Supercritical Carbon Dioxide Extraction of Useful Compounds from Japanese Citrus By-products

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Abstract: Citrus by-products contain compounds such as polyphenols, limonoids, carotenoids and essential oils, that are considered useful for the application in pharmaceutical, cosmetic and food industries. One of the promising techniques to recover these compounds is by supercritical carbon dioxide extraction. Taking sudachi residues as a representative sample, supercritical carbon dioxide extraction experiments were carried out in the temperature range of 40 to 80 °C and pressure range of 10 to 30 MPa. The effect of addition of ethanol (EtOH), as entrainer, was also studied. Results indicated that the yield increased with increasing pressure. Addition of 5mol% EtOH as entrainer increased the yield by more than three times of that without the entrainer (EtOH). Based on gas chromatography-mass spectrometry (GC-MS) analyses, the extracts consisted mainly of limonene, terpenes, some fatty acids and its esters. The amount of polyphenols in the extracts, estimated using the Folin-Ciocalteau method increased with increasing amount of EtOH. Experiments conducted on samples other than sudachi residues also showed promising results.

Keywords: Supercritical Carbon Dioxide Extraction, Citrus Residues, Polyphenols, Recycle

1. INTRODUCTION

Recycling of citrus processing by-products by recovery of useful components is being considered to avoid environmental consequences of its disposal. Citrus residues consisting of seeds and peels contain compounds such as polyphenols, limonoids, carotenoids and essential oils, that are useful for the application in pharmaceutical, cosmetic and food industries [1, 2].

One of the promising techniques to recover these compounds is by supercritical carbon dioxide extraction. With increasing concerns over the use of organic solvents and their disposal, supercritical fluid extraction is gaining in popularity. Of various gases and liquids studied, carbon dioxide remains the most commonly used fluid because of its low critical constants (Tc = 31.1 °C, Pc = 7.38 MPa), its non-toxic and non-flammable properties, and its availability in high purity with low cost [3]. To date, numerous works have been done on the application of supercritical carbon dioxide (scCO2) extraction in food processing industries and have been comprehensively reviewed by Rozzi and Singh [4], citing many advantages such as higher diffusion coefficient and lower viscosity than liquid solvents, possibility of manipulating selectivity by varying extraction temperature and pressure, and zero residues in the extracts. Supercritical carbon dioxide extraction technology has also been applied to fractionation of essential oils [5] and processing of biomaterials [6]. It has also proven its usefulness as a replacement for organic solvents with its application in large-scale extractions such as decaffeinating of coffee [4]. In the presence of entrainer such as ethanol (EtOH), scCO2 could enhance extraction of polar compounds like polyphenols [7], thus, increasing the usefulness and, in most cases, the therapeutic property of the extracts. These many advantages of scCO2 extraction gave us the motivation to apply the technique in the recovery of useful compounds from unutilized biomass resources.

Taking sudachi residues as a representative sample, the application of scCO2 technology to the extraction of useful compounds from citrus residues was investigated in this study. The effects of extraction conditions such as temperature, pressure and addition of EtOH as entrainer on the yield and the composition of the extracts were investigated. The total phenolic contents of the extracts were also determined.

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Figure 1. Schematic diagram of the supercritical fluid extraction apparatus.
**2. EXPERIMENTAL METHODOLOGY**

**2.1 Supercritical carbon dioxide extraction**

Powdered, dried *sudachi* sample was supplied by Vianove Co. Ltd., Japan. Extraction experiments at pressures up to 20 MPa were carried out using an experimental apparatus shown schematically in Fig. 1. Experiments at 30 MPa were done in another apparatus almost similar to the one in the figure except that carbon dioxide is introduced from the bottom of the extractor, passing through the sample in an upward direction. The temperature investigated was in the range of 40 to 80°C.

In a typical run, about 150 g of dried *sudachi* sample was placed in a 300-ml extractor (OM Labtech Co., Ltd). The extractor temperature was gradually set to the desired level, while pumping carbon dioxide (purity of 99.5 %) into the extractor to reach a system pressure of 20 MPa using a high-pressure pump (NS personal pump, NP-KX-500J). The system was held for 2 h at desired conditions, then carbon dioxide was allowed to flow continuously through the extractor for 5 h at a rate of 2 L/min (the room temperature was at 20-22°C). The extracts were collected in a flask immersed in an iced water bath at around 5°C.

In ethanol-modified supercritical carbon dioxide experiments, after reaching a pressure of 8 MPa, ethanol (Wako Chemicals, 99.5 %) was added gradually using a JASCO Intelligent HPLC Pump (PU-980) to reach the desired concentration.

**2.2 Gas chromatography-mass spectrometry (GC-MS) analysis**

GC-MS analysis of the sample was carried out using a Hitachi Gas Chromatograph (G-5000M) equipped with 3D Quadrupole detector (Hitachi M-7100). The column was a Chrompack capillary column (CP Sil 5 CB, 0.25mm x 30m, df=0.4µm) from GL Sciences Co. (Japan). The injector and the auxiliary temperatures were set at 280°C, and the carrier gas (He, 99.99 %) pressure was 0.59 MPa. The split gas flowrate was 1.04 x 10⁻² ml/sec during the analysis. The injection volume was 1 µL. Each peak was identified by the NIST database of mass spectra.

**2.3 Estimation of total phenolic contents**

The total phenolic contents of the extracts were estimated by the modified Folin-Ciocalteau method [8]. The procedure consisted of extracting about 55 mg sample with 5 ml of solvent (MeOH:H₂O = 50:50 v/v). About 100 µL of the resulting solution was mixed with 30 ml of water in a 50 ml volumetric flask. The 3-ml Folin-Ciocalteau reagent (Sigma-Aldrich, Japan) was added and mixed, followed by 5 ml of an aqueous solution containing 20 g of anhydrous Na₂CO₃ per 100 ml. The flask was filled to the mark with distilled water. The contents were mixed vigorously, and after two hours, the absorbance at 765 nm was determined using a UV-Visible spectrophotometer (Shimadzu UV-2400PC). The total phenolic content was calculated in gallic acid equivalents (GAE) by comparison with a standard curve similarly prepared with zero to 500 µg gallic acid (Wako Chemicals, Japan) per 50 ml flask.

**3. RESULTS AND DISCUSSION**

**3.1 Effect of extraction conditions on the yield**

Results of extraction experiments at 40°C, at a pressure range of 10-30 MPa are shown in Fig. 2. In a total of 4 runs, equivalent to about 20 h of continuous flow of carbon dioxide gas at a rate of 2L/min, the extraction yield at 10 MPa was 545 mg/100 g-sample. The cumulative yield increased with increasing pressure, obtaining up to 773 mg/100 g-sample at 30 MPa. At 20 MPa, addition of 5 mol% EtOH significantly increased the yield by more than three times of that with no EtOH addition. This increase in the extraction yield with the addition of EtOH could be attributed to the enhanced extraction of the oil components, as observed in our previous studies on okara [9] and co-extraction of some polar compounds like polyphenols. This will be discussed in details in section 3.3.

**3.2 Composition of the extracts by GC-MS analysis**

Fig. 3 shows the GC-MS chromatogram of the sample obtained at 40°C and at 20 MPa. The main component was limonene, a compound which gives the characteristic flavor of citrus. Other compounds such as terpenes, sesquiterpenes, fatty acids and their ester derivatives were also identified.
The compounds identified by GC-MS were subdivided into three main groups – limonene, terpenes, and fatty acids. Based on the total peak areas of these groups of compounds, the effect of extraction conditions on the relative composition of the extracts was investigated. As shown in Fig. 4, limonene was obtained predominantly at 10 and 20 MPa, but at a higher pressure of 30 MPa, terpenes and fatty acids were mostly obtained over limonene. Continuous extraction on the same sample with the addition of EtOH at different concentration (in mol%) yielded the composition behavior of the extracts as shown in Figure 5. Limonene was obtained initially with pure supercritical carbon dioxide, but it was not detected in the succeeding runs with the addition of EtOH as entrainer. The general trend for all the pressures studied shows a diminishing amount of terpenes, while the relative composition of fatty acids and some other polar compounds increased.

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3.3 Total phenolic content of the extracts

The estimates of total phenolic contents expressed in mg GAE (gallic acid equivalents) per gram of the extracts are shown in Fig. 6. The total phenolic contents of the extracts were higher at 20 MPa as compared to that at 10 MPa, but lower at 30 MPa. Addition of EtOH as entrainer dramatically increased the phenolic contents especially at 10 mol% EtOH. The presence of even small amount of phenolic compounds, known as antioxidants [10] and antimicrobials [11], not only offers health benefits [1, 2] but also serves to powerfully inhibit atmospheric oxidation [12].

4. CONCLUSION

Supercritical carbon dioxide was used to extract useful compounds from sudachi residues, as a representative of citrus-processing by-products. The effect of addition of ethanol, as entrainer, was also studied. Results indicated that the yield increased with increasing pressure. Addition of 5mol% EtOH as entrainer increased the yield by more than three times of that without the entrainer (EtOH). Based on gas chromatography-mass spectrometry (GC-MS) analyses, the extracts consisted mainly of limonene, terpenes, some fatty acids and their esters. The amount of polyphenols in the extracts, estimated using the Folin-Ciocalteau method increased with increasing amount of EtOH. Experiments conducted on samples other than sudachi residues also showed promising results. The prospect is high for the utilization of the obtained extracts in cosmetic, pharmaceutical and food industries.

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