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MAJOR CHEMICAL CONSTITUENTS OF CANDLE NUT OIL EXTRACT USING SUPERCRITICAL CARBON DIOXIDE

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Supercritical Fluid Extraction (SFE) is a new method of extraction which is very suitable to extract bioproducts from their natural matrices. Supercritical carbon dioxide was used as a solvent in the extraction of oil from ground candle nut seeds. The candle nut seeds and oil derived from it can be used for cooking purposes, and industrial use such as production of paint, resin, soap, medicine and cosmetics. In this study, candle nut oil was extracted at pressures ranging from 20.7 MPa to 48.3 MPa, and temperatures between 40°C to 80°C. Comparisons of the extracted oil yield under various conditions show that at pressure 34.5 MPa and temperature 40°C gives the highest yield of 52.58 g oil/100 g candle nut at a solubility of 0.89 g oil/100 g CO_2. Analysis of the oil extract using the GC and MS analysis showed that candle nut seeds contain saturated fatty acid such as palmitic acid (6.35%) and stearic acid (54.21%), and unsaturated fatty acid such as oleic acid (16.54%), linoleic acid (19.96%), and linolenic acid (2.80%). Supercritical carbon dioxide as an environmental friendly solvent can be used to produce high purity and high quality oil from candle nut seed.

Keywords: *Candle nut oil, Saturated and Unsaturated Fatty Acid, Supercritical Carbon Dioxide, Extraction*

INTRODUCTION

Candle nut (*Aleurites moluccana* Willd), also known as "kemiri" in Indonesia and "buah keras" in Malaysia, belongs to the family Euphorbiaceae, a native to the Molucca Islands and widely found in South East Asia. Because of the high oil content (about 60%), the nuts will burn with a smoky flame, hence the name candle nut. It is commonly used in Indonesian and Malaysian dishes, curry pastes and as meat tenderizer (Burkill 1935). The oil derived from the seeds of the candle nut can be used for industrial purposes such as the production of paint, resin, soap, medicine and also for cosmetics (Sunanto 1994).

Current conventional separation method of producing candle nut oil from the seed involves a series of processes such as peeling of kernels, screw press, separation and filtration which is time consuming, involves high operating cost and high energy consumption. Only 60% of the oil can be extracted using this technique. Furthermore, oil produced from this method usually is low quality and low purity.

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Supercritical fluid extraction (SFE) is a new and powerful technique in the extraction and separation processes. Several investigations have been made in recent years on probable industrial applications of the SFE. They offer some preferences over the conventional methods such as production of product of high quality and high purity, low operating temperature and cost, no contamination of chemical if carried out at optimum conditions, and sufficient extractor volume especially in the areas of food, pharmaceutical, chemical and oil industries (Bruno and Ely 1991; Sovova *et al.* 1994). SFE is being recognised as an important sample preparation method in the food sciences especially in the area of fats and oils, and has been used in the lipid analysis of various products (Arul *et al.* 1987; King *et al.* 1989; Bradley 1989; Lembke and Engelhardt 1993). Supercritical carbon dioxide (CO_2) is a natural solvent for the extraction of lipids because triglycerides and fatty acids are highly soluble in CO_2 (DeFilippi 1982; Ikushima *et al.* 1994). It is non-toxic, non-flammable, easily available and relatively cheap (Stahl *et al.* 1985; McHugh and Krukonis 1986; Luque de Castro *et al.* 1994). It is a promising solvent for extraction and fractionation of vegetable oil containing labile unsaturated fatty acids since these processes can be carried out at low temperature (Sovova *et al.* 2001). Free fatty acids and mono- and diglycerides which may be present in oils are more soluble in CO_2 than triglycerides (Goncalves *et al.* 1991; Nilson *et al.* 1991; Bharath *et al.* 1992). Solubility of olive husk oil, rich in free oleic acid, was observed to be up to several times longer than the solubility of triglycerides contained in the oil (Goncalves *et al.* 1991; Esquivel et al. 1993).

Supercritical fluid is a fluid whose pressure and temperature are above its critical point. The critical point of a pure substance is defined as the highest temperature and pressure at which the substance can exist in vapor-liquid equilibrium. For CO_2, its critical pressure is 7.38 MPa and its critical temperature is 31.1°C. Three factors have contributed to the recent attention given to supercritical fluids; (i) the environmental problem associated with common industrial solvents (mostly chlorinated hydrocarbon), (ii) the increasing cost of energy-intensive separation techniques such as distillation, and (iii) the inability of traditional techniques to provide the necessary separations needed for emerging new industries such as microelectronics and biotechnology (Bruno and Ely 1991).

Another advantage of using SFE is its selectivity to dissolve different substances depending on pressure and temperature. SFE of oil seeds has been thoroughly studied by many researchers (Friedrich 1984; Mannes *et al.* 1995; Ooi *et al.* 1996). At 48.3 MPa and 80°C, the solubility of soybean and cotton seed oil in SC-CO_2 is about 3% and rises dramatically at pressures and temperatures above 55.7 MPa and 60°C, respectively. Soybean triglycerides become infinitely miscible with SC-CO_2 at 81.1 MPa and 70°C (Friedrich 1984). The application of supercritical CO_2 as a solvent in the extraction of oil from oil seeds such as soybeans, corn germ, cotton seed and similar oil seeds have been reported and patented. Based upon these findings, Friedrich (1984) proposed a process for extracting soybeans, cotton seed and similar oil seeds with a minimum CO_2 of recycle using CO_2 at very high pressure. For example, solubility levels of 20—40 percent are reported at conditions of 60.8 MPa and 70°C.

Many applications of supercritical CO_2 extraction for recovery and refining of oils and fats from natural products have been investigated. Since the solvent power of supercritical CO_2 is relatively poor, it is often necessary to add a small amount of entrainer to improve the solubility and selectivity (Noh *et al.* 1995; Gonzalez *et al.* 2001).

This study aims to manipulate the pressure and temperature, which give optimum yield of the oil extracted from candle nut seed. The extracted oil will be analyzed using gas chromatography-mass spectrometry to identify its major components, and using gas chromatography for fatty acid constituents.

METHODS

Sample Preparation

The kernels of candle nut seed were removed from the hard skin. They were then cut into small pieces about 0.5 cm to 1 cm, thoroughly mixed and ground with a dry mixer (Blender Model 32 BL79; 8010; Warring Commercial. Torrington, Connecticut; 06057; USA) before they were dried for 4 hours at 100°C in an oven (Memmert ULM 400~800, Schwabach, Germany). Water constitutes about 4 percent of the fresh seeds. About 200 g each of the ground candle nut kernels were then packed in Magenta Box (7.5 cm × 6.5 cm × 6.5 cm) and kept in a refrigerator at temperature 4°C until ready for the extraction.

Supercritical Fluid Extraction

The experimental set-up of the extraction process is shown in Figure 2. It consists of a pump (American Lewa, Holistic, Massachusetts, USA) with a maximum capacity of 68.9 MPa, an oven (CCS Instrument System), a chiller (Yih Der BL-730) to cool down the CO_2 gas to liquid state, and 50 cm^3 extraction cell (Keystone Scientific Inc., Bellefonte, PA, USA) with internal diameter of 1.4 cm and 32 cm length and wet gas meter (W-NK-1A, Sinagawa Corp., Tokyo) to monitor the atmospheric flow rate of CO_2.

A 20 g sample of ground kernel was then charged into a 50 cm^3 extraction cell. CO_2 (MOX Sdn. Bhd., Penang, Malaysia) with more than 99% purity at selected pressures (20.7, 27.6, 34.5, 41.4, and 48.3 MPa) and temperatures (40, 60, and 80°C) were passed through the extraction cell. The extractor was kept to stabilize for 5 minutes before the exit valve was opened. The oil extracts were collected, and the volume of CO_2 passed through the extraction cell was recorded at atmospheric pressure and temperature, using a wet gas meter for 2 hours. Solubility of candle nut oil in SC-CO_2 was calculated based on the gram of oil/100 g candle nut.

Preparation of Methyl Esters of Fatty Acid

The preparation of methyl esters of fatty acids used the methods according to Palm Oil Research Institute of Malaysia (PORIM) Methods (Siew *et al.* 1995).

Characterization

The oil extracts were dissolved in hexane (T.J. Baker Inc., Philipsburg, NJ, USA), centrifuged at 4000 rpm for 10 minutes (Labofuge 200, Heraeus Inst., Germany), and characterized according to chromatographic derivatization using a GC Model 5890 Series II and HP5989A mass selective detector (Hewlett Packard, Palo Alto, CA, USA), and Chemstation data system. Electron impact-MS of the extracted components was performed at electron energy of 70 eV with a source temperature of 200°C, and a scan range of 20—550 amu at a rate of 0.81 scan per second. The column used was a 30 m × 0.25 mm × 0.25 μm (Quadrex Corp., New Heaven, USA) cross-linked methyl siloxane fused silica capillary column at 100°C for 5 minutes, then 10°C for 1 minute to 270°C for 40 minutes. Helium at a flow rate of 1 ml. min^{-1} was used as a carrier gas. The split less injector was kept at 250°C. Peaks of special interest (saturated and unsaturated fatty acid)

were reconfirmed by comparison to the retention times and spectra of the authentic standards with the gas chromatography (GC-17 A, Shimadzu). The column used was a 30 m x 0.25 mm, ID-BP1, 0.25 μm film thickness.

RESULTS AND DISCUSSION

The overall evaluation of the conditions used (temperature and pressure) to extract oil from candle nut can be seen in Table 1, Figures 1 and 2. Table 1 shows the oil yield (g oil/100 g candle nut) and the solubility of oil in CO_2 (g oil/100 g CO_2). Comparisons of the extracted yield under various conditions showed that the combined pressure 34.5 MPa and temperature 40°C provides the highest yield of 52.58 g oil/100 g candle nut at a solubility of 0.89 g oil/100 g CO_2. Figure 1 shows the graph of the yield of oil from candle nut at various pressures and temperatures. At specific pressures of 27.6, 34.5 and 41.4 MPa, there are not much variation in the oil yield and solubility of oil in CO_2 at the temperatures applied. However, there are variations in the yield and solubility between the pressures applied.

Table 1: Oil yield (g oil/100 g kernel) and the solubility of oil in CO_2 (g oil/100 g CO_2) at different combination of temperature and pressure

Pressure (MPa)	Temperature (°C)	Oil Yield (%) (g oil/100 g kernel)	Solubility (g oil/100 g CO_2)
20.7	40	22.39	0.18
	60	16.64	0.17
	80	9.59	0.11
27.6	40	22.73	0.34
	60	24.27	0.35
	80	22.07	0.32
34.5	40	52.58	0.89
	60	51.77	0.83
	80	50.28	0.88
41.4	40	50.53	1.14
	60	51.71	1.14
	80	46.89	1.18
48.3	40	24.38	1.52
	60	31.38	2.13
	80	45.31	2.92

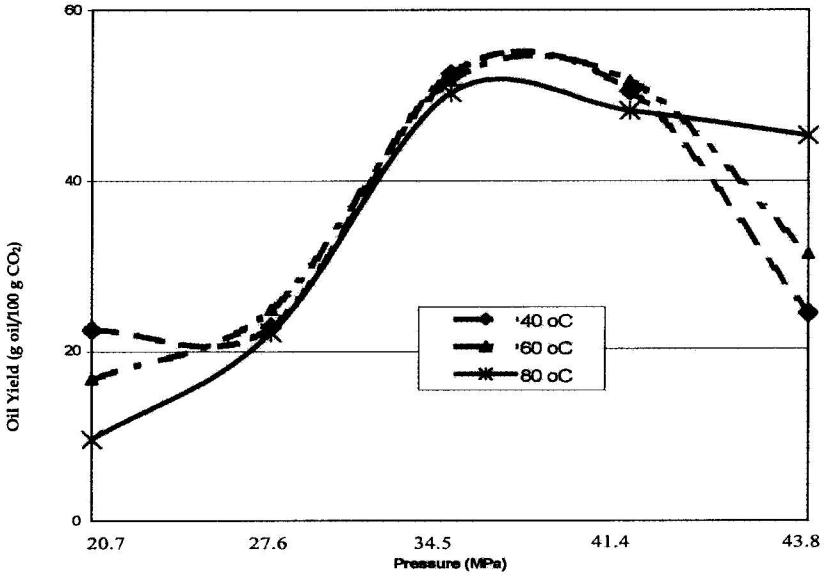


Fig. 1: Oil yield at various pressures and temperatures

The yield and solubility of the candle nut oil extracted using SC-CO_2 are dependent on both pressure and temperature. At lower and higher pressures of 20.7 and 48.3 MPa, temperature influences the yield and solubility. At 20.7 MPa, high temperature of 80°C exerts lower solubility of oil in CO_2, hence reducing the yield. While at higher pressures *viz* 27.6, 34.5 and 41.4 MPa, keeping each pressure constant while varying the temperature did not result in much change in the solubility. On the contrary, at a combined pressure of 48.3 MPa and temperatures of 40, 60 and 80°C, solubility is enhanced as the temperature is increased. The best yields were obtained when the pressure is set at 34.5 MPa followed by 41.4 MPa, independent of the effect of the temperature. Highest yield of oil was achieved at a combined pressure and temperature, 34.5 MPa and 40°C, respectively obtaining 52.58 g oil/100 g candle nut.

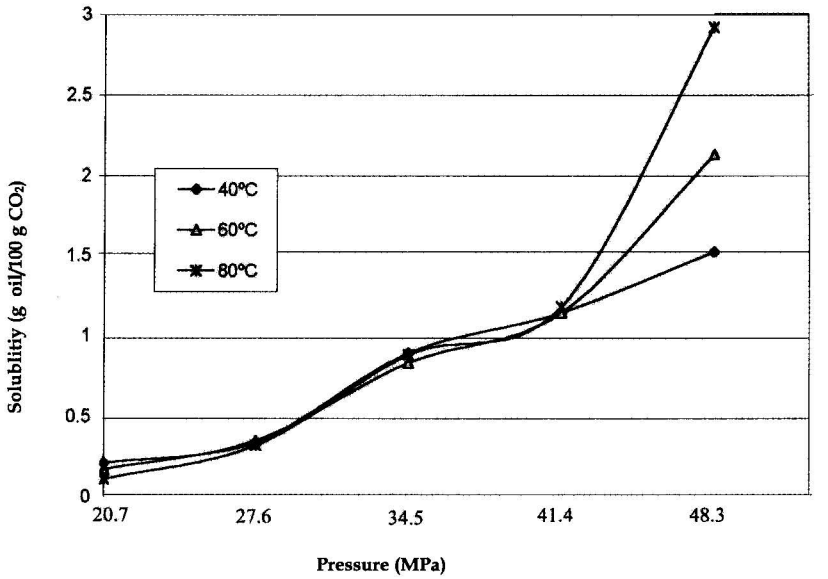
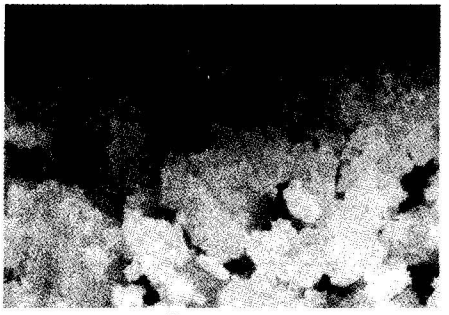
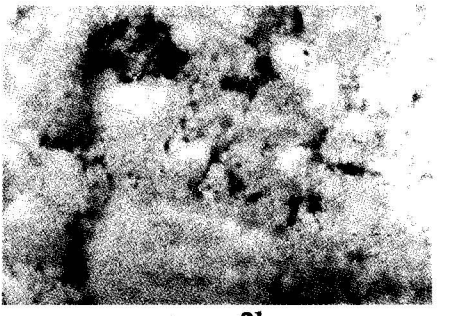


Fig. 2: Solubility of candle nut in SC-CO_2

Figure 2 shows that at 20.7 MPa, the solubility of oil decreases with an increase in temperature. At higher pressures of 34.5, 41.4 and 48.3 MPa, there appears to be no difference in the solubility with increasing temperature. At 48.3 MPa, the solubility of oil in SC-CO_2 almost tripled from about 1 to 3% as the temperature was increased from 40 to 80°C, however, the yield remained lower at pressure of 34.5 and 41.4 MPa (Fig. 2). Sovova *et al.* (2001) stated that the solubility is sensitive to the presence of mono- and diglycerides, and free fatty acids that may be present in oil which are more soluble than triglycerides. Solubility of olive husk oil, rich in free oleic acid, was observed to be up to several times longer than the solubility of triglycerides contained in the oil (Goncalves *et al.* 1991; Esquivel *et al.* 1993). According to Friedrich (1984), the solubility of soybean and cotton seed oil in SC-CO_2 at 48.3 MPa and 80°C is about 3% and rises dramatically at pressures and temperatures above 55.7 MPa and 60°C, respectively. At lower pressures, the solvent strength of SC-CO_2 is much affected by the changes in density. On the other hand, at higher pressures, the change in density with temperature is less significant compared with the changes caused by the increase in vapour pressure of the oil (Hassan *et al.* 2000). At combined temperature and pressure of 80°C and 48.3 MPa, greater solubility, 3 g oil/100 g CO_2 was achieved.



3a



3b

Fig. 3a: Ground candle nut before extraction with moist, lumpy and yellowish texture

Fig. 3b: Candle nut meal with dry, crumbly and white after extraction (34.5 MPa; 40°C).

Figures 3a and 3b show ground candle nut before and after extraction with SC-CO_2. Ground candle nut before extraction has a moist, lumpy and yellowish texture. However, after extraction at 34.5 MPa and 40°C, the candle nut meal has a dried crumbly and white in colour indicating that almost all the oils in candle nut had fully extracted.

Physical observation showed that the colour of the extracted oil was lighter than the oil extracted from soxhlet extraction using hexane as a solvent. The colour of the extracted oil also varied with extraction pressure and temperature. The oil extracted at higher pressures and temperatures were bright and yellowish.

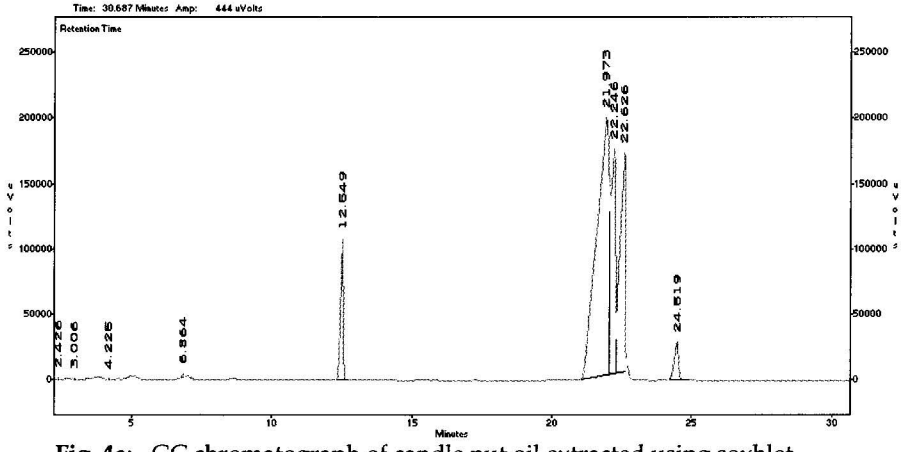


Fig. 4a: GC chromatograph of candle nut oil extracted using soxhlet (Component identified here at retention time between 2.43 and 24.52 minutes).

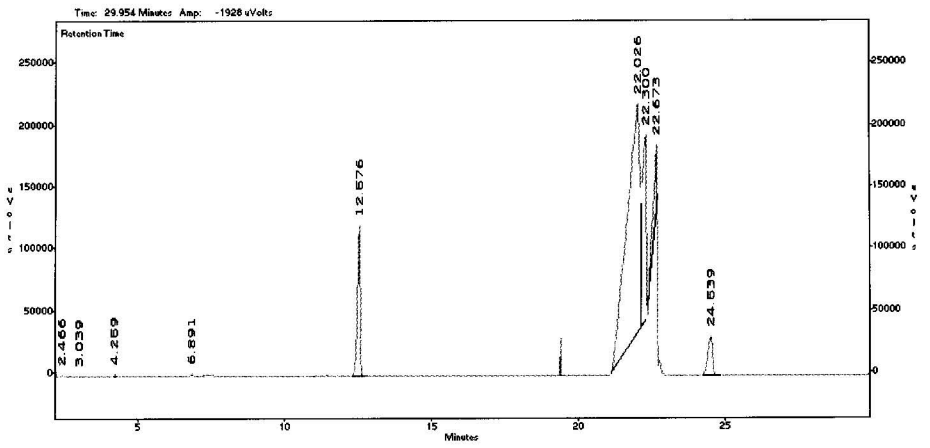


Fig. 4b: GC chromatograph of candle nut oil extracted using SC-CO_2 (Component identified here at retention time between 2.47 and 24.54 minutes).

Figures 4a and 4b show the total ion chromatogram of supercritical CO_2 extract of candle nut seed by using soxhlet and SC-CO_2 (34.5 MPa and 40°C), respectively. Hewlett Packard (HP-UX) Chemstation software with Wiley database library was used to obtain the mass spectra of compounds in the chromatogram. The peaks found at elution times of 2.47 to 24.54 minutes (Fig. 4b) were determined to be saturated and unsaturated fatty acids, respectively from mass spectral information by matching their retention times with the standards. It was likewise done for Figure 4a. Analysis of the oil extract using GC showed that candle nut seeds contain saturated fatty acids such as palmitic acid (6.35%) and stearic acid (54.21%), and unsaturated fatty acid such as oleic acid (16.54%), linoleic acid (19.96%) and linolenic acid (2.80%). Other identified components are listed in Table 2.

Table 2: Identified Compounds of Soxhlet and SC-CO_2 Extract of Candle Nut Seed With GC Analysis

No.	t_R (min)		Compounds	Carbon length	Percent (%)	
	Soxhlet	SC-CO_2			Soxhlet	SC-CO_2
1	2.43	2.47	Caprylic acid	C8:0	0.019	0.010
2	3.01	3.04	Capric acid	C10:0	0.017	0.011
3	4.23	4.26	Lauric acid	C12:0	0.022	0.041
4	6.86	6.89	Myristic acid	C14:0	0.051	0.051
5	12.55	12.58	Palmitic acid	C16:0	6.340	6.354
6	21.97	22.03	Stearic acid	C18:0	54.022	54.219
7	22.25	22.30	Oleic acid	C18:1	16.147	16.542
8	22.63	22.67	Linoleic acid	C18:2	20.391	19.964
9	24.52	24.54	Linolenic acid	C18:3	2.991	2.806
Total					100	100

Table 2 illustrates the comparable results obtained between both soxhlet and SC-CO_2 extracted oil. The percentage of fatty acid components do not vary too greatly between oil extracted via soxhlet and obtained through SC-CO_2 except for shorter chain fatty acids such as caprylic, capric and lauric acids with SC-CO_2 extracting 53, 65 and 186% of the free fatty acids respectively. Oil from candle nut contains mainly stearic acid (54%), followed far behind by linoleic acid (20%) and oleic acid (16%). In approximation, the proportion of saturation and unsaturation in fatty acid of candle nut oil is 60:40. Essential fatty acids are nutrients that we need in order to stay healthy. The linoleic acid and linolenic are essential fatty acids polyunsaturated and very much needed by the body. Lack of linoleic acid in our diet will result ailments such as eczema-like skin eruptions, loss of hair, liver degeneration, behavioural disturbances, kidney degeneration, excessive sweating accompanied by thirst, and drying up of glands.

CONCLUSION

The extraction and analysis of oil done showed that the optimum oil yield was achieved at temperature of 40°C and pressure of 34.5 MPa. The results obtained also showed that the solubility of oil in supercritical CO_2 was affected by the combination of both temperature and pressure. Analysis of fatty acid using gas chromatograph showed that candle nut oil was rich in stearic acid (54%), followed by linoleic acid (20%) and oleic acid (16%).

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