Simultaneous extraction of water and valuable substances from citrus leaves and peel using liquefied dimethyl ether

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ABSTRACT

Various kinds of sour citrus fruits are cultivated in Japan. Citrus fruit is used as the raw material for juice processing. However, most of the residue after juice processing is disposed, although it contains valuable substances such as flavonoids and essential oil. While most of the citrus flavonoids are present in the peel of the fruit, various valuable substances are also contained in the leaves. Extraction of flavonoids from plant materials has been typically accomplished by using an organic solvent whose polarity is comparable to that of the target compounds. Even though high yield of target compounds can be obtained by organic solvent extraction, residual solvent problem haunts the use of organic solvent. Moreover, the leaves and peel of citrus plants contain a large amount of moisture, and hence, dewatering is an essential part of the pre-treatment in the extraction of valuable substances.

In this study, we use liquefied dimethyl ether (DME) as the extractant for the dewatering step. In addition to acting a dewatering agent, DME can extract valuable substances from the given materials because it can dissolve a wide range of organic components. DME was used for dewatering and extraction of functional ingredient from citrus residue. With this process approximately 81.5–83.7% of the water from the samples was removed and valuable substances like flavonoids and essential oil in the dry samples could be isolated. The properties of the original samples, dewatered samples, and the extract were evaluated by elemental analysis, high-performance liquid chromatography, and gas chromatography-mass spectrometry.

INTRODUCTION

Various kinds of citrus fruits are cultivated in Japan. The yield of the citrus is about 130 million tons, which are used as the raw material for juice processing. However, most of pomace after juice processing is disposed, although it contains valuable substances such as flavonoids and essential oil. In addition, disposal cost of pomace is expensive because it contains large amount of water. Essential oil and citrus flavonoids are mainly contained in the pomace such as peel and leaves of citrus. These valuable substances are traded at a high price in the market. Essential oil is used as material of aromatherapy and high quality perfume. Citrus flavonoids are used as a raw material for health supplements. Commercially essential oil has been obtained from citrus peel by cold press and steam distillation method. However, extraction yield using cold press method is low because large amount of valuable oil is remaining in the residue. On the other hand, the quality of the essential oil by using steam distillation method is degraded due to distillation heat. Additionally, large amount of residue which has high contained water is disposed after those method. Citrus flavonoids cannot be extracted by the steam distillation method because of its high boiling point. Therefore, the flavonoids has been extracted by organic solvent such as menthol, dimethyl sulfoxide and hexane [1]. However, it has problems such as toxic, expensive, and hazardous to handle and remain in the product. In addition, those methods require several pre-processing such as drying, grinding and homogeneous of raw material.

Recently, alternative extraction method is demanded in food industries. Supercritical CO_2 (SC-CO_2) and liquefied dimethyl ether (DME) are receiving attention as extraction solvent because of those high abilities. SC-CO_2 extraction has merit of highly selectivity of the extract and it can be possible to fractionate of component kind by temperature and pressure control. However, it is necessary special apparatus to withstand high pressure of SC-CO_2 [2]. On the other hand, DME has high solubility with oil and also dissolve a wide range of organic components, and is partially soluble with water. Liquefied DME can extract water (dewatering) and oil. Normal boiling point of DME is -24.8^{o}C, and it exists in a gaseous state under normal conditions. Therefore it can be operated at lower temperature and pressure and easily separated from the extract at ambient pressure from residue [3]. Accordingly it can realize the weight reduction of the residue.

In this work, essential oil and flavonoids were extracted from citrus pomace by liquefied DME. *Citrus junos* (Yuzu) peel, leaves and *Citrus tangerine* (Ponkan) peel were used as raw materials and dewatering rate, yield of essential oil and citrus flavonoids were investigated.

MATERIALS AND METHODS

Outline of extraction method

Figure 1 shows a schematic diagram of the Lab-scale DME extraction apparatus.

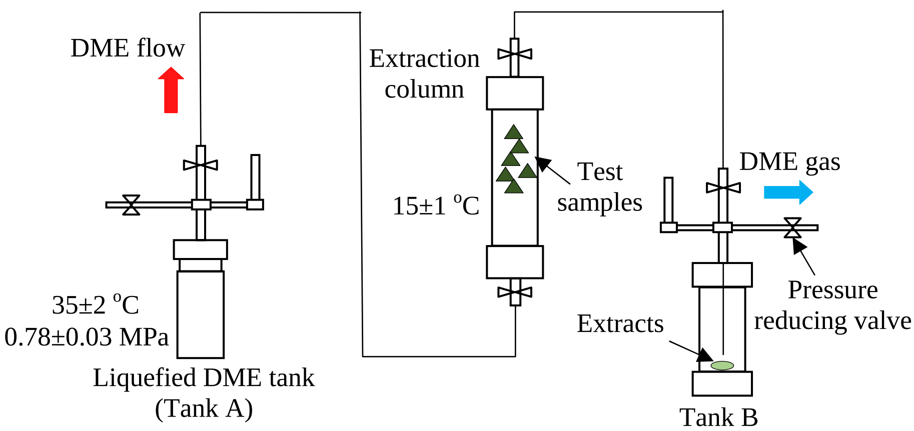

Figure 1 Lab-scale DME extraction apparatus

A storage tank containing liquefied DME (Tank A, volume: 100 cm^3), an extraction column (diameter: 11.6 mm, length: 190 mm; HPG-10-5, Taiatsu Techno Corp., Saitama, Japan), and a storage tank for the mixture of liquefied DME, water, and essential oil (Tank B, HPG-96-3, Taiatsu Techno Corp.) were connected in series. The extraction column was loaded with the test samples. On average of 3 tests, the amounts of yuzu peel, ponkan peel and yuzu leaves were 4.4 (±0.1) g, 5.0 (±0.1) g and 2.6 (±0.2) g, respectively. The initial water content of yuzu peel, ponkan peel and yuzu leaves were 82.7 wt%, 72.4 wt% and 82.6 wt%, respectively. The liquefied DME in the Tank A was controlled at 35±2 ^{o}C in a water bath, and the liquefied DME supplied for the extraction column. The DME flow rate was 10 cm^3/min, and the extraction temperature and pressure were 15±1^{o}C and 0.78±0.03 MPa, respectively. After the liquefied DME was passed through the extraction column at different time intervals, DME was evaporated by opening the pressure reducing valve of the Tank B. The amount of extracts left in the Tank B was equal to the total amount of the essential oil and water extracted from the test samples. The extract was weighted immediately after evaporation of DME, and the yields were determined by the following equations.

**Essential oil*

$$\text{Yield (\%)} = \frac{\text{Extracted oil [g]}}{\text{Dry samples [g]}} \times 100$$

**Water*

$$\text{Dewatering rate (\%)} = \frac{\text{Extracted water[g]}}{\text{Initial water amount of samples[g]}} \times 100$$

HPLC analysis

The amount of citrus flavonoids in the water phase were analysed by HPLC. Flavonoid extracts were analyzed using a HPLC gradient system (LC-20AD, Shimadzu Corp., Kyoto, Japan), equipped with Diode Array Detector (SDP-M10A, Shimadzu Corp., Kyoto, Japan). ODS column (Intertsil® ODS-3, GL Sciences Inc., Tokyo, Japan) was used for separation at 35^{o}C. The mobile phase consisted of solvent A, 0.1 % acetic acid in water, and solvent B, 0.1 % acetic acid in acetonitrile (acetonitrile/water = 75/25, v/v). The flow rate was 1.0 mL/min. The gradient elution was carried out according to the following steps: time 0 min A-B (88:12); time 5 min A-B (78:22); time 15 min A-B (72:28); time 22 min A-B (62:38); time 32 min A-B (52:48); time 37 min A-B (32:68); time 42 min A-B (0:100); time 45 min A-B (0:100); time 50 min A-B (88:12); time 60 min A-B (88:12). The yields were determined by the following equation.

**Citrus flavonoids*

$$\text{Yield (mg/g-sample)} = \frac{\text{Extracted citrus flavonoids [mg]}}{\text{dry sample [g]}}$$

RESULTS

Valuable substances extraction and dewatering using liquefied DME

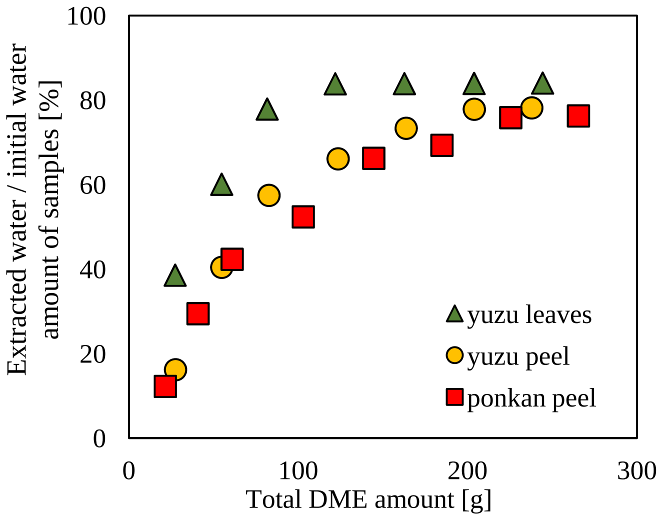

Figure 2 Amount of dewatering from the samples using liquefied DME.

Figure 2 shows amount of dewatering from the samples using liquefied DME. The amount of dewatering of yuzu peel, ponkan peel and yuzu leaves at maximum were 78.1±1.0%, 76.2±1.0% and 83.9±0.5%, respectively. Therefore, it was possible to remove the moisture of about 70% in the raw material by liquefied DME. Moreover, the color of the extraction residue became bright after DME extraction. Therefore, the pigments were also extracted from the samples by liquefied DME.

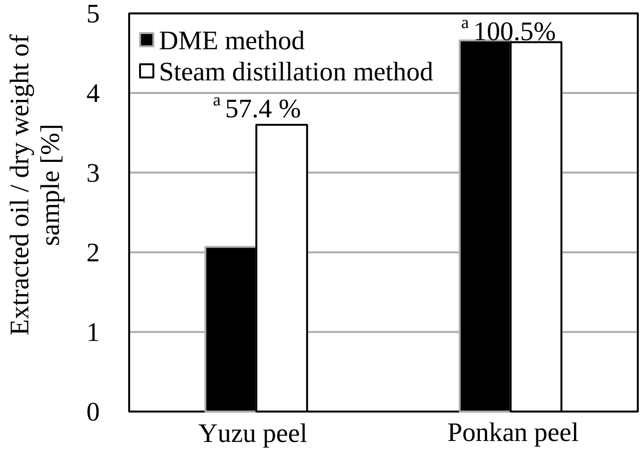

Figure 3 Essential oil extraction amount using the DME and steam distrillation method for samples.
[a] Extraction yield using the DME method relative to the steam distrillation method.

The extraction yields of essential oil from samples using the DME method were compared with the steam distillation (SD) method, as shown in **Figure 3**. Black columns indicate the DME extraction ratio based on the dry weight of the samples, whereas the white columns indicate the result of the SD method. The numbers beside the superscript ‘‘a’’ indicate the extraction yields by DME based on the SD method. In the case of ponkan peel, the same amount of essential oil with SD method was extracted by using DME method. Since the aroma of the extracts does not suffer thermal denaturation, it was close to the original aroma of the raw material. In the case of yuzu peel, since the peel is thicker than ponkan peel, the yield was lower than SD method.

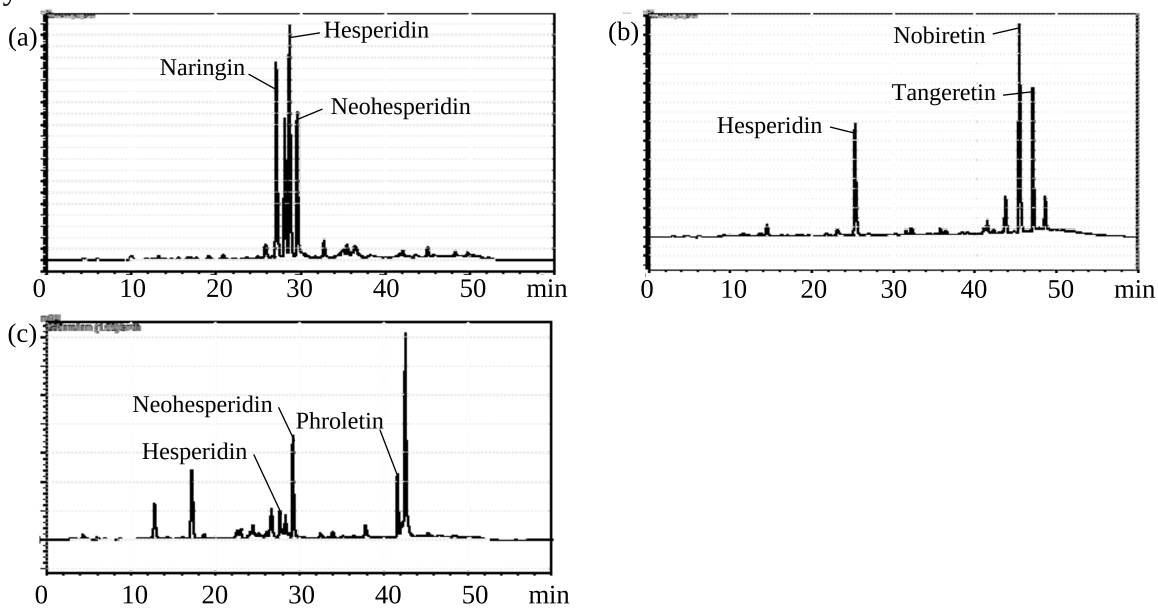

Figure 4 HPLC chromatogram of citrus flavonoids in the water phase at 285 nm. (a) : yuzu peel, (b) : ponkan peel, (c) : yuzu leaves

Table 1 The extraction amount of the citrus flavonoids in the water phase by DME extraction.

Citrus flavonoid	Yield [mg / g-dry sample]		
	Yuzu peel	Ponkan peel	Yuzu leaves
Naringin	2.9	-	-
Hesperidin	45.6	1.6	0.3
Neohesperidin	1.4	-	17.9
Phroletin	[a]-	-	0.2
Nobiretin	-	3.8	-
Tangeretin	-	1.2	-

[a] Not detected.

Figure 4 shows the chromatogram of citrus flavonoids extracted by liquefied DME. **Table 1** shows quantitative results of those flavonoids. In the case of yuzu peel, naringin, hesperidin and neo hesperidin were extracted and those yields were 2.9 mg, 45.6 mg and 1.4 mg, respectively. On the other hand, hesperidin, nobiretin and tangeretin were extracted from ponkan peel, and those yields were 1.6 mg, 3.8 mg and 1.2 mg, respectively. Additionally, hesperidin, neohesperidin and phroletin were extracted from yuzu leaves, and those yields were 0.3 mg, 17.9 mg and 0.2 mg, respectively.

CONCLUSION

More than 70 % water, essential oil and citrus flavonoids were extracted from citrus peel and leaves by DME extraction. In the case of ponkan peel, essential oil was extracted as same amount as steam distillation method. In addition, citrus flavonoids such as hesperidin, nobiretin and tangeretin were extracted as well. Those flavonoids cannot be extracted by SD method. Therefore, extraction of valuable substances and citrus pomace reduction were simultaneously accomplished by this process.

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