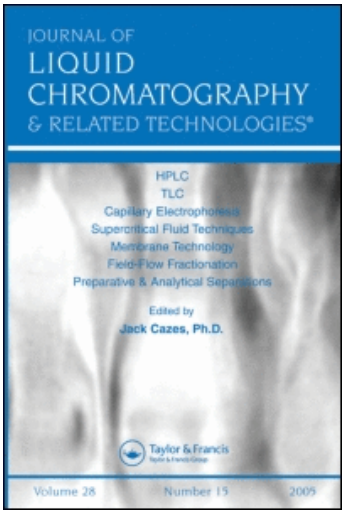

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SUPERCRITICAL FLUID EXTRACTION OF PERILLYL ALCOHOL IN KOREAN ORANGE PEEL

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ABSTRACT

A series of experiments for supercritical fluid extraction (SFE) were carried out to extract the perillyl alcohol (POH) from Korean orange peel. The yield of extract from the Korean orange peel powder was investigated under the temperatures of 30-60°C, the pressures of 150-200 bar, and the CO_2 flow rates of 1.5-3.5 L/min. It was found that the yield of SFE extract containing POH was obtained as 1% of the dry powder of the orange peel.

By GC-analysis of the peel oils obtained by SFE in the experimental ranges, the content of POH was 2.8×10^{-3} (%, wt.) based on the dry powder, which indicated that SFE was approximately 30 times more efficient than the solvent-extraction method previously reported. The SFE-extracts were further purified by grav-

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ity-flow packed column chromatography to confirm POH in the extracts.

INTRODUCTION

Perillyl alcohol (POH) is a cyclic monoterpene, which is a hydroxylated derivative of *d*-limonene, occurring in numerous species of plants, including mints, lavendar, perilla, citrus, and cranberries.[1] Perillyl alcohol has the potential to both prevent and treat a variety of cancers, including breast cancer.[2] POH is also used as a fragrance in perfumes, soaps, detergents, lotions, and creams.

Supercritical fluid extraction (SFE), which uses predominantly supercritical carbon dioxide as an extraction medium, has been used for a variety of industrial, environmental, food, and chemical applications.[3] The application of SFE to natural products has been reviewed.[4] The diffusivity of supercritical fluid is one to two orders of magnitude higher than those of other liquids, which permits rapid mass transfer and it can, thus, reduce the overall time required for the separation. CO_2 is nontoxic, nonflammable, and inexpensive, compared to a solvent method that uses a large volume of organic solvent, which increases the operating cost and presents a disposal problem. It has been suggested, that supercritical fluids can be used as a powerful alternative 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. Thus, SFE can be seen as a very attractive alternative procedure to classical extraction methods.

Over 300,000 tons of the Korean orange have been produced annually, but there is no special applications for its peel. The POH, which is a naturally occurring substance in Korean orange peel, had been extracted by solvent extraction.[5,6] In this work, POH in Korean orange peel was extracted by means of the supercritical fluid extraction technique. The experimental variables were temperature and pressure of the supercritical fluid, as well as the flow rate of CO_2. The research was focused to investigate the effects of the operating condition on the yield of POH.

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 Seoul. The standard chemicals of (*R*)-(+)-perillyl alcohol was purchased from Aldrich

Chemical Company. The extra-pure grade solvents of acetone were purchased from Dae Jung Chemicals & Metals Co., Ltd. (Korea).

Extraction by Supercritical Fluid CO_2

A supercritical system equipped with a cylindrical extractor (40 mm I.D, 60 mm O.D., 272 mm L) was used as shown in Figure 1. Ground peel was put into a cylindrical extraction vessel. CO_2 from the cylinder was delivered by an electric-driven high pressure compressor into the extraction vessel. The pressure in the extractor was controlled by a pressure control valve (No.11), as shown in Fig.1. The extracts dissolved in the supercritical solvent were separated from the CO_2 by pressure reduction and collected in the separator (40 mm I.D., 60 mm O.D., 272 mm L). The separators were equipped with a jacket. The operating pressure and temperature were 150 - 200 bar and 30 – 60°C, respectively, and the mass flow rate of CO_2 was varied 1.5 L/min to 3.5 L/min.

Analysis of Perillyl Alcohol by Gas Chromatography

The identification of peaks in the chromatogram was confirmed by the standard of perillyl alcohol. The extracts of Korean orange peel were analyzed using a Hewlett-Packard (HP) Model 5890 gas chromatograph equipped with an HP-5 (Crosslinked 5% PH ME Siloxane, 30 m × 0.32 mm I.D.) capillary column and a flame ionization detector (FID). The oven temperature program was gradually raised from 50°C to 80°C by increases of 10°C/min, followed from 80°C to 250°C by increases of 5°C/min. The injector and detector temperatures were 250°C and 300°C, respectively. The flow rate of the carrier gas N_2 was 1 mL/min and the split ratio set at 1:50.

Gravity-Flow Packed Column Chromatography

To separate and identify perillyl alcohol in SFE extract, a gravity-flow packed column (30 cm × 2.5 I.D.) with octadecylsilica (40-63 μm, Merck, Germany) was used. The mobile phase was chloroform. The extract, 2 mL, was injected into the gravity-flow packed column 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 C_{18} (RP-18F254s, Merck). The plates were developed with chloroform and detected by UV lamp at 254 nm.

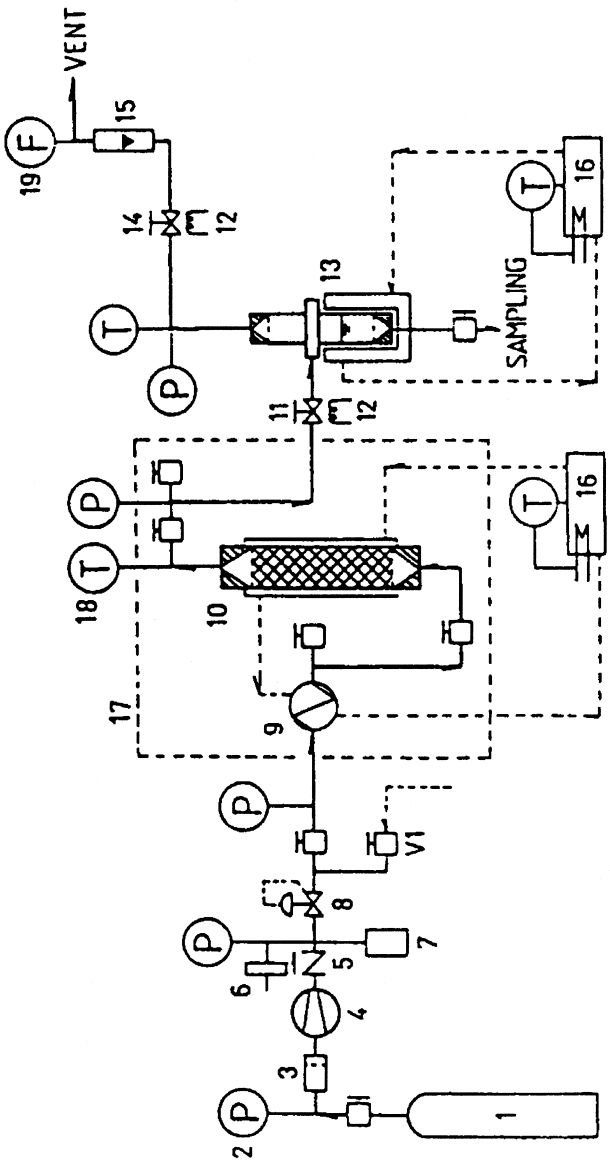

Figure 1. Schematic diagram of SFE system used in this study.

RESULTS AND DISCUSSION

The percentage yield was expressed as the mass of effluents to the total dry powder of orange peel loaded in the extraction vessel. Figures 2 and 3 show the relationship between the percent yield and CO_2 consumption with the pressure and temperature of supercritical CO_2. The flow rate of carbon dioxide was kept constant at 2.5 and 3.5 L/min, respectively. The pressure and temperature were changed from 150 to 200 bar, 30 to 60°C, respectively. In Figure 2, the extraction yields rapidly increased to 0.6 - 0.8% showing a slight difference with operating conditions. However, the yields of extraction over the range of pressure and temperature used in this work gradually approached to 0.9%.

Compared to Figure 2, the yields in Figure 3 were slightly higher, but there is no significant change. It was found that the yield at 150 bar and 30°C, which was slightly below the critical temperature 31.3°C, is higher than that at 200 bar and 30°C, and still a little higher than that at 200 bar and 60°C. This suggests that the extraction could be performed at room temperature with subcritical or liquid carbon dioxide at comparatively low pressures, around 150 bar. This condition is very similar to that of lemon peel proposed by Sugiyama and Saito.[7] The effect of flow rate of carbon dioxide on the yield was shown in Figure 4. From the

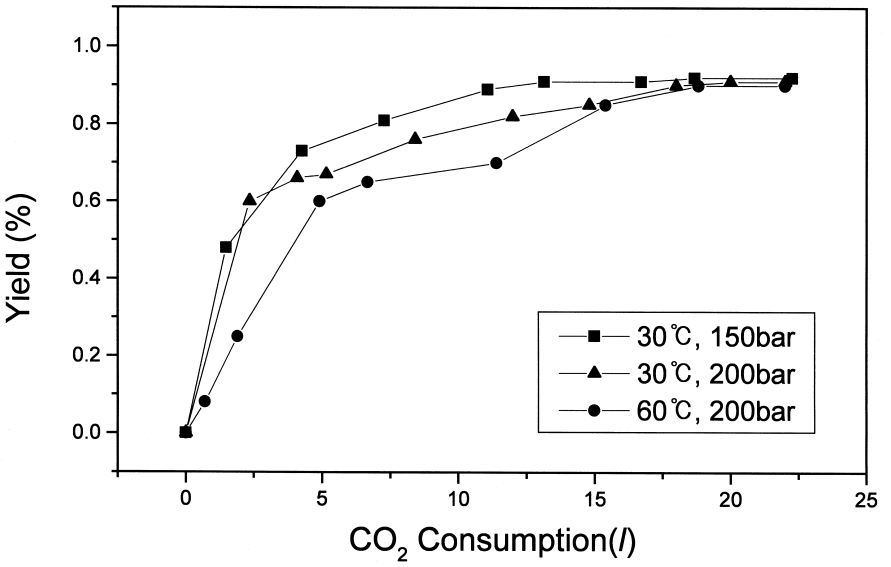

Figure 2. CO_2 consumption *versus* yield of extracts (2.5 L/min CO_2 flow rate).

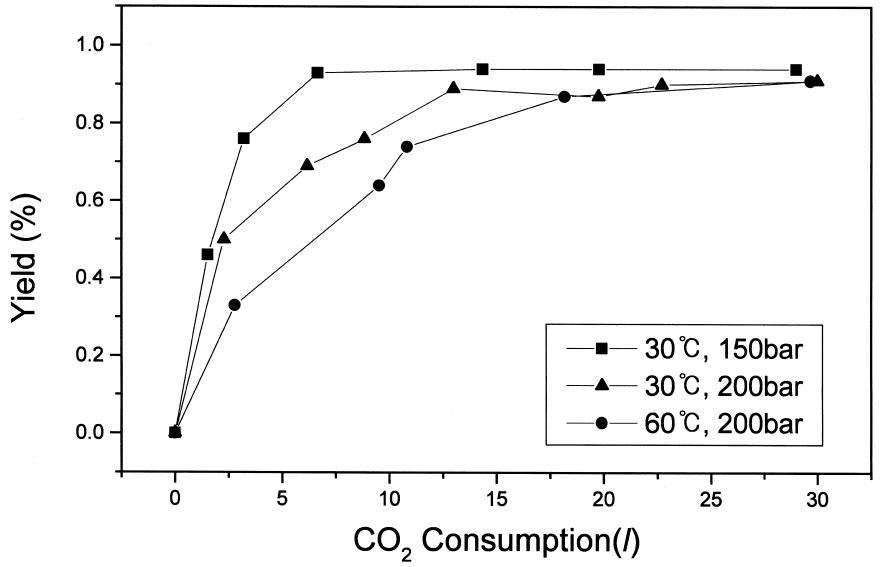

Figure 3. CO_2 consumption *versus* yield of extracts (3.5 L/min CO_2 flow rate).

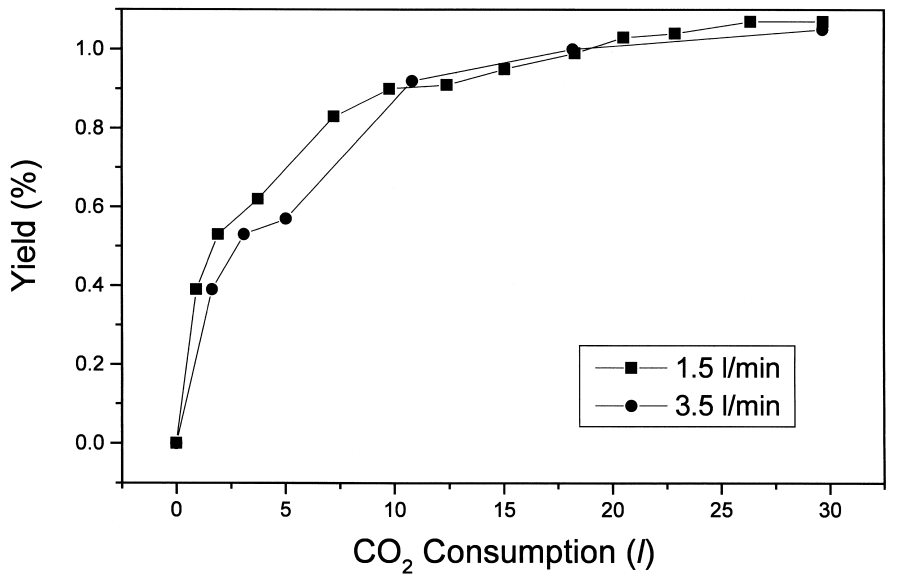

Figure 4. CO_2 consumption *versus* yield of extracts (60 °C, 150 bar).

experimental results in Figures 2, 3, and 4, these effects on the yield are almost negligible.

In Figure 5, the effluents obtained by SFE were diluted with acetone in order to analyze the sample by GC. The effluents were dissolved in 15 mL of acetone and the injection volume of the sample was 3 μL. The retention time of POH was 15.6 min. In our previous work with the solvent extraction method[5,6] the total yield was 10^{-4} (%, wt.). In this work with the supercritical CO_2, the total yield was determined as 2.8×10^{-3} (%, wt). The yield of POH by supercritical fluid extraction was about 30 times higher than that by the solvent extraction. The yield was obtained by a calibration graph. A linear calibration graph was produced for POH over the concentration range 10 - 1000 g/mL, and 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.

However, many other unnecessary components were also extracted with POH under the conditions used in this work. In general, solubility and selectivity are counterbalanced and, therefore, pressure and temperature appear to have a limited effect on the overall separation process. In the extraction of citrus oil in supercritical CO_2, a lower pressure gave a higher selectivity but a lower extraction yield, whereas a higher pressure gave higher yield but a lower selectivity.[8-10]

To identify the peak of POH in Figure 5, we purify the extracts by gravity-flow packed column, which was filled with reversed-phase C_{18} packing. The sample passed through the column by a gravitational flow. When the mobile phase of pure water was used, the total elution time was very long. Perillyl alcohol strongly adsorbed on the C_{18} packings, so it was not eluted out within 1 hr. Similar trends were shown with mobile phase of acetonitrile and acetone. Chloroform was chosen to separate POH in the extracts by gravity-flow packed column chromatography. During the separation, the fractions were monitored by thin layer chromatography (TLC) with UV detection at 254 nm. The fraction collected by gravity-flow packed column was analyzed by gas chromatography as shown in Fig. 6.

CONCLUSIONS

Supercritical carbon dioxide extraction has been shown to be an effective technique for the extraction of POH in Korean orange peel. The extract from the powder of Korean orange peel was obtained by CO_2 at supercritical state under various conditions. In the operating conditions used in this work, the yield was high, but the selectivity was low. The yield of POH by SFE was about 2.8×10^{-3} (%, wt.). It implied that significant enhancement was achieved over the solvent extraction, approximately 30 times. Gravity-flow packed column chromatography can be used as a pretreatment step for separating POH preparatively, and

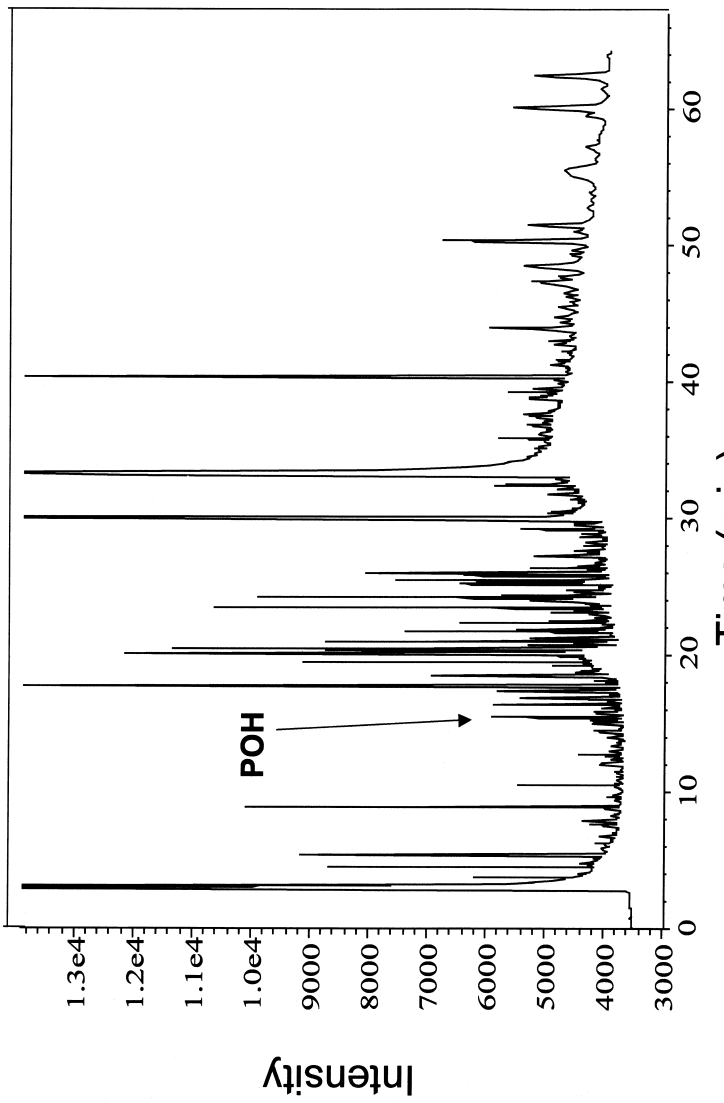

Figure 5. GC chromatogram of the SFE extracts (0.9 g of the extract in 15 mL of acetone, 3μL injection vol., 50°C, 200 bar).

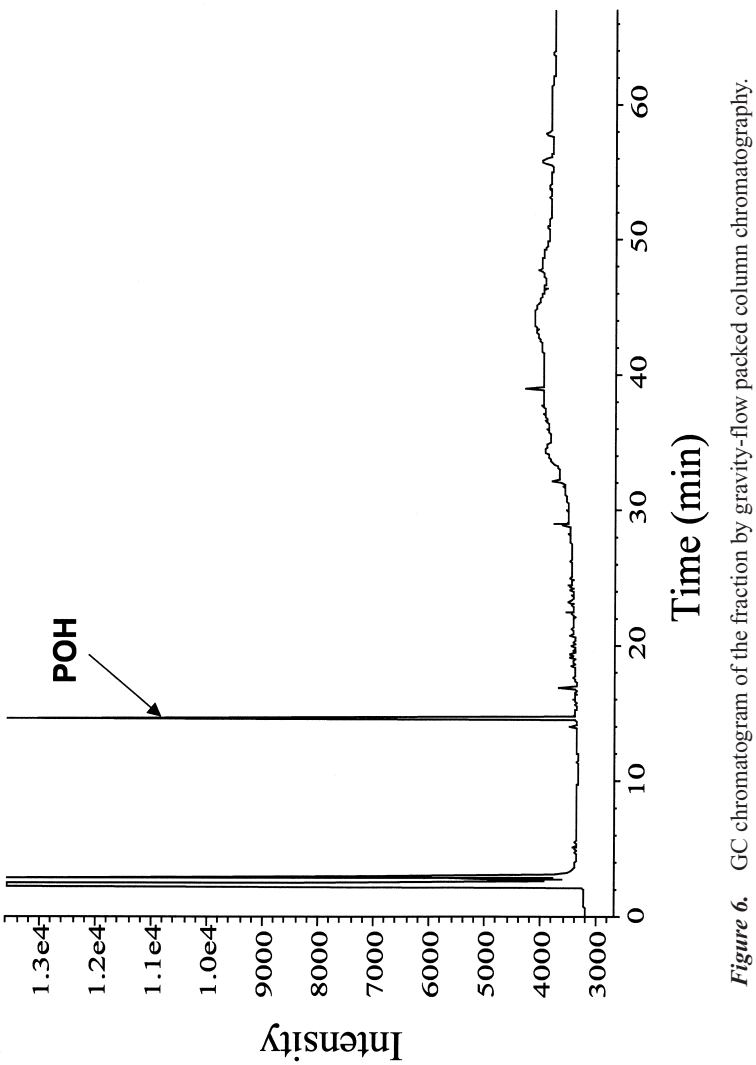

Figure 6. GC chromatogram of the fraction by gravity-flow packed column chromatography.

preparative HPLC will be utilized to resolve POH with high purity from the extracts.

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