EFFECT OF SUPERCRITICAL CO₂ EXTRACTION TEMPERATURE ON THE YIELD OF WAXES OF RADIATA PINE (*PINUS RADIATA* D.DON) NEEDLES *

(Pengaruh suhu ekstraksi superkritikal fluida CO₂ pada rendemen lilin dari daun Pinus radiata)

By/Oleh

Bambang Wiyono dan P.J. Jordan **

Ringkasan

Teknologi ekstraksi superkritikal fluida merupakan teknologi yang relatif baru yang saat ini sedang dikembangkan, terutama dalam ekstraksi senyawa kimia, seperti minyak atsiri, dari produk alami. Penelitian ini bertujuan untuk mengekstraksi lilin dari daun Pinus radiata D. Don dengan teknologi ekstraksi superkritikal fluida CO₂ pada tekanan ekstraksi 30 MPa dan pada beberapa tingkat suhu ekstraksi (40, 56 dan 72 °C) selama 30 menit. Lilin ditangkap oleh perangkap dingin pertama dengan kondisi tekanan sedang 6-7 MPa dan 12-13 MPa, dan 0°C. Sisanya ditangkap oleh perangkap dingin kedua pada kondisi tekanan sedang 0,1 MPa dan suhu 0°C.

Hasil penelitian menunjukan bahwa peningkatan suhu ekstraksi pada tekanan sedang 6-7 MPa menyebabkan rendemen yang dihasilkan menurun, baik yang diperoleh dari perangkap dingin pertama maupun kedua. Pada tekanan sedang 12-13 MPa, rendemen yang dihasilkan mula-mula turun, kemudian naik pada suhu ekstraksi 72°C, terutama pada perangkap dingin pertama. Penyebab peningkatan rendemen ini adalah setiap habis ekstraksi, pipa dan katup dimana lilin terperangkap disitu tidak dibilas dengan pelarut khloroform atau dibilas dengan cara diekstraksi lagi dengan superkritikal fluida tanpa sampel di sel ekstraksi. Untuk menunjang hasil penelitian ini, lilin hasil ekstraksi metode superkritikal fluida perlu dianalisis dengan metode High performance liquid chromatography (HPLC)

1. INTRODUCTION

In the two decades supercritical fluids have been the focus of active research and development programs, specially compounds intended to human beings consumption. Many research groups report extraction of a variety of compounds from natural products, such as food colours from dried grass and tumeric root, flavouring materials from leaves or needles (Subra, *et al.* 1991).

Leaves or needles, such as radiata pine needles contain, not only the flavouring materials but also other compounds, such as waxes which lay around the surface of the leaves or needles. Waxes which were extracted with a chloroform solvent from primary, secondary radiata pine needles and mixture of these needles were 0.27, 0.40 and 0.38 % based on fresh weight, respectively (Wiyono, 1994).

So far, liquid solvents, such as chloroform and n-hexane, were used to extract waxes from leaves or needles (Franich, *et al.* 1978; Tulloch, 1980). The other possibility to extract waxes is by using supercritical fluid technology, especially with CO₂ as a solvent. This technology offers advantages which are not achivable with older methods, such as liquid extraction, distillation, and preparative scale chromatography (Riek-

kola and Manninen, 1993). The advantages of this technology are as follows:

1. Selectivity of the extraction can be adjusted easily by varying the pressure and temperature, and the extractant can be directly subjected to other analytical procedures (Hills and Hills, 1993).
2. Solvent concentration often necessary in solvent extraction is eliminated (Snyder, *et al.* 1993).
3. Separation in this technology can be accomplished at a moderate temperature at asimilar low vapour pressure, so it can be applied to the components which are susceptible to heat degradation (Knez and Steiner, 1992).
4. The solvent can be removed from the extract and extracted materials (stahl, *et al.* 1988).
5. Fractionation is easily carried out by a stepwise change in solvent power in the loading or release steps, or both, without additional chemical species (stahl, *et al.* 1988).

The purpose of this work was to extract waxes from radiata pine needles with supercritical fluid CO₂ at a 30 MPa extraction pressure and at various temperatures for 30 minutes. Precipitation of waxes was conducted by lowering the extraction temperature to 0°C in the first

* Bagian dari tesis untuk memperoleh gelar Master of Science

** Senior lecturer at the Dep. of Chemical and Process Engineering, University of Canterbury, Christchurch, New Zealand.

cold trap at 6-7 MPa and 12-13 MPa intermediate pressure.

II. EXPERIMENTAL PROCEDURE

A. Source and Preparation on of Radiata Pine Needles

A mixture of primary and secondary radiata pine (*Pinus radiata* D. Don) needles was collected from five positions, from the top to the base of the crown of the tree (Franich, *et al*, 1977) taken from pruning activities in Burnham field, Canterbury, New Zealand. The needles was put in a plastic bag. After cutting off the sheaths of pine needles, the needles were cut into 2-3 segments, put into a container and stored in a freezer at -15°C , prior to be used as feed stocks in the supercritical fluid CO_2 extraction with a small scale extraction cell.

B. Supercritical Fluid CO_2 Extraction Process

Supercritical fluid extraction apparatus was designed and built by DR. Pat J. Jordan and DR. D Pearce from Department of Chemical and Process Engineering, University of Canterbury, Christchurch, New Zealand, and was constructed by the technical staff of the De-

partment. The maximum design pressure in this system is 34 MPa, although the compressor and a large extraction cell have a rated pressure of 68 MPa. The Configuration of this system is Figure 1.

les was placed in a small sample holder. After being closed, the sample holder was inserted in the pressure vessel (Hexagonal tube), and then the vessel was closed. The main and compressor water baths were half-filled from the high pressure water supply. The CO_2 cylinder was turned on and valve 1 were opened slowly until the pressure rose to 5 MPa. The cold traps (collecting vessels) were set up by being immersed in flask containing an ice/water mixture. After checking for leaks, if there was no audible hissing, the main bath was filled, the temperature indicator was set up, the compressor was switched on until the pressure rose to 10 MPa, and then the system was rechecked for leaks. If there was still no audible hissing, the electrical system, live steam, and stirrer were turned on after desired temperature was reached, the compressor was switched on, and the flow rate was set up at 7 scale by using micrometering and regulation valves at desired pressure and temperature for 30 minutes. Waxes were expected to be trapped in a cold trap (CT) I; however, any waxes which passed by CT I were expected to be trapped a cold trap (CT) II.

After finishing extraction, the compressor and electrical system were turned off, and the carbon dioxide cylinder and valve 1 were shut. The pressure was then let down. After reaching at ambient temperature, collecting vassels were taken out from the thermos flasks, and the extraction cell was released. Dissolved

Figure 1. Diagram of Supercritical Fluid CO_2 Extraction System.
Gambar 1. Diagram sistem ekstraksi superkritikal fluida CO_2 .

partment. The maximum design pressure in this system is 34 MPa, although the compressor and a large extraction cell have a rated pressure of 68 MPa. The Configuration of this system is Figure 1.

Approximately 4-5 of segmented radiata pine need-

waxes from CT I were collected in the flask. dissolved waxes from CT II was collected in other flask. Wax from CT I and CT II was collected in separate flask due to the possibility that essential oils would be trapped in cold trap II. The content of each flask was then

evaporated in a rotary evaporator, and each content was transferred into a preweighed vial. The remaining solvent in both vials was evaporated in vacuum oven at ambient temperature for 24 hours. Each vial was then weighed to determine the total weight of pine needle waxes. The percentage of the wax yield which was obtained from the cold trap was calculated based on fresh weight of pine needles.

C. Condition of Supercritical Fluid CO₂ Extraction

The supercritical fluid CO₂ extraction was performed at a 30 MPa extraction pressure and at various extraction temperatures (40, 56 and 72°C) for 30 minutes. Waxes coming from the extraction cell would be precipitated in the CT I at 0 °C and 6-7 MPa intermediate pressure. However, waxes being still soluble in the supercritical fluid CO₂ would be expected to be trapped in the CT II at 0 °C and MPa. With the same extraction conditions as before, but in the CT I, waxes coming from the extraction cell would be precipitated at 12-13 MPa.

III. RESULT AND DISCUSSION

Small scale supercritical fluid CO₂ extraction of waxes from radiata pine needles was conducted at a 30 MPa extraction pressure and at various extraction temperatures for 30 minutes. The extraction pressure was chosen to be 30 MPa since this is the highest pressure which can be reached in this Supercritical fluid equipment. It was assumed that this pressure at a given extraction temperature would give the highest yield of wax. The yield of waxes obtained from the first cold trap by lowering the temperature to 0°C at 6-7 MPa intermediate pressure decreased from 0.24 % to 0.11 %

(dy wt.) with an increase in extraction temperature. With the same extraction conditions, but 12-13 MPa intermediate pressure, waxes precipitated in the first cold trap initially decreased from 0.22 % to 0.20 % (dry wt) with an increase in temperature, and then increased to 0.80 % (Table 1).

Stahl, *et al.* (1988) stated that solubility behaviour of waxes in supercritical fluid CO₂ went down when the extraction temperature increased. He also mentioned that supercritical extracted waxes were precipitated in the cold trap by lowering the temperature to 0 °C at 7 MPa intermediate pressure. Lowering the temperature to 0°C led to decreased solubility of waxes in supercritical fluid CO₂; however, the solubility of the essential oils increased markedly. At this condition, the waxes could be precipitated in the first cold trap, but the essential oils and any waxes which could not be trapped in the first cold trap still flowed along with the supercritical fluid. Waxes from this cold trap mainly consisted of long-chain normal alkanes (Stahl, *et al.* 1988). Solubility of these compounds in supercritical CO₂ decreased when the extraction temperature increased (Reverchon, 1992). Waxes obtained in the first cold trap at 6-7 MPa intermediate pressure probably contained mainly long-chain normal alkanes which caused the decrease in wax as an increase in the extraction temperature (Figure 2).

Waxes which were still soluble in the supercritical fluid was expected to be precipitated in the second cold trap by lowering the pressure to 0.1 MPa at 0°C. At this condition, the solubility of these waxes decreased, so they could be trapped in the second cold trap. At 6-7 MPa intermediate pressure, the waxes which could be trapped in this cold trap decreased as the extraction temperature increased because at the high extraction temperature the waxes which were extracted from the sample decreased, and most of the were precipitated in

Table 1. Wax yields of supercritical fluid CO₂ extraction at various extraction temperatures

Tabel 1. Rendemen lilin hasil ekstraksi superkritikal fluid CO₂ pada beberapa tingkat suhu ekstraksi

Intermediate pressure (Tekanan sedang) MPa	Replication (Ulangan)	Extraction temperature (Suhu ekstraksi), °C					
		40		56		72	
		Wax from (Lilin dari) CT I, %	Wax from (Lilin dari) CT II, %	Wax from (Lilin dari) CT I, %	Wax from (Lilin dari) CT II, %	Wax from (Lilin dari) CT I, %	Wax from (Lilin dari) CT II, %
6 - 7	1	0.12	0.19	0.14	0.09	0.11	0.03
	2	0.35	0.06	0.11	0.07	0.10	0.03
Average (Rata-rata)		0.24	0.13	0.13	0.08	0.11	0.03
12 - 13	1	0.260	0.08	0.11	0.08	0.89	0.06
	2	0.170	0.09	0.19	0.01	0.71	0.04
Average (Rata-rata)		0.22	0.08	0.20	0.05	0.80	0.05

Note (Catatan) : CT = Cold Trap (Perangkap dingin)

Based on dry weight of pine needles (Berdasarkan berat kering daun tusam)

the first cold trap (Figure 2). When lowering the pressure to 0.1 MPa, the solubility of the essential oils decreased. This probably caused certain components in the essential oils were precipitated in the second cold

Figure 2. Supercritical extracted waxes at various extraction temperatures and at 6-7 MPa intermediate pressure.

Gambar 2. Hasil lilin yang diekstraksi dengan superkritikal fluida pada beberapa tingkat suhu ekstraksi dan pada tekanan sedang 6-7 MPa.

trap, and most of them released to the air and their smell could be recognized. These components appeared in the surface of wax solution as a rainbow and they could be seen during their evaporation process in the vacuum evaporation process in the vacuum evaporator, however they did not appear in the surface of wax solution from the first cold trap.

At 12-13 MPa intermediate pressure, waxes obtained from the first cold trap initially decreased and then increased as the extraction temperature increased; on the other hand, waxes which could still be trapped in the second cold trap decreased (Figure 3). Theoretically, waxes obtained in the cold trap I should decreased, because when the extraction temperature increased, wax solubility in the supercritical fluid decreased. This led to the decrease in wax yield at the first cold trap. However, the increase in wax yield at a 72°C extraction temperature was probably caused some factors: no flushing activities after each supercritical fluid extraction, and or undersired components, such as chlorophylls, precipitated in the first cold trap.

During the supercritical fluid CO₂ extraction, some of dissolved wax was precipitated in the line and valves, especially around the small water bath. This happened because when the supercritical fluid saturated

with wax came to the small water bath, the temperature of this supercritical fluid changed. At a certain pipe line it drop from the desired extraction temperature to ambient temperatures. The changing of the temperature to ambient temperatures caused the solubility of wax in supercritical fluid decreased, and some of dissolved waxes were precipitated in this line and valve. After the extraction, these waxes were not flushed by a chloroform solvent and supercritical fluid CO₂ extraction with no sample in the extraction cell at 72°C extraction temperature and at 30 MPa extraction pressure. This led to the problem of the wax yield for the next extraction, because the waxes which still remained in the line and valves could be dissolved with the supercritical fluid CO₂ in the next extraction and precipitated in the first or second cold trap depending where the line was. The result of the yield of waxes at the extraction would be higher than that the really was.

Jay, *et al.* (1988) mentioned that above 50°C extraction temperature solubility of chlorophylls in the supercritical fluid CO₂ increased as the extraction temperature arose. At a 72°C extraction temperature, much more chlorophylls could be dissolved in the supercritical fluid CO₂ and precipitated in the first cold trap. The presence of these compounds could be seen as a greenish tinge in the supercritical extracted waxes. These compound caused to increased the amount of wax yield which was obtained in the first cold trap. To prove that the increase in extraction temperature caused the decrease in waxes, the waxes which were obtained

Figure 3. Supercritical extracted waxes at various extraction temperatures and at 12-13 MPa intermediate pressure.

Gambar 3. Hasil lilin yang diekstraksi dengan superkritikal fluida pada beberapa tingkat suhu ekstraksi dan pada tekanan sedang 12-13 MPa.

from these extractions will be analyzed by using High Performance Liquid Chromatographic method.

As at 6-7 MPa intermediate pressure (Figure 2), probably in the second cold trap also contained certain components of the essential oils which could be seen in the surface of the wax solution during evaporation in the vacuum evaporator.

IV. CONCLUSIONS

The increase in extraction temperature at a 30 MPa extraction pressure and 6-7 MPa intermediate pressure for 30 minutes caused an increase in supercritical extracted waxes in the first and second cold trap. However, at the intermediate pressure of 12-13 MPa, the supercritical extracted waxes initially decreased, and then increased in the first cold trap as the extraction temperature increased. The cause of the increase in these waxes at a 72°C extraction temperature were no flushing activities after each supercritical fluid extraction, and much more chlorophylls dissolved in supercritical fluid CO₂ and precipitated in the first cold trap. Waxes obtained from these extraction will be analyzed with High Performance Liquid Chromatographic (HPLC) method.

REFERENCES

- Braun, G. and H. Schmidt. 1984. High Pressure Extraction of Crude Montan Wax. *Physical Chemistry*, (88) : 891-894.
- Franich, R.A. 1994. Personal communication.
- Franich, R.A., L.G. Wells and P.T. Wells, and P.T. Holland. 1978. Epicuticular wax of *Pinus radiata* needles. *Phytochemistry*, 17 1617-1623
- Hills, J.W. and H.H. Hills. 1993. Carbon Dioxide SFE with a Reactive Solvent Modifier for Determination of Polycyclic Aromatic Hydrocarbon. *J. Chrom. Scs.*, 31: 6-12.
- Jay, A.J., T.W. Smith and P. Richmond. 1988. The Extraction Of Food Colours Using supercritical Carbon Dioxide. *Proc. International Symposium on Supercritical Fluids*, October 17-19, 1988, volume 2:821-827. Nice, France.
- Knez, Z. and R. Steiner. 1992. Solubility of Capsaicin in Dense CO₂. *The Journal of Supercritical Fluid*, 5:251-255.
- Pearce, D.L. 1990. Solubility of triglycerides in supercritical CO₂. P. Hd Thesis. University of Canterbury, New Zealand.
- Revichon, E. 1992. Fractional Separation of SCF Extracts from Marjoram Leaves: Mass transfer and Optimization. *The Journal of Supercritical Fluid*, 5:256-261.
- Riekkola, M.L. and P. Manninen. 1993. Supercritical Fluid extraction as An Alternative Sample Preparation Method. *Trends in Analytical Chemistry*, 12(3):109-114.
- Stahl, E.K, W. Quirin, and D. Gerald. 1988. *Dense Gases for Extraction and Refining*. Springer-Verlag, Berlin.
- Subra, P., R. Tufeu, Y. Garrabos and P. Boissinot. 1991. Supercritical Fluid Extraction from a Mediterranean Brown Alga. *The Journal of Supercritical Fluids*, 4:244-249.
- Synder, J.L., R.L. Grob, M.E. McNally and T.S. Oostdyk. 1993. The Effect of Instrumental Parameters and Soil Matrix on the Recovery of Organochlorine and Organophosphate Pesticides from Soils Using SFE. *J. Chrom. Scs.*, 31:183-189.
- Tulloch, A.P. 1980. Composition of Epicuticular Waxes from 28 Genera of Graminae: Differences between subfamilies. *Can J. Bot.*, 59:1213-1221.
- Wiyono, B. 1994. Supercritical Fluid CO₂ Extraction of Waxes from Radiata Pine (*Pinus radiata* D. Don) Needles. Master Thesis in University of Canterbury, Christchurch, New Zealand.