PRODUCTION OF CRUDE PALM OIL THROUGH MICROWAVE PRETREATMENT *CUM* SOLVENT EXTRACTION

JASON TAN CHIA XERN

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UNIVERSITY OF MALAYA
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ABSTRACT

Conventional palm oil milling involves multiple steps after fruit collection and the oil clarification step involves introduction of large amount of water into the pressed oil, which results in large amount of wastewater. The present study investigated a combined process of pretreatment using microwave and solvent extraction to mill crude palm oil, with no introduction of water or steam. High oil yield was obtained from fresh palm fruits through microwave pretreatment in a 42 L, 1000 W and 2450 MHz microwave oven; and followed by hexane extraction. Besides obtaining up to 30% oil yield, the established optimum condition (microwave pretreatment of 10 min, solvent extraction of 12 h) also produced oil with low free fatty acid (<1.0%) and acceptable anisidine value (<3.0 meq/kg). The produced oil has a novel fatty acid composition that does not resemble conventional crude palm oil and crude palm kernel oil. The leached oil during microwave pretreatment has 6.3% lauric acid, while the subsequently solvent extracted oil has 1.5%. Factors affecting the oil quality were investigated and duration of the microwave pretreatment is the major attribution to poor oil quality. The duration has to be optimized to result in ruptured fruitlets to ensure solvent extraction effectiveness; but not to the extent of causing oxidation. The present study suggests that the microwave pretreatment should not be longer than 12 min as the oil produced after 15 mins contains oxidation products, namely, 1-methylcyclopentanol (12.96%), 1-tetradecanol (9.44%), 1-nonadecene (7.22%), nonanal (7.13%) and 1-tridecene (5.09%), which is postulated to be the results of fibre degradation to oxidation products. In summary, the findings in the present study show that microwave pretreatment is a potential alternative milling process for crude palm oil, with advantageous omission of wet treatment with steam in the current conventional and commercial process.

ABSTRAK

Pengilangan minyak sawit konvensional melibatkan penggunaan air dalam kuantiti yang besar yang akan menjadi air sisa pada akhirnya. Penyelidikan ini mengkaji kombinasi antara pemanasan menggunakan gelombang mikro dan pengekstrakan oleh pelarut untuk menghasilkan minyak sawit tanpa penglibatan air. Hasil minyak setinggi 30% dapat diperolehi melalui pemanasan gelombang mikro dalam sebuah ketuhar gelombang mikro (42 L, 1000 W dan 2450 MHz) dan pengekstrakan oleh heksana dalam keadaan optimum (10 minit pemanasan gelombang mikro dan 12 jam ekstraksi heksana). Selain memperolehi hasil minyak yang tinggi, minyak yang dihasilkan juga mempunyai asid lemak bebas (<1.0%) dan nilai anisidin (<3.0 meq/kg) yang rendah. Kandungan asid lemak bagi minyak yang dihasilkan tidak menyerupai minyak sawit konvensional dan adalah unik. Minyak yang terhasil semasa pemanasan gelombang mikro mempunyai kandungan asid laurik sebanyak 6.3% manakala minyak yang diekstrak oleh pelarut mempunyai kandungan asid laurik sebanyak 1.5%. Faktor-faktor yang akan mempengaruhi kualiti minyak dikaji dan didapati bahawa faktor utama adalah tempoh pemanasan gelombang mikro. Tempoh pemanasan gelombang mikro perlu dioptimumkan untuk memecahkan buah kelapa sawit semasa pemanasan supaya efisiensi pengekstrakan oleh pelarut dapat dioptimumkan. Hasil kajian mendapati bahawa pemanasan gelombang mikro tidak harus melebihi 12 minit kerana minyak yang terhasil selepas tempoh tersebut akan mengandungi produk-produk pengoksidaan seperti 1-metilsiklopentanol (12.96%), 1-tetradekanol (9.44%), 1-nonadekene (7.22%), nonanal (7.13%) dan 1-tridekene (5.09%). Produk-produk yang tersebut dihipotesiskan sebagai produk yang terbentuk oleh degradasi serat dalam buah kelapa sawit. Secara ringkasnya, penemuan dalam penyelidikan ini mencadangkan bahawa pemanasan gelombang mikro berpotensi untuk menjadi proses alternatif bagi pengilangan minyak

sawit. Proses alternatif ini dapat mengelakkan rawatan basah dalam proses pengilangan konvensional.

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%	per cent
°C	degree Celsius
µ	micron
AEE	aqueous enzymatic extraction
AnV	anisidine value
BOD	biological oxygen demand
CDM	Clean Development Mechanism
CE	conventional extraction
CER	Certified Emission Reduction
cm	centimetre
CMAE	continuous microwave-assisted extraction
COD	chemical oxygen demand
CPO	crude palm oil
CSE	conventional solvent extraction
DAG	diacylglycerols
EQA	Environment Quality Act
FAME	fatty acid methyl esters
FFA	free fatty acids
FFB	fresh fruit bunches
FID	flame ionization detector
g	gram
g/mL	gram per millilitre
GC	gas chromatography
GHG	greenhouse gases
GHz	gigahertz

h	hour
HRT	hydraulic retention time
i.d.	internal diameter
IR	infrared
IT	induction time
kg	kilogram
L	litre
m	meter
M	mol dm^{-3}
m/v	mass by volume
m/z	mass-to-charge ratio
MAE	microwave-assisted extraction
MAEE	microwave and aqueous enzymatic extraction
MAG	monoacylglycerols
MASE	microwave-assisted solvent extraction
mbar	millibar
meq/kg	milliequivalent of oxygen per kilogram
MHz	megahertz
min	minute
mL	millilitre
mm	millimetre
MPa	mega pascal
MPOB	Malaysian Palm Oil Board
NBD	neutralized, bleached, deodorized
nm	nanometre
P	power

PL	phospholipids
POME	palm oil mill effluent
ppm	parts per million
psi	pounds per square inch
PV	peroxide value
RBD	refined, bleached, deodorized
s	second
SFE	supercritical fluid extraction
SRT	solid retention time
T	temperature
TAG	triacylglycerols
TBARS	thiobarbituric acid reactive substances
TDS	total dissolved solids
TOTOX	total oxidation
TSS	total suspended solids
v/v	volume by volume
W	watt
α	alpha
β	beta
ω	omega

CHAPTER 1: INTRODUCTION

1.1 Oil palm

Oil palm (*Elaeis guineensis*) is native to western Africa. Malaysia was introduced to the cultivation of oil palm in 1870 as an ornamental plant, and since 1960, the plantation had grown at a fast pace. Oil palm tree can reach as high as 20 m. They grow optimally at 24 - 27°C and humid climate. Palm fruits can be harvested from 4 years old onwards oil palm tree for as long as 50 years (Bockisch, 1998).

1.1.1 Palm fruits

In Malaysia, the *Tenera* (a hybrid species between *Dura* and *Pisifera*) species is widely cultivated owing to their higher oil yield. Harvesting is easier to be carried out due to the shorter palm tree and *Tenera* species tend to produce better fruit bunch and bearing higher oil content (Noor Azian, 1995). Structure of *Dura*, *Pisifera* and *Tenera* are as shown in Figure 1.1. It is noted that *Dura* species actually have a rather thick shell surrounding the kernel whereas *Pisifera* species has no shell. The *Tenera* species, however, has shell of intermediate thickness. Shell thickness has a major effect on the oil content. The *Tenera* has 30% more mesocarp and respectively owning 30% more oil content in bunches comparing to that of the *Dura* (Corley and Tinker, 2003).

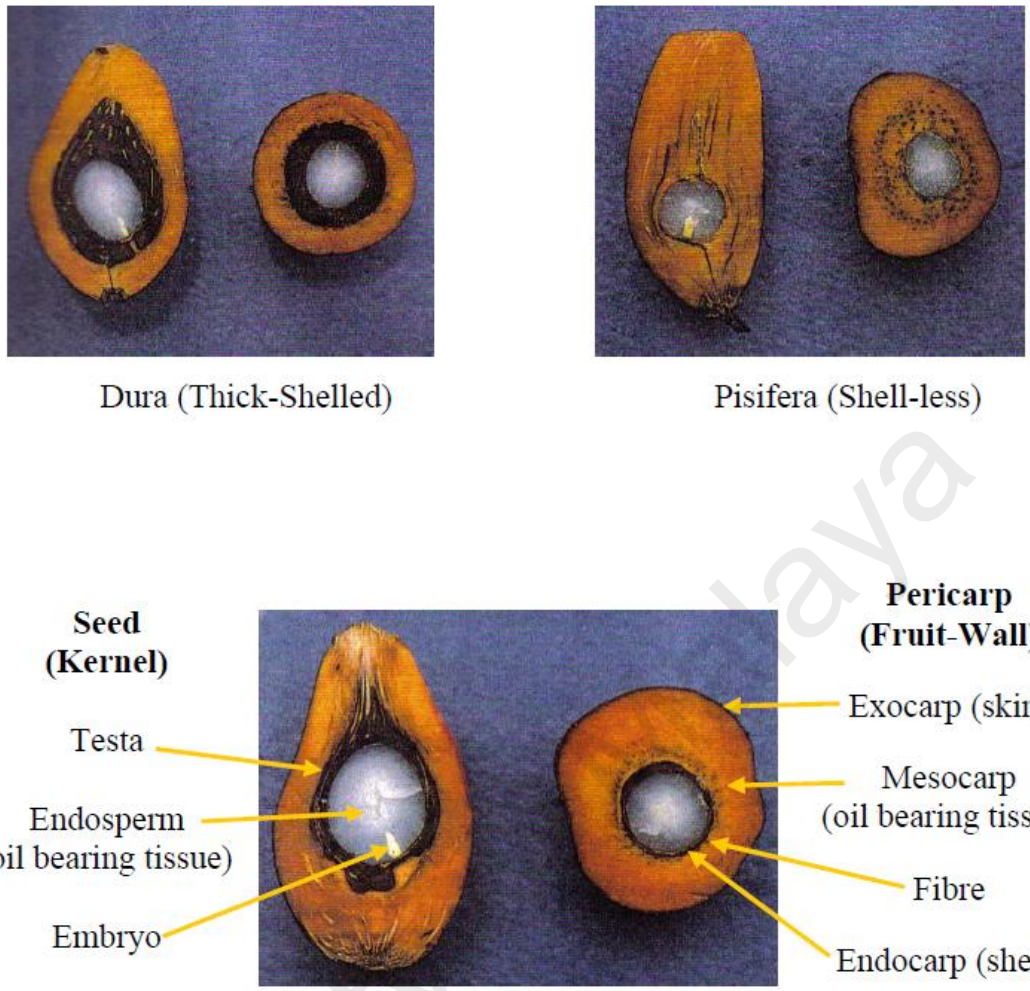

Figure 1.1: Structure of Dura, Pisifera and Tenera species (Noor Azian, 1995)

Bockisch (1998) reported that Asian palm fruits has a composition of 60% mesocarp, 29% skins and stems and 11% nuts. The mesocarp can be further broken down into 41% of water, 39% of oil and 20% of fibre. Natural fibres are composed of cellulose, hemicellulose and lignin, also known as lignocellulose.

1.1.2 Cellulose

Cellulose is a long chain polymer, a polysaccharide consisting of a few hundred to thousands of beta-glucoses (Jin, 2010). Figure 1.2 shows the molecular structure of a cellulose.

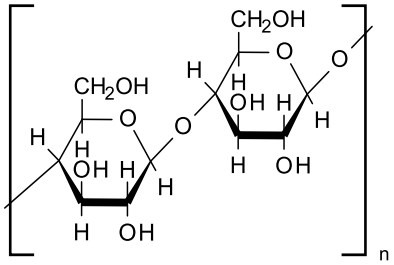

Figure 1.2: A portion of a cellulose chain

Cellulose is a polysaccharide that has a naturally arranged glucose units. Cellulose contains D-glucopyranoside units that are linked in (1→4) pattern in a very long, unbranched chain. Unlike starch and glycogen, which shared almost the same building blocks linked together through α(1→4) linkages, the linkages in cellulose are β-glycosidic linkages. This configuration of the anomeric carbon atoms of the cellulose makes cellulose chains linear (Solomons and Fryhle, 2007).

All organic compounds are vulnerable to degradation; cellulose is no exception to that. Though degradation of cellulose may take many forms, such as hydrolysis, enzymatic hydrolysis, anaerobic and thermal decomposition, all of them involves high cost, owing to its highly stable hemiacetal form. Jin (2010) also reported that the hydrolysis is difficult on cellulose due to its crystalline structure. However, these crystalline structures can be destroyed and turn into amorphous form under high temperature and pressure (>300°C, 25 MPa).

Hu, Wang and Yu (2004) reported that in low cellulolytic activities area, such as swamps, hydrolysis of cellulose is slow. A more efficient degradation of cellulose can be achieved through anaerobic degradation with rumen microorganisms in this situation. The highest efficiency value was achieved at pH 6.8 and 7.1 at 78%.

Valášková and Baldrian (2006) reported the usage of *P. betulinus* (a common wood-rotting fungus parasite) which caused the loss of dry mass up to 65% within 98 days. Its hydrolytic enzymes, which have wide substrate specifics, enable it to perform lignocellulose hydrolysis under a fast rate. The cellulolytic enzymes are produced during both fungal colonization of wood andwheat straw.

Dauenhauer, Colby, Balonek, Suzynski and Schmidt (2009) reported that thermochemical conversion process of biomass (contained up to 60% cellulose), such as pyrolysis, occurred at high temperatures (400 – 800°C). Figure 1.3 shows the formation mechanisms of furans and light oxygenates, explaining how major pyrolysis products are formed and the mechanisms for cellulose glycosidic bond cleavage, an important process in the depolymerisation of cellulose (Mettler *et al.*, 2011).

Shen, Fang and Chow (2006) reported that smoldering ignition of cellulosic materials is not yet well understood due to the lack of methods developed to deduce the necessary experimental data. Their result showed that spontaneous ignition of cellulosic materials varied from 210 to 497˚C. They also suggested that the time required to ignite different material is reliant on their relative values of thermal inertia, not from the ignition temperature.

Yan and Qi (2014) reported that hydrothermal degradation of homogeneous alkaline aqueous solution of cellulose at 160 – 300˚C produced malonic acid, lactic acid, formic acid and acetic acids through a simple chemical route (Figure 1.4).

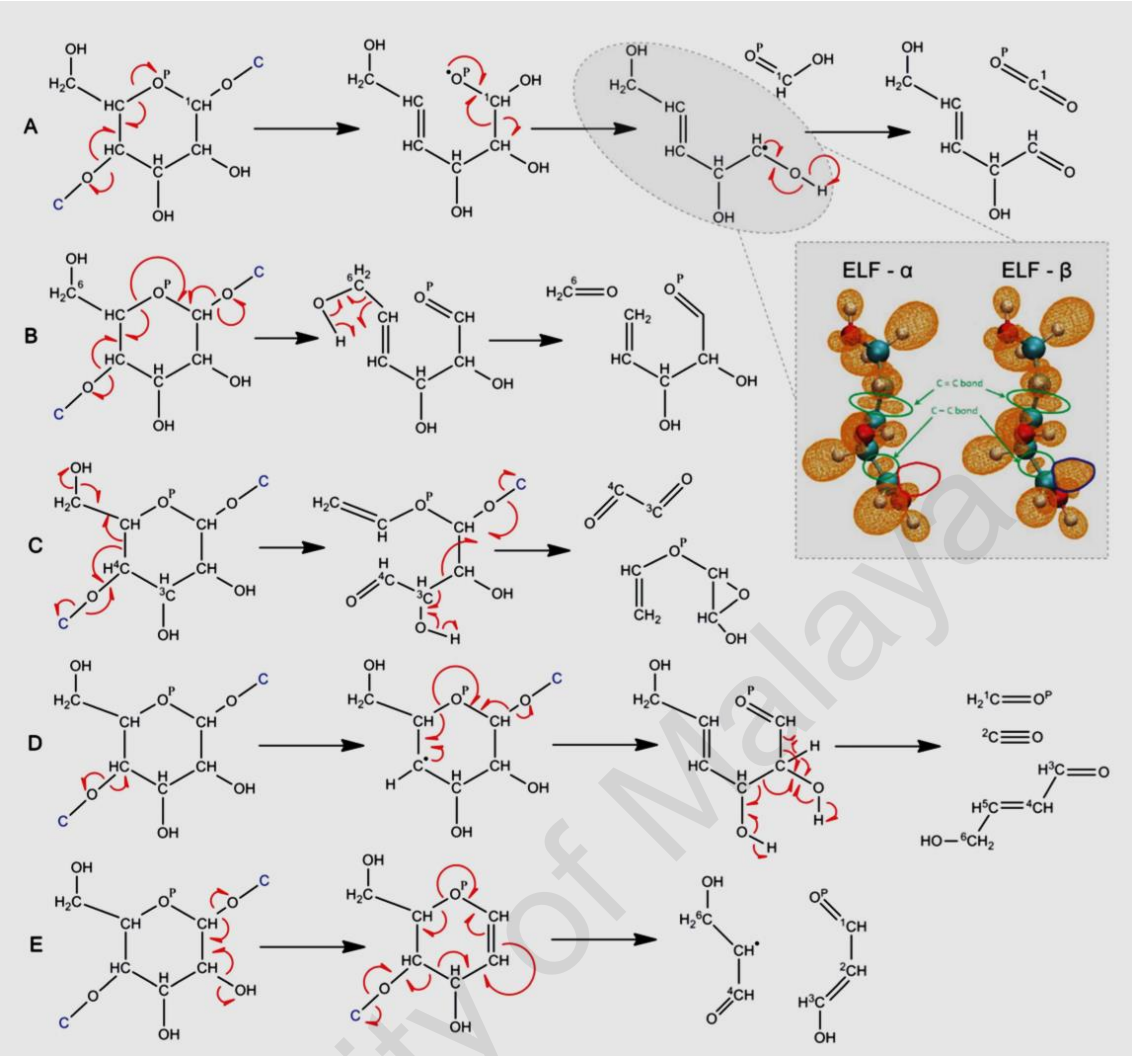

Figure 1.3: Cellulose pyrolysis pathway to volatile oxygenates (Mettler *et al.*, 2012)

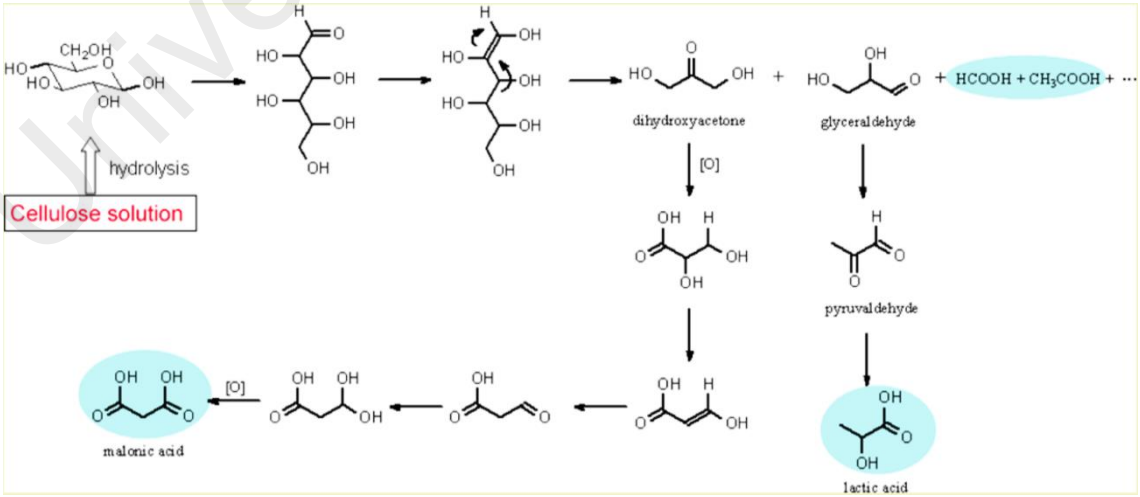

Figure 1.4: Hydrothermal degradation of homogeneous alkaline solution of cellulose (Yan and Qi, 2014)

1.2 Palm oil

1.2.1 Palm oil milling and refining in Malaysia

Conventional palm oil milling in Malaysia consists of a combination of physical and mechanical processes.

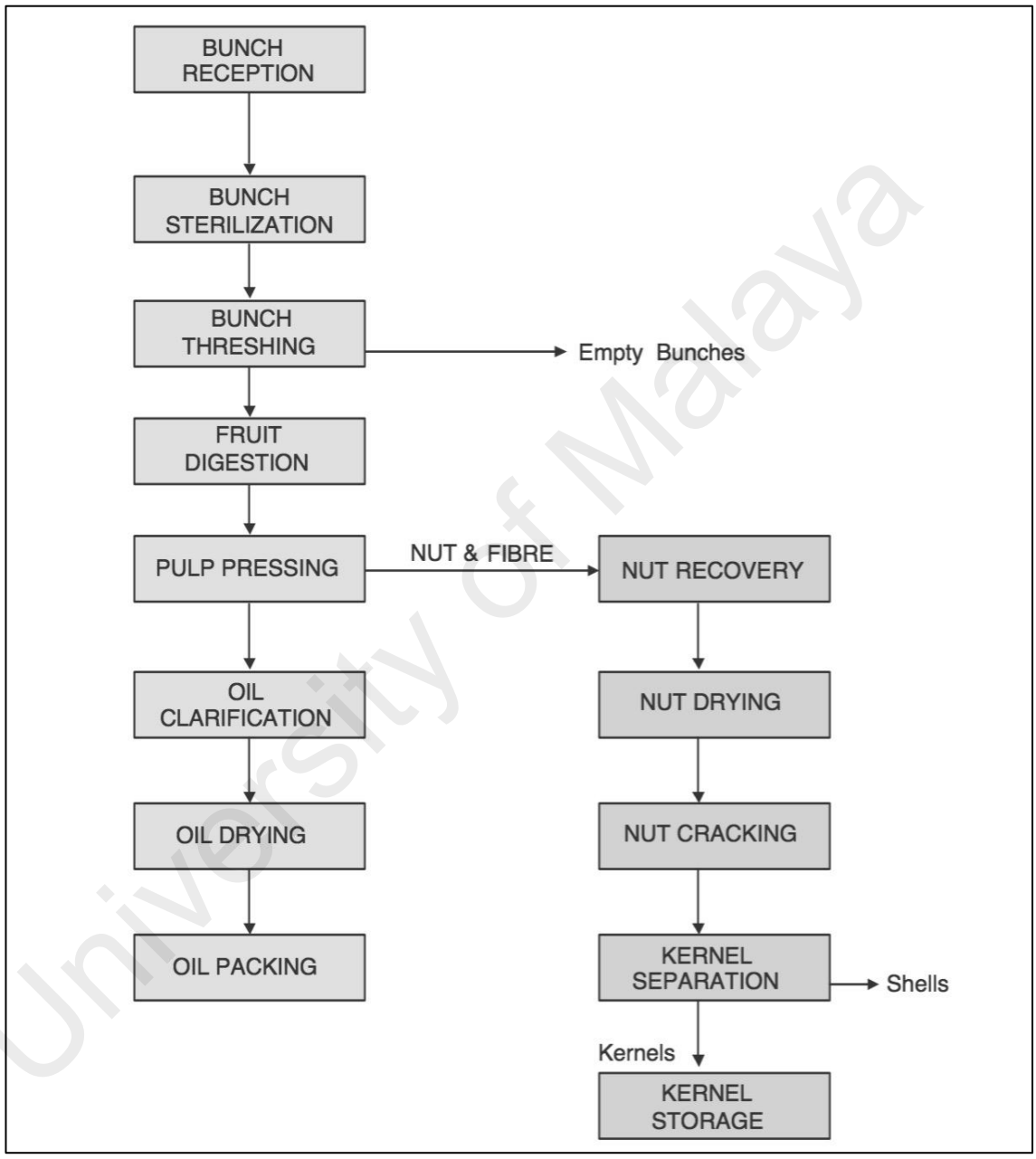

Figure 1.5: Flow chart of the conventional palm oil milling process (Poku, 2002)

Figure 1.5 depicts the multiple stages of conventional palm oil milling process in Malaysia. The first stage in the conventional milling is the sterilization process where fresh fruit bunches (FFB) are treated with saturated steam at 40 psi (140˚C) for 75 – 90

min (Sivasothy, 2000; Chow and Ma, 2007) to deactivate the oil splitting enzyme as well as to soften the palm fruits from the bunch. The heat applied breaks up the oil bearing cells from their mesocarp to ease oil release during the digestion stage later (Mahidin, 1998). The sterilized bunches are then threshed to separate the sterilized fruits from its bunch. The process continues with fruit digestion stage by moving them into digesters, steam heated vessels with stirring arms. High temperature pounding is carried out during this stage to complete the disruption of oil bearing cells initiated during sterilization process. The digested mesh is then pressed by a mechanical screw presser to mill out a mixture of oil, moisture, fibre and kernels. The kernels are milled for palm kernel oil in another separate process. The mixture of oil, moisture and fibre, on the other hand, is subjected to oil clarification process to separate the oil from its impurities. This is achieved by dilution with a large amount of water hot water, causing the heavier solid to sink to the bottom of the mixture. The heat applied causes the oil-water emulsion to break and due to the nature of oil being less dense than water, oil droplets will float to the top of the water. The oil from the top layer is then be skimmed off into a reception tank. The traces of moisture dissolved in the oil is removed by evaporation in dehydrator with or without the assistance of vacuum. Dried crude palm oil is then transferred to a tank for storage prior to dispatch from the mill.

During the sterilization and clarification process, a large amount of water, estimated at 5.0 – 7.5 tonnes is used to produce a tonne of crude palm oil (CPO). More than 50% of the water used will end up as waste water, known as Palm Oil Mill Effluent (POME), while the rest is lost as steam in the boilers, blow down, wash waters and leakage (Thani, Hussin, Wan Ibrahim, & Sulaiman, 1999).

Harvesting and transporting of FFB before the milling process is an important factor to determine the quality of the oil produced. When the fruits were young, the oil content of the fruits is very low. As the fruits approaches maturity, the formation of oil increases to about 50 percent of the mesocarp mass (Razali, Halim & Roslan, 2012). In a fresh ripe bunch, FFA content of unbruised fruit is below 0.3%. However, if the fruit is bruised, the FFA in the damaged part increases rapidly due to the enzymatic attack through hydrolysis (Chong & Sambanthamurthi, 1993). Bruising of the fruits cannot be avoided completely due to the nature of their exocarp which softens as it ripens. Lipase is the enzyme that hydrolyse lipids into FFA and glycerol. As the fruits ripen, the amount of lipase in the fruits increases. The rate of hydrolysis of palm oil by the lipase is linear on a logarithm scale (Khor, Tan and Chua, 1986). Thus, processing the fruits as soon as possible after harvest, within 48 hours is recommended (Poku, 2002).

CPO obtained by mechanical pressing contains desirable and undesirable compounds. Desirable compounds include triacylglycerols (TAG) and health beneficial compounds such as vitamin E (tocopherols and tocotrienols), carotenoids and phytosterols. Free fatty acids (FFA), phospholipids (PL) or gums and lipid oxidation products are the major undesirable compounds. Quality requirements for CPO is specified by Malaysian Standard (MS814:2007) (Table 1.1).

Table 1.1: Quality requirement for Crude Palm Oil (MS814:2007)

Characteristic	Crude palm oil	
	Special quality grade	Standard quality grade
Free fatty acid (as palmitic), % max	2.5	5.0
Moisture and impurities, %max	0.25	0.25
Peroxide value, meq/kg max	1.0	2.0
Anisidine value, meq/kg max	4.0	5.0
DOBI, min	2.8	2.3

In order to render the oil more suitable for consumption, the CPO must be refined to reduce the unwanted constituents to an acceptable level while minimizing losses of desirable components. Table 1.2 showed the specifications specified by Malaysian Standard (MS814:2007) for the quality of refined oils.

Table 1.2: Quality requirements for refined palm oil (MS814:2007)

Characteristic	Neutralized Palm Oil (NPO)	Neutralized, Bleached Palm Oil (NBPO)	Neutralized/Refined, Bleached and Deodorized (NBD/RBDPO)
Free fatty acid (as palmitic), %max	0.25	0.25	0.10
Moisture and impurities, % max	0.10	0.10	0.10
Peroxide value, meq/kg max	-	-	2.0
Anisidine value, meq/kg max	-	-	4.0
Colour, 133.35mm Lovibond, max	-	20.0 R	3.0 R

As shown in Table 1.2, a good quality oil must contain less than 0.1% of FFA. When the crude oil has high FFA, physical refining is preferred (Dunford, 2012; Gunstone, 2011). Čmolík and Pokorný (2000) reported that with CPO containing 12% of FFA, to reduce it to 1.3% FFA and to 0.5% FFA requires temperatures of 220˚C and 230˚C respectively by physical refining at a pressure of 0.8 kPa. It is noticed that the more

FFA required to be reduced, more energy has to be used, resulting in an increase in the refining cost. Thus, production of CPO with lower FFA value is more favourable.

There are two common methods used for refining, namely chemical and physical refining. Chemical refining is also known as alkaline refining where fatty acids are removed by neutralization with caustic soda. Although chemical refining is more suitable for treating most types of oil including poor quality crude oil, the oil loss is considerably higher than that of physical refining (Čmolík and Pokorný, 2000). Physical refining is also known as steam refining, achievable by distillation at low absolute pressure and high temperature. This method is widely adapted in palm oil refineries in Malaysia. Figure 1.6 shows the flow chart of both refining processes.

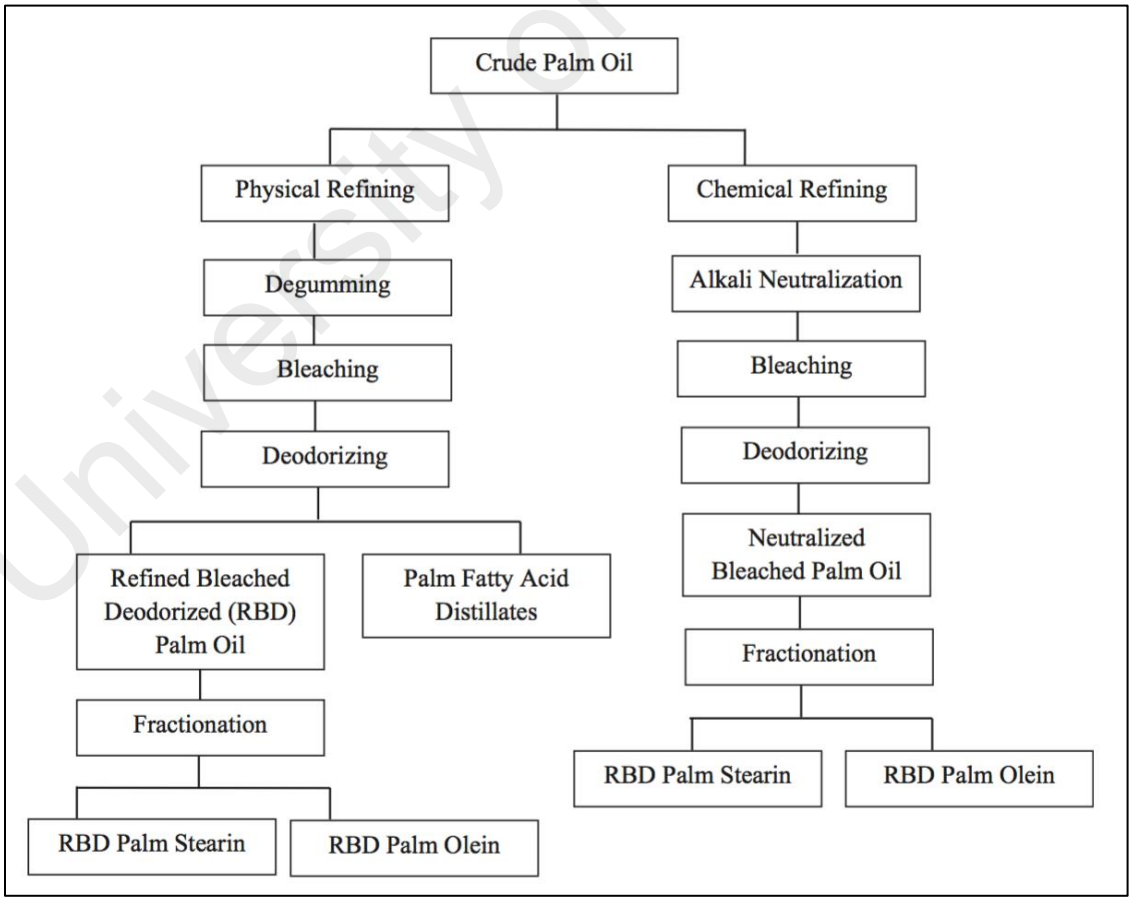

Figure 1.6: Flow chart of palm oil refining processes (Mba, Dumont & Ngadi 2015)

The refining process is categorized into three main steps, namely, degumming, bleaching and deodorizing. The quality and stability of the refined products are determined by how well the oxidative impurities, such as trace metal ions (iron, copper), PLs (gums) and oxidation precursors are removed prior to steam distillation. Impurities are inactivated and precipitated in the degumming step by adding food grade phosphoric acid (Dunford, 2012). The crude oil is first heated to the degumming temperature, which is about 75 – 85˚C in a plate heat exchanger using low pressure steam, or by heat exchange with hot deodorized oil, before being mixed with phosphoric acid proportionated by a dosing pump. Depending on the quality of the crude oil, the required amount of phosphoric acid varies (0.05% to 0.1%). Intimate mixing between oil and phosphoric acid is achieved in a high shear-homogenizing mixer followed by a retention vessel. Then the precipitated materials are removed in the bleaching step.

The practice of bleaching or adsorptive cleansing, involves the addition of activated clay (bleaching earth) to remove any undesirable impurities for improvement in initial taste, final flavour and oxidative stability of product. It also helps to overcome problems in subsequent processing by adsorption of soap traces, pro-oxidant metal ions, decomposes peroxides and adsorbs other minor impurities. The bleaching process is carried out under vacuum at a temperature of about 100°C and reaction time of 30 min. The amount of bleaching earth used varies with the type and quantity of starting oil and is usually in the range of 0.5 – 1.0%. As mentioned earlier the primary function of the bleaching earth is to reduce the unwanted impurities through adsorption. However, a certain amount of bleaching (colour reduction) by pigment adsorption occurs as a bonus effect. Colour reduction is actually observed in the next stage through high temperature thermal destruction of the pigments. The spent earth is separated from the oil by filtration. The slurry containing the oil and earth is then passed through the main filter

to give a clear, free-from-earth particles oil. Vertical pressure leaf filter is commonly used for this purpose. This type of filter can be easily automated. As steam blowing can dry the filter cake, it has a lower oil loss compared to the traditional plate-and-frame filter press used in some older refineries. In order to achieve a truly continuous flow of oil, two or more filters working alternatively are required. The filtered oil is passed through two or more units of polishing filter, working alternately, to remove fines and residual bleaching earth.

Free fatty acid, oxidation products, taste and odoriferous compounds are removed *via* steam distillation under low absolute pressure and high temperature. The breakdown of colour pigments and carotenoids due to thermal decomposition gives refined oil its final colour. The bleached oil is first deaerated to remove air and moisture. Heating of the bleached oil to the final processing temperature (250 – 265°C for palm oil) is achieved by energy recovery from hot deodorized oil in a spiral heat exchanger, followed by heating with high pressure steam or thermal oil. Removal of volatile free fatty acids is achieved by flash distillation to minimal level. The oil flows across a series of perforated sheets while steam bubbles through the holes into the oil, creating a large interfacial area for vapour/liquid contact. The required retention time for thorough deodorization in the holding trays is 50 – 60 min for palm oil. Agitation of the oil in the deodorizer is achieved by steam agitating. The formation of small steam bubbles with large interfacial area and intense mixing between oil and steam are crucial for the proper functioning of the deodorizer. Steam rising to the top provides intense agitation of the oil and carries with it the odoriferous and other volatile materials. In this way, the top surface is continuously and rapidly renewed with oil from the bottom of the tray therefore eliminating dead pockets of oil within the oil mass. The intense mixing of oil and steam thus achieved ensures thorough and uniform deodorization. Deodorization

can be carried out in batch, continuous or semi-continuous style. The present practices in Malaysia are continuous and semi-continuous processes due to its efficiency and less costly.

In a continuous alkaline refining route, the oil is generally heated 220 – 240°C under vacuum. By using ejectors and boosters, the vacuum pressure is maintained at the level of 2 – 5mbar. Heat bleaching of the oil will occur at this temperature through the thermal destruction of the carotenoid pigments. The residual free fatty acids, aldehydes and ketones that are responsible for unacceptable odours and flavours are essentially removed by the use of direct stripping steam. The oil leaves the deodorizer under vacuum and cooled down to less than 60°C. It passes through a polishing filter before it is sent to the storage tank.

As summary, physical and chemical refining differ basically in the way the fatty acids are removed from the oil. Physical refining, which eliminates the need for an effluent plant for the soap stock, involved subjecting the oil to steam distillation under high temperature and vacuum for removal of the free fatty acids. The physical refining is used to remove the free fatty acids. The refining of physical plant is practiced to subject the oil to steam distillation. However, the specification of refined, bleached, deodorized (RBD) oil and neutralized, bleached, deodorized (NBD) oil were same although undergo different process.

1.2.2 Palm oil mill effluent (POME) and its treatment

Palm oil mill effluent (POME) may cause environmental issues due to its high acidity, high biological oxygen demand (BOD) and chemical oxygen demand (COD); and

therefore, it must be treated and managed in an environmental and legislative acceptable manner.

A common way to treat POME is anaerobic digestions in ponding systems. This treatment can reduce the BOD level to below 100 mg/L. This treatment requires only low energy and the sludge from the process could be used for land application. However, this treatment will generate biogas containing methane, carbon dioxide (CO_2) and other greenhouse gases that contributes to global warming. This treatment also has a long hydraulic retention time (HRT) and consumes a vast land area. On the other hand, POME is also treated *via* aerobic ponding system, in contrast to the anaerobic ponding system; it has a short HRT and is more efficient in handling the toxic waste. The cons of this treatment would be the high energy required and the rate of pathogen inactivation is lower.

Several methods have been taken to reduce the emission of greenhouse gases (GHG) into the atmosphere. The Clean Development Mechanism (CDM) is a flexibility mechanisms defined in the Kyoto Protocol (Tong and Jaafar, 2006) that provides for emissions reduction projects, which generate Certified Emission Reduction (CER) units that may be traded in emissions trading schemes. Through this, industries may generate valuable end products that can be changed into revenue, such as methane gas. Methane gas can be cultivated and sell to other industry that is in need of methane gas. By utilizing the biogas for heat generation, can also reduce the industry's operational cost (Poh and Chong, 2009).

There are also some end-of-pipe treatments developed to treat the POME. Ultrafiltration membrane is able to reduce the total suspended solids (TSS), turbidity, total dissolved

solids (TDS) and COD up to 97.3%, 88.2%, 3.1% and 46.9%. About 45.3% of protein and 41.5% of carbohydrate are retained together which could be used as animal feeds. However, the down side of this treatment is the short lifespan of the membrane and the high cost of maintenance as compared to the ponding system (Wu, Mohammad, Jahim and Anuar, 2007).

Besides this, a further development revolving anaerobic system is the membrane anaerobic system (MAS). It actually increases the removal efficiency of COD from 96.6% to 98.4%. It also significant lowers the HRT and solids retention time (SRT). HRT is reduced from an average of 600 days to only about 7 days while SRT is reduced from 1000 days to only 12 days (Abdurahman, Rosli and Azhari, 2011).

Some natural coagulant like *Moringa oleifera* seeds is also utilized to reduce the COD in the ponding system. *M. oleifera* seeds after oil extraction (MOAE) is an effective coagulant with the removal of 95% of TSS and 52.5% reduction in COD. A further development by combining the MOAE with flocculent (NALCO 7751), the TSS removal increased to 99.3% (Bhatia, Othman and Ahmad, 2007).

The Environmental Quality Regulations 1977, put under the power of Section 51 of the Environmental Quality Act (EQA), are governing the regulations and contain the effluent discharge standard. An effluent discharge standards applicable to crude palm oil mills was set in accordance to the regulatory requirements by EQA 1974 (Rupani, Singh, Ibrahim & Esa, 2010). Despite all the controls setup by the government, Malaysia is still identified as the country that produces the largest pollution in the river (Variappan & Yen, 2008). Due to this fact, there is an urge to find a solution to preserve the environment as well as keeping the growth of the economy.

Although there are methods to manage POME in environment acceptable manners as afore described, it is best to avoid its production, disposal and management cost. Cheng, Mohd and Chuah (2011) reported that microwave pretreatment is a dry and clean technology for the extraction of palm oil due to the clarification step in conventional palm oil milling process was fully omitted. Without the presence of both steam sterilization and clarification steps, there are exactly zero water effluent being produced.

1.2.3 Constituents in palm oil

Palm oil consists of mainly triacylglycerols (TAG) that is made up of a range of fatty acids attached to the backbone of glycerol. TAG constitutes the major component, with small proportions of diacylglycerols (DAG) and monoacylglycerols (MAG) along with other minor constituents, such as free fatty acids (FFA) and non-acylglycerol components and all these constituents determine the oil's chemical and physical characteristics.

Acylglycerol is one of the important constituents in palm oil. Chong (1994) reported that palm oil contains a large number of different triacylglycerols (TAG) due to various placement of the fatty acids in glycerol backbone. According to Ong *et al.* (1995), TAG of palm oil consists of trisaturated (10.2%), disaturated (48%), monosaturated (34. 6%), and triunsaturated (6.8%) fatty acids. Siew and Ng (1997) reported that the composition of the DAG in palm oil is depending on the degree of fruit ripeness and the extent of hydrolytic degradation. More DAG are found in ripe and overripe fruit, and also in fruits which are not processed immediately.

Acyglycerols has wide applications. The most important commercial products are monostearate, monooleate and monolinoeate of glycerols. MAG are excellent emulsifier and has been widely used in the food industry. MAG also are being used in non-food applications as texturing agents, lubricants and plasticizers in pharmaceutical, cosmetics and textile (Mefferts, 1984). Enig (1996) reported that certain type of MAG such as monolaurin and monocaprin were used as anti-microbial agents or antiseptics agents in food and pharmaceutical industry.

Fatty acid is a carboxylic acid with long aliphatic chain, either saturated or unsaturated. Fatty acids are usually derived from TAG or PL through hydrolysis. Palm oil has a balanced fatty acid composition (FAC) in which the level of unsaturated fatty acid is almost similar to that of the saturated fatty acid. The FAC specification for CPO that is outlined in Malaysian Standard MS814:2007, is shown in Table 1.3.

Table 1.3: Fatty Acid Composition specification of Crude Palm Oil (MS814:2007)

Fatty acid composition, (mass % as methyl esters)	Observed range	Mean	Standard deviation
C12:0	0.0 – 0.5	0.2	0.10
C14:0	0.9 – 1.5	1.1	0.08
C16:0	39.2 – 45.8	43.5	0.95
C16:1	0.0 – 0.4	0.2	0.05
C18:0	3.7 – 5.1	4.3	0.18
C18:1	37.4 – 44.1	39.8	0.94
C18:2	8.7 – 12.5	10.3	0.56
C18:3	0.0 – 0.6	0.3	0.07
C20:0	0.0 – 0.5	0.2	0.16

MS814:2007 outlined that the FAC of processed palm oil has no significant differences from the CPO. As shown in Table 1.3, palmitic acid (C16:0) and oleic acid (C18:0) are the major fatty acids, followed by linoleic acid (C18:1) and traces amount of linolenic acid (C18:2). The low level of linoleic acid and linolenic acid made the oil relatively

stable to oxidative deterioration. Clegg (1973) reported that the ratio of palmitic/stearic acid content in *Eleais guineensis* oil varies was due to geographical influences. King and Sibley (1984) showed the minor difference and FAC from different countries of origin that was due to geographic variations.

Besides the major constituents as described, CPO also contains approximately 1% of minor components, such as carotenoids, vitamin E (tocopherols and tocotrienols), sterols, phospholipids, glycolipids, terpenic and aliphatic hydrocarbons and other trace impurities (Goh *et al.*, 1985). CPO is the world's richest natural plant source of carotenes in terms of retinol. The orange colour of palm oil is due to the presence of carotenes. The typical concentration ranged from 500 – 700 ppm for palm oil. In addition, it contains about 15 times more retinol equivalents (pro-vitamin A) than carrots and 300 times more than tomatoes. Carotene consists of β-carotene and α-carotene in which α and β-carotene comprise more than 90% in the CPO.

Methods to recover carotenoids from palm oil include saponification, adsorption, selective solvent extraction and transesterification followed by distillation. During the refining processes, where the temperature goes above 200˚C, the carotenes are often destroyed and only residual amount is left in the refined oil.

Carotene is commonly used as food colorant. They are also used in health products such as dietary supplement, which helps to prevent night blindness, eye problem and skin disorders (AREDS2 Research Group, 2013; Bayerl, 2008). Moreover, some of these products can also enhance immunity and protection against toxin, cold, flu, and infection. (Brambilla *et al.,* 2008; Gabriele *et al.,* 2000). The importance of carotenoids

is becoming more significant due to the more extensive use of natural compounds in foods, cosmetics, and pharmaceutical industries.

Dietary lipids, naturally occurring in raw food materials or added during food processing, play an important role in food nutrition and flavour. Meanwhile, lipid oxidation is a major cause of food quality deterioration, and has been a challenge for manufacturers and food scientists alike. Lipids are susceptible to oxidative processes in the presence of catalytic systems such as light, heat, enzymes, metals, metalloproteins, and microorganisms, giving rise to the development of off-flavours and loss of essential amino acids, fat-soluble vitamins, and other bioactive. Lipids may undergo autoxidation, photo oxidation, thermal oxidation, and enzymatic oxidation under different conditions, most of which involve free radical or oxygen species.

It is well known that lipids in edible oils are susceptible to autoxidation and photoxidation during processing and storage. This is a long recognized problem in the oil industry, leading to undesirable flavours and taste, lower nutritional quality and production of toxic compounds (Halvorsen and Blomhoff, 2011). Both oxidation processes are accelerated at higher temperatures, such as those experienced during deep frying, which is called thermal oxidation will increase the FFA, polar matter contents, foaming, colour, and viscosity in the oil. Unsaturated fatty acids are generally affected by such reactions, whether they are present as free fatty acids, triacylglycerols (as well as diacyglycerols or monoacylglycerols), or phospholipids. Both autoxidation and thermal oxidation of unsaturated fatty acids occurs *via* a free radical chain reaction that proceeds through three steps, namely, initiation, propagation, and termination. A simplified scheme explaining the mechanism of lipid peroxidation is shown in Figure 1.7.

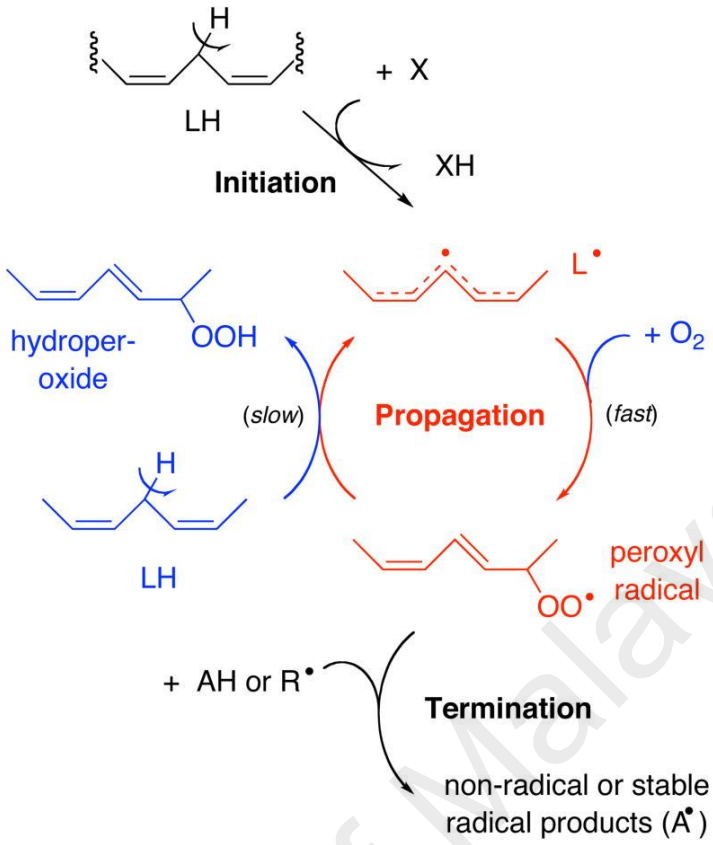

Figure 1.7: Reaction scheme for lipid peroxidation (Schneider, 2009)

Lipid peroxidation is the primary oxidation stage of lipids. As oxidation normally proceeds very slowly at the initial stage, the time to reach a sudden increase in oxidation rate is referred to as the induction period. Lipid hydroperoxides have been identified as primary products of autoxidation. Several methods have been developed to detect the peroxides to measure the extent the oxidation has undergone (peroxide value), iodometric titration being the most widely used method. This method determines the amount of peroxides by measuring the amount of iodine formed during the reaction of peroxides in lipids with iodide ion (Figure 1.8). Peroxide value is defined as amount of peroxide oxygen in milliequivalents per 1 kg of lipids (meq/kg). Other methods such as spectrophotometric ferric thiocynate method and conjugated diene determination method will not be discussed.

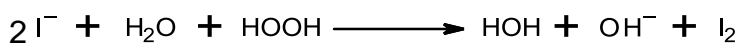

Figure 1.8: Liberation of iodine during iodometric titration

Lipid hydroperoxides is unstable in its form and has the tendency to decompose into a multitude of secondary oxidation products. These products include aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds. It is difficult to measure all the compounds simultaneously due to their small amounts formed and the large variations in chemical structures and properties. However, there are several methods developed to measure the secondary oxidation products, such as anisidine value and thiobarbituric acid reactive substances (TBARS) assay. The anisidine value method is based on the reaction between *p*-anisidine and the aldehydic compounds formed during the secondary oxidation present in the oil samples at acidic conditions. The reaction creates a yellow-coloured compound with absorbance at 350 nm.

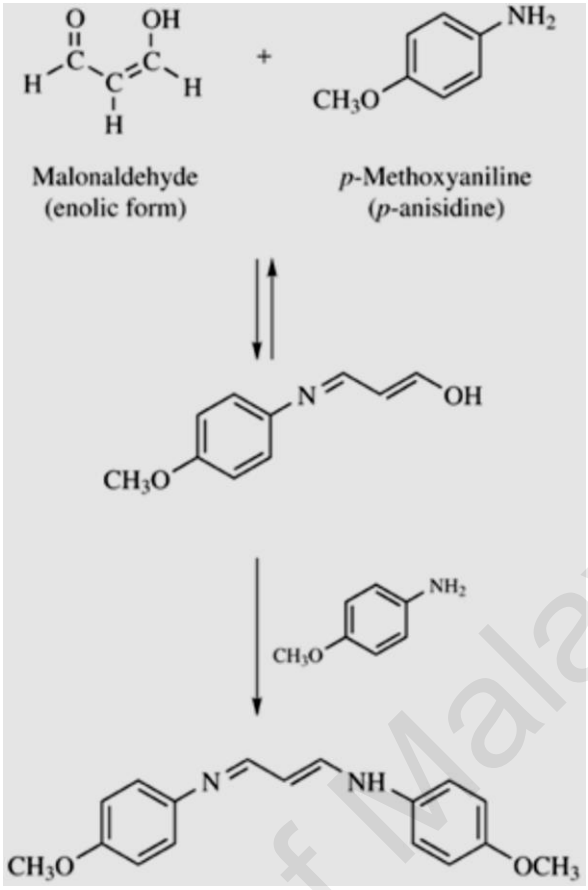

Figure 1.9: Proposed reaction between *p*-anisidine reagent and malonaldehyde (Shahidi and Wanasundara, 2002)

Figure 1.9 depicts the reaction between the *p*-anisidine reagent and malonaldehyde yielding the yellow-coloured compound (Shahidi and Wanasundara, 2002). This reaction does not involve the usage of any strong acids or high temperature thus minimizes the influence on the hydroperoxide decomposition (White, 1995). Anisidine value (AnV) is expressed as the absorbance of a solution made of 1g of fat in 100 mL isooctane solvent and *p*-anisidine agent (Frankel, 2005).

Holm (1972) suggested a combined expression of lipid peroxides and secondary oxidation products, hence developed the TOTOX value. Holm established Equation 1.1 through a demonstration that an increase of 1 PV unit corresponded to increase in 2 AV units, giving a value of total oxidation status in oil.

$$\text{TOTOX value} = 2\text{PV} + \text{AV} \quad \textbf{(1.1)}$$

1.3 Microwave energy and its application

1.3.1 Introduction

Microwave is a form of electromagnetic radiation with wavelengths ranging from one meter to one millimetre; with frequencies between 300 MHz and 300 GHz. Due to its broad range of wavelengths and frequencies, microwave is extensively used in multiple fields, including communication, navigation, radar, radio astronomy, spectroscopy, heating and power application.

In the past 20 years, the microwave oven has become an essential appliance in most kitchens. Faster cooking times and energy savings over conventional cooking methods are the primary benefits. Although the use of microwaves for cooking food is widespread, the application of this technology to the processing of materials is a relatively new development. The use of microwave energy for processing materials has the potential to offer similar advantages in reduced processing times and energy savings (El-Abassy, Donfack, & Materny, 2011).

In conventional thermal processing, energy is transferred to the material through convection, conduction, and radiation of heat from the surfaces of the material. In contrast, microwave energy is delivered directly to materials through molecular interaction with the electromagnetic field. In heat transfer, energy is transferred due to thermal gradients, but microwave heating is the transfer of electromagnetic energy to thermal energy and is energy conversion, rather than heat transfer. The difference in the way energy is delivered can result in many advantages to using microwaves for processing of materials. Because microwaves can penetrate materials and deposit

energy, heat can be generated throughout the volume of the material. The transfer of energy does not rely on diffusion of heat from the surfaces, and it is possible to achieve rapid and uniform heating of thick materials. In traditional heating, the cycle time is often dominated by slow heating rates that are chosen to minimize steep thermal gradients that result in process-induced stresses. For polymers and ceramics, which are materials with low thermal conductivities, this can result in significantly reduced processing times (Thostenson and Chou, 1999).

1.3.2 Literature Review

Thostenson and Chou (1999) reported that microwaves have the ability to couple energy directly to the material which is the primary advantage of microwave processing as compared to conventional techniques. The volumetric heating ability of microwaves allows for more rapid, uniform heating, decreased processing time, and often enhanced material properties. To optimize the process, the electromagnetic fields, microwave/material interaction, material transformations, and heat transfer is essentially needed to understand. Microwave can be applied in many fields, as long as it involves application of heat, such as, microwave oven, materials processing via microwave and extraction of essential oils.

Uquiche, Jeréz and Ortíz (2008) reported that microwave pretreatment could be applied to Chilean hazelnut oil extraction prior to pressing to improve the oil recovery and its quality. Microwave pretreatment enabled up to 45.3% of the oil to be extracted at the condition of pretreatment at 240 s and 400 W. Data also indicated that microwave radiation has a positive effect on oil quality. These oils obtained have higher stability to the oxidative deterioration (induction time, IT = 23.9 h) with respect to untreated seed (IT = 8.8 h). Microwave pretreatment seeds also yield oils of lower content of

unsaturated fatty acids (linoleic acid = 5.84%) compared to untreated seeds (linoleic acid = 7.16%).

Rui, Zhang, Li and Pan (2009) reported the effect of several extraction methods, namely, microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), aqueous enzymatic extraction (AEE) and a combination of microwave and AEE (MAEE)
under various conditions on the yield and characteristics of the oil obtained from seed kernel of white pitaya. The highest yield (7.78%) is obtained by using MAEE. Oil extracted using MAEE also possesses more variety of fatty acid compared to oils extracted using the other methods. On the other hand, MAE is found to be the fastest method among all the method tested (10 min to yield 6.63% of oil).

Amarni and Kadi (2010) compared the microwave-assisted solvent extraction (MASE) of oil from olive cake using hexane with the conventional solvent extraction (CSE). Under the condition that gives the best yields (P=720W for MASE and T=45°C for CSE), both method yield the same amount of oil for diffusion step (1.07%) but MASE yielded more amount of oil for washing step which is 4.42% compared to CSE which only yielded 4.28% for this step. Under the same condition as above, only 2 min are required to obtain the yield of 5.18% in MASE compared to CSE which required 11 min to obtain the same amount of yield.

Azadmard-Damirchi, Habibi-Nodeh, Hesari, Nemati and Fathi (2010) reported that it is advisable to pretreat rapeseed with microwave before extraction by oil press. This is because the microwave pretreatment of rapeseed can increase the oil extraction yield by 10%, phytosterols by 15% and tocopherols by 55% of the oil extracted by press. Oil

extracted by pretreatment of rapeseed with microwaves has high oxidative stability (8 h) compared to untreated rapeseed (1 h).

Balasubramanian, Allen, Kanitkar and Boldor (2011) reported that by using the microwave system, 76 to 77% of total recoverable oil of the Scenedesmus *obliqus* is extracted in 30 min compared to only 43 to 47% by water bath control. The MAE oil contained a more of unsaturated fatty acids, with more ω-3 and ω-6 essential fatty acids, which indicating a superior quality. The oil obtained from MAE process is suitable for either biodiesel production or nutrition.

Cheng *et al.* (2011) reported that the yield of palm fruits which pretreated with microwave for 3 min is comparable to the oil yield of the conventional palm oil milling process with an average of 20%. In addition, the quality of the palm oil produced by microwave pretreatment is superior compared to the palm oil produced by the conventional method. Free fatty acid (FFA) and moisture content of the oil produced is 0.26% and 0.05% respectively for the microwave pretreated oil. This oil also contains high concentration of carotene value (1585 ppm) and vitamin E (2345 ppm).

Terigar, Balasubramanian, Sabliov, Lima and Boldor (2011) developed a continuous microwave-assisted extraction (CMAE) method at laboratory and pilot-scale for rapid extraction of oil from soybean flour and rice bran using ethanol as solvent. These two systems were optimized and their extraction capabilities were compared to those of conventional extraction systems. For laboratory-scale CMAE, results indicated that more than 80% of extractable oil can be extracted in 21 min at 73°C for both feedstocks compared to the conventional extraction (CE) which needs to be carried out for 10h. As for pilot-scale CMAE, more than 90% of recoverable oil for both feedstock is extracted

in only 8 min at 73°C. The quality of these extracted oils met the biodiesel feedstock standard specifications.

Vincent, Shamsudin and Baharuddin (2014) had reviewed multiple pretreatment methods of oil palm fruitlets and concluded that microwave heating and oven heating are capable of replacing current sterilization process. These process are able to prevent POME from being produced.

1.4 Objectives of Present Study

Malaysian palm oil mills have been practicing the conventional palm oil milling process for decades without any major breakthroughs. The conventional palm oil milling processes have produced a lot of palm oil mill effluent (POME) as a consequence of the introduction of large amounts of water during the process. POME is a major pollutant to the environment due to its high chemical oxygen demand (COD) and biological oxygen demand (BOD).

Therefore, the main objective of this study is to investigate microwave technology as a dry sterilization process in palm oil milling, an alternative to the conventional steam sterilization process. This study also aims to investigate solvent extraction as a feasible subsequent stage, an alternative to the screw pressing process. The production of POME can be omitted through the combination of microwave pretreatment and solvent extraction, due to no introduction of water into the processes. The secondary objective of this study is to determine the quality of the oil produced.

CHAPTER 2: METHODOLOGY

2.1 Microwave Pretreatment of Palm Fresh Fruits

Conventional sterilization process involves a huge amount of water, which will end up as the unwanted POME. The present study utilized microwave heating to replace the conventional sterilization process by heating palm fruits. This replacement will involve no water at all stages thus preventing POME production. Microwave pretreatment of palm fruits can also provide multiple advantages over the conventional sterilization, such as a more uniform heating and energy saving.

2.1.1 Materials

Fresh palm fruits were obtained from MPOB Experimental Mill located in Labu, Negeri Sembilan. The fresh palm fruits were selected from the pile of fresh fruit bunches (FFB) that were harvested from palm tree that aged from 10 to 15 years old and about 15 kg in mass. The fruits were selected from it in accordance to the specific requirements needed for the present study. The fruits must be uncut, bruise-free and orange-red in colour. Selected fruits were stored in a refrigerator at 4°C.

2.1.2 Apparatus/Instrumentation

Microwave oven (SAMSUNG, M1600N, 600 W, 2450 MHz, 17 L) with a turn-table was used for small scale microwave pretreatment of the fresh palm fruits. Microwave oven (Panasonic, NN-CD997S, 1000 W, 2450 MHz, 42 L) with a turn-table was used for larger scale microwave pretreatment. Top-pan balance (Mettler-Toledo, model PB602-S/FACT) was used to measure the mass of the fruits. Infrared thermometer (Extech, model 42510A Wide Range Mini IR Thermometer) was used to measure the temperature of the fruits after microwave pretreatment. A set of rotary evaporator

(Buchi, R-114), water bath (Buchi, B-480), chiller and vacuum pump (Buchi, V-700, 210 W, vacuum pressure <10 mbar) was used for solvent removal.

2.1.3 Procedures

2.1.3.1 Small scale study

A 100 g of palm fresh fruits were placed in a set-up (Figure 2.1) and were then subjected to microwave pretreatment for 1, 2, 3, 4 and 5 min. The pretreatments were carried out in SAMSUNG M1600N microwave oven. Temperature on the surface of the fruits after pretreatment were measured and recorded on 5-points basis using the infrared thermometer. Oil leached out from the fruits were collected in the scintillation vial was dissolved in *n*-hexane (Systerm, 95%, analytical grade) and filtered through sodium sulphate anhydrous to remove traces of moisture and fibrous residues. Solvent was finally removed using the rotary evaporator. The leached oil yield was calculated according to Equation 2.1. Pretreatment was repeated by varying the microwave radiation duration at 2, 3 and 4 mins and all other procedures were similar as described previously.

$$\text{Yield of leached oil} = \frac{\text{mass of leached oil}}{\text{mass of fresh palm fruits}} \times 100\% \qquad \textbf{(2.1)}$$

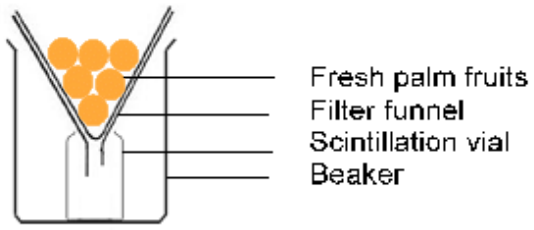

Figure 2.1: Microwave pretreatment setup

2.1.3.2 Larger scale study

Procedures for larger scale investigation are similar to that described in Section 2.1.3.1, replacing the 100 g of palm fresh fruits with 300 g of palm fresh fruits. The microwave oven used was Panasonic NN-CD997S. The effect of microwave pretreatment durations investigated were 1, 2, 3, 4, 5, 10, 12, 13, 14 and 15 min.

2.2 Solvent Extraction

Conventional palm oil milling process extracts palm oil from oil palm fruits through screw pressing process. This process produces palm oil along with a lot of impurities, which is then separated through clarification process. This process also involved a huge amount of water that will end up as POME. Present study used solvent extraction as a subsequent step to microwave heating. This omits the usage of water, which in turn fully omit the production of POME.

2.2.1 Materials

Pretreated palm fruits from Sections 2.1.3.1 and 2.1.3.2 were used in solvent extraction for small scale and larger scale, respectively. Solvent used for Soxhlet extraction was *n*-hexane (Systerm, 95%, analytical grade).

2.2.2 Apparatus/Instrumentation

Soxhlet extractor body (Quickfit), 150 mL and 600 mL, used for small scale and larger scale solvent extraction, respectively. Heating mantle (MTOP brand) 500 mL and 2 L were used as the source of heat for small and larger scale extraction respectively. A set of rotary evaporator (Buchi, R-114), water bath (Buchi, B-480), chiller and vacuum pump (Buchi, V-700, 210W, vacuum pressure <10mbar) was used for solvent removal.

2.2.3 Procedures

2.2.3.1 Small scale study

All pretreated fruits were placed in the Soxhlet extractor and hexane in a 500 mL round bottom flask, respectively. Fruits to solvent ratio used was 1:4 (m/v). The extraction was carried out for 6 hours. Upon extraction, solvent was removed using the rotary evaporator and extracted oil was placed in a desiccator until a constant mass was attained. Oil yield was calculated using Equation 2.2.

$$\text{Oil yield} = \frac{\text{mass of oil obtained}}{\text{mass of fresh palm fruits}} \times 100\% \qquad \textbf{(2.2)}$$

2.2.3.2 Larger scale study

Procedures for larger scale extraction is similar to that of Section 2.2.3.2, replacing the 500 mL round bottom flask with a 2 L round bottom flask and the effect of solvent extraction durations investigated were 6, 12, 15, 18 and 24 h.

2.3 Quality analyses

The Malaysian Standard set maximum requirements on a few of the important characteristics of the oil produced in palm oil milling process is given in Table 2.1. The characteristics of the oil produced in the present study is also analysed, namely, free fatty acids (FFA), carotene content, peroxide value (PV), anisidine value (AnV) and free fatty acid composition (FAC).

Table 2.1: Specification of crude palm oil (CPO) by MS814:2007

Specification	**MS814:2007**
Acid value (%)	< 5.0
Carotene content (ppm)	500 - 700
Peroxide value (meq/kg)	< 10.0
Anisidine value (meq/kg)	< 5.0

2.3.1 Free fatty acid (FFA) content

Free fatty acids (FFA) analysis is to determine the amount of the FFA contained in the oil produced. The principle of this analysis is to titrate the dissolved fatty acids in the alcohol with potassium hydroxide. Its value indicates the proportion of the triacylglycerols that have been hydrolysed.

FFA content was analysed in accordance to Kuntom (2005). A 1.0±0.1g of oil was weighed into an Erlenmeyer flask. A 50 mL of neutralized isopropyl alcohol was added. The flask was placed on a hot plate regulated at about 40°C. The sample was swirled gently while being titrated against standardised 0.1 M potassium hydroxide (KOH) until the first permanent pink colour was obtained. The result was expressed in percentage of palmitic acid according to Equation 2.3.

$$\text{FFA\% as palmitic acid} = \frac{25.6 \times M \times V}{m} \quad \textbf{(2.3)}$$

Where,

M is the molarity of standard KOH solution;

V is the volume, in mL of standard KOH used;

m is the mass, in g of sample used

2.3.2 Carotene content

Palm oil is naturally reddish in color because of its high beta-carotene content. This analysis is to analyse the amount of carotene that is contained in the oil produced by measuring the oil at the wavelength of light at 446 nm, which is the absorbance wavelength of beta-carotene. Higher value represents higher nutritional value of the oil.

Carotene content analysis was using the Kuntom (2005). A 0.10±0.01 g of oil was weighed into a 25 mL volumetric flask. The oil was dissolved in a few millilitres of iso-octane (Systerm, 95%, analytical grade) and was diluted to the mark. Ultraviolet-Visible Spectrophotometer (UV-vis) (Shidmadzu, UV-1650PC) equipped with a single beam and quartz cuvette was used to measure the absorbance at 446 nm. The carotene content was calculated according to Equation 2.4.

$$\text{Carotene content} = \frac{383\text{E}}{\text{lc}} \qquad \textbf{(2.4)}$$

Where,

E is the observed difference in absorption between the sample solution and the solvent;

l is the path length of the cell (in cm);

c is the concentration used for absorption measurement (in g/mL)

2.3.3 Peroxide value (PV)

Hydroperoxides are the products formed during the primary oxidation of oil. Peroxide value (PV) analysis is designed to measure the extent of the oil has undergone primary oxidation. Its principle is to measure the amount of free iodine formed during the reaction between the hydroperoxides with iodide ion. High value of PV represents high level of deterioration of the oil analysed.

PV analysis was performed in accordance to Kuntom (2005). A 1.0±0.1 g of oil was weighed into an Erlenmeyer flask purged with pure dry nitrogen just before use. A 50 mL of acetic acid/iso-octance solution (6:4, v/v) was added and the flask was stoppered. The flask was swirled until the sample was completely dissolved. Then a 0.5 mL of saturated potassium iodide was added using volumetric pipette and the flask was stoppered. The solution was allowed to stand for 1 min but was thoroughly shaken at

least 3 times during the stand time. Upon 1 min, 30 mL of water was added immediately and the solution was titrated against standard 0.01 M sodium thiosulphate solution until the yellow colour almost disappeared. 0.5 mL of starch solution was added and the titration was continued drop-wise until the blue colour disappeared. A blank determination was carried out concurrently. The peroxide value, expressed in miliequivalents of active oxygen per kilogram (meq/kg) was calculated according to Equation 2.5.

$$\text{Peroxide value} = \frac{1000(V - V_o)c}{m} \quad \textbf{(2.5)}$$

Where,

V is the volume, in mL, of sodium thiosulphate used for the sample determination;

V_o is the volume, in mL, of sodium thiosulphate used for the blank determination;

c is the concentration, in M, of the sodium thiosulphate;

m is the mass, in g, of the test portion

2.3.4 Anisidine value (AnV)

p-Anisidine value (AnV) is the common method used to measure the secondary oxidation of oils and fats. Its principle is to react *p*-anisidine with the aldehydes and ketones and form a product that absorbs wavelength of light at 350 nm.

Anisidine value analysis was done in accordance to Kuntom (2005). A portion of optically-cleared iso-octane was first prepared by percolating it through a glass column (3 cm – 5 cm internal diameter, and 100 cm long) filled with silica gel. A 0.40±0.01 g of oil was weighed directly into a 25 mL volumetric flask. A 5 mL of optically cleared iso-octane was added to dissolve the sample and the solution was made up to the mark with the same solvent. Two test solutions were prepared, namely, unreacted test solution

and reacted test solution. Unreacted test solution was prepared by transferring 5 mL of the sample solution to a 10 mL test tube and then, 1 mL of dried glacial acetic acid was added. The test tube was stoppered and shook well. The test tube was kept in the dark at 23±3°C for 8 min. Reacted test solution was prepared with the same manner by replacing the addition of glacial acetic acid by 1 mL of anisidine reagent. Blank solution was also prepared in the same manner by replacing sample solution with 5 mL of iso-octane. Within a further 2 min after the 8 min standing in the dark, each solution was transferred to a clean, dry spectrometer cell. Absorbance of each solution were measured against optically cleared iso-octane at 350 nm. The anisidine value was calculated according to Equation 2.6.

$$\text{Anisidine value} = \frac{1000\ \text{QV}[1.2(\text{A}_1 - \text{A}_2 - \text{A}_0)]}{\text{m}} \quad \textbf{(2.6)}$$

Where,

A_0 is the absorbance of the unreacted test solution;

A1 is the absorbance of the reacted test solution;

A2 is the absorbance of the blank;

m is the mass, in g, of the sample used;

Q is the sample content, in g/mL (Q = 0.1 g/mL), of the measure solution based on which the anisidine value was expressed;

V is the volume, in mL (V = 25 mL), in which the test sample was dissolved;

1.2 is the correction factor for the dilution of the 5 mL of the test solution with 1 mL of the reagent.

2.3.5 Fatty acid composition (FAC)

Every oil has its own unique fatty acid composition (FAC). This analysis is to determine the FAC of oil produced in the present study. It is done through the

comparison between fatty acid methyl esters (FAME) standards and the FAME of the oil produced.

Fatty acid methyl ester (FAME) was prepared in accordance to Christie (1993). 35mg of oil was dissolved in 1 mL of dry toluene in a test tube. A 3 mL of 0.5M sodium methoxide in anhydrous methanol (Fischer Scientific, 99%, analytical grade) was added and the solution was heated in a water bath at 50°C for 15 min. After that, 0.1 mL of glacial acetic acid was added and followed by the addition of 5 mL of distilled water. The mixture was then transferred into a separating funnel and the required esters were extracted by two additions of 5 mL of *n*-hexane (Fischer Scientific, 99%, analytical grade) using a Pasteur pipette. The hexane layer was dried over sodium sulphate anhydrous and the solvents were removed using rotary evaporator. FAME standards were prepared in the same manner. FAC was determined by gas chromatography (GC) (Shidmadzu, GC-2010A Series) equipped with capillary column BPX70 (30 m×0.32 mm i.d., 0.25 μm film thickness) with flame ionization detector (FID). The column temperature was programmed at 140°C and held for 2 min. After that, the temperature was increased at the rate of 8°C per min until 220°C and held for 5 min. Injector and detector temperatures were set at 240°C and 260°C, respectively. Helium gas was used as the carrier gas with a column flow rate at 1.10 mL per min. Peak identification was done by comparing the retention times of the sample with FAME standards. Percentage of FAC was calculated using the peak area.

2.4 Effect of Prolonged Microwave Pretreatment

In general, palm oil appeared as reddish orange in color. The oil produced in present study is no difference. However, prolonged microwave pretreatment beyond the optimal duration would burnt the palm fruits and yielded palm oil in black. The interest in this

study is to analyse the changes, physically or chemically, that cause the undesirable palm oil color.

2.4.1 Materials

Palm oil which was produced after prolonged microwave pretreatment (above 13 min) were investigated as it appeared black. Solvents used in this analysis were *n*-hexane (Merck, 95%, analytical grade) and acetone (Merck, 95%, analytical grade). Silica gel (Asset Point Scientific, 40-63 µ) was used for absorption studies.

2.4.2 Apparatus/Instrumentation

Nylon membrane filter (Sartorius Stedim biotech, 0.2 µm, 47 mm dia.) was used for filtration. Hotplate stirrer (Stuart, US152) along with a magnetic stirrer was used. A set of rotary evaporator (Buchi, R-114), water bath (Buchi, B-480), chiller and vacuum pump (Buchi, V-700, 210 W, vacuum pressure <10 mbar) was used for solvent removal.

Qualitative analysis was carried out by using SPME-GC-MS through a GC-MS system (Shimadzu, GCMS-QP2010) equipped with DB-WAX column (30 m×0.25mm i.d., 0.25 µm film thickness). The temperature was programmed at 40°C and held for 1 min. After that the temperature was increased up to 200°C at the rate of 10°C/min and kept for 20 min. Injector temperature was set at 220°C using a SPME device equipped with a 30 µ, polydimethylsiloxane (PDMS) coated fibre (Supelco, 100 µm). The MS was operated at 70 eV, total scan (TIC): *m/z* 42-300, scan rate 0.5 u/s.

2.4.3 Procedures

A 10 g of darken oil was dissolved in 100 mL of *n*-hexane in a 250 mL Erlenmeyer flask. A 5 g of silica gel was added into the flask. The suspension was stirred for 1 hour on a stirrer hotplate with assistance of a magnetic stirrer at room temperature and then left to stand for 30 min. Then, filtration through Nylon membrane filter was carried out. The residual silica was dried in a 60°C oven until a constant mass was obtained. Dried residual silica from the above was weighed and acetone was added in on the ratio of 1:20 (*m:v)*. The mixture was stirred for 1 hour on stirrer hotplate with assistance of a magnetic stirrer at room temperature. The suspension was then allowed to stand for 30 min. The suspension was filtered through the Nylon membrane. The filtrate after solvent removal was stored for SPME-GC-MS analysis.

2.5 Statistical Analysis

Data were subjected to Microsoft Excel 2016 (Microsoft®) for statistical analyses. The significant level of difference between microwave pretreatment duration and oil yield, solvent extraction duration and oil yield were accessed through regression test. The accepted level of significance was $P<0.05$.

CHAPTER 3: RESULTS AND DISCUSSION

3.1 Effect of Microwave Pretreatment and Solvent Extraction on Oil Yield

3.1.1 Physical Appearance of Palm Fruits After Microwave Pretreatment

Generally, fresh palm fruits are oval shaped, which are borne tightly in large bunches. The fresh palm fruits consist of three colours, red at the tip of palm fruits, orange at the middle part and the bottom part is yellowish due to low exposure to sunlight. However, a majority of the ripen palm fruits presented red and orange color (Tan, 1987). A similar observation was reported by Choo (1989), Rashid *et al.* (2002), Sundram *et al.* (2003) and Ahmed *et al.* (2009).

Palm fruits after microwave pretreatment had some ruptures on the mesocarp. The mesocarp fibre also became hard and crispy after pretreatment. This phenomenon occurred due to the loss of water during the pretreatment process. Water droplets formed on top the leached oil in the scintillation vial during the pretreatment showed that moisture escaped from within the palm fruits and flowed down the filter funnel into the beaker. For the small scale study, which used 100 g of fruits, at 1 and 2 min of pretreatment, the ruptures on the fruits were unnoticeable. Starting at 3 min to 4 min, the ruptures became more obvious to the naked eyes. Any duration beyond 4 min would burn the fruits and produce a black oil.

Figure 3.1(a): Palm fruit before microwave pretreatment

Figure 3.1(b): Palm fruits after microwave pretreatment for 4 min

3.1.2 Effect of Microwave Pretreatment Duration on Oil Yield

Both setups were subjected to solvent extraction (Soxhlet extraction) using *n*-hexane at fruits to solvent ratio of 1:4 (m/v) for 6 h after the pretreatment. The results for both small scale and larger scale experiments are reported in Table 3.1.

Table 3.1: Effect of microwave pretreatment duration on oil yield

Microwave Pretreatment Duration (min)	**Oil Yield (%)**					
	Small scale			Larger scale		
	Leached oil (LO)	Solvent extracted oil (SO)	Total	Leached oil (LO)	Solvent extracted oil (SO)	Total
0	-	0.1	0.1	-	0.1	0.1
1	0.1	2.5	2.6	0.0	0.2	0.2
2	0.7	5.2	5.9	0.0	1.0	1.0
3	1.3	10.7	12.0	0.0	1.9	1.9
4	4.3	16.5	20.8	0.1	2.4	2.5
5	5.9	15.2	21.1	0.1	3.1	3.2

As depicted in Table 3.1, small scale pretreatment (P=0.0009) for 5 min, yielded oil as much as 21.1%. However, the oil produced for this condition appeared black, which was undesirable. In contrast to this, oil produced from 1 to 4 min pretreatment appeared orange, the normal color of crude palm oil (CPO). A 4 min pretreatment was the optimized microwave pretreatment duration for the small scale study as it gave the best oil yield amount while keeping the appearance of the oil in the acceptable color range. Findings in the small scale experiment are in agreement with a similar study (Cheng,

Mohd & Chuah, 2011), which was conducted at the same scale while the present study was extended to larger scale at 300 g to further investigate the technical viability upon scaling up.

In the small scale microwave oven, the microwave radiation was able to penetrate and heat up the mesocarp of the fruits, thus causing the mesocarp to release water and rupture the fibre. Palm fruits are very suitable for microwave heating due to its composition (41% water, 39% oil and 20% fibre). When enough heat was supplied, water in the fruit will evaporate out of the mesocarp, drying and rupturing the fibre of palm fruit in the process. This process caused the oil contained in both mesocarp and kernel to leach out during the pretreatment and provided a higher surface area for the subsequent oil extraction, improving its efficiency.

Table 3.1 also shows that at the duration of 1 to 5 min, oil yield from larger scale pretreatment (P=0.0002) was almost negligible compared to that of the small scale. This was due to the lack of rupturing at the mesocarp of the fruits. However, the statistical analysis suggested that the microwave pretreatment has a significant impact towards the oil yield. Thus, optimization was needed for the larger scale pretreatment in order to achieve a similar oil yield obtained in the small scale microwave pretreatment. A few factors were taken into considerations, the sample size difference between small scale and larger scale (100 g vs. 300 g), microwave oven power difference (600 W vs. 1000 W) and the microwave oven capacity difference (17 L vs. 42 L). A comparison experiment of the pretreatment efficiency between both ovens were carried out (Table 3.2).

Table 3.2: Comparison of pretreatment efficiency between ovens used

Oven	Power (W)	Capacity (L)	Sample size (g)	Microwave pretreatment duration (min)	Oil yield (%)		
					Leached	Solvent extracted	Total
SAMSUNG	600	17	100	4	4.6	16.4	21.0
Panasonic	1000	42			4.2	16.3	20.6

This comparison was carried out under a few controlled parameters such as sample size (100 g), microwave pretreatment duration (4 min) and solvent extraction duration (6 h). As depicted by Table 3.2, both ovens performed rather similarly in terms of pretreatment efficiency, as both setups yielded almost similar oil yield. By comparing the leached oil yield from Table 3.1 to Table 3.2 at 4 min of microwave pretreatment, it was clear that the factor that affected the lower performance of larger scale microwave pretreatment in Table 3.1 was the sample size. Thus it is postulated that insufficient heat was applied to the larger scale sample size for the similar microwave pretreatment duration. The First Law of Thermodynamics (Equation 3.1) was applied in order to optimize the heat applied against the change of the sample size.

$$Q = mc\Delta T \quad \textbf{(3.1)}$$

where,

Q is the amount of heat energy, in J;

m is the mass, in g of substance;

c is the specific heat, in $\frac{J}{g \cdot K}$ of substance;

ΔT is the change of temperature, in K

As shown in Equation 3.1, the heat required in order to maintain the changes of the temperature within the oven is directly proportionate to the change in the sample sizes. Since the sample size was tripled when upscaling from small scale to larger scale, it was theorized that the microwave pretreatment duration must be tripled as well. Thus an experiment was carried out with extended microwave pretreatment duration in order to find the optimum duration for the larger scale extraction.

Table 3.3: Effect of extended microwave pretreatment durations to oil yield and the corresponding temperature

Microwave Pretreatment Duration (min)	**Oil Yield (%)**			**Oil Color**	**Temperature (˚C)**
	Leached oil (LO)	Solvent extracted oil (SO)	Total		
5	0.2	6.4	6.6	Light orange	98.4
10	3.2	8.7	11.0	Orange	101.0
12	4.0	9.9	13.9	Dark Orange	105.7
13	4.4	12.9	17.3	Black	110.0
14	5.0	15.0	20.0	Black	116.2
15	5.2	17.4	22.6	Black	120.2

As depicted in Table 3.3 ($P<0.0001$), 15 min of microwave pretreatment was able to produce comparable oil yield to that of the small scale. However, any microwave pretreatment duration after 10 min might risk to yield the undesirable black oil. Due to the existence of this risk, the optimum microwave pretreatment duration was 10 min, but the total oil yield (11.0% at 10 min) was incomparable from that of the small scale (20.8% at 4 min).

3.1.3 Effect of Solvent Extraction Duration on Oil Yield of the Larger Scale Extraction

Further optimization was carried out on the solvent extraction. Two factors were considered in the optimization process. However, only optimization of solvent extraction duration was investigated. This is because the fruits to solvent ratio, 1:4 (m/v) was the ratio that allowed more oil to be extracted, compared to 1:5 (m/v) and 1:6 (m/v) in a similar setup (Cheng, Mohd & Chuah, 2011).

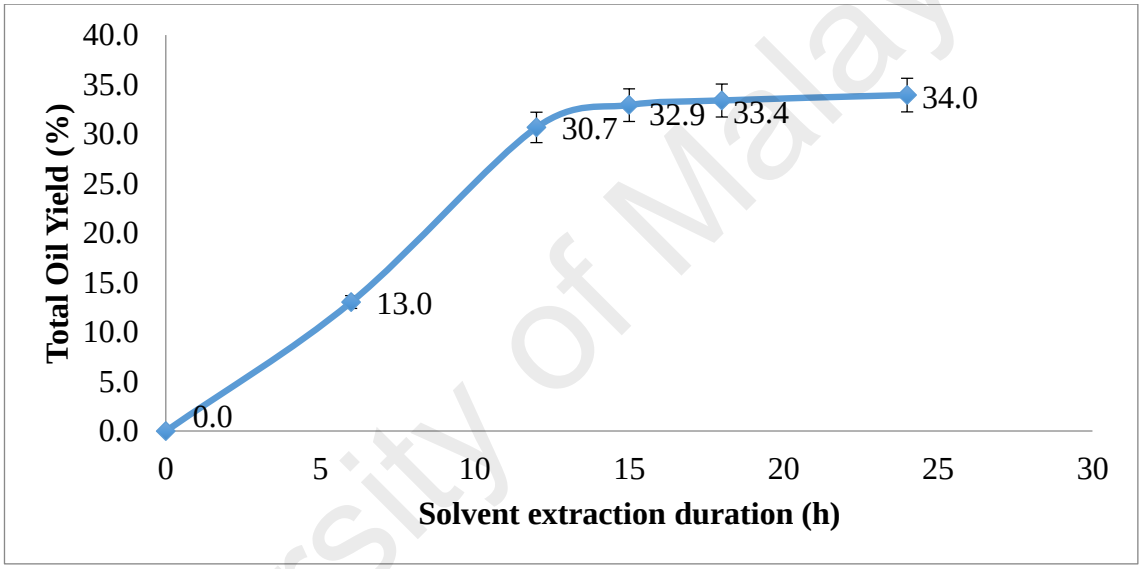

Figure 3.2: Effect of extended solvent extraction durations towards total oil yield in larger scale study

As depicted in Figure 3.2, the graph appeared as sigmoid shape and the oil yield was increasing and then reached a plateau after 12 h (P=0.125). Statistical analysis suggested that the oil yield obtained after 12 h extraction showed no significant different at P>0.05. The limiting factors is postulated to be the oil content in palm fruits. Bockisch (1998) reported that Asian palm mesocarp contains about 39% oil, 41% water and 20% fibre. After 12 h of extraction, 30.7% of oil was extracted and that accounted for 79% of available oil content in the fruit. This is a huge improvement over the small

scale extraction study. Although the oil yield was the highest after 24 h of extraction, any extraction beyond 12 h was not justifiable because the *p*-value suggested that there was no significant difference.

An extraction was carried out using the optimized conditions (microwave pretreatment of 10 mins and solvent extraction of 12 h). The yield for leached oil and solvent extracted oil were 1.3% and 29.0%, respectively, amounting for a total oil yield of 30.3%. The oil produced under this condition were subjected to quality analyses.

3.2 Quality of the Oil Produced in the Larger Scale Extraction

Quality analyses were carried out on the oil extracted of the optimized condition and its specifications compared to the specification by Malaysian Standard (MS814:2007) is reported in Table 3.4.

Table 3.4: Specification of the oil extracted and its compliance to MS814:2007

Characteristics	**MS814:2007**	**Oil produced in present study**
Free fatty acid (% as palmitic acid)	<5.0	0.6
Carotene content (ppm)	500 – 700	700
Peroxide value (meq/kg)	<2.0	3.5
Anisidine value (meq/kg)	<5.0	2.2

3.2.1 Free Fatty Acid (FFA)

The free fatty acid (FFA) content of the oil produced as reported in Table 3.4 was much lower than the specification stated by MS814:2007. This was majorly credited to the dry pretreatment process. The presence of moisture would promote the rate of hydrolysis of the acylgylcerols, thus forming free fatty acids as the product as depicted in Figure 3.3.

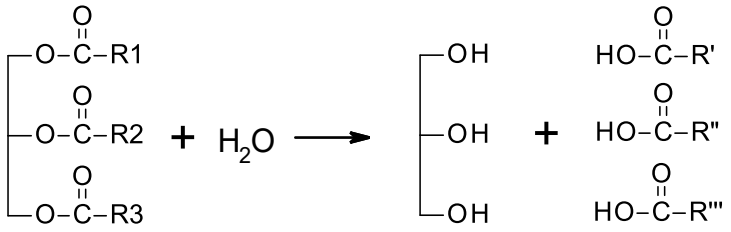

Figure 3.3: Formation of free fatty acid through hydrolysis

High acidity oil is unfavourable. Mba, Dumont and Ngadi (2015) stated that a good quality palm oil would consist of low FFA and moisture content, low levels of impurities and a good bleachability index. According to Malaysian Standard MS814:2007, FFA content of crude oil must be lower than 5% while refined oil lower than 0.1%. Čmolík and Pokorný (2000) reported that to reduce CPO with 12% FFA to 1.3% required 220˚C at 0.8 kPa pressure, and to 0.5% required 230˚C at the same pressure. More energy was required in order to reduce more FFA from the oil. Lowering the FFA content from the crude oil is important as it could reduce the cost of refining.

3.2.2 Carotene Content

As depicted in Table 3.4, the carotene content in the oil produced in current studies was around 700 ppm. This amount fell within the range provided by MS814:2007. Crude palm oil is the natural plant oil with the richest amount of retinol (provitamin A) equivalent, which accounted for its orange-reddish color. Higher amount of carotene content is desirable due to its provitamin A and antioxidant activities, contributing to the oil's nutritional quality.

Cheng *et al.* were able to obtain carotene content up to 1758 ppm through the same method on 2 min microwave pretreatment (pretreatment temperature at 101˚C) in a

lower powered microwave oven. They also found out that after 2 min of microwave pretreatment, the detected carotene content in the oil produced has decreased due to the increasing pretreatment temperature. There was a severe thermal effect on compounds with pro-vitamin A activities at high temperature during microwave heating (≥75°C) (Fratianni, Cinquanta & Panfili, 2009). Our present study was unable to obtain carotene content as high as the mentioned study due to more heat was accumulated during the longer microwave pretreatment duration. This had caused a higher pretreatment temperature (up to 120.2˚C) as shown in Table 3.3, hence decomposing a lot of carotenes in the oil produced.

3.2.3 Total Oxidation Value (TOTOX)

As shown in Table 3.4, the peroxide value (PV) recorded was 3.5 meq/kg, which does not comply to the specification of MS814:2007 (< 2.0 meq/kg). On the other hand, the anisidine value (AnV) of the oil produced was 2.2 meq/kg, complied with the specification of MS814:2007. This excessive amount of hydroperoxides formed might be due to the long exposure of the oil to high temperature during the pretreatment. As mentioned in 1.2.3, formation of hydroperoxide can occur under autoxidation. In addition to that, the elevation of the temperature during the microwave pretreatment sped up the formation of hydroperoxides when the oil was exposed to oxygen in the air.

Moigradean, Poiana and Gogoasa (2012) reported that palm oil with significant level of PV might still be odourless if secondary oxidation has not occurred. Secondary oxidation will result in products with low PV. Secondary oxidation decomposed the hydroperoxides into carbonyls and other compounds, particularly aldehydes. These products were what made up the rancidity as well as the bad odor in the oil. Low

TOTOX value is important because high TOTOX value could add burden to the palm oil refining process as extra resources are required to deodorize the oil.

3.2.4 Effect of Microwave Pretreatment Duration to Oil Quality

A study was carried out to investigate the effect of microwave pretreatment duration to oil quality. The said experiment was carried out manipulating various microwave pretreatment duration while controlling the solvent extraction duration at 12 h and the fruits to solvent ratio at 1:4 (m/v). The results are recorded in Table 3.5.

Table 3.5: Effect of Microwave Pretreatment Duration to Oil Quality

Microwave Pretreatment Duration (min)	Free Fatty Acid (% as palmitic acid)	Carotene Content (ppm)	Peroxide Value (meq/kg)	Anisidine Value (meq/kg)	Color
5	0.4±0.2	937±1	2.5±0.0	1.6±0.4	Light orange
10	0.4±0.0	781±3	3.5±0.0	1.2±0.0	Orange
13*	0.4±0.1	600±1	4.7±0.4	3.4±0.4	Dark orange
15*	0.3±0.00	445±2	6.7±0.4	4.4±0.0	Black
18*	0.3±0.00	375±0	9.2±0.3	5.2±0.4	Black
20*	0.4±0.00	320±2	10.2±0.3	7.1±0.4	Black

* Oil analysed after the black-coloured compound were filtered.

Table 3.5 shows the effect of microwave pretreatment duration on the amount of FFA in the oil produced. It was seen that the length of microwave pretreatment has no effect on the FFA content in the oil. This phenomenon is due to the initial inhibition of the lipase during the microwave pretreatment, similar to the inhibition of it in conventional milling process. Conventional palm oil milling induced the hydrolysis of acylglycerols even after the lipase inhibition due to the introduction of water. Unlike conventional palm oil milling, microwave pretreatment removed the moisture from the fruits during

the heating and solvent extraction did not introduced any form of moisture into the oil as well, causing no hydrolysis to occur, thus, lower the FFA amount.

Table 3.5 also shows the relationship between microwave heating duration and the carotene content. The longer the heating duration, the lower the carotene content detected. This was due to the increase in the accumulated temperature within the oven. Mba *et al.* reported that high temperatures will cause the carotene to be destroyed, thus reducing the oil's nutritional quality.

As shown in Table 3.5, PV and AnV increased in accordance to the microwave pretreatment duration. Prolonged microwave pretreatment caused the temperature to be increased and the elevated temperature will cause higher rate of autoxidation when the oil is in contact with oxygen in the air.

3.2.5 Fatty Acid Composition (FAC)

Every oils and fats have their own inherent fatty acid composition (FAC), enabling the identification of any oil and fat sample. The FAC of the oil produced was also analysed. The results were tabulated in Table 3.6.

Table 3.6: Fatty Acid Composition of the oil produced and comparison to MS814:2007

Fatty acid composition (FAC) (as % methyl ester)	Crude palm oil (CPO)	Crude palm kernel oil (CPKO)	Leached oil (LO)	Solvent extracted oil (SO)
C12:0	ND - 0.5	45.0 - 55.0	6.3	1.5
C14:0	0.5 - 2.0	14.0 - 18.0	2.9	1.4
C16:0	39.3 - 47.5	6.5 - 10.0	39.1	45.2
C18:0	3.5 - 6.0	1.0 - 3.0	3.2	3.1
C18:1	36.0 - 44.0	12.0 - 19.0	38.9	38.8
C18:2	9.0 - 12.0	1.0 - 3.5	9.6	10.0

As shown in Table 3.6, the oil produced using the optimized condition in the present study has a unique FAC. It does not fall into the range of neither CPO nor CPKO produced using conventional milling process (MS814:2007).

The commercial CPO has a very low level of lauric acid (C12:0), between undetectable to 0.5%. The is because the conventional palm oil milling process separates the palm kernel from the mesocarp prior to screw pressing. The oil produced in the present study has considerably high amount of lauric acid (6.3% in LO and 1.5% in SO) and out of the range of that in CPO and CPKO. This is because during microwave pretreatment, oil leached from the fruits may also contained oil leached from the kernel. The rupturing of the fibre also enabled the solvent to extract a certain amount of oil from the kernel, thus increasing the lauric acid content in the oil produced.

Lauric acid has been reported to exhibit antimicrobial properties in multiple scientific studies (Ouattara, Simard, Piette, Bégin & Holley, 2000; Hoffman, Han & Dawson, 2001; Dawson, Carl, Acton & Han, 2002). Besides that, lauric acid has the most profound effect in increasing the total cholesterol among all fatty acids and most of the increment is attributed to increase in high-density lipoprotein (HDL), the "good cholesterol" (Thijssen and Mensink, 2005). This resulted in a more favourable total:HDL ratio, which leads to lower atherosclerotic risk. High amount of lauric acid, along with a high amount of carotene content will add more nutritional value to the oil produced as it will have more beneficial effect to the human health as compared to the CPO produced using conventional milling process.

3.3 Effect of Prolonged Microwave Pretreatment

A study was carried out to investigate the effect of prolonged microwave pretreatment to the oil produced. A batch of black oil was produced under 15 min of microwave

pretreatment and 12 h of solvent extraction solely for this study. The oil yield obtained was 33.3% and the highest surface temperature of the burnt fruits recorded was 191±3˚C.

3.3.1 Physical and Chemical Changes

Even though prolonged microwave pretreatment will produce higher oil yield, it has adverse effect on the quality of the oil extracted, i.e. lower amount of carotene and higher value of peroxide value (PV) and anisidine value (AnV). Chemical and physical changes were noted upon prolonged microwave pretreatment. It was found that a microwave exposure for more than 12 min produced a black oil. Table 3.7 presents the identified compounds in the acetone fraction, which contained the desorbed and coloured compounds from SPME-GC-MS analysis.

Table 3.7: Compounds identified in acetone fraction using SPME-GC-MS analysis

Compound #	Compound	%
1	(E)-2-nonenal	0.47
2	(E)-5-tetradecene	0.91
3	1-(4-hydroxy-3-methoxyphenyl)-ethanone	1.16
4	1-bromo-3,3-dimethyl-2-butanone	0.39
5	1-methyl-1-(1-methylethyl)-2-nonylcyclopropane	0.66
6	1-methylcyclopentanol	12.96
7	1-nonadecene	7.22
8	1-nonadecene	2.01
9	1-octanol	1.15
10	1-octen-3-ol	0.55
11	1-tetradecanol	9.44
12	1-tridecene	5.09
13	1,1-hexylenedioxybutane	1.46
14	2-(prop-2-enoyloxy)-tetradecane	0.75
15	2-hexanone	1.31
16	2-methyl-1-pentadecene	0.50
17	2-methyl-1-pentadecene	0.80
18	2-methyl-1-pentanone	0.84
19	2-methyl-2-pentanol	1.47

Compound #	Compound	%
20	2-methylpyridine	4.03
21	2,2'-trimethylenebis-1,3-dioxolane	2.18
22	2,3-dimethyl-3-buten-2-ol	1.17
23	2,4-dimethyl-2-pentene	1.32
24	2,6-dimethoxyphenol	2.98
25	2,6,11-trimethyldodecane	0.46
26	3-hexanone	0.73

Table 3.7: Compounds identified in acetone fraction using SPME-GC-MS analysis, continued

Compound #	Compound	%
27	3-mehtylcyclopentanone	1.13
28	3-methyl-3-pentanol	2.95
29	3-methylbutanal oxime	0.72
30	3-methylcyclopentanol	1.47
31	3,4-dihydro-6-methyl-2H-Pyran	1.77
32	3,5-bis(1,1-dimethylethyl)-phenol	0.53
33	4-hydroxy-4-methyl-2-pentanone	1.13
34	4-nitro-pentanoic acid methyl ester	3.12
35	5-methyl-2-hexene	0.75
36	5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone	0.71
37	6-heptyltetrahydro-2H-Pyran-2-one	0.59
38	6-methyl-5-hepten-2-one	0.76
39	7-methyl-6-tridecene	0.51
40	7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	0.85
41	Acetic acid	1.30
42	Benzophenone	1.43
43	Butanal	0.74
44	Butyl acetate	0.24
45	Decanal	0.41
46	Dodecane	0.47
47	E-15-heptadecanal	0.39
48	Eicosane	0.34
49	Formic acid	0.62
50	Gamma-dodecalactone	0.86
51	Heptanal	0.91
52	Hexadecane	0.79
53	N-pentadecanol	0.85
54	Nonanal	7.13
55	Octanal	1.42
56	S,S-dioxide-trans-2-methyl-4-n-pentylthiane	1.27

57	Tetradecane	0.85
58	Tetrahydro-6-methyl-2H-Pyran-2-one	0.51
59	Trans-2,3-epoxyoctane	0.48

*Percentage was calculated based on area percentage under chromatogram.

From Table 3.7, there are a total of 59 compounds detected. Most of the compounds detected were alkanes, alkenes, alcohols, aldehydes and ketones. The aldehydes and ketones were deduced to be the secondary oxidation products. Multiple compounds were reported to be products formed during the pyrolysis of cellulosic materials in biomass, such as formic acid, acetic acid and 5-methyl-2(5H)-Furanone (Mettler *et al.*, 2011).

3.3.2 Cellulose Degradation

Yin (2012) reported that microwave pyrolysis was different from the conventional pyrolysis due to its unique features such as rapid, volumetric heating and inverted heat transfer. Therefore, high temperature requirement mentioned in Metter *et al.* (2011) in order to pyrolyse cellulosic materials was not required to pyrolyse the fibre in palm fruits.

The major compounds identified were 1-methylcyclopentanol (12.96%), 1-tetradecanol (9.44%), 1-nonadecene (7.22%), nonanal (7.13%) and 1-tridecene (5.09%) (Table 3.7). These compounds were deduced to be formed during the decomposition of cellulose in the palm fruits during microwave pretreatment. High temperature up to 191±3˚C promoted the burning and decomposition of cellulose (Figure 3.4). Microwave pyrolysis of biomass cellulose can happen at temperature lower than 350˚C, and estimated that 180˚C was identified as the key turning point in the microwave degradation of cellulose (Budarin, Clark, Lanigan, Shuttleworth & Macquarrie, 2010; Wu *et al.*, 2014).

Figure 3.4: Appearance of palm fruits upon prolonged microwave pretreatment

Cellulose is a linear polysaccharide polymer with 10,000 to 15,000 glucose monosaccharide units, hence it is deduced that the cellulose in palm fruits fibre first broke down into glucose monomers before further decomposed into products identified in Table 6 as depicted in Figure 3.5. Figure 3.6 showed one of the possible pathway for the formation of 2-hexanone, while Figure 3.7 showed its further decomposition into 1-methylcyclopentanol, which is the major compounds detected by SPME-GC-MS. Figure 3.8 showed the formation of 6-methyl-3,4-dihydro-2H-pyran further illustrated the existence of glucose in the decomposed products.

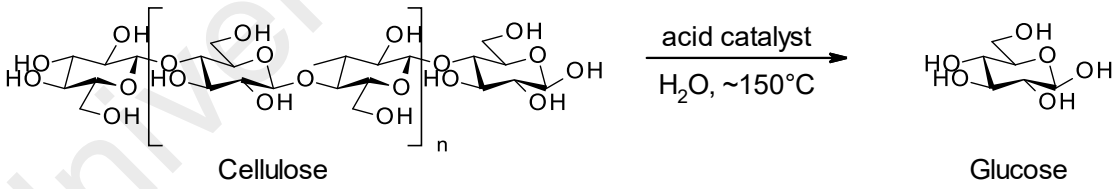

Figure 3.5: Formation of glucose from cellulose

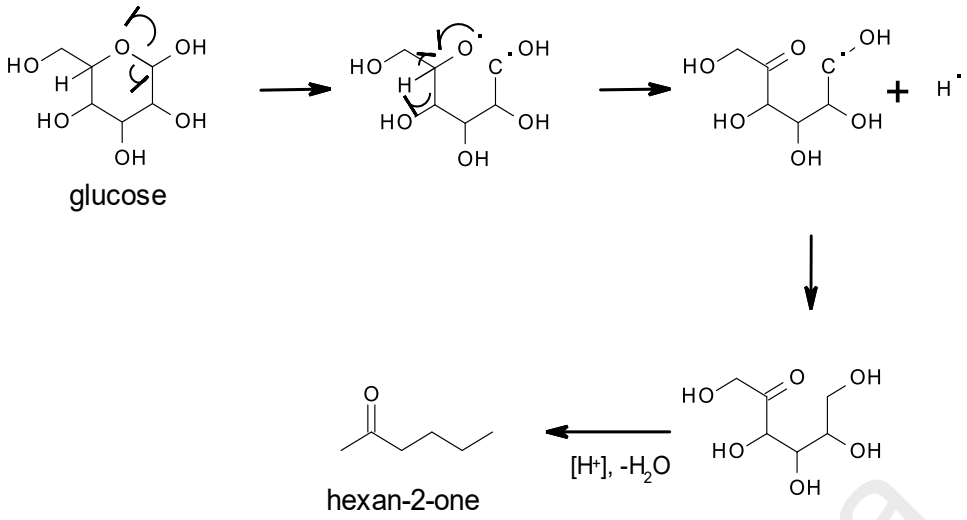

Figure 3.6: Formation of 2-hexanone from glucose

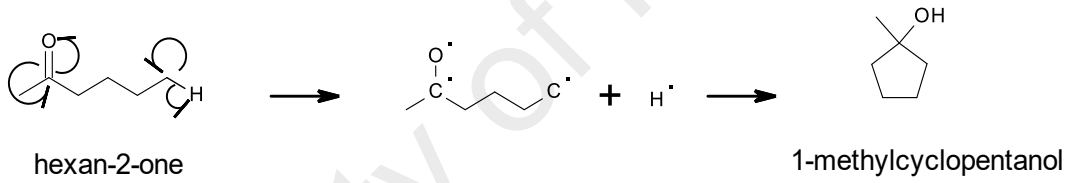

Figure 3.7: Formation of 1-methylcyclopentanol from 2-hexanone

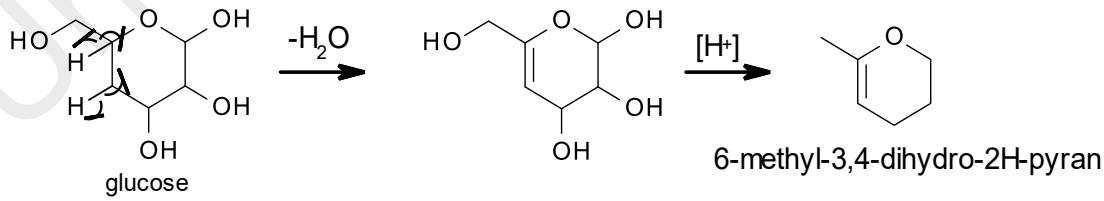

Figure 3.8: Formation of 3,4-dihydro-6-methyl-2H-Pyran from glucose

Due to the presence of moisture in the oven during the microwave pretreatment process, it is postulated that hydrothermal degradation of cellulose also occurred along with the

pyrolysis process. This is due to the presence of multiple organic acids in the identified compounds, namely, formic acid and acetic acid (Table 3.7).

Yan and Qi (2014) reported that at 180°C, a homogenous solution of cellulose degrade into 9.64% of formic acid and 4.53% of acetic acid. At a higher temperature of 200°C, 12.27% of formic acid and 9.47% of acetic acid was formed. Figure 3.9 showed the proposed mechanism for the formation of formic acid and acetic acid from glucose under hydrothermal reaction.

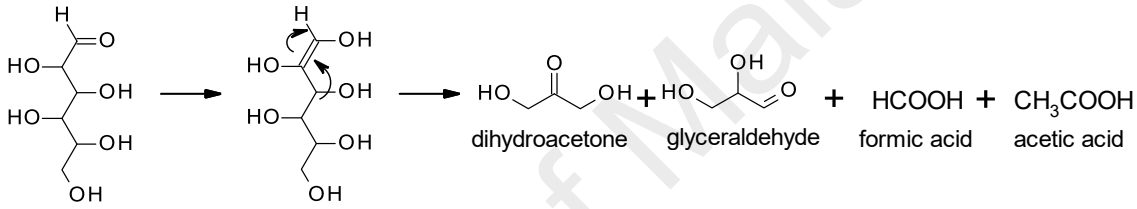

Figure 3.9: Proposed mechanism for the formation of organic acids (Yan and Qi, 2014)

CHAPTER 4: CONCLUSION

Findings from the present study supported that microwave pretreatment and solvent extraction can be a viable alternative method to extract palm oil. Statistical analyses showed that the microwave pretreatment duration had a significant impact towards the oil yield for both scales. However, for the larger scale extraction, prolonged microwave pretreatment beyond 10 min will give undesirable product. Solvent extraction duration longer than 12 hours did not bring significant improvement in oil yield as it hits a plateau after 12 hours. This is supported by a statistical analysis.

Under the optimized condition for the larger scale extraction (microwave pretreatment of 10 min, solvent extraction of 12 h) in the present study, high yield of oil (30.7%) was obtained with good quality (FFA=0.6%; AnV=2.2 meq/kg), which meet the specifications outlined by local authorities and palm oil industry, including the color and appearance except peroxide values (PV=3.5 meq/kg). Meanwhile, microwave pretreatment beyond 12 min on the other hand elevated the pretreatment temperature causing pyrolysis and hydrothermal degradation to occur at the same time forming degradation products that caused the undesirable color of the oil produced.

The proposed method is a cleaner method with no introduction of water into the process and hence produces no wastewater. Environmental issues arise from unacceptable management of wastewater can be omitted in the present processing method. Improvement in design of microwave oven chamber for a more thorough heating during the pretreatment stage may help to improve the unacceptable PV, which is worth to be further investigated. Nevertheless, the present study showed that alternative milling process is available and may be feasible in the future.

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