
PPL	Dipalmitoyl-3-linoleoyl glycerol
PPO	Dipalmitoyl-3-oleoyl glycerol
PPP	Tripalmitoyl glycerol
PPS	Dipalmitoyl-3-stearoyl glycerol
PS	Palm stearin
PUFA	Polyunsaturated fatty acid
RID	Refractive index detector
rpm	Revolution per minute
SBO	Soybean oil
SFA	Saturated fatty acid
SFC	Solid fat content
SHSs	Sunflower hard stearins
SMP	Slip melting point
SOO	1-stearoyl-dioleoyl glycerol
SOS	1,3-distearoyl glycerol
SPO	Stearoyl-palmitoyl-oleoyl glycerol
SSS	Tristearoyl glycerol
StStSt	Trisaturated
TAG	Triacylglycerol
USA	United State of America
USFA	Unsaturated fatty acid
UStSt	Disaturated
UUST	Diunsaturated
UUU	Triunsaturated
w/w	Weight per weight
WAXD	Wide angle X-ray diffraction
XRD	X-ray diffraction
α	Alpha
β	Beta
β'	Beta prime
μL	Microliter
μm	Micrometer

CHAPTER 1

INTRODUCTION

Animal fats are widely used as food ingredient for a long time. Apart from being used as a medium of deep frying and meat flavors, animal fats were also used as shortening in bakery products due to their availability and lower cost. Lard (LD) and tallow are well known animal fat with similar characteristics in terms of high saturated fatty acids (SFA). LD has been used in food applications in many countries in Europe (Antonietta *et al.*, 2004), America (deMan *et al.*, 1991) and in Asia such as China, Taiwan, Thailand, Cambodia and Vietnam (Omar *et al.*, 2010; Hsu and Yu, 2002; Morell and Enig, 2000). The main reason for this trend relates to its flavor and superior performance characteristics during food processing.

Although the use of LD is already popular in the food culture of certain ethnic groups, the consumption of LD is prohibited for some communities based on religious believes. Particularly, Islamic and Orthodox Jewish religions command the prohibition of consumption of both pork and LD in any products (Regenstein *et al.*, 2003; Montiel-Sosa *et al.*, 2000; Rashood *et al.*, 1996). Therefore, LD adulteration in food products is a concern for Muslim and Jewish people. Most of the manufacturers want to reduce production costs and to increase the amount of raw material because of high demand for some products which could be the main reason for adulteration. However, the fraudulent food claim, could lead to a loss of thousands even billions toward food industries, if they do not implement the right halal practices as being told by the advisory or authoritative bodies. In addition, if there is any contamination or non-halal substance detected in the product, it may be difficult to rebuild the trust and confidence among consumers. In this context, detection and estimation of LD adulteration in fats and oils has become an important aspect in food quality control due to growing public concern in many parts of the world. Therefore, the development of instrumental and analytical methods for halal authentication and detection was reported by several research groups (Rohman *et al.*, 2011; Juliana *et al.*, 2011; Mansor *et al.*, 2011; Marina *et al.*, 2010; Marikkar *et al.*, 2005; Che Man *et al.*, 2005).

In addition to religious prohibition, medical reports of unfavorable health effects of LD also prompted the general public to be more vigilant about pork and LD contamination in food products (Rashood *et al.*, 1996). According to previous studies, pork fat or LD could contribute to heart disease, obesity, hypertension and colon cancer (Wang *et al.*, 2013; Chicco *et al.*, 2008; Sinkeldam *et al.*, 1990; Rogers *et al.*, 1986). Owing to this, there has been a growing trend to formulate fat substitutes for the replacement of LD in many products (Rodrigues-Capena *et al.*, 2011; Degado-Pado *et al.*, 2011; Ospina-E *et al.*, 2010; Choi *et al.*, 2010; Serivini *et al.*, 2003; Muguerza *et al.*, 2003). However, there is still much potential to further research and innovation, especially for formulating halal alternative fats for LD using locally available plant lipids.

If modifications to fats and oils from plant sources could be done to mimic the physical properties of LD, it would be worthwhile. Blending is the simplest way to modify the physical properties of fats where it could be a mixture of different oils and fats (Siddique *et al.*, 2010; Miskandar *et al.*, 2005). Blending is also generally preferred to other modification techniques because it is less costly and the desired consistency can be reached by choosing the right mixture ratios (Nusantoro *et al.*, 2013). The fat substitute simulating the properties of LD could be done by mixing several fats and oils where the raw material should come from halal sources. Thus, plant lipids could be selected as the potential sources as they are usually not prohibited under halal laws.

In this study, fats and oils from plant sources such as oil palm (PO and PS), cocoa (CB), avocado (Avo), mee seed [*Madhuca longifolia* (MF)] and soybean (SBO) have been chosen for blending. PS and CB are categorized as hard fats. PO, Avo, and MF are categorized as semisolid fats while SBO is categorized as liquid oil. In order to obtain the simulating characteristics of LD, mixing of fats and oils from different plant sources can be achieved in the form of binary [mee fat:palm stearin (MF:PS)], ternary [avocado fat:palm stearin:cocoa butter (Avo:PS:CB)] and quaternary [palm oil:palm stearin:soybean oil:cocoa butter (PO:PS:SBO:CB)] mixtures at different ratios. The comparisons of physical properties of LD and the formulated plant based fat mixtures could be done by evaluating them with respect to slip melting point (SMP), iodine value (IV), solid fat content (SFC) and thermal properties. Besides these, the fatty acid (FA) and triacylglycerol (TAG) compositional analyses would also be carried out as they are key to understand the physical behavior of the formulated plant based fat substitutes. The selection of the most suitable mixture from each fat category namely binary, ternary and quaternary mixtures would be the preliminary step of the investigation. The selected plant based mixtures shall be processed into shortenings in the next step to cross-check their compatibility to LD shortening in terms of their physical characteristics, crystal behavior and polymorphism. In the final stage, the formulated plant based shortenings and LD shortening can be applied on to the preparation of cookies to find out their functional properties in actual product formulation.

1.1 Problem statements

- i. Whether it is possible to formulate fat substitutes for lard using binary, ternary and quaternary mixtures of selected plant fats
- ii. Whether the formulated plant-based fat substitutes for lard could really work as a fat ingredient in the preparation of good quality cookies

1.2 Hypothesis

It may be possible to formulate fat substitutes to simulate the properties of lard using binary, ternary and quaternary mixtures of selected plant fats.

1.3 Research objective

Hence, the overall objectives of this study were to formulate plant based fat substitutes in simulating the properties of LD as halal alternatives. The specific objectives of this research are:

- i. To formulate binary, ternary and quaternary mixtures of selected plant fats
- ii. To compare the composition and physico-chemical properties of the formulated mixtures with those LD
- iii. To compare the functional properties of LD shortening with those of the formulated plant based shortenings
- iv. To compare the cookie dough properties and cookie quality prepared from LD shortening with those using formulated plant based shortenings

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