Ethanol Modified Liquid Carbon Dioxide Extraction and Antioxidant Activity of Nobiletin and Tangeretin from Peels of *Citrus Poonensis*

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We extracted nobiletin and tangeretin from the dried peels of *Citrus Poonensis* (Ponkan) using ethanol modified liquid carbon dioxide (CO_2) and determined the best extraction condition. We conducted experiment under temperatures of 5, 20, and 25°C, pressures of 8, 10 and 14 MPa and different modifier (ethanol) mole fractions ($x_{ethanol}$ = 0, 0.06, 0.131, 0.262, 0.390 and 1) in the liquid CO_2 solution for 60 min extraction time. Furthermore, ultrasound with different irradiation power and supercritical CO_2 ($ScCO_2$, 31°C, 10 MPa) were also used for the comparison of the extraction yields of nobiletin and tangeretin. The highest nobiletin and tangeretin contents in dried Ponkan peels were achieved through the ethanol modified liquid CO_2 extraction condition at 25°C, 10 MPa, and mole fraction of ethanol in the liquid CO_2 solution (x_{EtOH} = 0.131) as well as $ScCO_2$ (31°C, 10 MPa).

1. Introduction

A number of citrus fruits are grown plenty in Japan. Most of the fruit is applied to juice processing and the peel has been disposed without any usage. However, these peels contain abundant amount of flavonoids. Methods for extracting flavonoids from citrus fruits have been carried out before. The most common method of them is using organic solvents. However, as many of these organic solvents are toxic and highly flammable, we must be taken to ensure that the solvent applied for the extraction is safe and eco-friendly. Also, the attention should be paid on the disposing of waste which has huge impact on natural environment. Regarding these, nowadays, an extraction method using liquid CO_2 is attracted. CO_2 is biologically friendly, and there is no risk of residual solvents like using harmful organic solvents. Alike other citrus fruits in japan, Ponkan (Citrus poonensis) is one of the high-yield sweet citrus. In this study, dried peels of Ponkan was used as a raw material to extract nobiletin and tangeretin which are expected to have anti-allergic properties by using liquid CO_2. Also the effect of the operating conditions on the extract yield was checked using the single-factor method.

2. Experimental

2.1 Reagents

Ponkan fruits were purchased from local markets in Fukuoka, and the peels dried at room temperature, and ground into a fine powder by using a freezer mill 6750 (SPEX CentriPrep Co. Ltd., New Jersey, USA). Nobiletin with purities >97 wt% were purchased from Sigma-Aldrich, Co, Japan. Tangeretin with purities >95.0 wt% were purchased from Tokyo Chemical Industry Co, Ltd, Japan.

2.2 Apparatus and Procedure

A schematic diagram of the experimental apparatus used for the liquid CO_2 extractions with or without ultrasonic irradiation is shown in Figure 1. The prepared peel powders were placed in an extraction cell (a high-pressure cell) of a liquid CO_2 mixture extraction apparatus (SFC; super200, JASCO Co. Ltd.). The extraction cell was approximately 150 cm3 in volume. The system pressure was controlled by a back-pressure regulator (880-81, JASCO, accurate to 0.1 MPa) and monitored by a digital pressure gauge (Shinwa Electronics Co., model DD-501, accuracy ± 0.3%). The temperature was controlled within ± 0.1 ºC with a water bath. The temperature of the water bath was maintained at 5, 20 and 25 ºC. The optimisation process was conducted under temperatures of 5, 20, and 25°C, pressures of 8, 10 and 14 MPa and different modifier (ethanol) mole fractions (xehanol = 0, 0.06, 0.131, 0.262, 0.393 and 1) in the liquid CO_2 solution for 60 min extraction time. Furthermore, ultrasound with different irradiation power and scCO_2 were used for the comparison of the extraction yields of nobiletin and tangeretin. Finally, the antioxidant activities were evaluated using the quenching activity of the free chromogenic radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

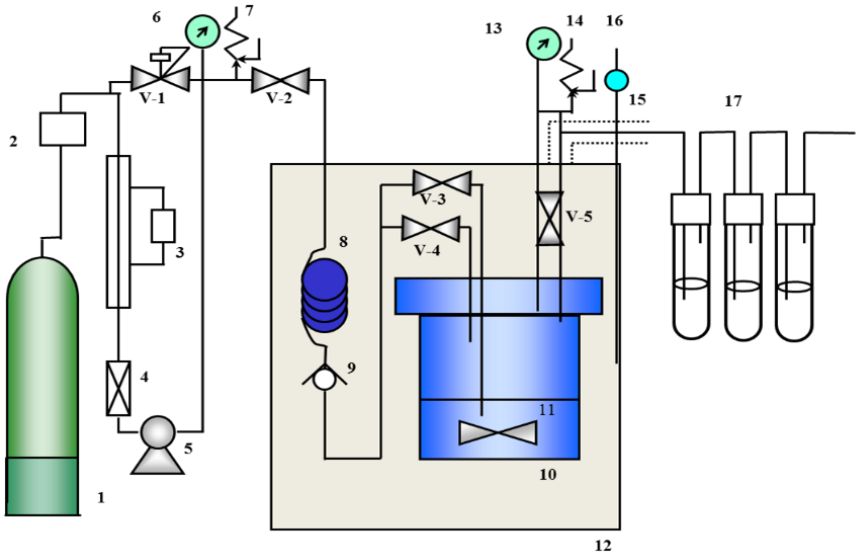

Figure 1. A schematic diagram of the experimental apparatus used for the liquid CO_2 extractions 1: gas cylinder, 2: dryer, 3: cooling unit, 4: filter, 5: pump, 6: pressure gauge, 7: safety valve, 8: preheating piping, 9: stopper, 10: high-pressure cell, 11: stirrer, 12: water bath, 13: pressure gauge, 14: safety valve, 15: heater, 16: thermometer, 17: trap, V-1: back pressure, and V-2–V-5: stop valves.

2.3 HPLC Analysis

The HPLC system consisted of a Tosoh LC-8010 system equipped with a UV detector. The detection wavelength was set at 343 nm and 40°C. Separation of the extract was performed at 40°C and at 1.0 mL/min flow rate. Methanol and 0.1% acetic acid in water were used during the separations. The methanol gradient profile is presented in Table 1.

Table 1. Time course of methanol gradient for HPLC analysis.

Time [min]	0	25	40	50	60	65	75
Methanol [vol%]	5	40	60	90	90	5	5

3. Results and Discussion

The solvent extraction behavior of nobiletin and tangeretin from the Ponkan peel powder was examined using methanol or ethanol at 25 ºC and extraction was performed at several time points. Qualitative and quantitative information was obtained via HPLC. It was found that the mass of extract of isovitexin generally increased over 0-60 min and were then saturated. Therefore, the appropriate time of treatment was set at approximately 60 min.

The effect of three operating parameters, temperature, pressure and the mole fraction of ethanol in a solution of CO_2, on the extraction yield was investigated. The effect of temperature and pressure on the extraction yield is shown in Figure 2. In general, the extraction yield clearly increased with increasing temperature or pressure at a constant temperature or pressure. The yields of both nobiletin and tangeretin are found to be the highest at 25 ºC and at 10 or 14 MPa. For these experimental conditions, the solvent molecular activity induced by the temperature is considered to be more predominant than that induced by the solvent density.

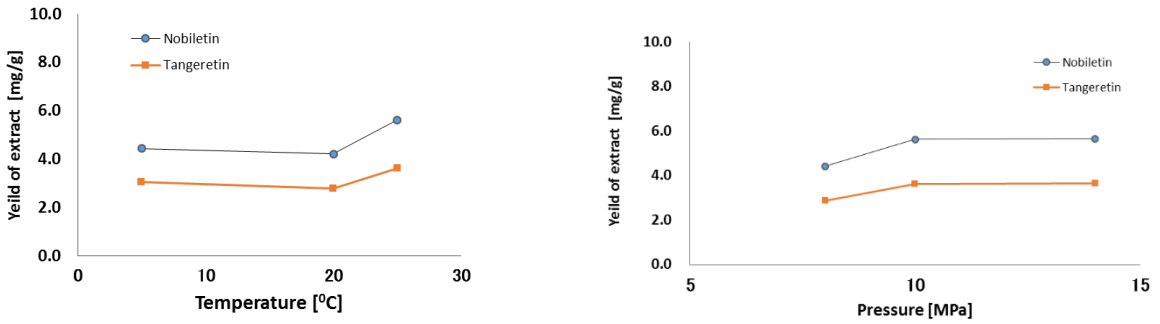

Figure 2. Temperature and pressure dependence of the extract yields using mixtures of liquid CO_2 and ethanol (ethanol mole fraction $x_{ethanol}$=0.131)

To elucidate the effect of changing the mole fraction of ethanol over liquid CO_2 (x_{EtOH}) on the yield, we performed the extraction by using ethanol and liquid CO_2 either solely or in combination. The pressure was set at 10 MPa, the temperature was set at 25ºC, and the extraction was performed for 60 min. The maximum yield was obtained at x_{EtOH} = 0.131. This result implies that lower or higher mole fractions of ethanol reduce the yield.

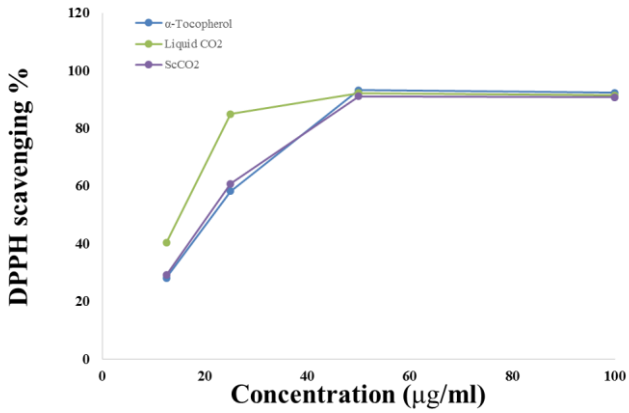

Figure 3. Comparison of the antioxidant activities of the extracts using DPPH

Finally, the antioxidant activities of the extracts were measured and the ethanol modified liquid CO_2 extract exhibited the highest scavenging activity of DPPH free radicals compared with α-Tocopherol (Figure 3). The presence of these activities is attributed to the flavonoid compounds such as nobiletin and tangeretin, revealed in HPLC.

4. Conclusion

Nobiletin and tangeretin were successfully extracted from Ponkan peels using mixtures of liquid CO_2 and ethanol. The yields of nobiletin and tangeretin in the liquid CO_2 extraction were improved by adjusting the operating temperature and pressure (25 ºC, 10 MPa), and mole fraction of ethanol in the liquid CO_2 solution (xethanol = 0.131). Furthermore, ethanol modified liquid CO_2 extract have considerable antioxidant activities value with respect to that of α-Tocopherol. Therefore, our results suggested that the ethanol modified liquid CO_2 extracts can be utilized as an effective and safe antioxidant source in the drug development.

Acknowledgement

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