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Research Article

Step-Wise Pressure Controlling Approach for Supercritical Carbon Dioxide Extraction of Essential Oil from Thick Peel of Bitter Orange (Citrus Aurantium)

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Abstract

Supercritical carbon dioxide (SC-CO_2) extraction has been applied for the recovery of essential oil from plant material. In conventional SC-CO_2 extraction, CO_2 is continuously flowed at a constant pressure from dried sample. In this work, step-wise pressure controlling approach was applied to essential oil from fresh bitter orange peel without drying pretreatment. The extracts contain water as well as essential oils. Extraction was carried out at temperatures of 40 to 80°C; and pressures of 10 to 30 MPa. The recovery of essential oil was considerably high at 95.4 %, more than 3.4 times the recovery obtained by conventional semi-continuous method.

Keywords: Citrus aurantium; Essential oil; Supercritical carbon dioxide; Pressure control.

Introduction

Citrus aurantium, bitter orange or called daidai in Japan, is a popular variety of sour citrus. Bitter orange has been mainly cultivated in Wakayama prefecture in Japan and is being harvested from December to January. Bitter orange fruit is covered with thick and hard peel which has pleasant flavor. Its juice has been used in food products such as sauces and chilled sweets because of its unique and desirable flavor. Most of citrus essential oils have been collected as byproducts in juice production process. Bitter orange juice has been produced in small scale, because only a few areas cultivate bitter orange trees. For this reason, the amount of bitter orange essential oil cannot be recovered in commercial scale. There are reports of its extraction using steam distillation, hydrodistillation and cold press method in laboratory scale [1-6]. The aromatic components contained in the essential oils of citruses are monoterpenes, sesquiterpenes, and oxygenated compounds. It is known that the monoterpenes, such as limonene, take a little part of the aroma. On the other hand, sesquiterpene and some of the oxygenated compounds contribute to the strong fragrances and peculiar characteristic flavor of the citrus. The characteristic flavor components of cold-pressed bitter orange essential oil have been investigated using GC-olfactometry and sensory analysis [4].

Essential oils from some citrus peel are focused as a medicinal applications [3,6,7]. The essential oil obtained from Citrus aurantium, have been used to study medicinal activity such as central nervous system action [6], anxiolytic and sedative effect [7], and cholinesterase inhibitory activity [3].

In the extraction of natural compounds such as essential oil, the use of supercritical fluid has been proposed. Supercritical fluid has liquid-like density and gas-like viscosity, thus it is considered to be a promising solvent that combines high solvent power and high transport capacity. Of the many possible fluids of choice, carbon dioxide is the most preferred because of its low critical temperature (31°C) suitable especially for thermally labile components such as aroma compounds. Besides, it is readily available and non-toxic. Unlike the conventional organic solvent extraction, the extraction process associated with the use of supercritical CO_2 does not leave harmful solvent residues in the extracts. Its presence in foods or beverages is generally regarded as safe and harmless for human consumption. CO_2 is nonflammable, non-toxic, environmentally-friendly, and cheap. In addition, SC-CO_2 has low critical temperature, low viscosity and it is easy to separate from the extract. Some researchers reported the supercritical extraction of essential oil from various citrus peels such as orange [8, 9], and grapefruit [10], bergamot [11], unshu [12], yuzu [13], and yuzu and kabosu [14,15]. In these reports, citrus peel was pretreated either by drying, crushing or a combination of these steps prior to a commonly used semi-continuous flow type of extraction.

In this work, a more efficient extraction method of the citrus essential oil from the unusually thick peel of bitter orange using step-wise pressure controlling approach was investigated. Scraped fresh bitter orange peel without pre-drying treatment was used as a sample material. The results were compared with the typical semi-continuous extraction method, and optimum extraction condition was investigated.

Materials and Methods

Materials and chemicals

Sample of bitter orange fruit was harvested in December 2009 at Fukuoka prefecture, Japan. The outer bitter orange peel was scraped automatically using a skinning machine (KR-ROBO-03, Mitsuwa Co. Ltd., Niigata, Japan) which separate the flavedo part containing albedo of approximately 2.4 mm thick. Its peel was scraped, placed inside an air-tight wrapping plastic bag, and then stored in a freezer at -20°C until extraction experiments were performed. No pre-drying treatment was carried out on the sample. The feed material contained

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water of approximately 80wt%. Prior to extraction experiments, the sample was gently thawed to room temperature. Carbon dioxide (99.9%) was obtained from Uchimura Sanso Co., Japan.

Steam distillation

Approximately 40g of scraped citrus peel was minced in 500 mL of distilled water, and placed into a 1L round flask. The flask was then connected to a distilling receiver for essential oil testing with Liebig condenser. Distillation was carried out at a temperature of 100°C for 24 h. The average amount of oil content in the wet peel obtained by steam distillation was 1.42%

Semi-continuous SC-CO_2 extraction

A semi-continuous flow SC-CO_2 extraction apparatus (AKICO Co., Ltd., Tokyo) shown in schematic diagram in Figure 1, was used in the experiments. Approximately 80 g of scraped citrus peel was placed in a 500 mL extractor having a height of 20 cm and inside diameter of 7 cm. Liquid CO_2 from a cylinder with siphon attachment was passed through a chiller kept at 0°C, and compressed CO_2 was flowed into the extractor covered by a thermostat heater that was maintained at the operating temperature. The supplied pressure of liquid CO_2 was controlled by Back-Pressure Regulator (BPR) 1, while the pressure in the extractor was controlled by BPR 2. The exit fluid from the extractor was expanded to a pressurized separator at 2 MPa and 0°C by BPR 3. CO_2 flow rate was measured by a flow meter and a dry gas meter. Extracted oil was collected from the pressurized separator every 60 min for a total extraction time of 300min, and weighed right after the collection. Extraction experiments were carried out at temperatures of 40, 60 and 80°C, pressures of 10, 20 and 30 MPa, with CO_2 flow rate of 0.95-1.05g/s.

Step-wise pressure controlling SC-CO_2 extraction

In the case of step-wise pressure controlling extraction approach, approximately 80 g of scraped citrus peel was placed in the extractor. Liquid CO_2 was then loaded into the extractor until the desired extraction conditions were attained. SC-CO_2 extraction was carried out under the same temperature and pressure conditions of semi-continuous experiments. The pressure was controlled in step wise consisted of a series of pressurization period, static high pressure period and depressurization period. In the static period, CO_2 flow was stopped. The static period of step-wise pressure controlling extraction was set to 10, 15, 30, 60 and 90 min. After the holding time, which allowed CO_2 to penetrate into the sample matrix has elapsed, the system was depressurized to ambient, while collecting essential oil at the separator. These series of step-wise pressurization-static-depressurization steps were conducted six times under various conditions.

Recovery of extracted oil

The recovery (%) was defined as the weight of extracted oils per weight of oil obtained by steam distillation as shown below.

Yield (%) = (weight of extracted oil)/(weight of steam distilled oil)×100

GC-MS analysis of essential oil

The essential oils obtained by supercritical CO_2 extraction and steam distillation were analyzed by GC-MS. The GC-MS is Agilent 7890N series gas chromatograph equipped with an Agilent 5975 mass selective detector and a HP-5MS column (30 x 0.25 mm (5%-Phenyl)-methylpolysiloxane column, film thickness x 0.25μm).

Results and Discussion

Components of essential oil

From the GC-MS chromatogram of essential oil extracted with supercritical CO_2, major components identified are shown in comparison with that obtained by steam distillation. About 76 peaks were observed by GC-MS for essential oil obtained by supercritical CO_2. The composition of essential oils between supercritical fluid extraction and steam distillation is slightly different, oils obtained by supercritical fluid extraction has higher content for limonene and linalool whereas lower content for α-pinene, myrcene, γ-terpinen, and α-farnesene. The components reported in literatures for the same citrus fruit are roughly similar; Maekawa et al. [1] (cold pressing: limonene 86.23%, β-pinene 5.50%, myrcene 1.60%, caprylaldehyde 1.93%, linalool 0.52%), Deterre et al. [5] (cold-pressing: limonene 90.47%, α-pinene 1.21%, b-pinene 0.21%, myrcene 4.37%, linalool 0.66%), Tundis et al. [3] (hydrodistillation: limonene 65.8%, α-pinene 1.8%, β-pinene 0.3%, myrcene 2.9%, ocimene 0.3%, γ-terpinene 0.1%, linalool 1.8%, β-caryophyllene 0.3%), and Pultrini et al. [6] (hydrodistillation: limonene 97.83%, myrcene 1.43%, octanal 0.45%). Considering the difference in harvesting season, geographical location, variety, and maturity, there is no significant difference in the composition and content.

Menichini et al. [16] compared the essential oil of Citrus medica L. cv. Diamante among hydrodistillation, cold-pressing, and supercritical carbon dioxide extraction. Although the oil composition

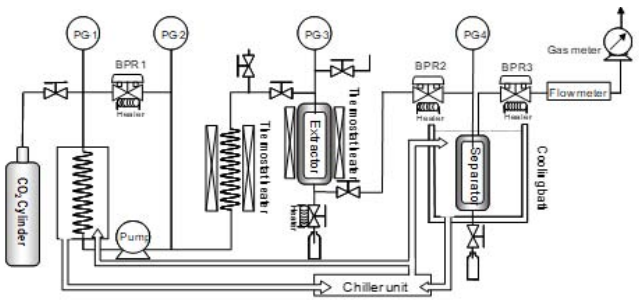

Figure 1: Schematic diagram of SC-CO_2 extraction apparatus.

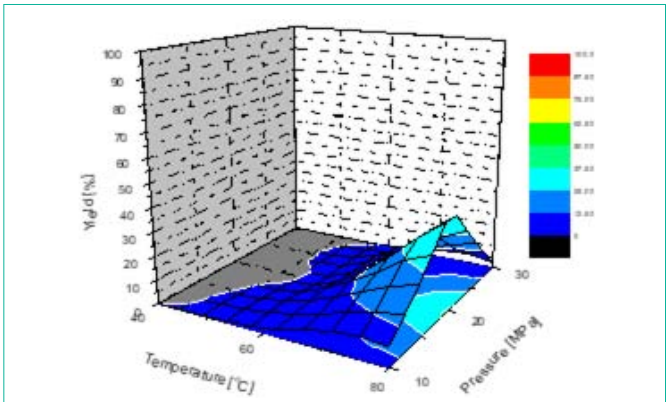

Figure 2: Yield of bitter orange essential oil through the semi-continuous extraction method.

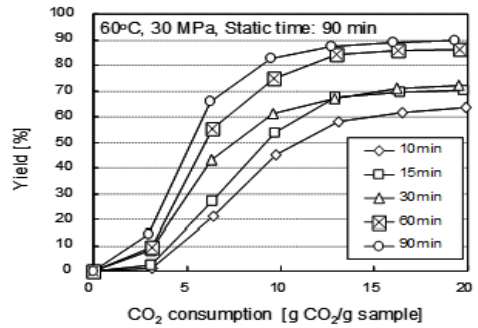

Figure 3: Effect of holding time on the yield of bitter orange essential oil at 60°C, 30 MPa.

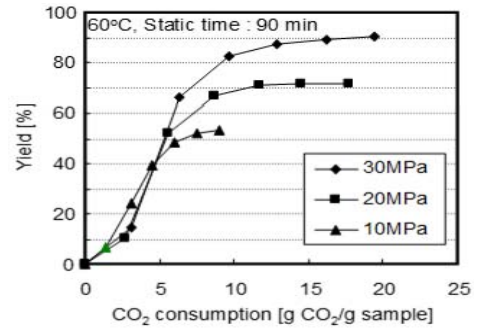

Figure 4: Effect of pressure on the yield of essential oil.

was similar between hydrodistillation and cold-pressing, components and composition of oil obtained by supercritical fluid extraction was significantly different. Most of essential oil components were not contained but 85% was citropten which was 2.5% in cold-pressing oil. One of the reason of this difference may be collection of extracted oil after the supercritical fluid extraction. Volatile components could not be easily collected in the collection vessel from gaseous carbon dioxide flow. In our work, the collection part was carefully designed and also water was extracted with essential oil which make the collection of volatile components easier.

Preliminary studies on semi-continuous extraction

Figure 2 shows the yield of total oil extract of bitter orange peel by semi-continuous extraction method at various temperature and pressure conditions for 300 min. The yield increased with increasing extraction temperature. The yield was the highest at a temperature of 80°C and pressure of 20 MPa. However, its yield was only 28% of those obtained by steam distillation. This relatively low yield compared conventional steam distillation method could be due to the difficulty of supercritical CO_2 to penetrate and diffuse through the sample matrix because of its thickness of the peel. The supplied CO_2 simply passed through the samples and could hardly isolate the target materials. Thus, in the succeeding experiments step-wise pressure controlling extraction approach was considered, instead.

Effect of holding time on step-wise pressure controlling extraction

The effect of holding time of the static period on step-wise pressure controlling extraction was investigated, and the results for the recovery of essential oils at a temperature of 60°C and pressure of 30 MPa are shown in Figure 3. The recovery increased with increasing holding time despite similar amount of CO_2 consumption. The difference in the recovery of bitter orange essential oils were hardly observed between 60 and 90 min. By treatment under static pressurized condition, thick bitter orange peel underwent swelling under supercritical CO_2 conditions. It was thought that SC-CO_2 penetrated and diffused through the cell matrix followed by dissolution of the target essential oil into CO_2, and was highly dependent on density changes during depressurization step. Longer holding time may lead larger amount of essential oil dissolved in CO_2. During depressurization step, CO_2 containing the essential oil penetrated in sample matrix expanded by pressure reduction and flow out to the fluid phase by convective flow. The mass transfer by the convective flow is much faster than diffusion process. In semi-continuous extraction at constant pressure, mass transfer is limited to diffusion, whereas it is both convective flow and diffusion in pressure controlling extraction process.

Effect of pressure and temperature on step-wise pressure controlling extraction

The holding time of the static time was fixed at 90 min to investigate the effect of pressure and temperature on the recovery of essential oil. Figure 4 shows the effect of pressure on the recovery of bitter orange essential oil. Under this isothermal condition, the amount of extracted essential oil increased with increasing pressure. This result indicates that the CO_2 density plays an important role in oil extraction. At higher pressure, more CO_2 penetrates into matrix, resulting in more essential oil dissolved in CO_2.

Figure 5 shows the effect of temperature on the yield of essential oil. Contrary to the effect of pressure as shown in Figure 4, the yield increased with temperature despite the decrease in CO_2 density at isobaric condition. Two factors relating to the properties of the target compounds and SC-CO_2 could contribute to this behavior. The first factor is the vapor pressure effect of essential oil with the rise in temperature, and the second relates to the mass transfer rate of essential oil from the sample matrix to the extracting SC-CO_2.

Optimum condition for batch-wise extraction

The holding time was fixed at 90 min to investigate the optimum extraction condition under various temperatures and pressures. Figure 6 shows the optimum condition to extract the bitter orange essential oil through the step-wise pressure controlling extraction approach. The increase in SC-CO_2 density as the pressure increased obviously had a positive effect on the recovery of essential oil. However, the

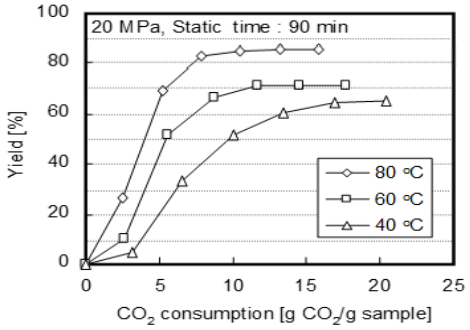

Figure 5: Effect of temperature on the yield of essential oil.

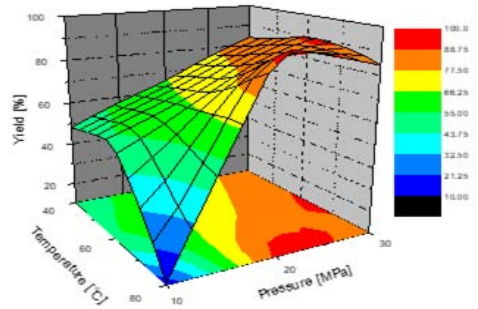

Figure 6: Optimum extraction condition obtained by batch-wise extraction.

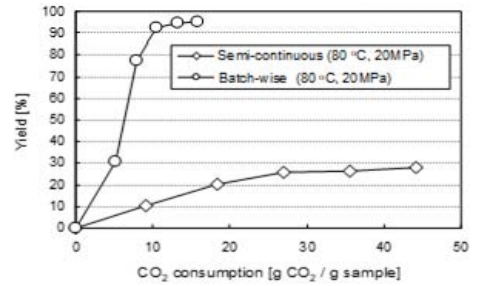

Figure 7: Comparison of the yield and CO_2 consumption between semi-continuous and batch-wise extraction approaches for 5 hours.

recovery of essential oil tends to increase at higher temperatures of 60 to 80°C, even though the SC-CO_2 density is lower in this temperature range compared to that at 40°C. The considerably high maximum recovery of 95.4% was observed at the temperature of 80°C and pressure of 20 MPa. It was thought that these extraction behaviors were related to the penetration of CO_2 into the sample matrix and dissolution of oil into CO_2, and the extracted oil accompanied by convective flow of CO_2 during depressurization step.

Comparison of the two extraction methods

Figure 7 shows the comparison of the yield and CO_2 consumption between semi-continuous and batch-wise extraction. By the batch-wise depressurization approach, the recovery was more than 3.4 times of that obtained using semi-continuous method at the same operating conditions. In addition, CO_2 consumption was reduced to 38%. The effectiveness of the pressure change technique has also been reported on the extraction of useful compounds from lovage and celery [17]. They found out that rapid depressurization of SC-CO_2 could significantly increase the yield of extraction.

Conclusions

The step-wise pressure controlling approach was found suitable for SC-CO_2 extraction of essential oil from unusually thick fresh citrus peel like that of bitter orange as compared to that of the typical semi-continuous method. Even from wet citrus peel, essential oil was able to be extracted. Since volatile compounds such as essential oil would volatilize and disappear during drying process, extraction from wet sample is valuable. Additionally, volatile components can be easily recovered with water. Optimum condition for extraction was obtained at a temperature of 80°C and pressure of 20 MPa, similar to that of a typical semi-continuous operation. At this condition, the recovery of essential oil was considerably high at 95.4%, more than 3.4 times the recovery obtained by semi-continuous method. In addition, CO_2 consumption was reduced to 38%. Future directions should consider a combination of semi-continuous and step-wise operation for a more efficient and effective extraction.

Acknowledgments

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