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Major Chemical Constituents of Supercritical Carbon Dioxide Extract of *Pandanus odorus* Leaves

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Abstract – Supercritical carbon dioxide was used as a solvent in the extraction of freeze-dried *Pandanus odorus* leaves. Analysis of the extract with GC-MS showed that *Pandanus odorus* leaves contain nutritional constituents such as α-tocopherol (Vitamin E) and squalene. The contents of α-tocopherol and squalene extracted from freeze-dried ground *Pandanus odorus* leaves at pressures ranging from 80 to 200 kg·cm^{-2} and temperatures between 40 to 80°C were 134~300 ppm and 750~1,200 ppm respectively. The highest yield was obtained at 200 kg·cm^{-2} and 40°C. Other major components identified were hexadecanoic acid, 9,12,15-octadecatrien-1-ol, campesterol, stigmasterol and β-sitosterol.
Keywords – α-tocopherol, *Pandanus odorus*, squalene, supercritical carbon dioxide.

Introduction

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 carbon dioxide, its critical pressure is 75.28 kg·cm^{-2} and its critical temperature is 31.1°C. Three factors have contributed to the recent attention given to supercritical fluids : (i) the environmental problems associated with common industrial solvents (mostly chlorinated hydrocarbons), (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).

Among the supercritical fluids, carbon dioxide is the most widely used and have been given special attention. It has a few advantages over other supercritical fluids and conventional solvents such as hexane and methanol. Its low vapor pressure allows it to be easily removed from the extract just by releasing the pressure. It is non-toxic, non-flammable and easily available at high purity at a reasonable price. It is selective in that its solvent power could be varied just by varying the pressure and temperature (McHugh and Krukonis, 1986).

Pandanus odorus Ridl. is a species of *Pandanus* in the family *Pandanaceae*. It is seldom more than 1.5 meters tall, with leaves about 0.75 meter long and 4 to 5 centimeters wide. It is propagated by cuttings and never flowers. It is commonly cultivated by Malays for its fragrant leaves. The leaves are used in cooking and in preparing bean curd. Chopped leaves are mixed with the petals of various flowers to make 'bunga rampai' a potpourri (Burkill, 1935; Purseglove, 1972).

It is also reported that *Pandanus odorus* has been used traditionally for health care and other purposes. It is used as a traditional medicine for the treatment of various diseases such as anaemia, gonorrhea, syphilis and sapraemia. Juice of pounded fresh leaves is used as a hair lotion while the ashes in vinegar is used as a lotion for the treatment of measles. The water of boiled *Pandanus odorus* leaves is used to bathe the mother after childbirth (Burkill, 1935). Other potential uses of *Pandanus odorus* leaves are as a cockroach repellant and as a source of green pigments which could be used as a coloring additive.

α-Tocopherol, $C_{29}H_{50}O_2$, stems from a Greek word meaning *'to bring forth offspring'*. At room temperature, α-tocopherol is a viscous oil, pale yellow in color. It is insoluble in water but soluble in aprotic solvents. The melting point of *R, R, R*-α-tocopherol was determined to be 2.5~3.5°C. It is thermally stable up to 200°C. When exposed to light and air, α-tocopherol slowly oxidizes and darkens (Sebrell and Har-

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ris, 1972). It is widely found in wheat germ oil (1153 ppm), corn oil (162 ppm), safflower oil (342 ppm), soybean oil (9.4 ppm), coconut oil (5 ppm) and olive oil (98 ppm) (Slover *et al.*, 1969; Slover *et al.*, 1983). The amount of α-tocopherol in various Mediterranean dry plant leaves was in the range of 0~846 ppm (Chevolleau *et al.*, 1993). α-Tocopherol is a powerful fat-soluble natural antioxidant and important for the stabilization of lipids. It also improves the fertility and reduces the effect of coronary heart disease. (Considine, 1976; Hui, 1992).

Squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14, 18,22-tetracosahexaene) is a highly unsaturated liquid triterpene with a molecular formula $C_{30}H_{50}$. It is easily purified by vacuum distillation (b.p. 240~242°C/ 4 mm). It occurs in large quantities in certain shark liver oil and the amount varied with the species (0.22~ 82.54 wt. %) (Borch-Jenson *et al.*, 1997). It has also been found in smaller amounts in various plant sources such as vegetable oils and various fungi (Parker, 1989; Pinder, 1960). Olive oil, wheat germ oil, rice bran oil and yeast contain about 0.1 to 0.7 percent squalene (Windholz, 1983). In olive oil deodorizer distillates, a by-product of olive oil refining which represents 0.05~0.1% of the total processed oil, the squalene content is between 10-30 percent (Bondioli, 1993). Squalene content in fruit and vegetables as determined by Lewis (1972) are : avocado (44.4 ppm), carrot root (0.96 ppm), apple (0.62 ppm), eggplant (2.5 ppm), squash flesh (0.15 ppm), squash seed (0.78 ppm), green banana (0.159 ppm), ripe banana (0.113 ppm), frozen green peas (1.24 ppm), mushroom (0.3 ppm).

Squalene plays a role in the biosynthesis of sterols and polycyclic terpenes. It is used as an intermediate in manufacturing of pharmaceuticals and cosmetics. It also has bactericidal activities (Parker, 1989; Windholz, 1983; Bondiolli *et al.*, 1993). At present shark liver oil is the primary source of squalene (Sun, 1997). Each year between 30 million and 100 million sharks are caught for their meat, fins, hides, jaws and their internal body parts. Shark liver oil seems to aid white-blood-cell production; it is also an active ingredient in haemorrhoid treatments (Lemonick, 1997).

Lately, because of the concern for marine animal protection, attention is given to the new source of squalene. Olive and amaranth seed oil are the two important crop sources. Olive oil contains about 0.3-0.7% squalene. The oil content of amaranth seed is only about 7%, but oil from amaranth grain contains 6~8% squalene, making it a particularly rich crop source of squalene (Sun, 1997). Lee reported that amaranth grain contains about 0.43% of squalene (Lee *et al.*, 1996).

In this study *Pandanus odorus* leaves were extracted with supercritical carbon dioxide (SC-CO_2) and the major non-volatile components were identified using gas chromatography-mass spectrometry technique. Quantification of α-tocopherol and squalene was done by gas chromatography using an internal standard method.

Experimental

Extraction – *Pandanus odorus* leaves (4 kg) were removed from stems and cleaned. They were then cut into small pieces (1~2 cm), thoroughly mixed and ground with a dry mixer (Moulinex, France) before they were dried for 40 hours at 35°C in a freeze-dryer (Unitop 600 L, The Virtis Co., Gardiner, N.Y., USA.). Water constituted about 80 percent of the fresh leaves. About 4 grams each of the ground freeze-dried leaves were then packed in 4 inch × 6 inch plastic bags. The air was removed by squeezing the bags and immediately sealing the open end. The bags were then kept in a refrigerator at 5°C until selected for the extraction. A sample was then charged into a 50 ml high pressure extraction cell (Keystone Scientific Inc., Bellefonte, PA, USA) with internal diameter of 1.4 cm and 32 cm length. Carbon dioxide (MOX Sdn. Bhd., Penang) at selected pressures (80, 100, 150 and 200 kg·cm^{-2}) and temperatures (40, 50, 60, 70 and 80°C) were passed through the extraction cell. The extracts were collected at every half-hour for 3 hours. The extraction system consists of a high pressure HPLC pump (PU980, Jasco Corp., Tokyo, Japan), back pressure regulator (880-81, Jasco Corp., Tokyo, Japan), oven (Memmert ULM 400~800, Schwabach, Germany)

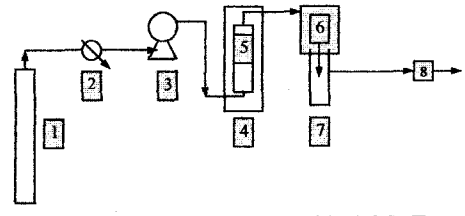

Fig. 1. Schematic diagram of Supercritical CO_2 Extraction System. 1. Carbon dioxide cylinder 2. Chiller 3. High pressure pump 4. Oven 5. Extraction cell 6. Back pressure regulator 7. Analyte receiver 8. Wet gas meter.

and chiller (BL-730, Yih-Der Inst. Co., Taiwan). Atmospheric flow rate of CO_2 was measured by a wet gas meter (W-NK, Sinagawa Corp., Tokyo, Japan). The experimental set-up is shown in Fig. 1.

Characterization – The extracts were dissolved in hexane (T.J. Baker Inc., Phillipsburg, NJ, USA), centrifuged at 4000 rpm for 10 minutes (Labofuge 200, Heraeus Inst., Germany) and characterized chromatographically without 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 an electron energy of 70 eV with a source temperature of 200°C

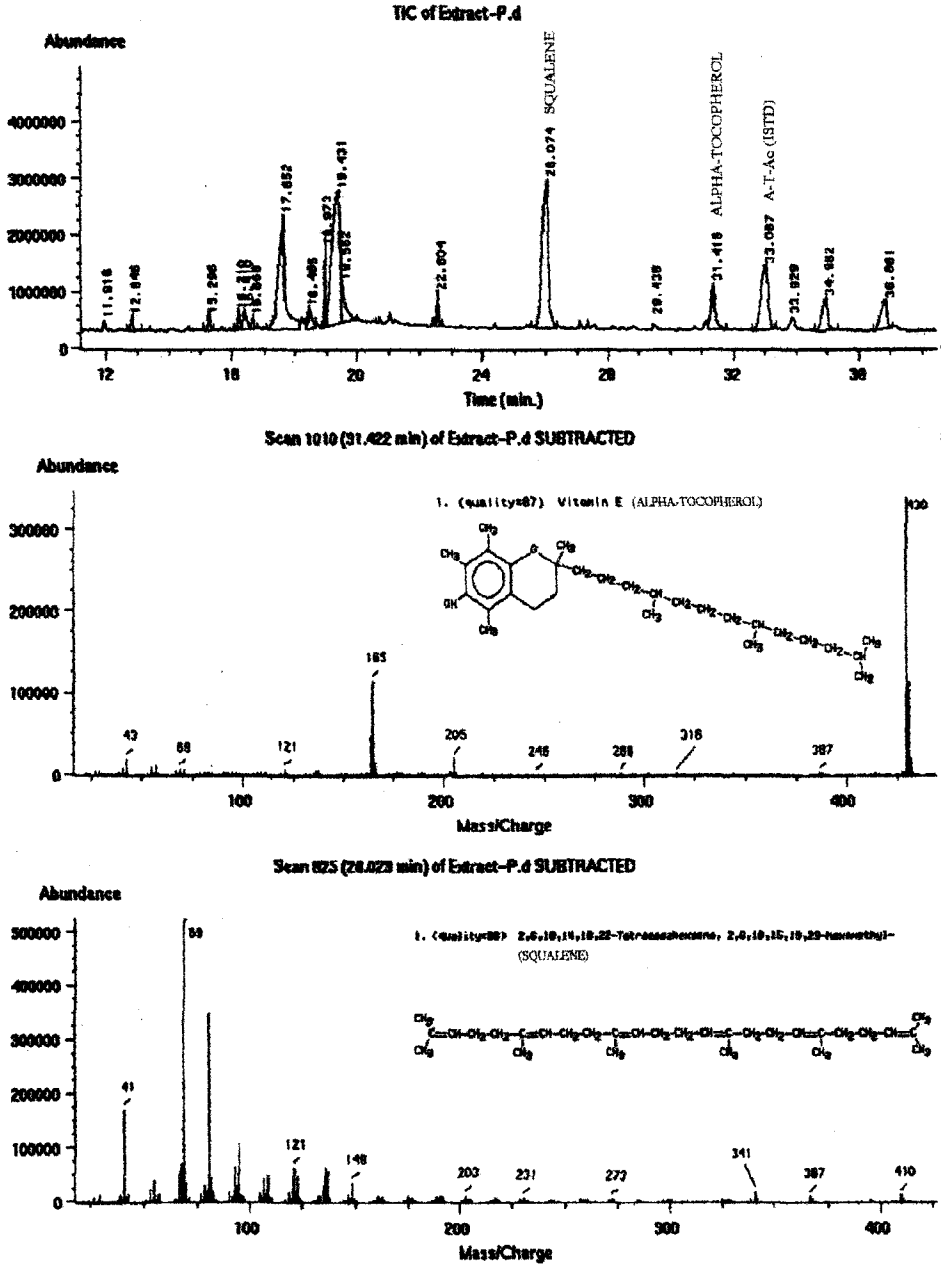

Fig. 2. Total ion current chromatogram of SC-CO_2 extract of *Pandanus odorus* leaves and mass spectra of α-tocopherol and squalene. GC and MS conditions : see text.

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 007-1, Quadrex Corp., New Heaven, USA) crosslinked methyl siloxane fused silica capillary column at 100°C for 5 min, then 10°C·min^{-1} to 270°C for 40 min. Helium at a flow rate of 1 ml·min^{-1} was used as a carrier gas. The splitless injector was kept at 250°C. Peaks of special interest (α-tocopherol and squalene) were reconfirmed by comparison to the retention times and spectra of the authentic standards.

α-Tocopherol and squalene analysis – Quantification of α-tocopherol and squalene in the extract was achieved by analysis with a GC/FID using an internal standard method. Hexane diluted extract together with the internal standard were injected without derivatization. A GC model Hitachi G-3000 with flame ionization detector and Hitachi D-2500 chromato-integrator (Hitachi Ltd., Tokyo, Japan) were used. The column was a 30 m × 0.25 mm × 0.1 μm TC-1 capillary (GL Sciences Inc., Tokyo, Japan) at an isothermal temperature of 270°C. Injection and detector temperatures were at 270°C and 300°C respectively. The carrier gas was nitrogen at a flow rate of 4.2 ml·min^{-1} (4 kg·cm^{-2}) and split ratio was 1 to 10. α-Tocopherol acetate (Fluka, Switzerland) was used as an internal standard.

Results and Discussion

Fig. 2 shows the total ion chromatogram of supercritical carbon dioxide extract of *Pandanus odorus* leaves. Hewlett Packard (HP-UX) Chemstation software with Wiley database library were used to obtain the mass spectra of compounds in the chromatograms. The peaks found at elution times of 26.074 and 31.416 minutes were determined to be squalene and α-tocopherol respectively from mass spectral information and by matching their retention times with those of the standards. These two nutritional compounds were given special attention and would be thoroughly studied. Their mass spectra and structures are shown in the same figure. Other compounds were identified by comparison to library spectra and were not confirmed by other means. The other identified components were listed in Table 1.

Identification of volatile scented compounds was not carried out in this study. The low boiling components were easily carried over by the flowing CO_2 gas and special trapping technique is needed to prevent the components from vaporizing.

Table 1. Identified compounds of SC-CO_2 extract of *Pandanous odorus* leaves

	t_R (min)	Compounds
1	11.916	Phenol,2,6-bis (1,1-dimethylethyl)-4-methyl
2	12.846	Dodecanoic acid (Lauric acid)
3	15.296	Tetradecanoic acid (Myristic acid)
4	16.410	Pentadecanoic acid (Pentadecylic acid)
5	17.652	Hexadecanoic acid (Palmitic acid)
6	18.973	2-Hexadecen-1-ol,3,7,11,15-tetramethyl
7	19.431	9,12,15-Octadecatrien-1-ol
8	22.604	1,2-Benzenedicarboxylic acid,bis(2-ethylhexyl)ester
9	26.074	(2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-(Squalene)
10	29.438	β-tocopherol
11	31.416	Vitamin E (α-tocopherol)
12	33.087	α-Tocopherol acetate (ISTD)
13	33.929	Campesterol
14	34.982	Stigmasterol
15	36.881	β-Sitosterol

The effect of pressure and temperature on the yields of α-tocopherol are shown in Figs. 3. and 4. At a moderate pressure of 80 kg·cm^{-2} and a temperature of 40°C, the yield of α-tocopherol was about 134 ppm and the extract was yellowish in color indicating low pigment content. The yield of α-tocopherol increases with an increase in pressure but decreases with increasing temperature. At 200 kg·cm^{-2} and 40°C, the yield of about 300 ppm was obtained in 3 hours.

The yields of squalene extracted follow the same trend as in the extraction of α-tocopherol. An approxi-

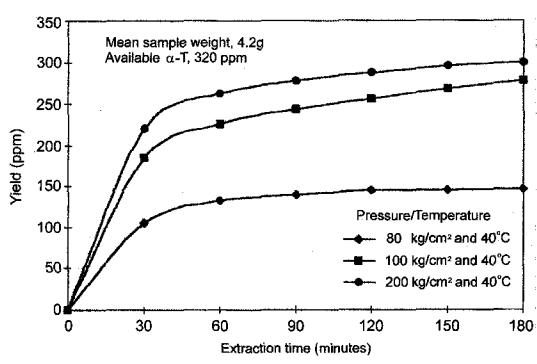

Fig. 3. Effect of pressure on the yield of α-tocopherol extracted at 40°C

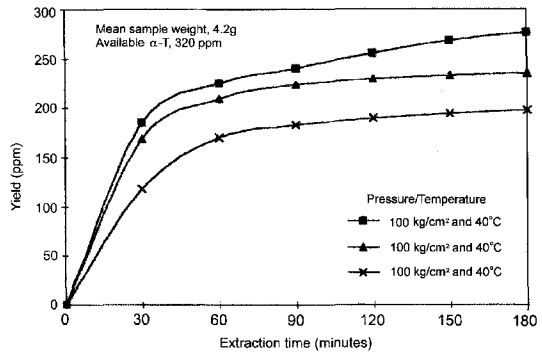

Fig. 4. Effect of temperature on the yield of α-tocopherol extracted at 100 kg·cm^{-2}

mately 1,200 ppm of squalene was obtained at 200 kg.cm^{-2} and 40°C. The yield decreases with a decrease in pressure but increases with a decrease in temperature. A conventional soxhlet extraction of the compounds at standard temperature and pressure using hexane as solvent was conducted to determine the initial content in the sample. The hexane extracted yields of α-tocopherol and squalene were 320 ppm and 1300 ppm respectively. The results show that about 93 percent of α-tocopherol and 92 percent of squalene were extracted from *Pandanus odorus* leaves using supercritical CO_2 at 200 kg·cm^{-2} and 40°C.

The contents of α-tocopherol and squalene in *P. odorus* leaves were relatively high when compared its presence in most of the oils, fats, leaves and grains. This study indicates that *P. odorus* leaves are a potential source of α-tocopherol, squalene and other natural products of the future and could be a suitable alternative for shark livers, which at present is the primary source of squalene. Unlike α-tocopherol, squalene has broader industrial applications especially in cosmetic industry. Further research on the extraction technique and applications should be undertaken and encouraged since the plant is easy to grow and matures quite fast.

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