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ABSTRACT

Supercritical fluid extraction (SFE) in a pilot plant was carried out to extract perillyl alcohol (POH) from Korean orange peel. The extract from the powder of Korean orange peel was obtained by supercritical CO₂ at operating conditions of 50°C, 200 bar, and 6 CO₂ kg/h/kg sample. Most of the POH was extracted within 14 hours in the experiment. The yield of extract containing POH was 2.5% based on the dry powder of the orange peel. The content of POH in the extracts was 2.8×10^{-3} (wt%) by GC analysis based on the dry powder, which indicated that SFE was approximately 30 times more efficient than the solvent-extraction method previously reported. A brief comparison of SFE and solvent extraction was made. The SFE extracts were further purified by open tubular chromatography to confirm POH in the extracts.

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INTRODUCTION

A number of natural products have proved to be extremely useful as drugs for the treatment of a range of medical conditions (1). New naturally produced compounds with interesting biological activities are regularly reported, and these often provide the basis for pharmaceutical or agrochemical development projects.

Perillyl alcohol (Fig. 1), NSC-641066, is a cyclic monoterpene. It is a hydroxylated derivative of *d*-limonene, and it occurs in numerous plants species including mints, lavender, perilla, citrus, and cranberries. The monoterpene is approved as a food additive by the Food and Drug Administration and the Council of Europe (2). Perillyl alcohol is also used as a fragrance in perfumes, soaps, detergents, lotions, and creams. Monoterpenes such as limonene and perillyl alcohol have the potential to both prevent and treat a variety of cancers including breast cancer (3). For example, *d*-limonene, which comprises over 90% of orange peel oil, has chemopreventive activity against rodent mammary cancer during the initiation phase as well as the promotion/progression phase. *d*-Limonene and perillyl alcohol, a more potent analog of limonene, have chemotherapeutic activity against rodent mammary and pancreatic tumors with low toxicity (4). As a result, their cancer chemotherapeutic activities were evaluated in Phase I clinical trials (5, 6).

Perillyl alcohol (POH) is currently being developed as a clinical candidate at the National Cancer Institute (NCI) because of its greater potency than limonene, which may enable potentially effective systemic concentrations of the active principals to be achieved at considerably lower doses. POH is 5 to 10 times more potent than limonene and has a similar therapeutic index. Consequently, POH finished Phase I human trials sponsored by the NCI at several centers and is currently undergoing Phase II clinical trials in advanced cancer patients.

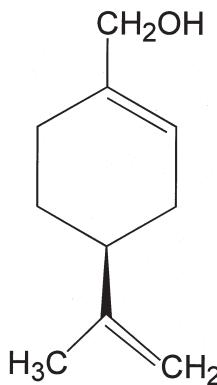


FIG. 1 Chemical structure of perillyl alcohol.



Supercritical fluid extraction (SFE), which predominantly uses supercritical carbon dioxide as an extraction medium, has been used for a variety of industrial, environmental, food, and chemical applications (7). The application of SFE to natural products, e.g., the extraction of carotenoids, lipids, flavor and fragrance compounds, steroids and triterpenes, alkaloids, and mycotoxins, has been reviewed (8).

Over 300,000 tons of Korean oranges are produced annually, but there are no special applications of its peel. POH, which is a naturally occurring substance in Korean orange peel, had been isolated by solvent extraction and chromatography (9, 10). In our work, POH in Korean orange peel was extracted by the SFE technique.

EXPERIMENTAL

Chemicals

Commercial grade carbon dioxide (99.95%) was used as a supercritical fluid. The Korean orange peel was purchased at a domestic market in Korea. The standard chemicals (*R*)-(+)perillyl alcohol and *d*-limonene were purchased from Aldrich Co. and Sigma Co., respectively.

Extraction by Supercritical Fluid CO₂

The pilot plant used in this work mainly consisted of an internal volume of 10 L, a separator, and a CO₂ recycle storage tank. Ground peel (2 kg) was put into a cylindrical extraction vessel (16 cm I.D. × 83 cm). CO₂ from the cylinder was delivered by a pump (Milroyal pump, MCP13D10B1M) into the extraction vessel. The pressure in the extractor was controlled by a pressure regulator. The extracts dissolved in the supercritical solvent were separated from the CO₂ by pressure reduction and collected in the separator. The separator was equipped with a cooling jacket. The extract-free CO₂ was cooled and recycled to the CO₂ recycle storage tank. The extraction pressure and the temperature were 200 bar and 50°C, respectively and the mass flow rate of CO₂ was set at 12 kg/h. The extraction was performed for 18 hours.

Analysis of Perillyl Alcohol and *d*-Limonene (GC)

The Korean orange peel extract was dissolved in acetone and analyzed by a Hewlett-Packard (HP) Model 5890 gas chromatographer (GC) equipped with an HP-5 (Crosslinked 5% PH ME Siloxane, 30 m × 0.32 mm), capillary column, and a flame ionization detector (FID). The oven temperature was gradually raised from 50 to 80°C by increases of 10°C/min, followed by increases from 80 to 250°C by increases of 5°C/min. The injector and detector temperatures were 250 and 300°C, respectively. The flow rate of the carrier gas N₂



was 1 mL/min, and the split ratio was set at 1:50. A calibration graph for perillyl alcohol was constructed by plotting the peak area against the concentration. The relationship between peak area and concentration of perillyl alcohol was linear. The identification of peaks in the chromatogram was confirmed by the perillyl alcohol standard.

Open-Tubular Chromatography

To separate and identify perillyl alcohol in the extract, open-tubular chromatography (30 cm \times 2.5 I.D.) with octadecylsilica (40–63 μ m, Merck, Germany) was used. The mobile phase was chloroform. The extract, 2 mL, was injected into the open-tube chromatograph, and the effluents were collected from the column outlet. The effluent was monitored by thin-layer chromatography (TLC) using glass plates covered with a 0.25-mm layer of C₁₈ (RP-18F_{254s}, Merck). The plates were developed with chloroform and detected by a 254-nm UV lamp.

RESULTS AND DISCUSSION

The diffusivity of supercritical fluid is one to two orders of magnitude higher than those of other liquids, which permits rapid mass transfer and can thus reduce the overall time required for the separation (11). These conditions make SFE faster and require less sample than solvent extraction. As shown in Fig. 2, as the amount of CO₂ per quantity of raw material (CO₂ kg/kg orange

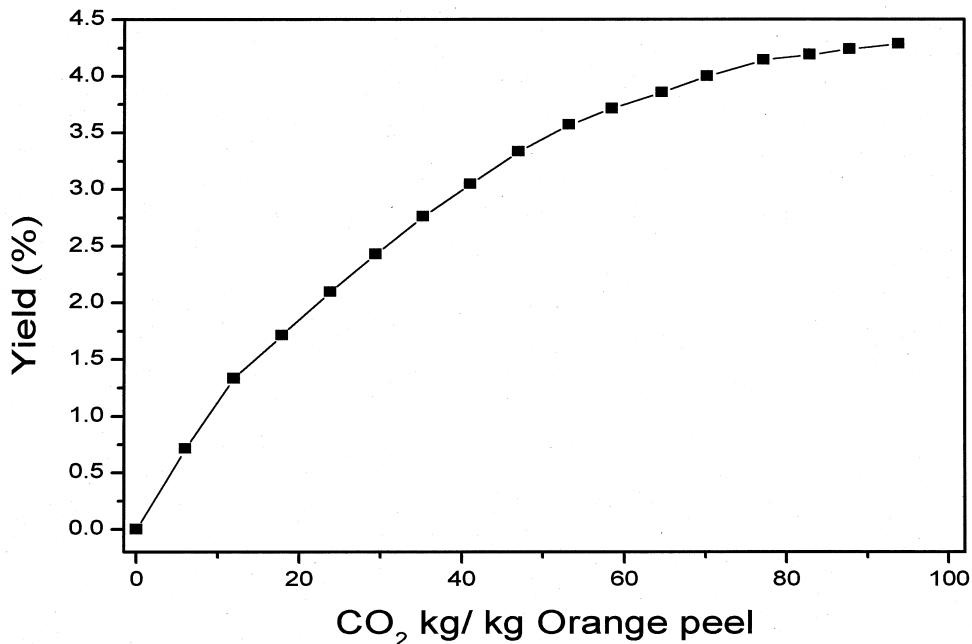


FIG. 2 Yield of extracts versus CO₂ per quantity of raw material (CO₂ kg/kg orange peel).



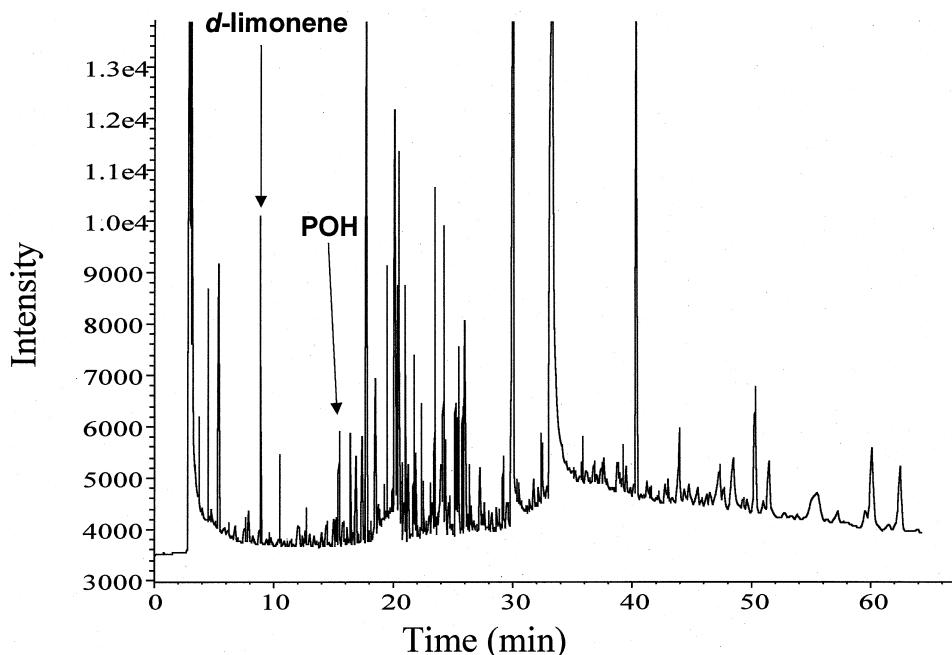


FIG. 3 Chromatogram of the extract by SFE (0.9 g of the extract in 15 mL of acetone, injection volume of 3 μ L).

peel) increased, the yield was increased. The temperature and pressure of CO_2 were 50°C and 200 bar, respectively. The percentage yield was expressed the mass of extracts to the total dry powder of orange peel loaded in the extraction vessel. The increase in POH yield is almost negligible after 14 hours.

As shown in Fig. 3, the extracts by SFE were diluted with acetone to analyze the sample by GC. The extracts were dissolved in 15 mL of acetone with a concentration of 0.06 g/mL, and the injection volume of the sample was 3 μ L. The retention times of *d*-limonene and POH were 8.9 and 15.6 minutes, respectively. The high solvating power of the supercritical fluid caused the complexity of the chromatogram. Quite a few oil and wax components were followed by *d*-limonene and POH. The oil components were eluted in less than 30 minutes while the wax components took more time.

In our previous work with the methanol extraction method (9, 10), 0.005 mg of POH was extracted from 5 g of dried Korean orange peel, so the yield was 10^{-4} (wt%). In this work with the supercritical CO_2 , 50 g of the SFE-extracts was collected from 2 kg of the dried powder of Korean orange peel. The extract contained 58.33 mg of POH, so its total yield was determined to be 2.8×10^{-3} (wt%). The yield of POH by supercritical fluid was about 30 times higher than that of POH by methanol extraction. The yield was obtained by a calibration graph. A linear calibration graph was produced for POH over the 10–1000 μ g/mL concentration range, and it gave a correlation coefficient (r)



of 0.9999 ($n = 4$, n : number of different concentrations). The slope and y -intercept for linear regression of the calibration graph for the POH were slope, 75.9525; y -intercept, -805.988.

To identify the peak of POH in Fig. 3, we purified the extracts by open-tubular chromatography in which reversed-phase C₁₈ packings were used. The sample passed down the column by gravitational flow. When a mobile phase of pure water was used, the total elution time was very long. Perillyl alcohol strongly adsorbed on the C₁₈ packings, so it was not eluted out within 1 hour. Similar trends were shown with a mobile phase of acetonitrile and acetone. Chloroform was chosen to separate POH in the extracts by open-tubular chromatography, and the experimental result is shown in Fig. 4. During the separation we monitored the effluent by TLC with UV detection at 254 nm.

However, many other unnecessary components were also extracted with POH at 50°C and 200 bar. In general, solubility and selectivity are counterbalanced, and therefore, the pressure and temperature appear to have a limited effect on the overall separation process. In the supercritical fluid extraction of oxygenated compounds such as aldehydes, alcohols, esters, and ketones in citrus oil, a lower pressure gave a higher selectivity but a lower extraction yield, whereas a higher pressure gave a higher yield but a lower selectivity (12–14). The higher pressure, 200 bar, used in this work gave high yield but low selectivity. We focused on the yield of POH in Korean orange peel, so the selec-

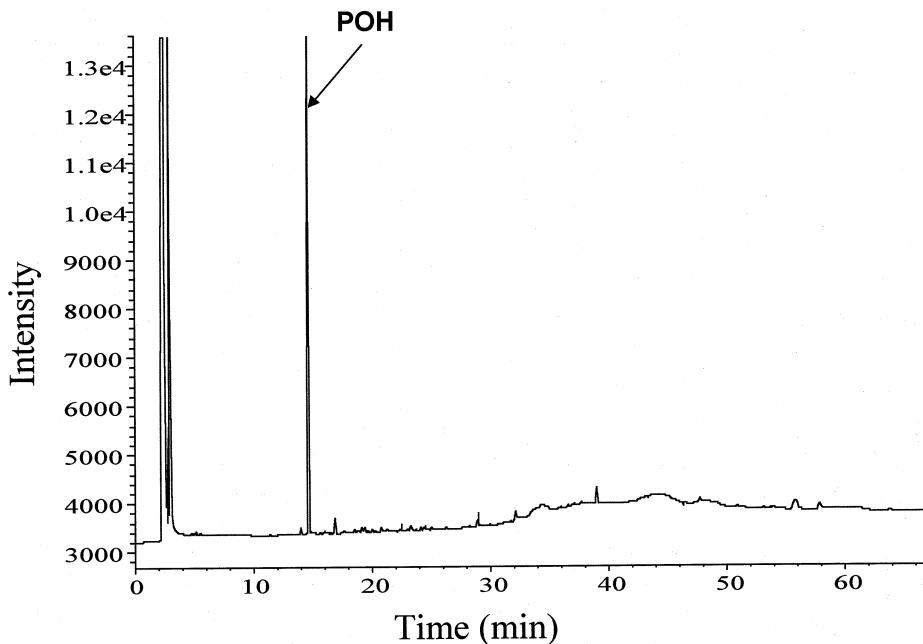


FIG. 4 Chromatogram of the extract after open-tubular chromatography (OTC) by C₁₈ with chloroform.



tivity was less important. The amount of POH in the peels is very small, so a higher pressure was applied in order to extract more POH.

CO_2 is nontoxic, nonflammable, and inexpensive compared to the large volume of organic solvent used in the solvent method which increases the cost of the process and presents a disposal problem. Supercritical fluids, which are highly efficient for extractions from complex matrices, are increasing in popularity as replacements for conventional solvents in extraction techniques. It has been suggested that supercritical fluids can be used as alternatives to organic solvents for the extraction of natural products, improving the purification process by achieving a selective extraction of the desired sample components through careful control of the extraction conditions. The SFE conditions, together with the supercritical properties of CO_2 , including its high diffusivity and low viscosity which increase the diffusion of the fluid into the matrix, facilitate extraction. The physical properties of carbons dioxide that make it widely used in extraction processes are low surface tension and viscosity, and high diffusivity. Thus, SFE can be a very attractive alternative procedure to classical extraction methods.

CONCLUSION

SFE with carbon dioxide has been shown to be effective for the isolation of perillyl alcohol (POH) in Korean orange peel. The extract from the powder of Korean orange peel (2 kg) was obtained by CO_2 at the supercritical state, 50°C and 200 bar. The mass flow rate of CO_2 was 12 kg/h. For a run time of 18 hours, the weight of extracts from the extraction vessel was 50 g. The yield of POH by SFE was 2.8×10^{-3} (wt%). An enhancement of approximately 30 times was achieved over solvent extraction. The extracts were analyzed by the GC-FID system. To isolate POH in a pure form, many interfering compounds need to be removed. Open-tubular chromatography can be used as a pretreatment step for separating POH, and preparative HPLC (high performance liquid chromatography) can be utilized to resolve POH of high purity from the extracts.

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