THE EFFECT OF TEMPERATURE, DURATION OF THE EXTRACTION AND DIFFERENCE DAY OF ANYLASIS ON ANTI-OXIDANT ACTIVITY IN PALM KERNEL CAKE (PKC)

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ABSTRACT

THE EFFECT OF TEMPERATURE, DURATION OF THE EXTRACTION AND DIFFERENCE DAY OF ANYLASIS ON ANTI-OXIDANT ACTIVITY IN PALM KERNEL CAKE (PKC)

Palm oil product has been known with its rich antioxidant when researches have been done on it and its by products such as palm kernel cake (PKC). A number of extraction techniques such as Spectrophotometric Peroxide Method and DPPH are used to extract the antioxidant from palm kernel cake. In this project, natural extraction will be used to extrac the antioxidant of palm kernel cake. DPPH Radical Scavenging Activity of palm kernel cake will be determined based on three main parameters which are temperature of extraction, duration of extraction and different time of analysis. For extraction, hexane will be used as the solvent to extract the antioxidant from palm kernel cake at 60°C and at 1 atmospheric pressure. Total amount of antioxidant that extracted by the three parameters will be compared during the discussion.

ABSTRAK

Minyak Kelapa Sawit telah dikenali kaya dengan anti-oxidant setelah kajian telah dibuat keatasnya. Pelbagai kaedah pengasingan anti-oxidant dari PKC telah dicuba seperti kaedah Spectrophotometric Peroxide and kaedah DPPH. Di dalam projek ini, hexane telah digunakan untuk mengasingkan anti-oxidant dari PKC berdasarkan tiga parameter iaitu kesan suhu, tempoh dan masa tangguh penganalisisan. Jumlah anti-oxidant yang diasingkan akan dibincangkan dalam bahagian perbincangan.

CONTENT

CHAPTER 1

Introduction

1.1 Overview

The oil palm sector is one of the major industries in Malaysia. Since the 1970s, Malaysia has been the largest producer and exporter of palm oil products in the world. In the year 2000, Malaysia produced 10.84 million tonnes of palm oil which accounted for 49.9% of the world palm oil production or about 9.4% of world total production of oils and fats. Malaysia exported 9.08 million tonnes of palm oil during the year 2000. Malaysia is also the largest producer and exporter of palm kernel products, especially palm kernel oil and palm kernel cake (PKC). In the year 2000, Malaysia produced 1.38 million tonnes of palm kernel oil and 1.64 tonnes of PKC. Of these, 0.52 million tonnes of palm kernel oil and 1.35 million tonnes of PKC were exported (Hishamuddin Mohd Aspar). Table 1 is showing the annual production of palm kernel and PKC:

TABLE 1. MALAYSIA: ANNUAL PRODUCTION OF PALM KERNEL AND PKC (t)

Year	Palm kernel	PKC
1975	232 821	n.a
1980	557 066	278 559
1985	1 211 887	633 316
1990	1 844 737	1 038 221
1995	2 395 586	1 293 144
1996	2 488 750	1 383 034
1997	2 636 000	1 435 104
1998	2 429 468	1 345 277
1999	3 025 690	1 624 134
2000	3 162 760	1 639 227
2001*	1 366 360	729 197

Table 1

Palm oil milling is a major industry in Malaysia. The total area under oil palm covers more than 2 million hectares. The industry, besides producing palm oil, also produces by-products usable as animal feed. These are palm kernel cake, palm oil sludge (POS) and palm pressed fibre (PPF). The most useful is palm kernel cake, which is the solid residue left behind after the extraction of oil from the kernels of the palm fruits. It is now well entrenched as a major feed ingredient in beef and dairy feed in the country.

The PKC is obtained out from two stages of oil extraction from the palm fruit. The first stage is the primary extraction of palm oil from the pericarp portion of the fruit, which also produces the kernel and by-products POS and PPF. The extraction of oil from crushed kernel then results in the production of PKC as by-product.

Two methods are used for the extraction of oil from the crushed kernels. These are the conventional mechanical screwpress method that results in the expeller pressed palm

kernel cake and the solvent (usually hexane) extraction method that results in the solvent extracted type(F.Y.Chin). The palm fruit (Elaies guineensis) yields palm oil, a palmitic-oleic rich semi solid fat and the fat-soluble minor components, vitamin E (tocopherols, tocotrienols), carotenoids and phytosterols. A recent innovation has led to the recovery and concentration of water-soluble antioxidants from palm oil milling waste, characterized by its high content of phenolic acids and flavonoids. This relatively new output from the oil palm fruit is the water-soluble phenolic-flavonoid-rich antioxidant complex. This has potent antioxidant properties coupled with beneficial effects against skin, breast and other cancers. Enabled by its water solubility, this is currently being tested for use as nutraceuticals and in cosmetics with potential benefits against skin aging(Sundram K, Sambanthamurthi R, Tan YA.,2003)

1.2 Antioxidant

Antioxidant compounds in food play an important role as a health-protecting factor. Scientific evidence suggests that antioxidants reduce risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants.

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases(Aruna Prakash, PhD, 2001).

Various antioxidant activity methods have been used to monitor and compare the antioxidant activity of foods. In recent years, oxygen radical chemiluminescence assays have been used to evaluate antioxidant activity of foods, serum and other biological fluids. These methods need special equipment and technical skills for the analysis. These types of methods published in the literature for the determinations of antioxidant activity of foods involve electron spin resonance (ESR) and chemiluminescence methods. These analytical methods measure the radical-scavenging activity of antioxidants against free radicals like the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, the superoxide anion radical (O2), the hydroxyl radical (OH), or the peroxyl radical (ROO). The various methods used to measure antioxidant activity of food products can give varying results depending on the specificity of the free radical being used as a reactant.

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2,2-Diphenyl-1- picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods. It has also been used to quantify antioxidants

in complex biological systems in recent years. The DPPH method can be used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample. A measure of total antioxidant capacity will helps us understand the functional properties of food.

Antioxidant activity has been expressed in various ways including the percentage of the reagent used, the oxidation inhibition rate and so on. An easier way to present antioxidant activity of foods would be to reference a common reference standard. One common reference standard, (S)-(-)-6-hydroxy-2,5,7,8-tetramethylchroman-2- carboxylic acid, also known as Trolox, serves as such a common reference standard.

1.2.1 Phenolic Compounds

Phenolic compounds are present in plants, fruits and even foods as different functions. The types of phenolic contained in plants may be simple phenolics, phenolic acids, coumarins, flavonoids, stilbenes, hydrolyzable and condensed tannins, lignans and lignins. Phenolics acts as phytoalexins, antifeedants, attractants for pollinators, contributors to plant pigmentation, antioxidants and protective agents against UV light.

The distribution of phenolics in plant can be found in the cell wall for the insoluble phenolic and within the plant cell vacuoles for soluble phenolics. under stress conditions such as UV radiation, infection by pathogens and parasites, wounding, air pollution and exposure to extreme temperatures, the content of phenolics may increase. The level of phenolics in plant sources may vary depend on some factor such as cultivation techniques,

cultivar, growing conditions, ripening process, as well as processing and storage conditions.

Phenolics present in many fruits such as apple, blueberries, cranberries, grape berries and grape berries. The phenolics compounds present in fruit are caftaric acid, coutaric acid, *trans*-fertaric acid, gallic, caffeic, *p*-coumaric, ferulic, ellagic acids and etc. Phenolic acids, catechins, flavonols, anthocyanins and proanthocyanidins which are antioxidative in nature can be found richly in blueberries. Blueberry leaves were also found to serve as a good source of phenolics that possess high antioxidant activity.

1.2.2 Flavonoids Compunds

Flavonoids are one of the important classes of phenolic compounds. Studies have shown that flavonoids have significant antioxidant activity like phenolic as well. Anthocyanins, proanthocyanidins, and flavanols are the flavonoids used in the diet. Blueberries, blackberries, strawberries, raspberries, eggplant are usually rich in these compounds. In plants, phenolics may act as phytoalexins, antifeedants, attractants for pollinators, contributors to plant pigmentation, antioxidants and protective agents against UV light (B.B. Li, B. Smith and Md. M. Hossain, 2005)

1.3 Extraction Method

Antioxidant compounds may be water-soluble, lipid-soluble, insoluble, or bound to cell walls. Hence, extraction efficiency is an important factor in quantification of antioxidant activity.

1.3.1 DPPH Method

A simple method that has been developed to determine the antioxidant activity of foods utilizes the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The structure of DPPH and its reduction by an antioxidant are shown above. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with a hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured.

Trolox (as the reference standard) and the sample are reacted with DPPH solution in methanol/water for four hours at 35°C in a vessel mounted on a rotary shaker and the absorbance changes are measured at 517 nm. The quantity of sample necessary to react with one half of the DPPH is expressed in terms of the relative amount of Trolox reacted. Antioxidant activity of a sample is expressed in terms of micromole equivalents of Trolox (TE) per 100 grams of sample, or simply (TE) per 100 gm of sample or Trolox units per 100 gm.

A difference between this method and other published methods is carrying out the reaction of the sample itself with DPPH in methanol/water. Reacting an aqueous methanolic DPPH solution with the sample for 4 hours at 35 °C facilitates the extraction of antioxidant compounds from the sample thereby increasing the measured antioxidant activity of the sample. Determination of antioxidant activity of various types of foods using DPPH is comparable to other methods. It is probable each of these methods measure a somewhat different profile of antioxidant compounds. Antioxidant analysis by other published methods is limited to those compounds soluble in the selected solvent. Antioxidant activity of insoluble compounds was not accounted in a single extraction method. Extraction techniques using different solvents and concentrating the solvent is time consuming. In this method, DPPH is allowed to react with the whole sample. Sufficient time allows DPPH to react slowly with weak antioxidants.

1.3.2 Soxhlet

Soxhlet extraction method can be used in extraction of the antioxidant components inside the palm kernel cake. A Soxhlet extractor is a type of laboratory glassware invented in 1879 by Franz von Soxhlet. It was originally designed for the extraction of lipid from a solid test material, but can be used whenever it is difficult to extract any compound from a solid.

Typically, dry test material is placed inside a "thimble" made from filter paper, which is loaded into the Soxhlet extractor. The extractor is attached to a flask containing a solvent (commonly diethyl ether or petroleum ether) and a condenser. The solvent is

heated, causing it to evaporate. The hot solvent vapor travels up to the condenser, where it cools and drips down onto the test material. The chamber containing the test material slowly fills with warm solvent until, when it is almost full, it is emptied by siphon action, back down to the flask. This cycle may be allowed to repeat many times. During each cycle, a portion of the lipid dissolves in the solvent. However, once the lipid reaches the solvent heating flask, it stays there. It does not participate in the extraction cycle any further. This is the key advantage of this type of extraction; only clean warm solvent is used to extract the solid in the thimble. This increases the efficiency of the extraction when compared with simply heating up the solid in a flask with the solvent.

At the end of an extraction, the excess solvent may be removed using a rotary evaporator, leaving behind only the extracted lipid (Wikipedia, 2006). A schematic representation of a soxhlet extractoris shown as below:

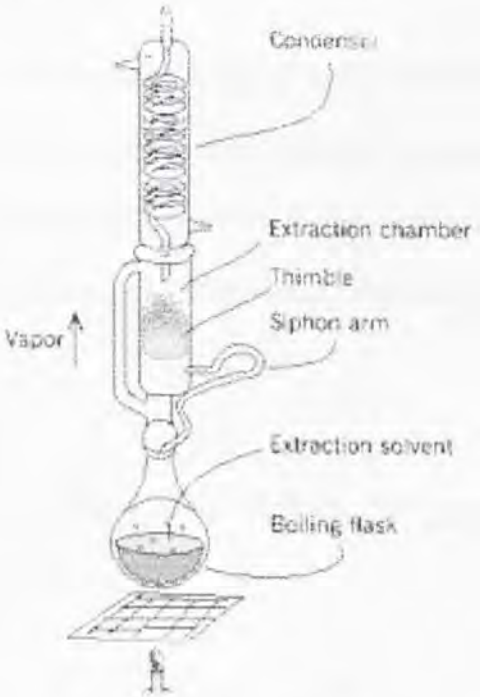

Figure 1: Model of Soxhlet

1.4 Objective

The objective of this project is to determine the total amount of antioxidant components inside the palm kernel cake and also determine how many amount of antioxidant can be extracted from palm kernel cake through solvent extraction method. Different temperature will be applied on the experiment to optimize the amount of antioxidant from extraction.

1.5 Thesis Organization

Chapter 1 is the introduction of this project. The topic included in this chapter is overview of this project, introduction of palm kernel cake, antioxidant, phenolic and flavonoids. The extraction method that will be used in the project is also discussed in this chapter.

Chapter 2 is literature review. All the journals and information that support this project is included in this chapter. Journals and internet sources were searched based on the title of the project. Included in this chapter are the detail information of antioxidant of palm kernel cake, parameters that affect the result of extraction and types of extraction method.

Chapter 3 is methodology and apparatus. All possible methodologies and apparatus are discussed in this chapter.

Chapter 4 is result, data analysis and discussion. Experiments' result and calculations are shown here. Discussions are made according to the experiments had done.

Chapter 5 is the conclusion for the whole report and the discussion for the whole the project.

CHAPTER 2

Literature Review

2.1 Antioxidant properties of palm fruit extracts

Phenolic compounds have been shown to exhibit bioactive properties, and in particular antioxidant effects. A phenolic-rich fraction has been isolated from the aqueous by-product obtained during the milling of oil palm fruits. The objectives of the study were to determine the phenolic content of the crude and ethanolic extracts of oil palm fruits and to evaluate the antioxidant properties of these extracts. The total phenolics content of the crude and ethanol extracts as determined by the Folin-Ciocalteu method were found to be 40.3 ± 0.5 and 49.6 ± 0.6 mg GAE/g extract (dry basis), respectively. The radical scavenging activity of the extracts determined using 2,2 diphenyl-1-picrylhydrazyl radical (DPPH.) indicated that both crude and ethanol extracts exhibit hydrogen-donating capacity, and have antiradical power (ARP) comparable to ascorbic acid. The DPPH radical scavenging activity of the extracts were less than that of gallic acid, but the time-course variations of the scavenging curves suggest that the extracts acted by a mechanism similar to that of gallic acid. The electron-donating potentials of the extracts were inferred from the hydrogen peroxide scavenging and reducing power assays. The reducing power of crude and ethanol extracts at 1 mM GAE were found to be comparable to that of 0.3 mM gallic acid. The extracts indicated complete scavenging of hydrogen peroxide at concentrations above 0.4 mM GAE. These findings suggest that the crude and ethanol

extracts are able to scavenge free radicals, by either hydrogen or electron donating mechanisms, and can therefore act as primary antioxidants (Mohamed A Bayorh, Imad K Abukhalaf and Agabaa Ganafa, 2005).

2.2 Phenolics in cereal, fruits and vegetables

A series of extraction research of phenolics in cereal, fruits and vegetable had been conducted by Marian Naczk and Fereidoon Shahidi(2006). The content of some phenolics may increase under stress conditions such as UV radiation, infection by pathogens and parasites, wounding, air polution and exposure to extreme temperatures.

The level of phenolics in plant sources also depends on such factors as cultivation techniques, cultivar, growing conditions, ripening process, as well as processing and storage conditions, among others. Solvents, such as methanol, ethanol, propanol, acetone, ethyl acetate, dimethylformamide and their combinations have also been used for the extraction of phenolics, often with different proportions of water.

Quantification of phenolic compounds in plant materials is influenced by their chemical nature, the extraction method employed, sample particle size, storage time and conditions, as well as assay method, selection of standards and presence of interfering substances such as waxes, fats, terpenes and chlorophylls. In their research, they have used Folin reagent to determine the total amount of phenolic and spectrophotometric used to determine the amount of phenolic extracted. simple phenolics have absorption maxima between 220 and 280 nm, , but their absorption is affected by the nature of solvent

employed and the pH of the solution. Moreover, the possibility of interference by UV-absorbing substances such as proteins, nucleic acids and amino acids should be considered. Therefore, development of a satisfactory UV assay is a rather cumbersome and difficult task. Moreover, it is difficult to find a specific and suitable standard for quantification of phenolics. This is due to the complexity of plant phenolics as well as existing differences in the reactivity of phenols towards reagents used for their quantification.

2.3 Parameters that effect the results of extraction

B.B.Li, B.Smith and Md.M.Hossain have conducted research on the extraction of citrus peels based on several parameters using Folin-Ciocalteu reagent. The main parameters that affected the yield of phenolics include the condition of the peels, temperature of the extraction, types of enzymes, enzyme concentration.

The extraction of the peel powder was done by placing the peel powder in a 50 ml centrifuge tube and 16 ml of aqueous enzyme solution at the desired concentration was added. The preparation was left to stand at different temperature (varies from 20 to 60 °C) for 3 h. The mixtures were then centrifuged using a Mistral 1000 centrifuge at 500 g for 10 min at room temperature. After centrifugation, the supernatants were filtered through Whatman No. 42 filter paper. Following filtration, a 10 ml aliquot of the filtrate was concentrated by evaporation of the solvent, using a rotary evaporator under partial vacuum at 40 °C until less than 1 ml of filtrate remained. The extract was then re-dissolved in 10 ml of Milli-Q water and stored at 4 °C prior to purification step (up to 1

day). All the extracts were prepared in triplicate (B.B.Li, B.Smith and Md.M.Hossain, 2005).

Total phenolic contents in citrus extract were evaluated using the Folin–Ciocalteu reagent. Briefly, 250 µl of citrus extract (in triplicate), a gallic acid calibration standard, or Milli-Q water (as blank) was placed in a separate 25 ml volumetric flask, followed by the addition of 15 ml Milli-Q water and 1.25 ml Folin–Ciocalteu reagent. The contents were swirled to mix and allowed to stand for 5–8 min at room temperature. Next, 3.75 ml of a solution of sodium carbonate (7.5%, w/v) was added. Then, Milli-Q water was added to the flask to volume. Solutions were mixed and allowed to stand for 2 h at room temperature before measurement of the absorbance at 765 nm using UV–vis spectrophotometer. Results were expressed as mean total phenol content (mg of gallic acid equivalents per 100 g of citrus peel) ± S.D. for triplicates (B.B.Li, B.Smith and Md.M.Hossain, 2005).

2.3.1 Effect of the time of contact for extraction

Figure 2.1 shows the total phenolic contents of Meyer lemon peel obtained using different extraction times. An incubation period of 3 h was sufficient for enzyme-assisted aqueous extraction. For aqueous extraction, only the highest recovery was achieved for 6 h duration and beyond that time, there was little difference in recovery. It is observed in results that enzyme-assisted aqueous extraction improves the extraction efficiency over and above the aqueous only extraction.

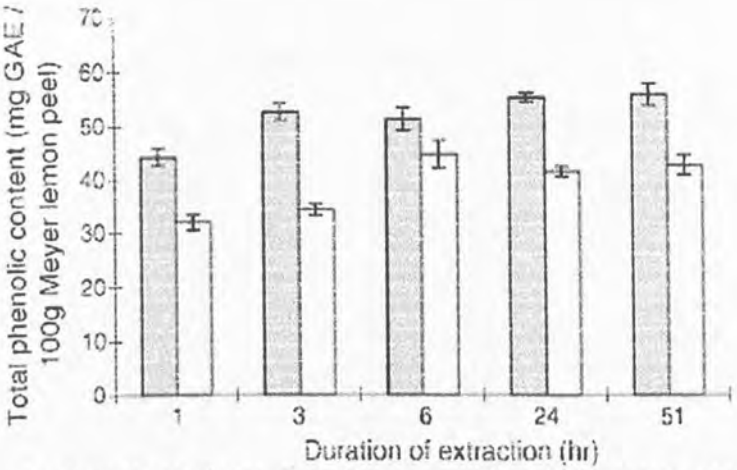

Figure 2.1: Effect of contact time: (■) Enzyme-assisted aqueous extraction and (□) aqueous extraction. Results are presented as means ± S.D. for triplicate analyses.

2.3.2 Effect of temperature on extraction

Figure 2.3 shows the total phenolic contents obtained following extraction with 1.5% (weight of peels) Celluzyme CL, 1.5% Celluzyme MX or 1.5% Kleepase AFP 106L and water at 19, 37, 50, 65 and 80 °C. Generally, 1.5% Celluzyme MX showed the greatest total phenol extraction overall and this increased with increasing temperature. However, at 80 °C, there was little difference between any of the treatments including water, whereas at the lower temperature, the aqueous treatment was not as effective as the enzyme-assisted extraction. The optimum temperature for activity of Celluzyme CL is in the range of 50–80 °C (Zymus, enzyme data sheet) and this corresponds to the results shown in Fig. 2.2.

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