DEVELOPMENT OF A NOVEL PALM KERNEL TESTA REMOVAL METHOD AND CHARACTERIZATION OF ITS EFFECTS

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2016

DEVELOPMENT OF A NOVEL PALM KERNEL TESTA REMOVAL METHOD AND CHARACTERIZATION OF ITS EFFECTS

by

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Thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

July 2016

ACKNOWLEDGEMENTS

In the name of Allah, the Merciful, the Compassionate.

All praise and thanks is due to Allah S.W.T., and His blessings be upon His Beloved Prophet Muhammad, peace be upon him, and his family and companions. I would like to thank my family for their patience, fortitude and support in the course of my Doctoral studies – my mother, Norshela Mansor; my father, Harry Zuknik (25^{th} December 1935 – 16^{th} July 2014, may Allah S.W.T. have Mercy upon him, and grant him entry into The Garden); and my wife, Ruzanna Muhammad.

I would like thank my supervisor, Professor Ir. Dr. Mohd Omar Abdul Kadir, for his patience and invaluable guidance throughout the course of my Doctoral studies. I would also like to thank Professor Dr. Nik Norulaini Nik Abd, Rahman, of the School of Distance Education, for her valuable input and advice concerning my Doctoral studies. I would like to thank Professor Dr. Zhari Ismail, of the School of Pharmaceutical Science, for his valuable input and advice concerning my Doctoral studies. Finally, I would like to thank Shalima Thamarakshan, for processing all the bureaucratic affairs which were required throughout the length of my studies.

Thank you.

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LIST OF SYMBOLS

		Unit
C_1	Concentration of sodium carbonate	%
C_2	Concentration of hydrogen peroxide	%
t_1	Treatment duration of sodium carbonate	minutes
T_1	Treatment temperature of sodium carbonate	°C
t_2	Treatment duration of hydrogen peroxide	minutes
T_2	Treatment temperature of hydrogen peroxide	°C

LIST OF ABBREVIATIONS

FAME	Fatty Acid Methyl Esters
FTIR	Fourier Transform Infrared
GC-MS	Gas Chromatography–Mass Spectrometry
H_2O_2	Hydrogen peroxide
Na_2CO_3	Sodium carbonate
NMR	Nuclear Magnetic Resonance
PKK	Palm kernel without testa
PKK_W	Whole palm kernel without testa
PKT	Palm kernel testa
RPK	Raw palm kernel
RPK_W	Whole raw palm kernel

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PEMBANGUNAN KAEDAH BARU UNTUK PENYINGKIRAN TESTA ISIRUNG SAWIT DAN PENCIRIAN KESANNYA KE ATAS ISIRUNG SAWIT

ABSTRAK

Dalam kajian ini, satu kaedah baru untuk penyingkiran testa dari isirung sawit telah dibangunkan menggunakan rawatan berjujukan dengan natrium karbonat (Na_2CO_3) dan hidrogen peroksida (H_2O_2). Untuk menjalankan rawatan paling berkesan bagi 100g isirung sawit, syarat-syarat untuk memastikan penyingkiran testa secara menyeluruh adalah seperti berikut: kepekatan Na_2CO_3 (C_1) dan kepekatan H_2O_2 (C_2) pada tahap 30%; suhu Na_2CO_3 (T_1) dan suhu H_2O_2 (T_2) masing-masing pada suhu 90°C dan 80°C; manakala tempoh rawatan Na_2CO_3 (t_1) dan tempoh rawatan H_2O_2 (t_2) masing-masing pada jangka masa 80 minit dan 50 minit. Kaedah baru untuk penyingkiran testa isirung sawit yang telah dibangunkan dalam kajian ini turut digunakan dalam penyediaan sampel isirung sawit tanpa testa (PKK) dan sampel testa isirung sawit (PKT) yang kedua-duanya diperolehi daripada sampel isirung sawit mentah (RPK). Kandungan kompaun kimia ketiga-tiga jenis sampel RPK, PKK dan PKT kemudiannya ditentukan melalui proses pengekstrakan soxhlet menggunakan *n*-heksana dan metanol sebagai pelarut. Ekstrak yang diperolehi daripada sampel-sampel tersebut kemudiannya dianalisis menggunakan mesin kromatografi gas-spektrometri jisim (GC-MS), spektroskopi inframerah transformasi Fourier (FTIR) dan spektroskopi resonans magnet nukleus (NMR). Analisis ekstrak RPK menunjukkan kehadiran lima asid lemak bebas, tiga sebatian fenolik, dan trigliserida. Manakala, analisis ekstrak PKK pula menunjukkan kehadiran hanya dua asid lemak bebas, trigliserida, dan tiada sebatian fenolik. Keputusan ini menunjukkan bahawa penggunaan proses penyingkiran testa ini menyebabkan penurunan dalam

kandungan asid lemak bebas dan sebatian fenolik di dalam isirung sawit. Analisis ekstrak PKT pula tidak menunjukkan kehadiran sebarang sebatian fenolik, dan ini menunjukkan bahawa penggunaan proses penyingkiran testa ini mengakibatkan kehilangan sebatian fenolik dari testa isirung sawit. Melalui analisis yang dijalankan ke atas struktur bersel bagi sampel RPK dan PKK menggunakan mikroskop pengimbas elektron (SEM), didapati bahawa struktur bersel isirung sawit kekal kukuh selepas menjalani proses penyingkiran testa ini. Selain itu, aplikasi kaedah penyingkiran testa ini ke atas isirung sawit mentah juga menyebabkan peningkatan dalam hasil minyak isirung sawit yang diekstrak daripada isirung sawit; dimana sampel RPK dan PKK masing-masing menunjukkan purata hasil minyak 43.49% dan 64.68%. Analisis asid lemak metil ester (FAME) turut dijalankan ke atas minyak isirung sawit yang telah diekstrak daripada sampel-sampel RPK dan PKK. Keputusan dari analisis FAME menunjukkan bahawa profil asid lemak RPK dan PKK adalah serupa. Analisis kandungan protein di dalam sampel RPK, PKK dan PKT pula menunjukkan bahawa penggunaan proses penyingkiran testa ini menyebabkan penurunan kandungan protein dalam isirung sawit. Purata kandungan protein bagi sampel-sampel RPK, PKK dan PKT masing-masing adalah 8.35%, 7.00% dan 15.19%. Ujian mampatan kuasistatik yang dijalankan ke atas sampel RPK sempurna (RPK_W) dan sampel PKK sempurna (PKK_W), menunjukkan bahawa purata beban maksimum yang diperlukan untuk memampatkan sampel RPK_W dan PKK_W sehingga mencapai titik pecah masing-masing adalah 635.1N dan 143.2N. Ini menunjukkan bahawa kekuatan struktur fizikal isirung sawit menurun selepas melalui proses penyingkiran testa yang dibangunkan dalam kajian ini.

DEVELOPMENT OF A NOVEL PALM KERNEL TESTA REMOVAL METHOD AND CHRACTERIZATION OF ITS EFFECTS

ABSTRACT

A new method was developed in this study for the removal of palm kernel testa from palm kernels utilizing sequential treatment with sodium carbonate (Na_2CO_3) and hydrogen peroxide (H_2O_2). For the treatment of 100 g of palm kernels, the most effective treatment conditions which resulted in a complete removal of the testa were as follows: Na_2CO_3 concentration (C_1) and H_2O_2 concentration (C_2) of 30%; Na_2CO_3 temperature (T_1) and H_2O_2 temperature (T_2) of 90°C and 80°C, respectively; and Na_2CO_3 treatment duration (t_1) and H_2O_2 treatment duration (t_2) of 80 and 50 minutes, respectively. The new palm kernel testa removal method developed in this study was then applied to raw palm kernel (RPK) samples to produce palm kernel without testa (PKK) and palm kernel testa (PKT) samples. The chemical compound content of RPK, PKK and PKT were then determined by subjecting the respective samples to soxhlet extraction using *n*-hexane and methanol as solvents. The extracts of the respective samples were then subjected to analysis by gas chromatography-mass spectrometry (GC-MS), Fourier transform infrared (FTIR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy. Analysis of RPK extracts detected the presence of five free fatty acids, three phenolic compound, and triglycerides. Analysis of PKK extracts indicated the presence of only two free fatty acids, triglycerides and no phenolic compounds, indicating that application of the testa removal process developed in this study resulted in a decrease of the content of free fatty acids and phenolic compounds of the palm kernels. Analysis, of PKT extracts could not detect the presence of any phenolic compound, indicating that application of the testa removal process resulted in a loss of phenolic compounds

from the palm kernel testa. Scanning electron microscopy (SEM) analysis of the cellular structure of RPK and PKK samples found that the cellular structure of the palm kernels remained intact after undergoing the testa removal process. Subjecting raw palm kernels to the testa removal method developed in this study also resulted in greater palm kernel oil yields which were subsequently extracted from the palm kernels, with RPK and PKK having an average oil yield of 43.49% and 64.68%, respectively. Palm kernel oil extracted from RPK and PKK samples were also subjected to fatty acid methyl ester (FAME) analysis, with the results indicating that the fatty acid profile of RPK and PKK were similar. Protein content analysis of RPK, PKK and PKT samples found that the application of the testa removal process developed in this study resulted in a decrease of the palm kernel protein content. The average protein content of RPK, PKK and PKT was 8.35%, 7.00% and 15.19%, respectively. Quasi-static compression tests performed on whole RPK (RPK_W) and whole PKK (PKK_W) samples found that the average maximum load required to compress the RPK_W and PKK_W samples until rupture point was 6.351 N and 143.2 N, respectively, thus indicating that the palm kernels were physically weakened after undergoing the testa removal process developed in this study.

Chapter 1

Introduction

1.1 Introduction

The oil palm (*Elaeis guineensis*) is one of the main important cash crops cultivated in Malaysia, with Malaysia accounting for 85% of the world's total production of palm oil. Malaysia produced a total of 19.22 million tonnes of crude palm oil in 2013, a 2.28% increase from the total of 18.79 million tonnes of crude palm oil produced in 2012. The oil palm is the most efficient crop in terms of oil production with a single hectare of oil palm plantation having the capacity to produce an average of 3.7 tonnes of oil per year in Malaysia.

The fruit of the oil palm is a sessile drupe and is comprised of an outer skin called the exocarp, a middle pulp called the mesocarp and a shell which covers the kernel called the endocarp. The oil content of the mesocarp varies from under 40% to over 60% (Corley and Tinker, 2003). Removal of the soft, oil-bearing mesocarp from the oil palm fruit will reveal a nut, which is the seed of the fruit. The seed of the oil palm is comprised of an endocarp, or outer shell, which encloses the kernel. From a botanical perspective, the actual seed of the oil palm is the kernel itself. However, the term 'seed' is commonly used to refer to the entire nut due to the fact that in agriculture, it is the nut which is stored, germinated and planted (Corley and Tinker, 2003). Like the mesocarp, the kernel of the oil palm fruit is also rich in oil, which is commonly extracted using the screw press method yielding between 40 to 43% (g oil/100g kernel) of palm kernel oil (PKO). On a wet basis, palm kernel contains 45 to 50% oil (Tang and Teoh, 1985). Removal of the oil from the palm kernel via the screw press method will yield palm kernel cake (PKC), which is ground palm kernel

that has been shorn of a majority of its oil content. Raw PKC has a total dietary fibre content of about 60.71% and crude fibre content of 15.17%.

The kernel of the oil palm fruit is surrounded by a dark brown testa which constitutes 3 to 5% of the kernel weight (Sreedhara et al., 1992). The dark colour of the testa is due to the presence of phenolic compounds within the testa. Food products made from palm kernel, such as PKO and palm kernel flour, will in turn be coloured due to the phenolic compounds from the palm kernel testa being present within these products. According to Sreedhara et al. (1992), the presence of phenolic compounds within the testa restricts the utilization of palm kernel oil and meal due to the dark colour which is imparted to these products by the phenolic compounds. Similarly, PKC also has a dark colour due to the presence of the testa within the matrix of the cake. When used as animal feed, the presence of phenolic compounds and tannins within the testa of PKC has the adverse effect of lowering the availability of its protein content when ingested by livestock. As feedstock, PKC is ranked higher than copra cake and cocoa pod husk. However, it is ranked lower compared to fish meal and groundnut cake, particularly in its protein value (Wong and Wan Zahari, 1997). Tannins within the palm kernel testa may bind to or react with proteins via hydrogen bonding, ionic and hydrophobic interactions, all of which cause the tridimensional structure of palm kernel protein to be altered (Barry, 1989; Ramaswamy and Rege, 1976; Griffiths and Mosely, 1980; Oloyo, 1991; Sastry and Narasinga Rao, 1991; Yu et al, 1995). Phenolic compounds within the testa may be oxidized into quinones which in turn form insoluble complexes via reaction with the thiol and amino groups of proteins, which results in the decreased digestibility of the proteins present in the palm kernel when ingested by livestock (Pierpoint, 1969; Siebert et al., 1996; Sosulki 1979; Vithayathil and Gupta, 1981).

Due to the adverse effects of the palm kernel testa on the nutritional quality of products derived from palm kernel, removal of the testa should potentially improve the nutritional quality of subsequent products derived from palm kernel. However, the testa of the palm kernel is strongly bound to the kernel by a layer of gum or lignin, which makes removal via physical methods extremely difficult. Within the literature review that has been done, the only study that has been carried out concerning the removal of the testa from palm kernel is by Sreedhara et al. (1992). Within the study, Sreedhara et al. (1992) utilized a wide array of different solvents to attempt the removal of the testa from palm kernel including hydrochloric acid (HCl), sulphuric acid (H_2SO_4), phosphoric acid, acetic acid, sodium hydroxide (NaOH), potassium hydroxide (KOH), ammonium hydroxide, barium hydroxide and calcium hydroxide, amongst others. The solvents were applied at different concentrations, treatment times and temperatures. The only solvents that were successful in completely removing the testa from palm kernels were HCl, NaOH and KOH. However, the utilization of NaOH and KOH for the removal of the testa from palm kernel resulted in the kernels being coloured brown by the treatment process. Only treatment with HCl was ultimately successful in removing the testa from the palm kernels and yielding white coloured kernels. According to Sreedhara et al. (1992) treatment of palm kernels with 1N HCl at temperature of 95°C for 45 minutes resulted in complete removal of testa from palm kernels. When higher concentrations of HCl were applied for treatment of palm kernels, shorter treatment times were required.

In another study, Sreedhara and Kurup (1998) compared the protein absorption of rats that were fed a diet of either pure casein, treated palm kernel meal or untreated palm kernel meal, all for a period of 10 days. Rats fed the casein diet

had the highest gains in body weight, gaining an average of 64.2 g while rats fed the treated and untreated palm kernel meal had an average body weight gain of 59.5 g and 42.5 g, respectively. Therefore, the results of the study show that the removal of palm kernel testa improved the characteristics of the palm kernel meal as animal feed. However, the study by Sreedhara et al. (1992) remains the only piece of literature available to date on the removal of testa from palm kernel, and which unequivocally states that HCl is the best solvent for removing the testa. However, HCl is a dangerous and highly corrosive chemical. Therefore, alternative methods for the removal of testa from palm kernel are desirable from a safety standpoint, preferably using safer materials.

Within a patent made by George et al. (2000), a method was detailed regarding the removal of skins or hulls from seeds used for human consumption including legumes, grains, drupes, achenes and silques. The method comprises of two major steps. In the first step, the seeds are wetted with an alkaline solution such as sodium carbonate, sodium bicarbonate, sodium silicate or sodium phosphate, at various concentrations, temperatures and time durations. In the second major step of the method, seeds are then wetted with a peroxygen compound at various concentrations, temperatures and time durations. The reaction between the alkaline solution and the peroxygen solution will cause the formation of gas which will then blister the skin of the seeds, thus loosening the skin and facilitating its removal. For the first step involving the alkaline solution, the possible ranges for concentration, temperature and time periods are 0.1% to 15%, 40 to 200°F (4.4444 to 93.3333°C) and 5 seconds to 300 minutes, respectively (George et al., 2000). For the second step involving the peroxygen solution, the possible ranges for concentration, temperature

and time periods are 2% to 35%, 8 to 180°F (-13.3333 to 82.2222°C) and 30 seconds to 300 minutes, respectively (George et al., 2000).

Based on the method outlined by George et al. (2000), a new method was developed in this study for the removal of testa from palm kernel, which was used in this study. Since this method has never been applied to the removal of the testa from palm kernel, a range of concentrations, treatment temperatures and treatment times must be tested in order to determine the most effective combination of parameters for testa removal.

However, the effects of the utilization of the new palm kernel testa removal method, which is to be developed in this study, on the palm kernels are currently unknown. Since the removal of the testa has been shown to improve the quality of feed made from palm kernel due to the elimination of phenolic compounds from the testa, it is probable that the removal of the testa using the new method that has been developed in this study will also result in a lessening of the phenolic compound content of the kernels.

Since palm kernels have been extensively utilized as a source of edible oils, information on its fatty acid content is available within the literature. Nik Norulaini et al. (2004) carried out fatty acid content analysis of commercial PKO using gas chromatography (GC) and found that the majority of fatty acids within PKO are made up of lauric acid (C_{12}), which accounted for 48.30% of the total fatty acids. Other fatty acids present within PKO are caprylic acid (C_8), capric acid (C_{10}), myristic acid (C_{14}), palmitic acid (C_{16}), stearic acid (C_{18}), oleic acid ($C_{18:1}$) and linoleic acid ($C_{18:2}$), which accounted for 4.40, 3.70, 15.60, 7.80, 2.00, 15.10 and 2.70% of the total fatty acids composition, respectively (Nik Norulaini et al., 2004). However, the effect of the testa removal method that has been developed for this

study on the fatty acid content of palm kernel is unknown and no such data exists within the literature to date. Furthermore, the effect of the application of the testa removal method that has been developed in this study on the oil yield of palm kernels is also unknown.

According to Sreedhara and Kurup (1998), the removal of the testa from palm kernel using the method developed by Sreedhara et al. (1992) increased the protein content of palm kernel. Compared to whole untreated palm kernels which had a protein content of 79 g/kg, treated palm kernels which had their testae removed had a protein content of 85 g/kg. Similarly, defatted flour made from untreated palm kernels had a protein content of 183 g/kg while defatted flour made from treated palm kernels had a protein content of 198 g/kg. However, the effect of the testa removal method that has been developed for this study on the protein content of palm kernel is currently unknown.

1.2 Problem statements

As previously mentioned, the study by Sreedhara et al. (1992) remains the only piece of literature available to date on the removal of testa from palm kernel, and which unequivocally states that HCl is the best solvent for removing the testa and obtaining white palm kernels. However, HCl is a dangerous and highly corrosive chemical. Furthermore, wastes containing HCl (which is an inorganic acid) are classified as “scheduled wastes” under the Malaysian Environmental Quality Act (1974), and thus require special and disposal procedures. Therefore, alternative methods for the removal of testa from palm kernel are desirable from a safety standpoint, preferably using safer materials. Both sodium carbonate and hydrogen peroxide are currently used in the food processing industry, which make them

attractive alternatives for the removal of testa from palm kernels. However, to date, no literature exists on the removal of palm kernel testa form the kernels utilizing sequential treatment with sodium carbonate and hydrogen peroxide.

Previous studies have been carried out on the fatty acid content of palm kernels (Hassan et al., 2000; Nik Norulaini et al., 2004; Zaidul et al., 2006; Zaidul et al., 2007a; Zaidul et al., 2007b). Sreedhara and Kurup (1998) found that the removal of the testa from palm kernel increased the protein content of the kernels. However, the effect of the application of the new palm kernel testa removal method that is to be developed in this study on the palm kernel oil yield, fatty acid content and protein content of the kernels is currently unknown. Furthermore, no data is currently available on the chemical compound content of palm kernel, whole or otherwise. The physical effect of the application of the new palm kernel testa removal method that is to be developed in this study on the palm kernels is also currently unknown.

The current research is therefore proposed in order to fill in the gaps in the present body of knowledge and fulfil the current needs of palm kernel processing technology.

1.3 Objectives of study

The objectives of this study are:

1. To develop a new method for the complete removal of the testa from palm kernel utilizing sequential treatment with sodium carbonate and hydrogen peroxide.
2. To analyze the micro structure of raw palm kernel, palm kernel without testa and the testa itself utilizing scanning electron microscopy (SEM).

3. To determine the chemical compound content of raw palm kernel, palm kernel without testa and palm kernel testa, and to thus determine the overall changes in the chemical compound content of treated palm kernel.
4. To determine the palm kernel oil yield, fatty acid content and protein content of raw palm kernel and palm kernel without testa.
5. To determine the physical effect of the new palm kernel testa removal process on the palm kernels, via quasi-static compression tests.

1.4 Hypotheses

Phenolic compounds are present within the testa of palm kernels. Removal of the testa utilizing the new removal method that is to be developed in this study should, therefore, alter the content of the various chemical compounds within the palm kernels. Due to this, the chemical compound content of raw palm kernel should be different from that of palm kernel without testa.

The development of the new testa removal method utilizing sequential treatment with sodium carbonate and hydrogen peroxide, could also involve the application of heat during the immersion of the kernels within the solutions. This application of heat in addition to altering the micro structure of the kernels, could also potentially affect the oil yield and fatty acid content of the kernels.

1.5 Significant contribution

The significant contribution of this research is to provide a new method for the removal of palm kernel testa from raw palm kernels using Na_2CO_3 and H_2O_2, which has hitherto not been attempted. This research also contributes academic knowledge of the chemical compound content of palm kernels, as well as changes in

the chemical compound content due to the removal of the palm kernel testa utilizing the new removal method developed for this study. Using soxhlet extraction with hexane and methanol as solvents, a chemical compound content range encompassing both polar and non-polar compounds may be obtained.

Another significant contribution of this study is the furnishing of information of the oil yield, fatty acid content and protein content of raw palm kernel and palm kernels after testa removal by the new method developed by this study. Studies have shown that the removal of testa form palm kernels improves the protein content of the kernels by making it available for absorption when consumed by livestock. Therefore, information of the protein content of palm kernel, both before and after testa removal by the new method developed for this study, can be used by the food and agricultural industry for the development of new products made from palm kernel.

Similarly, the information furnished by this study on the palm kernel oil yield and fatty acid content of palm kernels, both before and after testa removal by the new method developed for this study, can be used by the food and agricultural industry for the development of new products made from palm kernel.

Chapter 2

Literature Review

2.1 Introduction

The oil palm, *Elaeis guineensis* Jacq., received its botanical name from Nikolaus Joseph von Jacquin in 1763, and is grouped in the subfamily Cocosoideae, along with *Cocos* (coconut) and other genera (Corley and Tinker, 2003). The first name, *Elaeis*, is derived from the Greek word *elaion*, meaning 'oil'. The specific name *guineensis*, was chosen by Jacquin due to the fact that he believed the origin of the plant to be from the coast of Guinea in Africa (Corley and Tinker, 2003). The oil palm was first introduced to Brazil and other tropical countries in the 15th century by the Portuguese, who brought the plant from its native habitat of the west coast of Africa. A second more intensive phase of oil palm propagation took place in 1848, when the plant was introduced by Dutch tobacco planters from its native habitat in West Africa into Indonesia via four seedlings that were planted in Bogor on the island of Java. In 1875, the subsequent progenies of the oil palms introduced by the Dutch were introduced into Singapore at the Botanical Gardens, and from thence, the oil palm was eventually introduced into the Malay Peninsula in 1878. It is interesting to note that the oil palm was introduced into the Malay Peninsula as an ornamental plant. Cultivation of the oil palm for commercial purposes would not occur until 1917 in the Malayan state of Selangor (Sambanthamurthi et al., 2000).

Today, Malaysia and Indonesia account for 85% of the world's total palm oil production. Oil palm is also commercially cultivated in other countries including Thailand, Papua New Guinea, Nigeria, Colombia and Ecuador (Sime Darby, 2014). In 2013, a total of 5.23 million hectares of land in Malaysia was utilized for the commercial cultivation of palm oil, out of which 2.59 million hectares (49.6%) was

in Peninsula Malaysia, and 2.64 million hectares (50.4%) was in Sabah and Sarawak (MPOB, 2014). Malaysia produced a total of 19.22 million tonnes of crude palm oil in 2013, a 2.28% increase from the total of 18.79 million tonnes of crude palm oil produced in 2012. Out of the total crude palm oil produced in Malaysia in 2013, 10.33 million tonnes (53.75%) was produced in Peninsula Malaysia, while 8.89 million tonnes (46.25%) was produced in Sabah and Sarawak (MPOB, 2014).

Out of all oil-producing seed crops, the oil palm is the most efficient in oil production. One hectare of oil palm plantation produces an average of 3.7 tonnes of oil per year in Malaysia, while the most efficient plantations can produce up to 8.0 tonnes of oil per hectare oil palm (Edem, 2002; Sambanthamurthi et al., 2000; Sime Darby, 2014). In 2012, out of the total global land use for oilseeds cultivation, oil palm comprised only for 5.5% of the total. However, from this percentage of global land use, palm oil accounted for 32% of the world's total output of oils and fats, illustrating the efficiency of the oil palm plant in oil production (Sime Darby, 2014).

The oil palm is a large, pinnate-leaved palm, and possesses a single columnar stem with short internodes (Corley and Tinker, 2003). The tree is characterized by an untidy appearance due to the separate upper and lower ranks of leaflets on the rachis. Both within the fruit bunch and on the leaf petiole are short spines. The oil palm is a monoecious plant, producing both male and female flowers on the same tree, with the inflorescences positioned in the axils of the leaves (Corley and Tinker, 2003). The oil palm tree can achieve a maximum height of 20 to 30 metres, and can obtain an economic lifespan of 25 to 30 years (Edem, 2002).

The fruits of the oil palm are produced and carried on a large, compact bunch. A single fruit bunch may weigh between 30 to 40 kilograms and contain up to 2000 individual fruitlets. The fruit of the oil palm is black when first produced, and then

will slowly attain an orange, reddish colour when it achieves ripeness (Edem, 2002). The fruit is a sessile drupe and is comprised of an outer skin called the exocarp, a middle pulp called the mesocarp and a shell which covers the kernel called the endocarp. Structurally, the seed is formed by both the endocarp and the kernel. The exocarp and mesocarp are included together in measurements of the thickness of the pulp. The oil content of the mesocarp of ripe fruit can vary widely – from under 40% to over 60% (Corley and Tinker, 2003).

Removal of the soft, oil-bearing mesocarp from the oil palm fruit will reveal a nut, which is the seed of the fruit. The seed of the oil palm is comprised of an endocarp, or outer shell, which is encloses the kernel. The number of kernels within a nut ranges from one to three. However, within the tricarpellate ovary, two of the three ovules will usually abort, which results in most nuts bearing only one kernel (Corley and Tinker, 2003).

From a botanical perspective, the actual seed of the oil palm is the kernel itself. However, the term ‘seed’ is commonly used to refer to the entire nut due to the fact that in agriculture, it is the nut which is stored, germinated and planted (Corley and Tinker, 2003).

2.2 Phenolic compounds within the testa of palm kernel

The kernel of the oil palm fruit is surrounded by a dark brown testa, which constitutes 3 – 5% of the kernel weight and is strongly bound to the kernel by a thin layer of gum or lignin (Sreedhara et al., 1992). The testa of the oil palm kernel is surrounded by a network of fibres, while the kernel is comprised of layers of hard, oil-bearing endosperm and is greyish-white in colour. According to Alang et al. (1988), the endosperm contains 47% lipid and 36% galactomannan. The endosperm

provides the energy needed for the seedling's growth, upon which it completely depends on during the first few weeks of growth. The galactomannan within the endosperm is utilized more rapidly than the lipid during early stages of germination (Alang et al., 1988).

According to Sreedhara et al. (1992), the presence of phenolic compounds within the testa restricts the utilization of palm kernel oil and meal due to the dark colour which is imparted to these products by the phenolic compounds. According to Waniska (2000), among the factors affecting food colour are grain colour, pericarp colour, endosperm colour, pigmentation of the testa, presence of tannins, degree of milling and food system pH. Similar to the case of palm kernel, the acceptance of sorghum grain and flour are greatly influenced by their colour, with the most acceptable sorghum food products being made from white sorghums (Waniska, 2000).

Furthermore, palm kernel meal obtained from undehulled palm kernel is also dark brown in colour due to the presence of testa, the presence of which has been reported by several studies to lower its acceptability by animals (Babatunde et al., 1975; Barry and Duncan, 1984; Cornelius, 1983; Sreedhara and Kurup, 1998). As feedstock, palm kernel cake, which is produced from palm kernel, is ranked higher than copra cake and cocoa pod husk. However, it is ranked lower compared to fish meal and groundnut cake, especially in its protein value (Wong and Wan Zahari, 1997). A similar case has been reported regarding the effects of testa constituents on the nutritional quality of sorghum food products. In the case of sorghums, in addition to mold resistance, dark colours and astringency, higher levels of tannins and phenolic compounds have been known to also decrease the nutritional value of foods and feeds made from sorghums (Earp et al., 1983; Hahn et al., 1984). Brown or

tannin sorghums are consumed by livestock animals at identical or higher rates but, when compared to sorghums without tannins, do not gain as much weight. The digestibility and the efficiency of utilization of absorbed nutrients in sorghums are reduced from 3% to 15% by the presence of tannins (Waniska, 2000). The available literature indicates that although phenolic compounds in the testae of plant seeds may exert strong antioxidant effects, they also exert strong antinutritional effects which in turn affect foods that are derived from the seeds of such plants.

Since the presence of phenolic compounds within the testae of palm kernel adversely affects the utilization of the kernels as a food source, the removal of the testae from the kernels should have a positive effect on the food products that are subsequently made from such kernels. However, the testae of palm kernels are bonded very tightly to the endosperm and are extremely difficult to remove via conventional dehulling methods (Sreedhara et al., 1992). The only available piece of literature available on the subject of the removal of the testae from palm kernels is by Sreedhara et al. (1992), which reports the various attempts to remove the testae from palm kernels via different physical and chemical means.

2.3 Removal of the testa of palm kernel

Sreedhara et al. (1992) attempted to loosen the palm kernel testa by soaking palm kernels in hot water at temperatures of 85 to 100°C and for time periods of 30 to 120 minutes followed by air or oven drying. However, the combination of treatments with hot water was unsuccessful at removing the testa. In palm kernels, the caps of the palisade walls are surrounded by a contiguous layer of gum and lignin, whilst quinones are also present in a continuous layer of cells within the lumen and cell wall. The presence of these structures renders the testa impermeable

to water which in turn renders any treatment by water ineffective for the purpose loosening of the testa (Sreedhara et al., 1992).

Similarly, a range of organic solvents were utilized at room temperature to attempt removal of the testa but were ultimately ineffective. The solvents used by Sreedhara et al. (1992) in these attempts were hexane, methanol, chloroform, 90% methanol and methanol/hexane/water (44:40:16, vol/vol/vol).

Heat treatment, using both dry and wet heat at temperatures of 80 to 160°C and time periods of 1 to 6 hours, was also ineffective at removal of palm kernel testa. The use of autoclaving at 15 psi for 5 to 30 minutes also failed to remove the palm kernel testa (Sreedhara et al., 1992).

Sreedhara et al. (1992) also attempted the use of a range of acids to remove palm kernel testa, namely, sulphuric acid, phosphoric acid, acetic acid and hydrochloric acid. Out of the range of acids mentioned, only hydrochloric acid (HCl) was successful in completely removing the testa from palm kernel. Treatment with phosphoric acid and acetic acid were unsuccessful in removal of testa, while treatment with sulphuric acid (H_2SO_4) was only partially successful, with 25% of palm kernels undergoing testa removal after being treated with 8N H_2SO_4 for 30 minutes. For the kernels with the testa still intact after treatment with H_2SO_4, only the surface of the testa may have undergone dehydration since only the surface of testa appeared affected by the treatment process.

Total separation of testa from palm kernels was achieved by Sreedhara et al. (1992) when they were treated with 1N HCl at temperature of 95°C for 45 minutes. When the concentration of HCl was increased to 2N, a shorter treatment period of 30 minutes was sufficient for total testa removal from the palm kernels. For HCl concentrations of 3N and 4N, treatment times required were 13 to 15 minutes and 6

to 7 minutes, respectively. At HCl concentrations of 7N and 8N, the treatment time required for total testa separation from palm kernel was only about 30 seconds. These observations would seem to suggest that for separation of testa from palm kernel, HCl concentration was inversely proportional to the treatment time when treatment temperature was held constant.

When HCl concentration was held constant at 4N, it was found that complete testa removal was achieved at treatment times of 60, 40, 20 and 15 minutes for temperatures of 70, 80, 85 and 90°C, respectively. The decrease of treatment time with increase in temperature indicates that treatment temperature was inversely proportional to treatment time when the concentration of HCl was held constant.

Sreedhara et al. (1992) also found that treating palm kernels with sodium hydroxide (NaOH) at concentration of 1N and temperature of 95°C for 7 to 10 minutes was also effective in totally removing the testa from palm kernels. Similar to HCl treatment, when temperature was held constant, treatment times decreased with an increase in NaOH concentrations. At a constant temperature of 95°C, NaOH concentrations of 4N, 5N and 6N required 4, 3 and 2 minutes, respectively, for complete palm kernel testa removal. When treatment was carried out at room temperature, NaOH concentrations of 6N to 7N and a treatment time of 3 hours were required for complete removal of palm kernel testa. Similar results were obtained when potassium hydroxide (KOH) was used for treatment. Sreedhara et al. (1992) also attempted using other alkaline solutions for the removal of the testa from palm kernel such as ammonium hydroxide, barium hydroxide and calcium hydroxide but these solutions were ineffective.

Though treatment with NaOH and KOH were effective for removing the testa from the palm kernels, Sreedhara et al. (1992) observed that the kernels were

coloured brown by the treatment process. Treating the kernels subsequently with HCl solutions of differing concentrations after alkali treatment slightly lightened the kernels' appearance, but did not give completely pearl-white kernels, as was the case with the kernels that had undergone treatment only with HCl. However, if HCl concentrations of 4N or higher were used at treatment periods longer than those stated previously, the kernels were coloured yellowish-brown. Palm kernel that had undergone HCl treatment to remove the testa were used to make defatted palm kernel flour by grinding the pearl-white palm kernels in a plate mill to pass though a 44 mesh screen and then subsequently defatted with hexane. The palm kernel flour obtained from pearl-white kernels was likewise white in colour and was described as having an "appealing appearance" (Sreedhara and Kurup, 1998; Sreedhara et al., 1992).

2.4 Antinutrional effects of palm kernel testa

Sreedhara and Kurup (1998) utilized the method developed by Sreedhara et al. (1992) to produce defatted palm kernel flour by treating raw palm kernels in 4M HCl for 6 to 7 minutes at 95°C. After treatment, the palm kernels were washed with water, dried at 45°C ground in a plate meal and subsequently defatted with hexane. The nutritional quality of the defatted palm kernel flour was compared with palm kernel flour that had not undergone prior treatment with HCl to remove the testa. When compared to untreated palm kernel flour, it was found that treated palm kernel flour had higher protein content, with the untreated palm kernel flour containing 183 g/kg protein and the treated palm kernel flour having a protein content of 198 g/kg (Sreedhara and Kurup, 1998). Sreedhara and Kurup (1998) attributed the difference

in protein content between the treated and untreated samples to the absence of testa in treated palm kernel flour.

Sreedhara and Kurup (1998) also conducted *in vivo* digestibility studies, which were conducted on rats fed a diet of either: pure casein, treated palm kernel meal or untreated palm kernel meal, all for a period of 10 days. It was found that rats fed the casein diet absorbed 94% of food nitrogen, while for rats that were fed diets containing treated and untreated palm kernel meal, 80% and 65% of food nitrogen was absorbed, respectively. Rats fed the casein diet had the highest gains in body weight, gaining an average of 64.2 g. Rats fed the treated palm kernel meal had an average body weight gain of 59.5 g while rats that were fed untreated palm kernel meal had average body weight gain of 42.5 g. The increase in the protein content of the treated palm kernel flour when compared to untreated palm kernel flour, as well as increased protein absorption and weight gain in rats when fed treated palm kernel meal compared to untreated palm kernel meal, all indicate that HCl treatment to remove palm kernel testa from palm kernel improved the flour and meal that was subsequently manufactured from the kernels.

According to Sreedhara and Kurup (1998), the differing nutritional quality of the different kinds of palm kernel flour used in the study is directly related to the phenolic compounds and tannins contained within the testa of the palm kernel. In addition to functioning as a defense against insect consumption, tannins also bind to or react with proteins via hydrogen bonding, ionic and hydrophobic interactions, all of which cause the tridimensional structure of palm kernel protein to be altered (Barry, 1989; Ramaswamy and Rege, 1976; Griffiths and Mosely, 1980; Oloyo, 1991; Sastry and Narasinga Rao, 1991; Yu et al, 1995). The phenolic compounds may be oxidized into quinones which in turn form insoluble complexes via reaction

with the thiol and amino groups of proteins, which results in the decreased digestibility of the proteins present in the palm kernel when ingested by living organisms (Pierpoint, 1969; Siebert et al., 1996; Sosulki 1979; Vithayathil and Gupta, 1981). In a study on the functional properties of proteins in palm kernel flour derived from palm kernel with and without testa, Uvarova and Barrera-Arellano (2005) found that when the testa of palm kernels were removed using HCl, the polyphenol content of the palm kernel flour decreased. According to Uvarova and Barrera-Arellano (2005) the colour of the palm kernel flour derived from palm kernel without testa was also light cream in colour, similar to the results reported by Sreedhara and Kurup (1998).

2.5 Solvent extraction of chemical compounds from plants

Within the literature that has been surveyed, most studies would seem to suggest that phytochemicals may be extracted from plants using different kinds of solvents, mostly in the form of organic solvents. The term ‘solid-liquid’ extraction is commonly used to refer the method of using a solvent or combination of solvents to extract an analyte or a combination of analytes from a solid sample matrix. According to Luque de Castro and García-Ayuso (1998) however, a more accurate physicochemical term to use for such a method would be ‘leaching’ or ‘lixiviation’, and as such has been among one of the oldest methods of solid sample pre-treatment.

Boskou et al. (2006) conducted a study to determine the profile of phenolic compounds of a variety of table olives of Greek origin and utilized methanol as the solvent of choice to extract the polyphenolic compounds from both the olive kernel and flesh. Davendran and Balasubramanian (2011) used 70% ethanol (v/v) to extract phytochemicals from *Ocimum sanctum* L. leaves. Prior to the extraction the leaves

were cleaned, shade-dried and pulverized into powder using a mechanical grinder. Ezhilan and Neelamegam (2012) utilized ethanol to extract phytochemicals from the whole plant of *Polygonum chinense* L., which were shade-dried and then ground into powder in a mechanical grinder prior to extraction. Similarly, Gopalakrishnan and Vadivel (2011) utilized ethanol to extract phytochemicals from whole-plant samples of *Mussaenda frondosa* L. which were shade dried and then ground into powder in a mechanical grinder prior to extraction. Jing et al. (2012) used extracted antioxidants from pomegranate seed flour using 80% methanol and 50% acetone, which were carried at ambient temperatures under ultrasonic wave. Maruthupandian and Mohan (2011) used ethanol to extract phytochemical constituents from the wood and bark of *Pterocarpus marsupium* Roxb., which were air-dried and powdered prior to solvent extraction. Paranthaman et al. (2012) utilized 95% ethanol to extract phytochemical constituents from the leaves of *Amaranthus caudatus* L., which were shade-dried and powdered prior to solvent extraction. De Souza et al. (2008) extracted phenolic compounds from the leaves of *Maytenus ilicifolia* with water under reflux. After the extracts were concentrated under vacuum evaporation, cold ethanol was added to the extracts to precipitate macromolecules. The extracts were then concentrated under vacuum evaporation once more and freeze-dried (De Souza et al., 2008).

Khaopha et al. (2012) applied solvent extraction to extract phenolic compounds from the testa and testa-removed kernels of 15 different genotypes of Valencia-type peanuts (*Arachis hypogaea* L.). In the study, the solvent used was methanol, 40mL of which was added to a beaker containing 1g of ground peanut testa or 10g of ground testa-removed peanut kernels. The solvent extactions were carried out while stirring for 2 hours at room temperature. Khaopha et al. (2012) identified six compounds in the peanut testa and peanut kernel extracts namely; *p*-

coumaric acid, vanillic acid, ferulic acid, *p*-hydroxybenzoic acid, sinapinic acid and syringic acid. Of the six phenolic acids which were identified, *p*-coumaric acid was the predominant phenolic acid in all the extracts from the testa-removed kernels. Furthermore, no correlation was found between the colour of the peanut testae and the total phenolic content of the testa-removed peanut kernels. Compared to peanut testae with gray and yellow colours, peanut testae with pink colour exhibited significantly greater content of phenolic acids. The results of the study conducted by Khaopha et al. (2012) concurred with previous research which indicated that the total phenolic content of peanut kernels has a strong correlation to the colour of the testa (Chukwumah et al., 2009). Furthermore, the total phenolic acids of the testa of all peanut genotypes tested was greater compared to the testa-removed peanut kernels, which comprised cotyledons and embryonic axes. Khaopha et al. (2012) found that the predominant phenolic acids in all the testae of the peanut genotypes in the study were *p*-coumaric acid and vanillic acid. However, the most common phenolic compound in the testa of the KK4 genotype was *p*-hydroxybenzoic acid, which was the sole exception in this study. The methanolic extract of testae from the KK4 genotype also contained the highest content of phenolic compounds of all the testa extracts in the study.

2.6 Soxhlet extraction of chemical compounds from plants

All of the studies that have been listed in the preceding paragraph utilized the solvents in a conventional solid-liquid extraction mode in which the samples were simply immersed in the solvent of choice for a specified amount of time. Some other studies however, utilized a more specific form of solid-liquid extraction known 'Soxhlet' extraction.

Soxhlet extraction is essentially a hybrid continuous-discontinuous solvent extraction technique (Luque de Castro and García-Ayuso, 2000). In Soxhlet extraction, a specific amount of the sample is placed within a cellulose extraction thimble, which is in turn placed within the Soxhlet extraction chamber. An extraction flask containing the solvent of choice is connected to the bottom part of the extraction chamber, while a condenser is connected to the top part of the extraction chamber. The flask is then placed into a heating mantle. The heating mantle will slowly heat the solvent, which will then volatilize and flow into the condenser, where the solvent vapours will then condense and flow into the Soxhlet extraction chamber. The Soxhlet extraction chamber will slowly be filled with the extraction solvent. Solid-liquid extraction will slowly take place between the sample matrix within the cellulose thimble and the extraction solvent. When the solvent reaches the designated overflow level, it will then be aspirated via a siphon back into the flask. The extracted analytes from the sample will thus be carried with the solvent and will be transported back into the bulk liquid in the extraction flask. As long as the entire apparatus is connected to the heating mantle, the entire process will repeat itself (Luque de Castro and García-Ayuso, 2000). Figure 2.1 shows the common set-up of a conventional Soxhlet extraction apparatus.

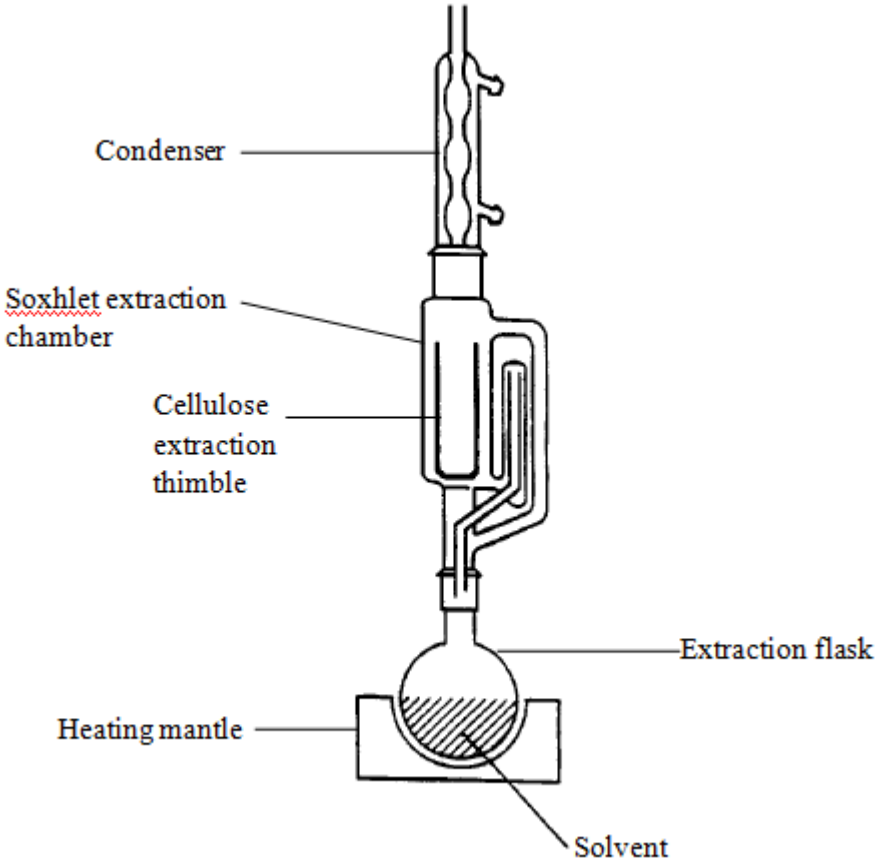

Figure 2.1: Common set-up of a conventional soxhlet extraction apparatus.

Source: Luque de Castro and García-Ayuso (2000).

Conventional Soxhlet extraction was initially devised for the determination of fat from milk samples. Currently, conventional Soxhlet extraction is used mainly in two ways. Firstly, it can be used as an extraction step in a given procedure. Secondly, it can also be used as the extraction method by which to compare other newer or more novel extraction methods, often in terms of extraction efficiency (Luque de Castro and García-Ayuso; 2000).

Several studies have been carried out using Soxhlet extraction with the aim of isolating phytochemicals from various samples. Ahmad et al. (2010) utilized Soxhlet extraction to extract phytochemicals from ground *Herba Leonuri* samples. The extractions were carried out using *n*-hexane and methanol and at three extraction

times of 6, 9 and 12 hours. Anand and Gokulakrishnan (2012) used Soxhlet extraction with 70% ethanol as the solvent to extract phytochemical compounds from whole plant samples of *Hybanthus enneaspermus* L. which were shade-dried and ground prior to extraction. Arunkumar and Muthuselvam (2009) used Soxhlet extraction with distilled water, ethanol and acetone as solvents to extract phytochemicals from the leaves of *Aloe vera* L. which were air-dried and powdered prior to exraction. Ganesh and Venilla (2011) utilized Soxhlet extraction with methanol as the solvent to extract phytochemical constituents from the leaves of *Acanthus ilicifolius* L. and *Avicennia officinalis* L. Before extraction, the leaves were washed with water, shade-dried and powdered with an electrical blender. Kumar et al. (2010) utilized 70% ethanol as solvent to conduct Soxhlet extraction on the stem samples of *Hibiscus micranthus* L. The stems were cut into small pieces and shade-dried prior to extraction. Ravikumar et al. (2012) utilized several different solvents for Soxhlet extraction of powdered stem bark *Zanthoxylum tetraspermum* samples. The solvents used were petroleum ether, chloroform, ethyl acetate, ethanol and water, while the weight percentage of the extracts were 1.25%, 1.37%, 1.96%, 3.75% and 4.06%, respectively. It is interesting to note that in the study, the highest extraction percentages were obtained by using ethanol and water, which were two most polar solvents used. This indicates that the most phytochemicals within the samples were of high polarity. Vijay et al. (2011) conducted Soxhlet extraction on the leaves of *Gmelina arborea* L. to determine the phytochemical compounds present within the leaves. The solvent utilized in the study was 95% ethanol.

In all the studies listed in the preceding paragraph, the compounds of interest were stated in the studies as “phytochemicals” or “phytochemical compounds”. However, in other studies the compounds of interest was more specific. For example,