Moringa oleifera Lam. KERNEL OIL REFINING AND APPLICATIONS IN EMULSION-BASED FOOD PRODUCTS

By

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Food security is a complex issue to resolve, where it is important to increase food production at the same time reducing harmful effect on the environment. Moringa oleifera (MO) is potentially one of the most valuable crops in addressing food security issues. Specifically, the mature seed kernels contain Moringa oleifera kernel oil (MOKO) which is a high-oleic oil composed of 70-75% oleic acid. The oil needs to undergo refining process prior to its application in food products. Thus, this study aimed to: 1) determine the effect of individual refining stages of degumming (water- and acid-), neutralization (10 and 15) °Bé NaOH, bleaching (acid-activated bleaching earth [BE] type A and B; and a natural one), and deodorization (200, 235 and 270) ˚C on the physicochemical and oxidative properties of MOKO, 2) determine the effect of multiple refining stages on the physicochemical and oxidative properties of MOKO of different grades, and 3) determine the effect of different refined MOKO-soybean oil (SBO) blend ratios on the physicochemical properties of mayonnaises during storage. Acid degumming contributed to better oxidative properties as compared to water degumming, except for AV ($p < 0.05$) which were 4.233 ± 0.021 mgKOH/g and 4.158 ± 0.027 mgKOH/g, respectively. Neutralization using 15 °Bé NaOH performed better for all oxidative properties than that of 10 °Bé. Acid-activated BE type B resulted in lowest AV (3.965 ± 0.022 mgKOH/g) and MC (0.007 ± 0.001 %) as compared to other BEs. Deodorization at 270˚C resulted in lowest AV (2.439 ± 0.096 mgKOH/g) and MC (0.002 ± 0.000 %) than the use of other lower temperatures. Neutralization (15 °Bé NaOH) was better than deodorization (270˚C) in lowering the AV. Therefore, a combined series of refining stages comprising acid degumming, neutralization by using 15 °Bé NaOH, and bleaching using acid-activated BE Type B were further carried out on two crude MOKO batches (Batch Y and Z) of different grades. Significant decrease ($p < 0.05$) was observed in phosphorus content (98.20%; 97.18%), PV (66.67%; 50.00%), MC (85.71%; 85.71%) and AV (99.57%; 99.57%) in both refined Batch Y and Z, respectively. The IV (80.94-81.03) and FAC were unaffected except for the saturated and unsaturated fatty acids of Batch Z. The refined MOKO color

was lighter based on significant changes ($p < 0.05$) in the L*, a*, and b* values, while the total tocopherol content significantly increased ($p < 0.05$) in both Batch Y (52.28 %) and Batch Z (23.25 %). Different blend ratios of refined MOKO-SBO did not significantly affect ($p > 0.05$) the physicochemical properties of mayonnaises except for their color and rheological properties at storage week 0 and week 6. The MOKO-SBO blend at 50:50 (w/w) ratio (i.e., MOKO 50%) exhibited desirable properties especially due to its lowest droplet size on day 0, insignificantly difference ($p > 0.05$) in droplet size with other MOKO-based mayonnaises after 6 weeks, and its highest crossover value. In overall, the establishment of refining processes for MOKO is advantageous for the oil to be applied in various types of food products.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENAPISAN MINYAK ISIRONG *Moringa oleifera* Lam. DAN APLIKASI DALAM PRODUK MAKANAN YANG BERASASKAN EMULSI

Oleh

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Keselamatan makanan adalah isu yang komples untuk diselesaikan, di mana ia adalah penting untuk meningkatkan pengeluaran makanan pada masa yang sama mengurangkan kesan berbahaya kepada alam sekitar. Moringa oleifera (MO) berpotensi sebagai salah satu tanaman yang paling berharga dalam menangani isu keselamatan makanan. Minyak isirong Moringa oleifera (MOKO) adalah minyak beroleik tinggi, dengan kandungan asid oleik sebanyak 70-75%. Minyak perlu menjalani proses penapisan sebelum digunakan dalam produk makanan. Oleh itu, kajian ini bertujuan untuk: 1) menentukan kesan penapisan individu iaitu penyahgaman (air- dan asid-), peneutralan (10 dan 15) °Bé NaOH, pelunturan (peluntur bumi [BE] diaktifkan asid jenis A dan B; dan semulajadi), dan penyahbauan (200, 235, dan 270) ˚C keatas sifat fizikokimia dan oksidatif MOKO, 2) menentukan kesan pelbagai peringkat penapisan ke atas sifat fizikokimia dan oksidatif MOKO daripada gred yang berbeza, dan 3) menentukan kesan perbezaan nisbah campuran MOKO ditapis-minyak kacang soya (SBO) keatas sifat fizikokimia mayonis semasa penyimpanan. Penyahgaman asid menyumbang kepada sifat oksidatif yang baik berbanding dengan penyahgaman air, kecuali untuk AV ($p < 0.05$) iaitu masing-masing dengan 4.233 ± 0.021 mgKOH/g and 4.158 ± 0.027 mgKOH/g. Peneutralan menggunakan 15 °Bé NaOH menunjukkan prestasi yang lebih baik daripada 10 °Bé untuk semua sifat pengoksidaan. BE diaktifkan asid jenis B menghasilkan AV (3.965 ± 0.022 mgKOH/g) dan MC terendah (0.007 ± 0.001 %) berbanding BE yang lain. Penyahbauan pada 270˚C menghasilkan AV (2.439 ± 0.096 mgKOH/g) dan MC terendah (0.002 ± 0.000 %) berbanding penggunaan suhu lain yang lebih rendah. Peneutralan (15 °Bé NaOH) adalah lebih baik daripada penyahbauan (270˚C) dalam menurunkan AV. Oleh itu, satu siri gabungan peringkat penapisan yang terdiri daripada penyahgaman asid, peneutralan dengan menggunakan 15 °Bé NaOH, dan pelunturan menggunakan BE diaktifkan asid

jenis B telah dijalankan pada dua kumpulan MOKO mentah (Kumpulan Y dan Z) daripada gred yang berbeza. Bagi kedua-dua Kumpulan Y dan Z yang ditapis, masing-masing menunjukkan penurunan yang signifikan ($p < 0.05$) dalam kandungan fosforus (98.20%; 97.18%), PV (66.67%; 50.00%), MC (85.71%; 85.71%) and AV (99.57%; 99.57%). IV dan FAC tidak terjejas kecuali asid lemak tepu dan tak tepu pada Kumpulan Z. Warna MOKO yang ditapis adalah lebih cerah berdasarkan perubahan signifikan ($p < 0.05$) dalam nilai L*, a*, dan b*, manakala jumlah kandungan tokoferol meningkat dengan signifikan ($p < 0.05$) dalam kedua-dua Kumpulan Y (52.28 %) dan Kumpulan Z (23.25 %). Perbezaan nisbah campuran MOKO ditapis-SBO tidak memberi kesan signifikan ($p > 0.05$) ke atas sifat fizikokimia mayonis kecuali pada warna dan sifat reologinya pada minggu penyimpanan 0 dan 6. Campuran MOKO-SBO pada nisbah 50:50 (b/b) (iaitu, MOKO 50%) menunjukan sifat yang diingini terutamanya disebabkan saiz titisannya yang paling rendah pada hari 0, saiz titisan yang tidak signifikan ($p > 0.05$) dengan mayonis yang lain yang juga berasaskan MOKO selepas 6 minggu, dan nilai silangnya yang tinggi. Secara keseluruhannya, penubuhan proses penapisan untuk MOKO adalah bermanfaat untuk minyak tersebut untuk digunakan dalam pelbagai jenis produk makanan.

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LIST OF ABBREVIATIONS

Ab	Absorbance
AD	Acid degumming
ANOVA	Analysis of variance
AnV	Anisidine value
AOCS	American Oil Chemists' Society
AV	Acid value
Bé	Baume caustic
BE	Bleaching earth
BO	Butter oil
C-H	Carbon-hydrogen
$D_{4,3}$	Volume weighted mean diameter
DPPH	1,1-diphenyl-2-picrylhydrazl
DNB	Degumming-neutralization-bleaching
EVOO	Extra virgin olive oil
FA	Fatty acids
FAC	Fatty acid composition
FAME	Fatty acid methyl esters
FFA	Free fatty acids
G'	Storage modulus
G"	Loss modulus
GC-MS	Gas chromatography-mass spectrometry
h	Hour
HCL	Hydrochloric acid
HDL	High density lipoprotein
HPL	Hydratable
HPLC	High-performance liquid chromatography
LDL	Low density lipoprotein

LVE	Linear viscoelastic
IV	Iodine value
KOH	Potassium hydroxide
MC	Moisture content
Mins	Minutes
MO	*Moringa oleifera*
MOKO	*Moringa oleifera* kernel oil
MPOB	Malaysian Palm Oil Board
MUFA	Monounsaturated fatty acids
NaOH	Sodium hydroxide
NHPL	Nonhydratable
NR	Not reported
O/W	Oil-in-water
p-AV	*p*-Anisidine value
pH	Potential of hydrogen
PKDG	Palm kernel based-diacylglycerol
PKO	Palm kernel oil
POL	Palm olein
POL-DAG	Palm olein-based diacylglycerol
PO	Palm oil
PS	Palm stearin
PUFA	Polyunsaturated fatty acid
PV	Peroxide value
RBD	Refined, bleached and deodorized
SBO	Soybean oil
SFA	Saturated fatty acids
SFC	Solid fat content
SFO	Sunflower oil

TAG	Triacylglycerol
TBA	Thiobarbituric acid
TOP	Total degumming process
TOTOX	Total oxidation
VCO	Virgin coconut oil
WD	Water degumming
w/v	Volume concentration
w/w	Mass fraction (mass/mass)

CHAPTER 1

INTRODUCTION

1.1 Background

Moringa oleifera (MO) is the most widely cultivated species of the Moringaceae family (Kou et al., 2018). The plant is native to northern India, and is widely distributed throughout the tropics and subtropics including Africa, Asia, Central and South America due to its strong resistance to drought (Falowo et al., 2018). Almost every part of the plant (leaves, root bark, stem bark, immature pods, and mature seeds) is edible and valuable. The upsurge in demand of food requires the need to boost the overall production of different source of edible oils (Manzoor et al., 2007). From an economic point of view, MO is potentially one of the most valuable crops for food, industrial, agricultural, and medicinal uses, especially for addressing food security issues in countries where hunger and malnutrition are major problems (Kou et al., 2018). Therefore, we mainly bring our focus on MOKO as it has many health benefits whilst addressing the food security issues.

One of the key parts of MO is its seed kernel, which can contain up to 35% (w/w) protein and 40% (w/w) edible oil (Mat Yusoff et al., 2016). Many studies reported on the physicochemical and oxidative properties of MO kernel oil (MOKO). These studies attributed its high stability to the presence of up to 75% oleic acid which is comparable to olive oil, and significant amounts of tocopherol and sterol (Dinesha et al., 2018; Mat Yusoff et al., 2020). A diet rich in monounsaturated fat as a substitute for saturated fat is a strong and continuing trend due to its beneficial impact on coronary heart disease and overall heath (Gillingham et al., 2011). With reference to these findings, MOKO can potentially be developed and commercialized as healthy and functional food product. It can also be promoted as a new source of oil in tropical and subtropical countries since the tree grows rapidly under a wide range of climatic conditions with little or no agricultural input (Falowo et al., 2018). Nadeem & Imran (2016) incorporated the oil in food products including vanaspati and shortening, while Dollah et al., (2016) used it in a margarine formulation. Additionally, in terms of frying stability, the MOKO was comparable with that of palm olein and was better than that of both canola oil and soybean oil (Abdulkarim et al., 2007).

Despite these advantages, very limited studies reported on how MOKO was refined prior to its use, thus limited commercialization of fat-based food products incorporated with the oil. The need to refine MOKO has not been addressed systematically in literature. Different types of oil will require different refining methods. Refining is critical to produce and preserve oil of edible quality (Vaisali et al., 2015). Robust refining processes retain the most desirable components in the oil, and eliminate detrimental and off-flavor-producing compounds which, in turn, allows the oil to remain bland and neutral in taste over the course of its shelf

life lasting several months. Sánchez-Machado et al. (2015) have reported on degumming and neutralization of MOKO originating from South Sonora, Mexico. But there is a significant knowledge gap. In addition to degumming and neutralization, it is also necessary to investigate deodorization, and the effect of different refining stages on the oil's acid value which is a critical parameter in an oil refining. Furthermore, MOKO contains significant levels of tocopherol, and the effects of different refining stages on tocopherols is not known. A systematic study of refining alternatives and their effects on the final MOKO quality is therefore necessary in order to exploit its full potential.

The refined MOKO can further be used in development of fat-based food products. Therefore, in this study, the refined MOKO was further blended with soybean oil (SBO) for potential development of high-oleic mayonnaise with enhanced nutritional properties particularly vitamin E. Mayonnaise is an oil-in-water emulsion-based food product with minimum of 65% (w/w) fat content (Food Regulations, 1985). The fat portion plays critical role in affecting the physical properties of mayonnaise which may diversify over storage (Patil & Benjakul, 2019), besides contributed to its desirable texture and flavor upon consumption (Rahmati et al., 2014). In 2021, the global market size of mayonnaise had reached US$ 11.8 Billion. The market is looking forward to reach US$ 15.1 Billion by the year 2027 (Businesswire, 2022). This increasing consumption trend has led to the selection of mayonnaise as the product of interest in this study.

1.2 Objectives

Given the above background, the main objectives of this study were:

i) To determine the effect of individual refining stages, mainly degumming, neutralization, bleaching, and deodorization on the physicochemical and oxidative properties of MOKO.
ii) To determine the effect of multiple refining stages on the physicochemical and oxidative properties of MOKO of different grades.
iii) To determine the effect of different refined MOKO-SBO blend ratios on the physicochemical properties of mayonnaises during storage. There may be a preamble at the beginning of a chapter. The purpose may be to introduce the themes of the main headings.

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