EFFECTS OF SC-CO_2 PROCESS PARAMETERS AND TESTA REMOVAL ON THE NUTRITIONAL QUALITY OF PALM KERNEL CAKE

by

MOFTAH MASSAUD BENNAMA

Thesis submitted in fulfillment of the requirements for the requirements

for the degree of

Doctor of Philosophy

February, 2014

ACKNOWLEDGEMENT

In the name of Allah, the Beneficent, the Merciful

First and foremost, I express my deep gratitude to the Almighty Allah for giving me the course and strength to complete this study.

I would like to express my grateful thanks to my main supervisor Professor Dr. Nik Norulaini Nik Abd. Rahman for her guidance, support, advice and encouragements throughout the study. I admire her invaluable comments and keen insight in the research. I also express my healthful gratitude to my co-supervisor Professor Ir. Mohd. Omar Ab Kadir for his thoughtfulness guidance, invaluable comments and suggestions amidst his busy schedule. I am gratefully acknowledged to Associate Professor Dr. Abbas for the valuable discussions on the statistical part.

I am grateful to staffs and lab technicians of the School of Distance Education and School of Industrial Technology, USM for their assistance and access the facilities. I am also thankful to the Ministry of the Science and Education, Libya for awarding me the scholarship as a financial support through out my study.

I owe my greatest gratitude to my parents Massaud Bennama and Ashya, my siblings Farag, Mahamud, Fajra and Mariym, their love and prayer this dream would not have come true. I gratefully acknowledge to my beloved wife, my son Mohamed Bennama and my daughter Dawya, due to their moral support and care. I do remember my friends, those who have helped me diverse way upon this study- Md. Bazlul Mubin, Md. Sohrab Hossain, Md. Jahurul Hauque, ……..and many more. ‘Thank you’ is just not enough to say. I appreciated your kind help throughout the journey.

Moftah Massaud Ben Nama

February, 2014.

TABLE AND CONTENTS

CHAPTER FOUR : RESULT AND DISCUSSION

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND SYMBOLS

AAS	Atomic Absorption Spectrometer
ANOVA	Analysis of Variance
AOAC	Association of official Analytical chemists
As	Arsenic
ATR	Attenuated Total Reflectance
BAM	*Bacteriological Analytical Manual*
C10:0	Capric acid
C12:0	Lauric acid
C14:0	Myristic acid
C16:0	Palmitic acid
$C_{18:1}$	Oleic acid
C18:2	Linoleic acid
C18:2	Linoleic acid
$C_{6:0}$	Caproic acid
$C_{8:0}$	Caprylic acid
Cd	Cadmium
FAME	Fatty acid methyl ester
FDA	Food and Drug Administration
FFF	Field flow fractionation

FID	Flame ionized detector
FTIR	Fourier Transform Infrared Spectroscopy
GC	Gas chromatography
GCMS	Gas chromatography-mass spectroscopy
HCN	Hydrogen cyanide
Hg	Mercury
HPLC	High-Performance Liquid Chromatography
MPa	Mega Pascal
MPN	Most Probable Number
Pb	Lead
PKC	Palm Kernel Cake
PKC_M	Defatted PKC collected from palm oil mill.
SC- PKC_MO-	Oil extracted from palm kernel cake collected from palm oil mill using SC-CO_2 extraction.
Sox-PKC_MO	Oil extracted from palm kernel cake collected from palm oil mill using Soxhlet extraction.
PKO	Palm Kernel Oil
PKt	palm kernel with testa
SC-PKtO	Oil extracted from palm kernel with testa using SC-CO_2 extraction.
PKtO-Sox	Oil extracted from palm kernel with testa using Soxhlet extraction.
PKw	Palm kernels without testa

SC-PKwO	Oil extracted from palm kernels without testa using SC-CO_2 extraction.
PO	Palm Oil
Sb	Antimony
SC-CO_2	Supercritical carbon dioxide
SCF	Supercritical Fluid
SC-PKtO	Extraction oil from palm kernel with testa using supercritical carbon dioxide
SC-PKt	Defatted PKC obtained after oil extraction from PKt using SC-CO_2
SC-PKwO	Extraction oil from palm kernel without testa using supercritical carbon dioxide
SEM	Scanning electron microscope
SFE	Supercritical Fluid Extraction
Sn	Tin
Sox -PKtO	Defatted PKC obtained after oil extraction from PKt using Soxhletextraction
Sox- PKwO	Defatted PKC obtained after oil extraction from PKw using Soxhletextraction.
SSF	Solid state fermentation
V8	Prepared mixed vegetable juice agar

KESAN TESTA KE ATAS HASIL MINYAK EKSTRAK SC-CO_2 DALAM KANDUNGAN NUTRIEN DAN ANTI-NUTRIEN DALAM GENTIAN ISIRUNG SAWIT

ABSTRAK

Hampas isirung kelapa sawit (PKC) merupakan hasil sampingan proses pengeluaran minyak kelapa sawit. Sebahagian besar bahan ini telah digunakan sebagai bahan makanan kepada ruminat kerana kos yang agak rendah dan ketersediaan bahan tersebut. Walau bagaimanapun, penggunaan PKC_M terhalang kerana dianggap sebagai bukan ruminat kerana mengandungi anti-nutrien, logam toksik dan makanan yang terhasil daripada mikroorganisma patogen. Fokus utama kajian ini adalah untuk menentukan keberkesanan kaedah pengekstrakan karbon dioksida lampaugenting (SC-CO_2) dalam pengeluaran minyak isirung kelapa sawit dan pengeluaran PKC_M nyahlemak sebagai sumber berkualiti serat pemakanan untuk kegunaan manusia daripada isirung kelapa sawit dengan testa (PKt), isirung kelapa sawit tanpa testa (PKw) dan hampas isirung kelapa sawit yang diperoleh daripada kilang kelapa sawit (PKC_M). Kaedah pengekstrakan soxhlet juga digunakan untuk mengekstrak minyak kelapa sawit daripada PKt dan PKC_M. Eksperimen ini telah dijalankan untuk membandingkan keberkesanan kaedah pengekstrakan SC-CO_2 dengan kaedah konvensional yang digunakan dalam industri minyak kelapa sawit. Selain itu, eksperimen ini juga membandingkan kandungan kimia SC-CO_2 ekstrak PKC dan PKC_M, yang dikumpulkan daripada industri, dalam usaha menentukan kaedah pengekstrakan SC-CO_2 yang sesuai bagi tujuan perindustrian. Pengekstrakan SC-CO_2 minyak telah dilakukan di bawah tekanan yang

berbeza iaitu 27.6, 34.5 dan 41.4 MPa, dengan suhu 40, 60 dan 80 ^{0}C dan masa pengeluaran 30, 40 dan 60 minit pada kadar aliran 2 ml /min. Didapati bahawa pengekstrakan oil daripada PKt, PKw dan PKC_M banyak dipengaruhi oleh saiz zarah. Komposisi nutrisi dan anti-nutrisi SC-CO2 hampas isirung kelapa sawit terawat seperti SC-PKt, SC-PKw dan SC-PKC_M telah dijalankan. Keputusan komposisi nutrisi dalam PKC dinyahlemak menunjukkan bahawa SC-PKt mengandungi nilai nutrisi paling tinggi daripada SC-PKw dan SC-PKC_M. Sebaliknya, penentuan kandungan anti-nutrien dalam hampas isirung kelapa sawit yang dinyahlemak menunjukkan bahawa komposisi anti-nutrien dalam PKC_M adalah lebih tinggi daripada SC-PKt. Walau bagaimanapun, penentuan kandungan vitamin, kandungan asid amino, karbohidrat, kandungan logam berat, komposisi alphatoksin dan kehadiran mikroorganisma dalam SC-PKt, SC-PKw dan SC-PKC_M turut dilakukan dalam kajian ini. Dengan memaksimumkan pengeluaran glukosa daripada hampas isirung kelapa sawit selepas kaedah penapaian pepejal membuktikan bahawa SC-PKC_M yang dinyahlemak boleh digunakan sebagai bahan makanan untuk haiwan ruminan dalam industri makanan ternakan. Hasil kajian ini mencadangkan bahawa mengeluarkan minyak menggunakan SC-CO_2 dapat mengurangkan bahan buangan dengan menghasilkan hampas isirung kelapa sawit dinyahlemak, yang boleh digunakan sebagai sumber serat murah yang bernutrisi.

EFFECTS OF SC-CO_2 PROCESS PARAMETERS AND TESTA REMOVAL ON THE NUTRITIONAL QUALITY OF PALM KERNEL CAKE

ABSTRACT

The main focus of the present study is to determine the effectiveness of the supercritical carbon dioxide (SC-CO_2) extraction of residual oil from PKC_M and production of defatted PKC_M. This could be a quality source of the dietary fibre for human consumption either from PKC_M SC-CO_2 defatted palm kernels with testa (PKt), and without testa (PKw). Soxhlet extraction method was also applied to extract the oil from the PKt and PKC_M. The SC-CO_2 extraction of oil was performed under different pressures of 27.6, 34.5 and 41.4 MPa, temperatures of 40, 60 and 80 ^{0}C and extraction time of 30, 40 and 60 min at flow rate 2 ml/min. It was found that the oil extraction from PKt, PKw and PKC_M was greatly influenced by particle size, pressure and temperature. The highest extracted oil yield was gained about 13%, 51% and 35% from PKC_M, PKt and PKw, respectively; using SC-CO_2 at particle size 0.05 mm, pressure 41.4 MPa, temperature 60^{o}C for 40 min extraction time. The nutritional and anti-nutritional composition in the SC-CO_2 treated palm kernel cake (i.e., SC-PKt, SC-PKw) and PKC_M were determined. The results of the nutrient compositions in defatted PKCs show that SC-CO_2 maintained the nutrient, and it does not affect the nutrient content in extracted sample by undergoing degradation. The highest protein content was determined in SC-PKt (18.7%) flowed by the PKw (15%) and then PKC_M (13.5%). The total dietary fiber content was found to be 61.58%, 58.69 % and 60.71% in SC-PKt, SC-PKw and PKCM, respectively. The percentage ash contents were determined to be 4.43% in SC-PKt, 3.55%

in SC-PKw and 13.92% in PKC_M. The highest Crude fat content (10.2%) was detected in PKC_M, wherein it was found to be 2.5% and 1.6% in SC-PKt and SC-PKw, respectively. Determination of the anti-nutrients content in defatted palm kernel cakes revealed that the anti-nutrient composition in PKC_M was higher than SC-PKt and SC-PKw. It was observed that SC-CO_2 effectively influenced the anti-nutrient content in produced PKCs. The highest alpha amylase inhibitory of 98% was detected in defatted PKC_M, where the alpha amylase inhibitory in SC-PKt and SC-PKw was 35% and 12%, respectively. The phythic acid content in defatted palm kernel cake was 3.18 mg/g, 0.84 mg/g and 4.07 mg/g for SC-PKt, SC-PKw and PKC_M. The presence of tannin content in SC-PKt and PKC_M were 0.26 mg/g and 0.31 mg/g, respectively. No tannin was detected in SC- PKw. The highest insoluble oxalate content was found to be 6.8 mg/g in PKC_M followed by 2.3 mg/g in SC-PKt, whereas it was almost nil to SC-PKw. In the case of soluble oxalate content, the soluble oxalate content was almost nil in SC-PKw. The soluble oxalate content in SC-PKt and PKC_M were 0.12 mg/g and 0.46 mg/g, respectively. HCN content in PKC_M was measured to be 0.41 mg/g, it was undetectable in SC-PKt and SC-PKw. The vitamin, amino acid, carbohydrate, heavy metal, alphatoxin composition and the presence of microganisms in SC-PKt, SC-PKw and SC-PKC_M were also examined in this study. Maximizing glucose production from palm kernel cake following solid state fermentation method proved that the defatted SC-PKC_M could be used as a feed ingredient for ruminant animals in the livestock feeds industry. The results of this study suggested that extracting oil using SC-CO_2 could minimize the waste by producing superior defatted palm kernel cake, which could be used as a cheap source of fibre with good nutrition.

CHAPTER ONE

INTRODUCTION

1.1 Supercritical Fluid Carbon Dioxide

Public concern has increased with regard to the health, safety, and environmental hazards associated with the conventional solvent extraction method for oil processing. Hence, a potential niche exists for the development of a new safer solvent medium for oil extraction. The use of organic solvents can also lead to contamination of the product by solvent residues (Zaidul et al., 2006a). Supercritical fluids have gained the interest of many researchers as an attractive organic solvent (Salto, 1995). The dissolution of solutes in a supercritical fluid (SFE) medium was first observed by Hannay and Hogarth (1879). Supercritical fluid extraction (SFE) has been extensively examined for commercial process applications over the past five decades (Steytler, 1996). In addition, conventional methods are generally conducted at high temperatures, which can destroy valuable substances in the product.

The SFE method has been proven to have many advantages: a clean product free of solvent, the avoidance of compound degradation, better extractability for oil, high yield, and varied compositions. All of these can be obtained under different extraction conditions (Kim et al., 1999, Chen et al., 2008; Nik Norulaini et al., 2004). Above a certain temperature, a gas cannot be liquefied regardless of the applied pressure; this temperature is the critical temperature. The pressure required to liquefy the gas at the critical temperature is the critical pressure. A fluid subjected to a temperature and pressure above the critical temperature and pressure is an SFE (McHugh, 1994). Products (such as oils) obtained using supercritical carbon dioxide (SC-CO_2) is completely free of solvent residue. SC-CO_2 can remove fat in palm kernel meal; therefore, it can be used to produce low-calorie food (Akanda et al., 2012; Kriechbaumer et al., 2012). The crude oils obtained by SC-CO_2 extraction contain less impurities and are easier to refine than conventionally extracted oils (Nik Norulaini et al., 2004). Extraction using the SFE technique is becoming an important processing procedure and is employed in a wide range of applications. SFE has been effectively used to produce high-quality essential oils, separate essential oils, as well as for other applications in the food derivatives (Reverchon, 1997), cosmetics (Ozer et al., 1996), pharmaceutical, and other related industries.

SFE has been proven to be an alternative refining process for extracting enriched solutes in particular compounds, such as wheat germ oil (Eisenmenger and Dunford, 2008), rice bran oil (Kim et al., 1999, Chen et al., 2008), and crude PO and its components (Birtigh et al., 1995 ; Davarnejad, et al., 2008). SC-CO_2 has been widely used to extract oil from dehulled and unhulled ground palm kernels (Nik Norulaini et al., 2001). Studies have also been successfully conducted on the deacidification of olive oils (Goncalves et al., 1991), oilseeds/lipids in natural products (Eggers, 1996), and sunflower seed oil (Cocero and Calvo, 1996) using the SFE process.

1.2 Palm kernels and palm kernel cakes

The oil palm (*Elaeis guineensis* Jacq) is native to West Africa. It has been successfully planted in tropical regions between the latitudes 10°N and 10°S around the equator (Obahiaqbon, 2012). The palm fruit consists of pericarp and palm kernels. The pericarp contains three layers: the exocarp (skin), mesocarp, and endocarp. Two types of oil can be extracted from the oil palm fruit: palm oil (PO), which is extracted from the fibrous mesocarp; and palm kernel oil (PKO), which is extracted from the kernels (seeds) of the fruit. Although both types of oils are extracted from the palm fruit, the chemical and nutritional properties of PO and PKO are different. Generally, palm kernel contains about 45 to 50% oil on a wet basis (Tang and Teoh, 1985). PKO is rich in lauric acid (C_{12}) (about 50% lauric acid). The remaining major fatty acids are myristic (C_{14}) and oleic acids ($C_{18:1}$) (Hristov et al., 2004; Tang and Teoh, 1985; Zaidul et al., 2006b).

Palm kernel cakes (PKCs) are the major byproduct of the PO industry after oil is extracted from the palm kernel (Akpan, 2005). PKC is the solid residue left behind after extraction of oil from the palm kernels. PKC is estimated to comprise 10% unextracted residual oil on average, with a mean moisture content of 7.1%. PKC also contains about 18.6% crude protein, 37% dietary fiber, and 4.5% crude fiber and ash (Ramachandran et al., 2007). In Malaysia, three different extraction methods are currently being used to extract PKO from palm kernels: screw pressing, solvent extraction, and pre-pressing followed by solvent extraction (MPOB, 2009). These conventional methods usually remove insoluble oil impurities, including fruit fibers, nut shells, and free moisture. However, these traditional methods also extract other constituents along with the PKO, such as soluble non-glycerides, including free fatty acids, trace metals, phospholipids, carotenoids, tocopherols, sterols, and oxidation components. These soluble non-glycerides are difficult to remove from the PKO; therefore, various refining steps are required (Mohd Suria, 2001). In addition, the traditional methods for PKO extraction have several limitations, such as pollution concerns, time consumption, high energy costs, and the requirement for organic solvents (Norhuda and Jusoff, 2009).

The challenge for the palm oil -producing nations of Malaysia, Indonesia, and Nigeria is to utilize PO mill wastes such as palm kernel cakes, and optimize residual oil extraction from the PKCs. The mass of PKCs is increasing with the growth of the PO industry in many parts of the world, particularly in Asia and Africa (Jessada, 2007). Therefore, the PO industry is searching for better ways to use of its waste in particular in animal feed as well as food. There is ample opportunities in the oil palm industry for conversion of the biomass into pulp and paper, particle boards, medium-density fiber boards, furniture, etc. (Kamaruddin et al., 1997; Singh et al., 2013).

1.3 Palm kernel testa

The seed of the oil palm is the nut which remains after the mesocarp has been removed from the fruit. The nut consists of an endocarp, or outer shell, and a kernel. It is the kernel that constitutes the seed proper from a botanical perspective. However, the term “seed” is commonly used to refer to the nut, which constitutes both endocarp and kernel. This is due to the fact that in agriculture, it is the nut of the palm fruit which is stored, germinated and planted. The kernel lies within the endocarp and is constituted of greyish-white endosperm surrounded by a dark-brown testa covered with a network of fibres. The endosperm is hard and contains palm kernel oil. Palm kernel meal obtained from undehulled palm kernel is 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 (Sreedhara and Kurup, 1998).

The tannins and phenolic compounds within the palm kernel testa contribute to its characteristics as animal feed. For example, tannins act as a defence against insect consumption and can react and bind with proteins within the kernel (Barry et al., 1989; Yu et al., 1995). Also, quinones, which result from the oxidation of phenolic compounds within the testa, form insoluble complexes with thiol and amino groups of proteins within the kernel (Vitayathil and Gupta, 1981). The reactions of these tannin and phenolic compounds with proteins within the kernels thus lower the availability of the proteins for intake when consumed by agricultural livestock (Wolffram et al., 1995). Sreedhara and Kurup (1998) found that rats that were fed palm kernel meal with the testa removed absorbed more protein compared to rats that were fed palm kernel that included the testa. Hence, protein digestibility of palm kernel meal appeared to improve with HCl treatment to remove the testa (Sreedhara and Kurup, 1998).

1.4 Factors Influencing Extractability of Oil in Supercritical Carbon Dioxide

Modifying and optimizing the parameters for SC-CO_2 extraction facilitate the separation of different fractions of oil (Bernardo-Gil and Casquilho, 2007). At the optimum values of the variables that influence SC-CO_2, the extraction of the PKO gave yields with both high quality and large quantity. Various parameters that can influence the oil extraction using SC-CO_2 include temperature, mass transfer, solubility, extractability, matrix, and pressure (Bernardo-Gil and Casquilho, 2007; Terada et al., 2010; Zaidul et al., 2006a). The oil yield from SFE can be affected by temperature in two ways. Increasing the temperature decreases the density of the solvent, SC-CO_2, which can decrease the solubility of seed oil in SC-CO_2 as a result of the solvent-solute contact being minimized. On the other hand, increasing the temperature at high pressure improves the solubility of the seed oil in SC-CO_2 (Terada et al., 2010). Similarly, raising the pressure augments the oil yield since this makes the density of SC-CO_2 heavier giving it ample contact time with the solute to dissolve in the solvent, raising the solubility of the solute (Terada et al., 2010; Zaidul et al., 2006b, 2007a). Zaidul et al. (2007b) reported a significant increase in the extraction yield with increased pressure. Guan et al. (2007) showed that the yield of PKO increased with increase in pressure from 34.5 to 48.3 MPa at higher temperatures owing to the increase in the solubility of the oil components. Thus, an increase in the SC-CO_2 density increases its dissolving capability.

Some researchers have established extractability performance by varying different processing parameters for the SC-CO_2 extraction technique. Careri et al. (2001) showed that the temperature of SC-CO_2 does not influence the extraction efficiency for carotenoids from *Spirulina pacifica* algae. Conversely, Reverchon et al. (1997) varied the pressure and temperature when extracting volatile oil from rose concrete using SC-CO_2. The study showed that large quantities of steroptens and paraffins were co-extracted with the rose volatile oil at a pressure of 100 bar and temperature of 40 ^{0}C (Reverchon et al., 1997).

Hamburger et al. (2004) determined the effect of supercritical pressure on extracted yields from three medicinal plants: marigold, hawthorn, and chamomile. They found that the yields of the total extracted substances increased above pressure 30 MPa. Similarly, Gaspar (2002) studied the effects of the pressure and temperature on the extraction of essential oils from oregano bracts (*Origanum virens* L.) by compressing CO_2 at pressures of 5 to 30 MPa and temperatures of 300 to 320 K. He found that moderate experimental conditions with a solvent CO_2 density of 0.3 to 0.5 kg m^{-3} were adequate for the maximum extraction of essential oils (Gaspar, 2002). Among the processing parameters, the extraction time can influence the amount of plant based oils yield. This has been shown by Pourmortazavi et al. (2004), who reported on the influence of the extraction time on the essential oil obtained from *Juniperus communis* leaves using SC-$CO_{2.}$ The researchers used extraction time of 20, 25 and 30 min. Their results showed that increasing the extraction time at a constant pressure of 35.46 MPa enhanced the plant oil extraction.. Bisunadan, (1993) observed that high pressure, temperature, density and solubility increase the vapor pressure of the oil exponentially. Likewise, Bharath et al. (1993) reported that in the fractionation of fatty acid constituents of vegetative oil into C6, C12, C16, and C18:1, raising the pressure on SC–CO_2, the solubility of the components correspondingly increased. However, at constant pressure, the solubility decreased with increasing carbon number.

1.5 Palm kernel fiber as Human Food

In Malaysia, PKCs are used as feed for a variety of animals, such as ruminants, because of its crude protein content of about 18.6%, moisture content of 9.2 %, total dietary fiber of 37%, and crude fiber and ash of 4.5% (Ramchandran, et al., 2007). A conventional method of extracting PKOs from palm kernels, such as mechanical screw pressing, yields PKC as the byproduct. However, this technique cannot extract oil completely, leaving an average residual oil content of 5 to 10% (Johnson and Lusas, 1983). The main problem with utilizing PKC for human consumption is the residual oil remaining in its matrix after conventional extraction methods such as mechanical screw pressing (Zahari & Alimon, 2004). The residual oil in PKC gives it a rancid taste after a period of storage, which reduces its potential as a food additive for human consumption. It has not been a practice to recover the residual oil from PKC. However, SC-CO_2 offers an effective and cleaner mode of removing the residual oil to generate two useful products: PKO (residual oil) and defatted PKC (Akanda et al., 2012; Nik Norulaini et al., 2009). Defatted PKCs can be a good and cheap source of dietary fiber for human consumption.

Foods high in fiber content prevent constipation, lower cholesterol, facilitate weight loss, and have many more benefits. Lau et al. (2006) evaluated the application of SC-CO_2 as a novel technique to recover residual oil and its associated nutritional component. SC-CO_2 has been employed successfully as a solvent to replace other traditional solvents in food industries owing to its non-toxicity, non-flammability, reasonably low cost and environmental friendliness.

Palm kernel fibers can be stored for longer periods of time than PKCs. Both are rich sources of protein and energy and are mainly used as cattle feed, especially in Europe. PKCs contain 14.5% crude protein, which is a much higher ratio than rice bran, and high fiber content (Akpanabiatu et al., 2001; Chee et al., 2012). PKC contains low moisture content, an average amount of protein, and high fiber content. It has low nutrient solubility owing to its high fiber content (Hii et al., 2012). About 27% of the PKC is made up of hemicellulose, especially polysaccharide (mannan) comprising mannose with some galactose and 78% non-polysaccharides (Siew and Berger, 1986). PKCs have an average level of crude protein and fibre. It is a useful source of energy for livestock.

1.6 Problem Statement

To achieve the optimum extraction of the residual PKO from PKCs and realize PKCs as a potential raw material a fiber source, a novel technique of oil extraction is needed. As stated above, oil is conventionally extracted from palm kernels via mechanical screw pressing to yield PKCs, which is not effective enough to completely extract the oil and thus leaves an average residual oil content of 10%. The oil in the resulting PKC needs to be further extracted by solvent to become a defatted PKC with enhanced nutritional value (Kolade et al., 2005). The resulting PKC can be used as a raw material to enhance ruminant animal feed owing to its nautrative values and high level of fibre (Akpan, 2005; Ramachandran et al., 2007). Defatted PKCs can also be a possible cheap source of dietary food fiber for human consumption.

Sreedhara et al. (1992) developed a method for the removal of testa from palm kernel which involved treating the palm kernels with hydrochloric acid (HCl) at high temperatures followed by mechanical action to remove the testa. However, the effect of testa removal on nutrients and anti-nutrients compositions in defatted PKCs has not been studied, especially for its potential use as food ingredient.

The present study was conducted to determine the feasibility and possibility of maximizing the extraction parameters using SC-CO_2 as solvent for the extraction of residual oil from PKC_M and PKO from palm kernels with testa (PKt) and Palm kernels without testa (PKw). SC-CO_2 which acts as solvent will interact and solubilize the solute, which is the residual oil in PKC to yield defatted PKC. This will eventually produce oil-free or defatted PKC as a potential fiber source for human consumption. To ascertain its nutritive value, the nutrient and anti-nutirent composition of the defatted PKC will be analyzed.

1.7 Objectives of the research

1. To determine the combined effect of pressure and temperature (P and T) and particle size on the supercritical CO_2 (SC-CO_2) extraction of yield from palm kernel with testa (PKt) and without testa (PKw) and palm kernel cake from mill (PKC)
2. To ascertain the differences in the nutrient contents in PKt, PKw and PKC after and before SC-CO_2 extraction
3. To determine the the presence of anti-nutrient compositions in SC-CO_2 treated PKt and PKw, and PKC from mill (PKC_M).
4. To investigate the effect of solid state fermentation with *Trichoderma sp*. in palm kernel fiber obtained after SC-CO_2.

CHAPTER TWO

LITERATURE REVIEW

2.1 Palm Oil Industry in Malaysia

Palm oil (PO) production has the most efficient process among all vegetable oils. The largest tonnage of oil per hectare was produced in 2007 (OilWorld, 2008). PO accounts for 20% and 46% of the global oil and fat production and trade, respectively. In recent years, almost half of Malaysia’s cultivated land consists of oil palm trees. The country is one of the largest producers and exporters of PO (Jessada, 2007). Currently, Malaysia’s PO industry produces about 1.5 million tons of PKC as a byproduct each year (Chin, 2001).

PO was first introduced in Malaysia in the late nineteenth century. However, the Malaysian government did not intervene to support and expand the sector until the 1960s. Soon after, the Palm Oil Research Institute of Malaysia (PORIM) was created in the mid 1970s. PORIM was further supported under the auspices of Industrial Master Plans (IMPs) from 1986 to 1996. In 1986, IMP 1 was introduced to set specific targets and provide institutional support to improve refinery technology, stimulate PO research and development (R&D), and develop corresponding domestic industries. Later on, PORIM was expanded to include R&D and technician training for a range of PO products, such as oleo-chemicals, fats, processed PKO, and biofuels (Catherine, 2011).

The Malaysian PO industry has continued to dominate and maintain its position as the global leader in PO supply for both consumption and domestic production of biofuels over the past three decades. Today, the PO sector of Malaysia accounts for nearly 7% of the gross domestic product (GDP) of the country and provides more than 1.4 million jobs (MPOB, 2009). The PO industry of Malaysia uses three methods to extract PKO: screw pressing, direct solvent extraction, and pre-pressing followed by solvent extraction. These methods normally remove insoluble oil impurities such as fruit fibers, nut shells, and free moisture. However, these conventional methods also co-extract oil-soluble non-glycerides. Thus, the extracted PKO must undergo various stages of chemical or physical refining such as degumming, bleaching, and deodorization to bleach and deodorize the PKO (Norhuda and Jusoff, 2009). The extracted oil obtained from those conventional extraction methods also require further processing by fractionation and hydrogenation for different food uses (Akanda et al., 2012; Norhuda and Jusoff, 2009; Zaidul et al., 2006a). Each of these refining processes affects the nature of the oils produced (Norhuda and Jusoff, 2009). Thus, conventional methods are inefficient for oil extraction because not all PKO can be extracted from the palm kernels and the soluble constituents of the oil cannot be adequately removed.

Along with these limitations of the various conventional methods of extracting PKO, there is an urgent need for an updated PKO extraction method with little or no effect on the nature and structure of the extracted oil components at a reduced cost of production. A potential alternative for better PKO extraction is the SC-CO_2 method. The application of supercritical fluid extraction (SFE) to palm-pressed fiber was evaluated as a novel technique to recover oil and its associated nutrition (Lau et al., 2006).

2.2 Conventional method for Palm kernel oil extraction

Palm kernels contain about 45 to 50% of PKO on a wet basis. Several methods have been utilized around the world to extract PKO. Malaysian PO industries used the mechanical extraction to extract PKO in earlier days. In recent years, most PO industries in Malaysia extract PKO from palm kernels using three conventional methods: mechanical extraction using a high-pressure screw press, direct solvent extraction, and pre-pressing followed by solvent extraction (MPOB, 2009). Figure 2.1 illustrates the flow chart for existing conventional methods for PKO extraction.

2.2.1 Mechanical extraction using high-pressure screw press

Mechanical extraction is one of the most widely used conventional methods to extract oil from its oil-bearing materials (Sriti et al., 2011). This extraction method offers the advantages of a relatively low initial cost, minimum operational cost, the possibility of using the cake residue, and the production of uncontaminated oil (Fasina and Ajibola, 1989). However, the mechanical extraction process involves three basic steps: kernel pretreatment, screw pressing, and oil clarification.

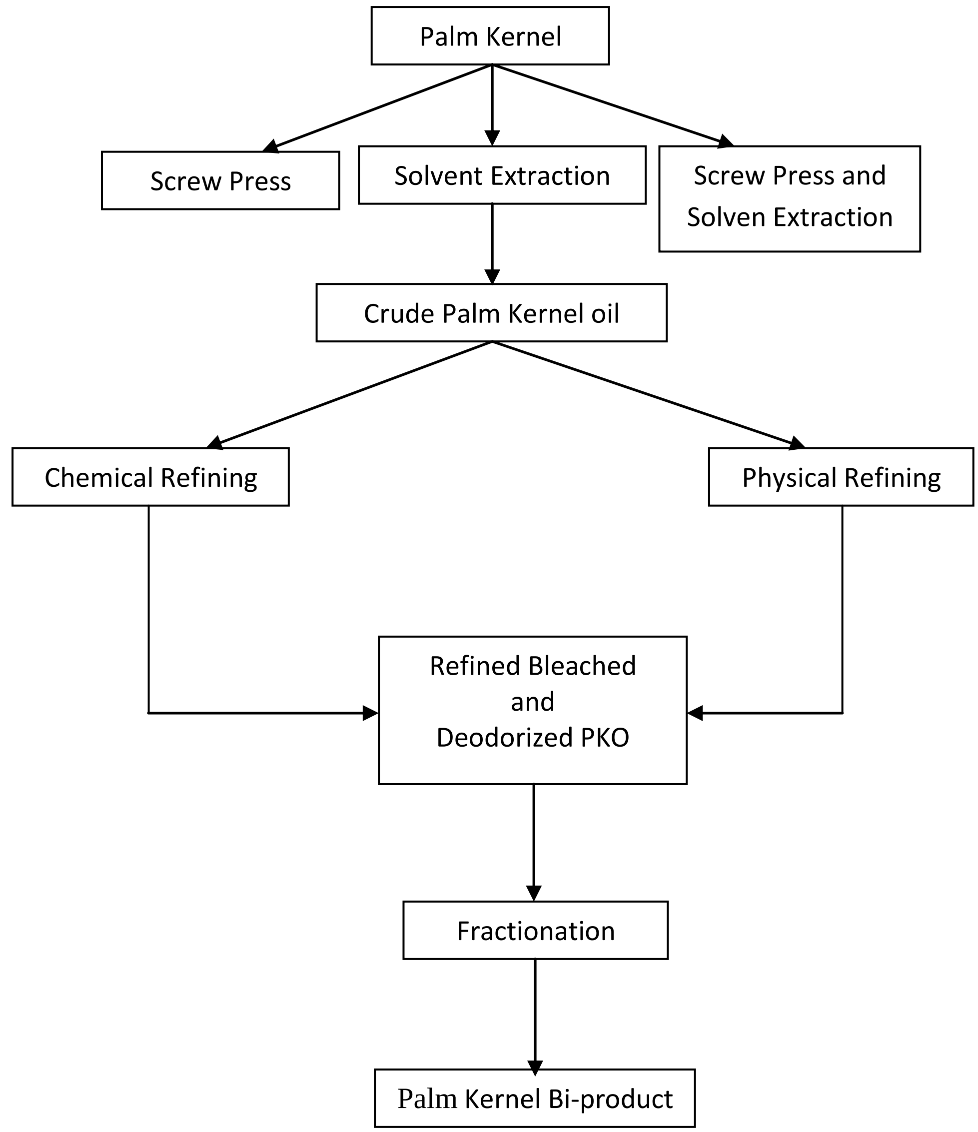

Figure 2.1 The Flow chart of conventional method of palm kernel oil extraction (Source: Norhuda and Jusoff, 2009).

2.2.1.1 Palm kernel pretreatment

This involves cleaning the kernels of foreign materials to prevent contamination of the product and damage to the screw press device. A magnetic separator on the device acts to remove metallic impurities. A vibrating screen is used to sieve out sand, stones, and other similar debris. The palm kernels then break into small fragments by a swinging hammer grinder, breaker roller, or a combination of the two. The kernel fragments are then flaked in a roller mill that revolves at 200 to 300 rpm. The flaked kernels are then transferred to a stack cooker for steam conditioning to adjust the moisture content, reduce the viscosity of the oil, rupture the kernel cell wall, coagulate the protein content in the meal, and allow for easy separation of the oil from its protein.

2.2.1.2. Screw pressing

The pretreated palm kernel is then fed into a screw press. This consists of a helical thread (worm) that revolves within a stationary perforated cylinder. The palm kernel meal is forced through the barrel by the action of the revolving worms. The extracted oil drains through the perforations in the lining of the barrel, and PKC is discharged through an outlet. Large amounts of heat are generated during screw-pressing of the kernel feed by both the worm and the barrel. However, the worm-shaft is kept cool by circulating water, and the barrel is cooled with the recycling of some cooled oil to protect the oil quality against the generated heat.

2.2.1.3. Oil clarification

The expelled oil from the mechanical extraction process consists of various solid impurities that need to be purified. The oil from the screw press drains into a reservoir and is either pumped to a decanter or the revolving coarse screen to remove a larger part of the solid impurities. A filter press is used to remove the remaining impurities. The cakes produced from the oil presses are taken out for storage. Mechanical extraction using a high-pressure screw press is considered to be an inefficient palm oil extraction process owing to the lower oil extraction efficiency (less than 70% oil extraction). Figure 2.2 summarizes the extraction of PKO by mechanical extraction using a high-pressure screw press.

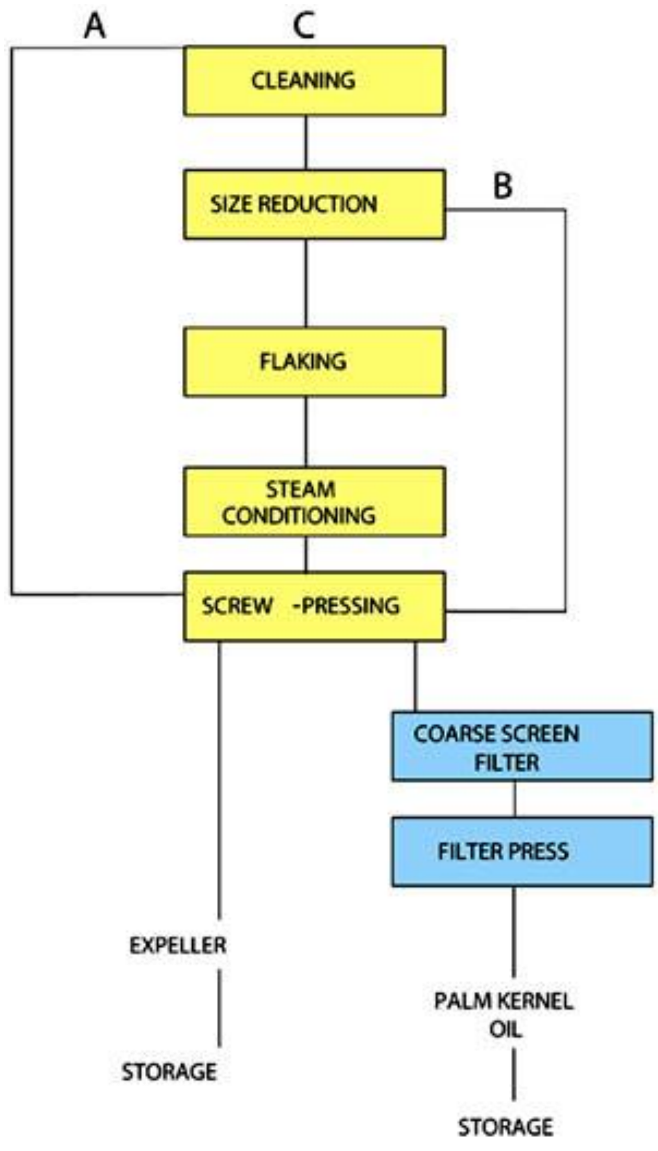

Figure 2.2 Mechanical extraction of palm kernel oil. Line (A) is for direct screw-pressing without kernel pre-treatment; Line (B) is for partial kernel pre-treatment followed by screw-pressing; and Line C is for complete pre-treatment followed by screw-pressing (Source: Tang and Teoh, 1985).

2.2.2 Single screw press method of palm kernel oil extraction

Screw pressing of PKO is a simple, flexible, and a continuous mechanical extraction process (Singh and Bargale, 2000); where the kernels are mechanically screw pressed to extract the oil. A single-screw press machine consists of a single screw with variable pitch and depth channel that slowly rotates in a cage-type barrel (Isobe et al., 1992). There are two types of oil screw press expellers: a full press expeller and pre-press expeller. The full press expeller is used in small and medium oil mills. Pre-press expellers are used for large-scale oil extraction purposes. Full press expellers can extract higher amount oil from palm kernels than pre-press expellers so that about 5 to 8% of oil content remains in the PKC. The palm kernels are fed into one side of the press and enter the pressing chamber and the pressure and friction from the screw move and compress the palm kernels. The extracted oil is then channeled through the small openings of the machine to prevent any solid object or PKC from moving with the extracted oil. PKC exits from the other end of the expeller.

2.2.3 Solvent extraction method

Solvent extraction is usually used to recover a component from either a solid or a liquid sample. Generally, the sample is in contact with a solvent so that the solutes of interest can be dissolved. The solvent extraction method is the most efficient method for separating valuable products from complex feed stocks. It is widely used in the chemical and biochemical industries (Birch, 2000). Solvent extraction of PKO from palm kernels involves the extraction of oil by treating palm kernels with a solvent such as hexane.

Solvents expel PKO from palm kernels to leave a minimum residual oil of about 0.5 to 0.7% in the PKC. This extraction can be conducted either continuously or in batches.

Solvent extraction is principally a solid–liquid extraction process. This extraction process diffuses a solvent into oil-bearing cells of the palm kernel to result in a solution of the oil in the solvent. The solvent can be a vapor, SFE, or liquid and the sample can be solid, gas, or liquid. Generally, hexane (a petroleum byproduct) is used as the solvent for oil extraction from oilseeds as a non-polar solvent. Solvent extraction of PKO from palm kernels is divided into three steps: diffusion of the solvent into the solid, dissolution of the oil droplets into the solvent, and diffusion of the oil from the solid particles to the surrounding liquid (FAO, 1992). This extraction process can be separated into operational units: kernel pretreatment, oil extraction, and solvent recovery from the oil and meal (Poku, 2002).

2.3 Limitations of conventional palm kernel oil extraction methods

The major disadvantages of the existing conventional PKO extraction methods are environmental pollution, increased energy costs, and intensive separation technology (Norhuda, 2009). Furthermore, these methods are time consuming, costly, and require organic solvents to obtain refined, bleached, and deodorized PKO (Zaidul et al., 2007b). Another drawback is the organic solvents used for extraction can contaminate the oil with solvent residues (Zaidul et al., 2006a). There are government restrictions on the use of organic solvents in consumer products owing to concerns about food safety and environmental pollution (Temelli, 2009). The temperature required in conventional methods is a setback because temperature can ruin the oil quality and other essential substances in the oil (Zaidul et al., 2006a).

2.4 Types of oil palm fruits

Oil palms are mainly cultivated in the inter-tropical regions of Latin America, Asia, and Africa. There are two parental varieties of palm fruit *Elaeis Guineensis*: Dura and Pisifera. Cross-breeding of these two breeds yields the hybrid Tenera. The commercial oil palms in Malaysia are Tenera hybrids (*E. guineensis*) (Kok et al., 2011). Dura palms have a thick and hard shell, while Pisiferas have no shell but a small kernel surrounded by a fibrous ring. The Dura palm has a low oil extraction ratio (OER) of 12 to 16% unlike the Tenera palm, which has a much improved OER of over 25%.

Basiron and Abdullah, (1995) reported that some of the planting materials based on the improved Tenera produced 100% more PO than the standard Tenera with continuous breeding and selection work. Owing to the rise in global demand and multipurpose use of PO, the selected oil palm clones were engendered to increase oil yield. The selected oil palm clones were developed from highly fruitful individual Tenera hybrids by the use of tissue culture techniques (Brenardo-Gil et al., 2007). However, cloning of oil palms was conducted by inducing somatic embryogenesis on Calli copied from various tissue sources (Jouannic et al., 2005). Figures 2.3 and 2.4 show a palm fruit and its structure, respectively.

Figure 2.3 Palm fruits, (a) Individual spikelet, (b) Detached ripe fruits (Henderson, 2000).

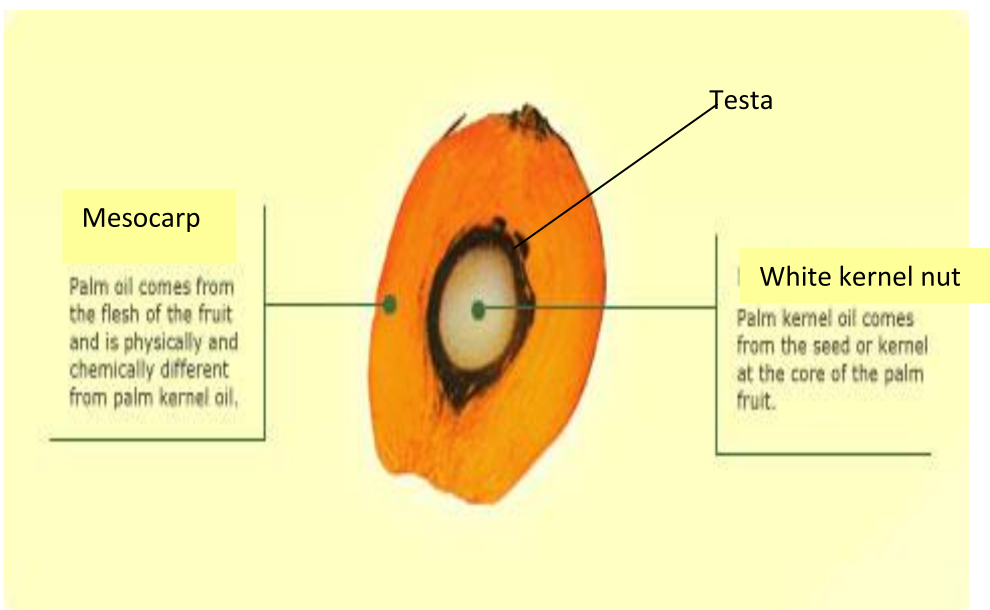

Figure 2.4 Palm fruit cut open showing the mesocarp and white kernel nut with testa

2.5. Palm kernel without testa (PKw)

Global production of palm oil has increased considerably over the years and is likely to increase further, resulting in large quantities of palm kernel. Little use is made of the palm kernels at present. Palm kernel oil is akin to coconut oil in its fatty acid composition. The palm kernel seed is covered by thin dark brown skin (0.1 - 0.2mmthick) called testa, weighing about 3-5% of the kernel. The layer of the gum/lignin that binds the seed coat to the kernel appears to be thin. The testa contains tannins and phenolic constituents which are responsible for the dark color of the palm kernel oil (Sreedhara and kurup1998).

Palm kernel has a thin, single seed coat weighing 3-5% of the kernel. The layer of gundlignin that binds the seed coat to the kernel appears to be thin. The testa is so strongly attached to the kernel that its removal by dehulling processes is not easy. The dehulling process usually practiced include water soaking, treatment with various chemicals, such as soda ash or lime, sodium bicarbonate, sodium carbonate, sodium borate sodium hypochlorite alkali or a combination of alkali-acid (Sreedhara et al., 1992).

The function of the testa is primarily to protect the seed from damage that may be inflicted upon it by the environment and also to control the germination of the seed itself via such mechanism such as its impermeability to water and oxygen and mechanical resistance to protrusion of the radicle (Debeaujon et al., 2000). Furthermore, according to Debeaujon et al. (2000), these properties are related to the colour of the testa of the seed of diverse plant species, which is itself the product of the presence of phenolic compounds. Waniska (2000) has stated that phenolic compounds are primarily responsible for the pigmentation in the pericarp and testa of sorghum grains. A study carried out by Khaopha et al. (2012) indicated that the testa of peanuts themselves contain greater total phenolic content compared to the peanuts without testa. Uvarova and Barrera-Arellano (2005) produced two types of palm kernel flour: one type from palm kernels with the testa intact and a second type from palm kernel that had undergone treatment with hydrochloric acid to remove the testa. The study reported that removal of the testa resulted in a decrease of the polyphenol content of the palm kernel flour. Also, the flour produced from palm kernels which had undergone testa removal was a light cream colour (Uvarova and Barrera-Arellano, 2005). In an earlier study, Sreedhara and Kurup (1998) treated palm kernels with acid to remove the testa and found that the resulting palm kernels, as well as the flour produced from them, were pearl-white in colour. Such results would seem to suggest that very little of the compounds which contribute to pigmentation are found within the palm kernel itself, with the majority of the pigmentation being concentrated within the testa in the form of phenolic compounds.

2.6 Palm kernel cake (PKC)

Palm kernel cake (PKC) is the byproduct (solid residue) of palm kernel oil processing, which is left behind after the palm kernel oil (PKO) has been extracted from the palm kernels through mechanical processes using screw press (Akpan, 2005). According to Tang and Teoh, (1985), palm kernel cake (PKC), also known as palm kernel expeller since the palm kernels are passed through the expeller extraction process to get the oil and generate the PKC at the same time. Its usefulness as animal feed has been known for decades now, and first reported by Collingwood in 1958 (Ravindran and Blair, 1992). Continuous research has shown that PKC is suitable for ruminants as well as for pigs, poultry and horses (Alimon, 2006; Hossain et al., 2011).

2.6.1 Production and export of palm kernel cake in Malaysia

The global production of PKC has increased tremendously owing to the rapid growth of the oil palm industry in many parts of Asia and Africa. Malaysia in 2001 produced 1.4 million tones of palm kernel oil together with 1.6 million tones of Palm kernel cake. However, the production of palm kernel oil and palm kernel cakes increased to 4.0 and 2.2 million tonnes by 2011, respectively (Atasie and Akinhanmi, 2009). Table 2.1 shows the annual production of palm kernel and palm kernel cake in Malaysia since 1975 (MPOB, 2009). The yield of PKC from the palm kernel is about 50%. Most of the PKC produced in Malaysia is exported, especially to European countries for use as an ingredient in animal feed formulations. It was being estimated that the European Union (EU) countries absorb more than 85% of Malaysian PKC annually and the Netherlands