UNIVERSITI PUTRA MALAYSIA

AN ENZYMATIC TRANSESTERIFICATION OF REFINED, BLEACHED AND DEODORIZED PALM OIL DURING DRY-FRACTIONATION IN A PILOT-SCALE FRACTIONATION PLANT

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By

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Enzymatic transesterification of refined, bleached and deodorized (RBD) palm oil during dry-fractionation was studied using commercially prepared immobilized lipase from *Rhizomucor miehei* (Lipozyme IM 60, Novozyme, Bagvaerds, Denmark). A 5-liter pilot-scale fractionation apparatus was used. The enzyme concentrations used were of 1, 1.5, 2.0, 2.5, 3.5 and 4.5 wt%, and was added into the crystallizer of the fractionation apparatus along with the RBD palm oil. Each transesterification process was carried out in five replicates for 12 hours based on a typical cooling profile for palm oil fractionation in palm oil refineries. The initial temperature of the cooling process was 65°C and the final temperature was 25°C. After the cooling process, the liquid fraction was extracted from the transesterified RBD palm oil through filtration using a piece of commercial filter-cloth of 600μm porosity (Netzsch, Selb, Germany).

Samples of RBD palm oil (Control 1), transesterified (enzyme-reacted) RBD palm oil, commercial RBD palm olein (Control 2) and the liquid fraction extracted from the transesterified RBD palm oil were taken after every batch-run. Prior to analysis, the immobilized enzyme was removed from the transesterified materials by filtration using a piece of commercial filter-cloth of 600μm porosity (Netzsch, Selb, Germany). The samples were then analyzed for their physical and chemical properties: iodine value (IV), slip melting point (SMP), cloud point (CP), free fatty acid content (%FFA), triglyceride (TG) profile, fatty acid (FA) composition and thermal behavior.

The results show that the RBD palm oil obtained after transesterification was not significantly different ($P > 0.05$) when compared to Control 1. The liquid fraction extracted from the transesterified palm oil also did not show any significant differences ($P > 0.05$) when compared with commercial RBD palm olein. The chemical and physical properties of the transesterified palm oil show that the quality oil was similar to the RBD palm oil. Similarly, the chemical and physical properties of the liquid fraction extracted from the transesterified palm oil showed similar results with those of commercial RBD palm olein.

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

TRANSESTERIFIKASI BERENZIM BAGI MINYAK KELAPA SAWIT TERTULEN, TERNYAH WARNA AND TERNYAH BAU SEMASA FRAKSINASI-KERING DALAM LOGI FRAKSINASI SKALA-PEPANDU

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Transesterifikasi berenzim minyak kelapa sawit yang tertulen, ternyah warna dan ternyah bau [refined, bleached and deodorized (RBD)] semasa fraksinasi-kering telah dikaji dengan menggunakan lipase tersekat-gerak dari *Rhizomucur miehei* (Lipozyme 1M60, Novozyme, Bagvaerds, Denmark). Logi fraksinasi skala-pepandu 5 liter digunakan dalam kajian ini. Enzim berjumlah 1, 1.5, 2.0, 2.5, 3.5 dan 4.5 berat % ditambah ke dalam penghablur ('crystalizer') bersama dengan minyak kelapa sawit RBD di dalam alat fraksinasi. Setiap proses transesterifikasi dijalankan sebanyak 5 replikasi selama 12 jam berdasarkan kepada profil penyejukan yang tipikal untuk fraksinasi minyak kelapa sawit di kilang minyak kelapa sawit. Suhu awal dalam proses penyejukan adalah 65°C dan suhu akhir pula adalah 25°C. Selepas proses penyejukan, pecahan cecair dari minyak

kelapa sawit RBD tertransesterifikasi berenzim dikeluarkan secara penapisan dengan menggunakan kain penapis saiz liang 600μm (Netzsch, Selb, Germany).

Sampel minyak kelapa sawit RBD (Kawalan 1), minyak kelapa sawit RBD tertransesterifikasi berenzim, minyak olein RBD komersil (Kawalan 2) dan pecahan cecair dari minyak kelapa sawit RBD tertransesterifikasi berenzim di keluarkan selepas setiap proses fraksinasi dilakukan. Sebelum analisis, enzim dikeluarkan dari minyak transesterifikasi secara penapisan dengan menggunakan kain penapis saiz liang 600μm (Netzsch, Selb, Germany). Sampel-sampel tersebut dianalisis bagi sifat-sifat fizikal dan kimianya: nilai iodin (IV), takat lebur gelincir (SMP), takat awan (CP) serta nilai asid lemak bebas (%FFA), profil trigliserida (TG), komposisi asid lemak (FA) dan sifat termal.

Keputusan menunjukkan bahawa minyak kelapa sawit RBD tertransesterifikasi berenzim tidak menunjukkan perbezaan ($P > 0.05$) yang ketara apabila dibandingkan dengan Kawalan 1. Pecahan cecair dari minyak kelapa sawit RBD tertransesterifikasi berenzim juga tidak menunjuk perbezaan ($P > 0.05$) yang ketara apabila dibandingkan dengan minyak olein RBD komersil. Ciri-ciri kimia dan fizikal minyak kelapa sawit RBD tertransesterifikasi berenzim menunjuk bahawa mutunya adalah sama dengan minyak kelapa sawit RBD. Ciri-ciri kimia dan fizikal pecahan cecair dari minyak kelapa sawit RBD tertransesterifikasi berenzim juga menunjuk persamaan dengan ciri-ciri minyak olein RBD komersil.

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ABBREVIATIONS

α	Alfa
β	Beta
β′	Beta prime
CN	Carbon number
CPO	Crude palm oil
DG	Diglyceride
DSC	Differential Scanning Calorimetry
FA	Fatty acid
FAME	Fatty acid methyl ether
FFA	Free fatty acid
GC	Gas chromatography
HPLC	High performance liquid chromatography
IV	Iodine value
L	C18:2; linoleic acid
La	C12; lauric acid
M	C14; myristic acid
MG	Monoglyceride
O	C18:1; oleic acid
OLL	Oleoyl-dilinolein
OOL	Linoleoyl-diolein
OOO	Triolein

P	C16:1; palmioleic acid
PLP	Linoleyl-diplamitin
POL	Palmitoyl-oleoyl-linolein
POO	Palmitoyl-diolein
POP	Oleoyl-dipalmitin
PORLA	Palm Oil Registration and Licensing Authority
PORIM	Palm Oil Research Institute Malaysia
PPP	Tripalmitin
RBD	Refined, Bleached and Deodorized
S	C18; stearic acid
SOO	Stearoyl-diolein
SOS	Oleoyl-distearin
TG	Triglyceride

Chapter 1

Introduction

Palm oil is derived from the mesocarp of oil palm fruit. The palm, known as *Elaeis Guineensis*, originates from West Africa. The palm is the most prolific oil producing plant and the national average yield in Malaysia is 4 tones per hectare per year with 11% of that quantity as palm kernel oil, a co-product (Ong, 1995). The production of palm oil in the world for 2001 was around 16 million tones of which Malaysia and Indonesia are the two largest producers (Lim, 2002). Currently, it was stated that Malaysia has its palm output of 9.6 million tones during 2001 (Lim, 2002). With the growth production yield of 6.78% per year, it is expected that by the year 2002, palm oil will be as important as soyabean oil (Ong, 1995; Lim, 2001; Lim, 2002).

The usage of palm oil has also developed through out the years. It has become a prime product in the field of food industry as well as oleo-chemical industry. However, 90% of palm oil is used in food products (Ong, 1995). Its physical properties as a semi-solid vegetable oil make it particularly suitable for margarines, bakery shortenings and some confectionery fats (Ong, 1995).

With the daily increase in demand for palm oil, refineries have been thriving to produce the best quality oil in the industry, as well as providing a

marginal profit through the efficiency of the refining process The refineries received crude palm oil (CPO) from the miller CPO is composed mainly of triglyceride (TG) molecules, to which various fatty acids (FA) are attached The CPO is set through various stages of refining process until the quality of the oil is achieved The refining process involve is degumming, bleaching, deacidification and fractionation of the CPO (Krishnamurthy and Kellens, 1996) Degumming and bleaching process are processes that removed non-glyceride compounds such as phosphatides and other contaminants The deacidification process is used to remove or to reduce free fatty acids (FFA) that cause undesirable odor in the oil (Cowan, 1976, Lawson, 1995) Once the CPO has gone through these steps, it is called refined, bleached and deodorized palm oil or RBD palm oil in short The last step of refining would be fractionation, where RBD palm oil is separated into its liquid and solid fraction, palm olein and palm stearin, respectively (Krishnamurthy and Kellens, 1996)

Commercially, fractionation of palm oil can be separated into two different processes, dry fractionation and detergent fractionation The dry process uses direct filtration of the crystals, and the detergent process uses an aqueous detergent solution to separate the crystals from the olein by centrifugation However, for detergent process palm oil is fractionated in its crude form, since the olein and stearin will require full refining to remove traces of detergent (Deffence, 1985)

Currently, dry fractionation process is the most popular process among the refiners due to its low operating cost.

The dry fractionation process plants include Bernadini, De Smet and Tirtiaux fractionation plant (Bek-Nielsen & Krishnan, 1977; Deffence, 1985). All three plants emphasize on cooling the oil until the liquid and the solid can be differentiated. The mixture is then sent to a vacuuming filter to separate the liquid and the solid.

The fractionation process of palm oil refinery has improved through out the years. The temperature sensor of the oil has been linked to a computerized recorder, which has a cooling profile for the oil programmed, thus, enabling a more efficient and cost saving fractionation plant. The Florentine filter has also developed for the past years. Currently, the membrane filter press has replaced the Florentine filter for its separating purpose. The membrane filter press consists of a high-pressured vertical cell chambers. The RBD palm oil is sent through these cells. Once the cell is loaded, the olein is purged out through the use of compress air, thus, leaving the stearin inside the chambers, which is transferred to another section of the plant. The membrane filter press is an alternative to provide a higher yield of olein for the refinery. However, at the end of the day, what is important is still the cooling profile that is programmed within the computerized recorder and the quality of the crude palm oil received to grant a better fractionation.

The composition of the CPO produced in Malaysia consists of 6.8% trisaturated TG, 48% disaturated TG, 34.6% monosaturated TG and 10.2% triunsaturated TG (Timms, 1987). According to DeMan (1999), trisaturated TG and triunsaturated TG are easier to fractionate compared to disaturated and monosaturated TG. Triunsaturated TG and monosaturated TG are liquid, while trisaturated TG and disaturated TG are solid at room temperature. However, in palm oil fractionation, it is difficult to obtain an olein fraction that is free from disaturated TG, as disaturated TG and monosaturated TG have a relatively close melting point (DeMan, 1999). Disaturated TG and trisaturated TG are the main cause of cloudiness is palm olein at low temperature (Swe et al., 1995; Siew and Ng, 1996; Sulaiman et al., 1997). To overcome such problem given by the chemical structure of the oil, many have turned to double fractionation or even transesterification process (Affandi, 1993; Pantzaris, 1987).

The transesterification process can change the chemical structure of the oil. The process involves the exchange of a FA from one TG molecule with a fatty acid from another TG molecule (Macrae, 1983; Marangoni et al., 1993). This process can be done through the use of chemicals or through the use of enzymes. The use of enzyme to catalyze transesterification reactions has received considerable attention lately because of certain advantages over chemical processes. By using enzyme, reactions can be done at lower temperatures, the product obtain are cleaner

and waste production is reduced (Rosendal, 1989; Yamane, 1987; Mittelbach, 1990). According to Macrae (1983), enzyme that is used in fats and oils, can be 1,3-regiospecific or non-specific towards the position of the acyl group of TG during reaction, or they can posses selectivity toward particular types of FA. By using 1,3-specific lipases, the exchange of acyl moieties is reported to confine to the *sn*-1- and *sn*-3- positions of the glycerol molecule. Ghazali et al. (1995) reported that by using lipases, at low-moisture content, the transesterification process resulted in formation of trisaturate, as well as an increase in the concentration of triunsaturate.

By using lipases to rearrange the fatty acid position of the TG molecules of the oil, it is possible to separate the saturated and the unsaturated part of the oil, thus, enabling better fractionation. At present, there are no refineries using lipase as part of their process to improve the quality of the oil. Therefore, it is the main objective of this study to use food grade lipase to transesterify the RBD palm oil within the palm oil fractionation plant to improve the composition of the oil. The model laboratory work of this study was experimented by Zakeri (2002). In Zakeri's (2002) work, the effects of various organic solvents with different log P values such as dimethylsulphoxide, dimethylformamide, isopropanol, tetrahydrofuran, toluene, cyclohexane, heptane on lipase-catalyzed transesterification of palm oil was used. Thus, based on Zakeri (2002) work, this study will concentrate on a larger experimental scale without the use of solvent,

which is not the common practice in refineries The procedures are to be carried out at a pilot scale fractionation plant and it is to follow exactly how normal fractionation plant would operate Thus, any changes of the 'new' oil can be detected through its chemical and physical properties This study may be the key to improve the quality of the oil, as well as providing the refiners a better and cheaper way of refining palm oil

CHAPTER 2

LITERATURE REVIEW

2.1 Palm Oil

2.1.1 Structure of Oil

Fats and oils have been considered to be one of many sources in homemade as well as industrial food preparations. Glycerols and FAs (long chain aliphatic mono-carboxylic acids) mainly form the structure of fats and oil. There are three FA chains (represented by HOOCR1, HOOCR2 and HOOCR3), which can be esterified, with one molecule of glycerol, as shown in Figure 1. Thus, fats are in short TGs (Hoffmann, 1989).

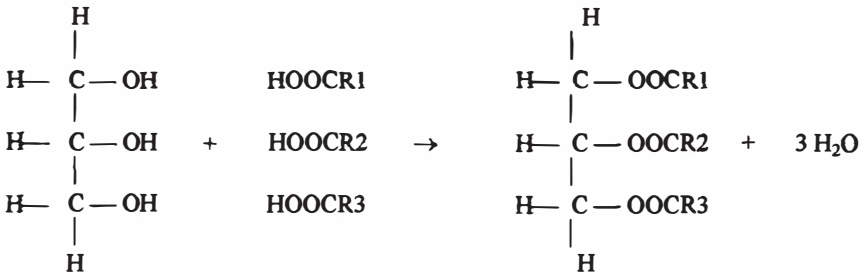

Figure 1. Formation of a TG

(Source: Swern, 1964)

2.1.2 Fatty Acid (FA).

The definition of FAs can be best described as straight chain aliphatic carboxylic acids with four or more atoms (Hoffmann, 1989). FAs can be separated to two different types; saturated and unsaturated FAs. Both have different physical and chemical properties.

2.1.2.1 Saturated FAs

Saturated FAs are termed as FAs with all the carbon valencies that can be positioned independently, except in the carboxylic acid group (Hoffmann, 1989). Physically, FA changes state from liquid to solid through its chain length. Thus, the longer the chain length, the higher the melting point and the boiling point. However, the density would decrease (Hoffmann, 1989). The short chain saturated FA can be distilled with ease at atmospheric pressure, when compared with long chain FAs. It can be distilled easily through the use of steam (Hoffmann, 1989). This is due to the fact that as the CN increases, solubility in water decreases. In short, the solubility of FA mainly depends upon the affinity of the acid to the solvent; different solvents being more or less polar in character (Hoffinann, 1989).

The chemical properties of FAs can be easily transformed by the use of soaps, which can be defined as the corresponding alkalis. This reaction can be performed even in heterogeneous phases. Even though saturated FAs have their