MODIFICATION OF RAMBUTAN (Nephelium lappaceum L.) SEED FAT BY CHEMICAL INTERESTERIFICATION TO PRODUCE VANASPATI-LIKE FAT

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By

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Chairman : Professor Hasanah Mohd Ghazali, PhD
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The rambutan (*Nephelium lappaceum* L.) is a common and popular fruit grown in Malaysia. The flesh is processed industrially to produce juices, jams, jellies and marmalade, while the seed is considered as an agro-industrial waste. A novel way to reduce the wastage is to use rambutan seed as alternative natural source of edible fat with possible food applications as it has been reported that the seed possesses a relatively high proportion of fat (between 14% and 41%). Hence, the goals of this study were to characterize the physicochemical properties of fat from the seeds of three varieties of locally grown rambutan (R4, 47 and Serjan) and select the best variety as the source of fat to optimize the chemical interesterification reaction between rambutan seed fat and palm stearin. From the reaction, the best interesterified fat blend was selected for development of a vanaspati-like fat which was then evaluated and compared with commercial vanaspati during three months of storage period at 25 ˚C. Results obtained showed that there was a significant difference ($p < 0.05$) between the crude fat content (34.25-37.62%), iodine value (44.26-47.49 g I_2 /100g oil) and melting point (24.54-26.60 ˚C) among the three varieties. Variety R7 was chosen to be subjected to chemical interesterification process as its seed contained the highest ($p < 0.05$) crude fat content (37.62 %).

Response surface methodology (RSM) was employed based on a five-level, three-factor central composite design (CCD**)** to optimize the chemical interesterification conditions to produce a vanaspati-like fat with the desired solid fat content (SFC). In this study, rambutan seed fat (RSF) was blended with palm stearin (PS). The effect of three factors namely rambutan seed fat ratio (50-100 w/w), reaction temperature (60–90 ˚C) and reaction time (30–60 min) on responses such as SFC at 20 ˚C, 25 ˚C, 30 ˚C and 35 ˚C were investigated. The response optimizer plot suggested that rambutan seed fat ratio, reaction temperature and time should be 60 w/w, 66 ˚C and

30 min, respectively, to produce a vanaspati-like fat with optimal SFC. Interesterification significantly ($p < 0.05$) increased the free fatty acid content and reduced melting point and SFC of blends.

The physicochemical and textural properties of experimental vanaspati (EV) prepared from chemically interesterified RSF:PS (60:40) blend at 66 ˚C for 30 min and commercial vanaspati (CV) throughout three months of storage at 25 ˚C were evaluated and compared. Both EV and CV had significant ($p < 0.05$) increments in peroxide value (PV), p-anisidine value (p -AV), solid fat content and hardness throughout storage. PV of the EV and CV after three months of storage were within acceptable levels of 2.23 and 2.52 meq O_2/kg, respectively. The initial p -AV of EV was found to be 0.84 and for CV it was 1.2, and after storage period of three months, there was a 205.9% and 206.6% increase, respectively. The thermal behavior of EV and CV remained unchanged during storage. The β crystal form became predominant in EV and CV after 4 and 6 weeks of storage, respectively. It was observed that post-hardening occurred in both experimental and commercial vanaspati during storage. In general, the study successfully showed that rambutan seed fat may be used to produce a vanaspati-like fat when interesterified with palm stearin. Vanaspati produced from the blend undergo some physico-chemical changes, similar to changes that occurred when commercial vanaspati was stored under similar conditions.

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

PENGUBAHSUAIAN LEMAK BIJI RAMBUTAN (*Nephelium lappaceum* L.) MELALUI INTERESTERIFIKASI KIMIA UNTUK MENGHASILKAN PRODUK SEPERTI VANASPATI

Oleh

FATEMEH GHOBAKHLOU

Januari 2019

Pengerusi : Profesor Hasanah Mohd Ghazali, PhD
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Rambutan (*Nephelium lappaceum* L.) adalah sejenis buah popular yang ditanam di Malaysia. Isinya diproses secara industri bagi menghasilkan jus, jem, jeli dan marmalad, sementara bijinya dianggap sebagai sisa agroindustri. Kaedah baharu bagi mengurangkan pembaziran adalah dengan menggunakan biji rambutan sebagai sumber baharu bagi lemak semulajadi yang boleh dimakan beserta aplikasi makanan yang mungkin kerana telah dilaporkan bahawa bijinya mengandungi kadar lemak yang agak tinggi (antara 14% dan 41%). Oleh itu, objektif kajian ini adalah untuk mencirikan sifat-sifat fizikokimia bagi lemak dari biji-biji tiga jenis rambutan yang ditanam tempatan (R4, 47 dan Serjan) dan memilih varieti terbaik sebagai sumber lemak untuk mengoptimumkan tindakbalas interesterifikasi kimia antara lemak biji rambutan dan stearin sawit. Dari tindak balas tersebut, campuran lemak teresterifikasikan yang terbaik telah dipilih untuk pembangunan produk seperti vanaspati yang kemudiannya dinilai dan dibandingkan dengan vanaspati komersil sewaktu tiga bulan tempoh penyimpanan pada 25 ˚C. Keputusan menunjukkan bahawa terdapat perbezaan ketara ($p<0.05$) antara kandungan lemak kasar (34.25-37.62%), nilai iodin (44.26-47.49 g I_2 /100g oil) dan takat lebur (24.54-26.60 ˚C) di antara ketiga-tiga varieti. Varieti R7 telah dipilih untuk menjalani proses interesterifikasi kimia kerana bijinya mengandungi kandungan lemak kasar (37.62%) yang tertinggi ($p<0.05$).

Metodologi tindak balas permukaan berdasarkan kepada rekabentuk komposit pusat lima aras, tiga faktor telah digunakan untuk pengoptimuman bagi kondisi interesterifikasi kimia untuk menghasilkan produk seperti vanaspati dengan kandungan lemak pepejal (SFC) yang dikehendaki. Dalam kajian ini, lemak biji rambutan (RSF) telah diadun dengan stearin sawit (PS). Kesan dari tiga faktor seperti nisbah lemak biji rambutan (50-100w/w), suhu tindak balas (60–90 ˚C) dan masa tindak balas (30-60min) terhadap respon seperti SFC pada 20 ˚C, 25 ˚C, 30 ˚C dan 35 ˚C telah diperiksa. Plot pengoptimuman tindak balas telah mencadangkan bahawa nisbah lemak biji rambutan, suhu tindak balas dan masa seharusnya masing-masing 60 w/w, 66 ˚C dan 30 min, bagi menghasilkan produk seperti vanaspati dengan SFC yang optimum. Interesterifikasi meningkatkan kandungan asid lemak bebas dan merendahkankan takat lebur dan SFC bagi campuran dengan ketara ($p<0.05$).

Sifat-sifat fizikokimia dan tekstur bagi vanaspati eksperimen (EV) yang disediakan dari campuran RSF:PS (60:40) teresterifikasikan secara kimia pada 66 ˚C selama 30 min dan vanaspati komersial (CV) sepanjang tempoh penyimpanan selama tiga bulan pada 25 ˚C telah dinilai dan dibandingkan. Kedua-dua EV dan CV mempunyai kenaikan yang ketara ($p<0.05$) dalam nilai peroksida (PV), nilai p-Anisidin (p-AV), kandungan lemak pepejal dan kekerasan sepanjang penyimpanan. PV bagi EV dan CV selepas tiga bulan penyimpanan berada dalam tahap yang boleh diterima masing-masing 2.23 dan 2.52 meq O_2/kg. p-AV awal bagi EV adalah 0.84 dan 1.2 bagi CV, dan selepas tempoh penyimpanan selama tiga bulan, terdapat kenaikan masing-masing 205.9% dan 206.6%. Sifat terma bagi EV dan CV kekal tidak berubah semasa penyimpanan. Bentuk kristal β menjadi dominan dalam EV dan CV masing-masing selepas 4 dan 6 minggu penyimpanan. Ia didapati bahawa berlakunya pengerasan pada kedua-dua vanaspati eksperimen dan vanaspati komersial semasa penyimpanan. Secara umumnya, kajian ini membuktikan bahawa lemak biji rambutan boleh diguna untuk menghasilkan lemak seperti vanaspati selepas diinteresterifikasi dengan stearin sawit. Vanaspati yang dihasilkan daripada campuran tersebut mengalami beberapa fizikokimia perubahan dan perubahan ini juga dialami oleh vanaspati komersial apabila ia disimpan padu keadaan yang sama.

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Finally, my deepest thanks go to my both beloved parents and family for their support and encouragement.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AOAC	Association of official Analytical Chemists
AOCS	American Oil Chemists' Society
C	Degree centigrade
C/min	Degree centigrade per minutes
DSC	Differential Scanning Calorimetry
FAME	Fatty acid methyl ester
FAO	Food and Agricultural Organization
FDA	Food and Drug Administration
FFA	Free fatty acid
g	Gram
GC	Gas chromatography
h	Hour
HPLC	High performance liquid chromatography
i.e.	That is
IV	Iodine value
Kg	Kilogram
KOH	Potassium hydroxide
L	liter
mg	*Milligram*
mol/L	Mole per litre
min	Minute
mL	milliliter
mL/min	millilitre per minutes

mm	millimetre
MPOB	Malaysian palm oil board
MUFA	Mono unsaturated fatty acid
NaOH	Sodium hydroxide
p-AV	p-anisidine value
PV	Peroxide value
psi	Pound per square inch
PUFA	Poly unsaturated fatty acid
SFC	Solid fat content
SMP	Slip melting point
TAG	Triacylglycerol
USDA	*United States Department of Agriculture*
v/v	volume per volume
w/w	Weight per weight
µm	Micrometre

CHAPTER 1

INTRODUCTION

Nowadays, natural vegetable oils are increasingly used as raw material for medicines, cosmetics production and industrial food (Solís-Fuentes et al., 2010; Yara-Varón et al., 2017). Increase in the world population has resulted in increase in demand and price of edible oils which leads to search for novel source of natural oil particularly among the non-conventional and underexploited oil source like agro-industrial residues (Diemeleou et al., 2014). Besides, no oil from a single source has been found to be suitable for all applications because oils from different origins are basically different in their composition. This also necessitates the need to find new sources of oils. Fruit seed are usually thrown out after processing as an agro-industrial residue, while, they can be used potentially as a novel natural oil source with high nutritional, pharmaceutical and industrial significance.

Rambutan (*Nephelium lappaceum* L.) is a seasonal fruit of the Sapindaceae family which is native to Malaysia (Solís-Fuentes et al., 2010; Romain et al., 2013). This fruit is usually consumed fresh, but in Malaysia and Thailand, rambutan is industrially processed into juice, jam, jelly and marmalade. In addition, rambutan fruits are also processed as rambutan stuffed with a chunk of pineapple and canned in syrup (Sirisompong et al. 2011). Rambutan seed (~4-9g/100g) is the major waste by- product from rambutan fruit processing industry (Zzaman et al., 2014). Some studies reported that rambutan seed possesses a relatively high amount of fat (between 14% and 41%). The disposal of this agricultural waste can lead to a serious environmental issue. The challenge is, therefore, to transform the seed waste of the processed crop product into ecological friendly or sustainable material suitable for industrial purposes. A more in-depth knowledge of the fruit seed will encourage its utilization and, thus, wastage may be reduced. Rambutan seed also has the potential to be utilized in the cosmetic and personal care industries with the ability to produce stable bar and liquids soaps that are not only stable in terms of physical appearance but have a beneficial ingredient where the fat content is comparable with other vegetable oils and cosmetic ingredient and is compatible with other cosmetic ingredients (Lourith et al. 2016). While the rambutan seed fat could be used as a cocoa butter substitute in chocolate manufacturing (Issara et al. 2014; Zzaman et al. 2014), however to date, there has been no such application in the food industry. Although there have been some reports on the seed fat, more study is needed as there is a huge potential for the fat to be used in various sectors of the food industry.

Rambutan are consumed, fresh, canned or processed and its consumption results in the production of vast amount of rambutan waste from their seeds and rind, disposal of these rambutan wastes can have a serious environmental impact which is becoming harder to solve. Much effort will therefore be needed to develop the nutritional and industrial potential of by-products waste and these underutilized agricultural products [6]

Vanaspati is a shortening-like product, which is considered as a cheap substitute for ghee in the same way that margarine is for butter. It is such a fat that can basically be consumed for all purposes particularly in frying, cooking and baking applications in India, Pakistan and Middle East countries (Farmani et al., 2015). Vanaspati is produced by partial hydrogenation of vegetable oils blends such as palm oil, soybean, rapeseed and cottonseed (Idris and Dian, 2005). However, industrial partial hydrogenation of oil leads to the production of *trans* fatty acids (Albuquerque et al., 2017). Many nutritional and epidemiological studies indicate that a high-*trans* fatty acid diet may cause several adverse effects on human health including cardiovascular disease, coronary heart disease, diabetes mellitus, cancers, abnormalities in infant development and inflammation (Thompson et al., 2011; Hung et al., 2016). Based on the Food Drug Administration (FDA) and the United States Department of Agriculture (USDA) laws, *trans* fat does not have to be listed if the total fat in the food is less than 0.5 g per serving and no claims are made about the fat, fatty acids or cholesterol content (Sampurna and Reddy, 2011). In this respect, vanaspati may be regarded as a main source of *trans* fatty acid which ranges from 1.04% to 50% (Krishnaswamy et al., 2016; Dorni et al., 2018).

Interesterification is used as a variant process to partial hydrogenation to produce low- or no-*trans* fats which modifies the TAG composition and, therefore, changes the physical properties. The two types of interesterification are chemical interesterification and enzymatic interesterification. Chemical interesterification, which is also known as random interesterification, is ideal in modifying oils and fats because of the lower investment and production costs of finished product, easier to carry out and it is a tried-and-true approach (Norizzah et al., 2016 and 2018). However, disadvantageous of enzymatic interesterification include higher enzyme, operating and equipment costs, longer reaction time and it produces more free fatty acids in the product than chemical process (Lampert, 2000; Farmani et al., 2009). Chemical interesterification is currently the main alternative to prepare plastic fats containing no *trans* fatty acids (Ribeiro et al., 2009; Shetty et al., 2014).

Trans-free vanaspati could be made using palm stearin and other liquid oils either by employing interesterification or by direct blending. Majumdar and Bhattacharyya (1986) explored *trans*-free vanaspati production by transesterification of blends of palm stearin with some vegetable oil. The method of *trans*-free vanaspati production was reported in the Nor Aini et al. (1999) study through physical blending of ternary blends of palm oil/palm stearin/palm olein and palm oil/ palm stearin/palm kernel olein. In another study which was accomplished by Farmani et al. (2006, 2007 and 2008), the production of *trans*-free vanaspati using chemical and enzymatic transesterification of vegetable oil blends addressed. They found that the transesterification of blends of vegetable oils resulted in SFC suitable for zero-*trans* Iranian vanaspati production with improved oxidative stability.

Palm stearin and rambutan seed oil were used to produce vanaspati fat. Palm stearin (PS) was selected as it is a source of high-melting solid fat. Since vanaspati is produced through hydrogenation process, it generates undesirable *trans* fatty acids which have adverse effects on body health (Farmani et al., 2007). However, by using palm stearin in vanaspati production, no *trans* fatty acids were generated. By chemical interesterification blend of palm stearin and rambutan seed fat, physico-chemical properties of the fat blends were modified and no *trans* fatty acid was produced. Rambutan seed fat was selected as a source of arachidic acid with high melting temperature (76°C) which do not absorb in the body. Therefore, the vanaspati fat can be considered as a low-calorie fat. Several studies showed that chemical interesterification has been utilized to prepare low- or zero-*trans* plastic fats with great potential applications from blend of hard fats and liquid vegetable oils (Farmani et al., 2007; Mayamol et al., 2009; Soares et al., 2009; da Silva et al., 2010; Guedes et al., 2014; Naeli et al., 2016). In this study *trans*-free vanaspati was produced by chemical interesterification of binary blends of rambutan seed fat and palm stearin. Thus, the objectives of this study were as follows:

1. To determine the physiochemical properties of seed fat of three rambutan varieties (R4, R7 and Serjan) and select the suitable variety as the source of rambutan seed fat.
2. To optimize the reaction parameters of chemical interesterification of binary blends of rambutan seed fat (RSF) with palm stearin (PS) namely rambutan seed fat ratio (100:0, 90:10, 75:25, 60:40 and 50:50%), reaction temperature (60, 66, 75, 84 and 90 ^{o} C) and reaction time (30, 36, 45, 54 and 60 min) using RSM to produce a vanaspati-like modified fat.
3. To evaluate and compare the storage stability of experimental vanaspati produced from chemically-interesterified fat blend of rambutan seed fat and palm stearin with a commercial vanaspati through three months of storage at 25 ^{o} C.

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