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Supercritical CO_2 extraction of essential oil from Kabosu (*Citrus sphaerocarpa* Tanaka) peel

Takuya Suetsugu[1,3], Masahiro Tanaka[2*], Hideo Iwai[1,3], Teruaki Matsubara[1,3], Yukihiro Kawamoto[3,5], Chiho Saito[4], Yoshito Sasaki[4], Munehiro Hoshino[2], Armando T Quitain[1], Mitsuru Sasaki[1], Junshi Sakamoto[4] and Motonobu Goto[5]

Abstract

Background: *Citrus sphaerocarpa* Hort. ex Tanaka is one of many popular sour citruses in Japan. Its juice processing peel residues contain a lot of useful compounds including essential oil. Our interests mainly focused on the extraction of this essential oil using supercritical carbon dioxide (SC-CO_2), an environmentally benign and generally regarded as safe solvent that has many advantages such as low critical temperature, low viscosity, and easy separation from the extract. In this research, essential oil was extracted from *Citrus sphaerocarpa* Tanaka peel using SC-CO_2 at extraction temperatures of 313 to 353 K and pressures of 10 to 30 MPa.

Results: A maximum yield of 1.55% (by weight of wet sample) was obtained at the temperature of 353 K and the pressure of 20 MPa. The yield obtained by SC-CO_2 method was over 13 times higher than that of the conventional cold-press method. Extracted essential oil was qualitatively analyzed using GC/MS, identifying 49 compounds including several non-polar and weakly polar hydrocarbons such as terpenoid, free fatty acid, and coumarin. Compared to the extracts obtained by the conventional methods, the extracts by SC-CO_2 had lower content of monoterpenes and higher content of oxygenated compounds, sesquiterpenes, which strongly contribute to the aromatic characteristics of the extracts. Auraptene, a bioactive compound was also identified in the SC-CO_2 extract.

Conclusions: Kabosu essential oil with a fresh natural fragrance was effectively extracted using SC-CO_2 compared to the conventional extraction method. In addition, it was found that the extract contained higher content of aromatic components that characterize Kabosu. This work provides an important sequential method for the recovery of valuable compounds from citrus fruit waste using an environmentally friendly technique.

Keywords: *Citrus sphaerocarpa*, Essential oil, Supercritical carbon dioxide, Auraptene

Background

Citrus sphaerocarpa Hort. ex Tanaka, referred to as 'kabosu' in Japanese, is one of many popular sour citruses in the same genus lemon. Kabosu trees have been mainly cultivated in Oita prefecture, and the fruits are usually being harvested in August to September while they are still unripe. The fruit ranged from 100 to 150 g in weight and from 50 to 65 mm in diameter during the harvesting time. Annual production of the fruit is around 5,000 tons, about 25-30% of which are utilized for juice processing, leaving 70-75% in the fresh-fruit market. Kabosu juice contains about 5.7% citric acid and 5.0% Brix [1], water soluble solid content such as sugar mainly. Kabosu fruits have thin and smooth peel, possessing a very pleasant smell. Due to this characteristic flavor of kabosu, its essential oils are widely used to add distinct flavor in food, beverage, pharmaceutical, perfumery, and cosmetic industries.

The development of gas chromatographic techniques of analyses has brought about significant research progress in the chemistry of citrus essential oils. Essential oils of widely well-known citruses, sweet orange, grapefruit, mandarin, lemon, lime, bitter orange, and bergamot, had been analyzed quantitatively [2]. Recently, the technique has also been applied to the composition analysis of essential oils obtained from different citrus varieties such as Tunisian citrus [3], Diamante peel [4], bitter orange [5], and commercially available citrus essential oils [6]. Results indicated that citrus essential oil consists of mostly aromatic components such as monoterpene, sesquiterpene, and some oxygenated compounds.

* Correspondence: tanaka-m@asciicorporation.co.jp
[2]Maruboshi Vinegar Co., Ltd., 2425 Tabara, Kawasaki-machi, Tagawa-Gun, Fukuoka 827-0004, Japan
Full list of author information is available at the end of the article

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It is known that the monoterpenes, such as limonene, contribute a little to the aroma, while sesquiterpenes and some of the oxygenated compounds are mainly responsible for the strong fragrances and peculiar characteristic flavor of the citrus despite low contents. Many researchers have also reported about the components of kabosu peel. Sixty-six volatile components of kabosu cold-pressed oil were identified [7]. Recently, four distinctive aroma components (citronellal, neral, linalyl acetate, and octyl acetate) like kabosu have been investigated using gas chromatography/olfactometry (GC/O) analysis [8]. The essential oil also contains a bioactive compound called auraptene [9], which is considered to be a major coumarin of citrus plants, and has proven to possess anticancer activities [10,11] and to improve metabolic syndrome [12].

Industrially, citrus essential oils are being collected from the byproducts of juice production processes. Trace amount of essential oil is also confirmed present in supernatant of juice. Various approaches to the extraction of citrus essential oils have been reported. Especially, steam distillation method has been employed for purposes of comparison with SC-CO_2 extraction method [13,14]. Their study showed that extraction method has an influence on the content of aroma compounds and yield of essential oil. Steam distillation method has also been reported to cause partial hydroxylation [15]. In the extraction of natural essential oil, the use of supercritical fluid has been proposed. Of the many possible fluids of choice, carbon dioxide is the most preferred because of its low critical temperature (304 K) suitable especially for thermally labile components. Besides, it is readily available and non-toxic. Unlike the conventional organic solvent extraction, the extraction process associated with the use of SC-CO_2 does not require complicated process to remove the solvent. Its presence in foods or beverages is generally regarded as safe and harmless for human consumption. With these properties, SC-CO_2 has been applied to extraction of natural compounds such as essential oil [16-19], seed oil [20,21], pigment [22], and bioactive compounds [23]. In general, raw materials for SC-CO_2 extraction undergo pretreatment process such as milling and drying before extraction in order to increase extraction efficiency. Excess water in the raw material has been considered to act as a barrier in the transport of the extract to the fluid [24]. However, these pretreatment steps will expose the materials for possible air oxidation due to the increase in surface area, and furthermore enzymatic denaturation caused by disruption of cell tissues [25].

In this work, SC-CO_2 extraction of essential oil from scraped wet peel of kabosu was carried out without any pretreatment. The optimum extraction condition was investigated based on the yield of essential oil, and the composition of the extracted oil was compared with the oil obtained by cold press and steam distillation methods. The work contributes further to the knowledge of SC-CO2 extraction of essential oil as applied to Japanese citrus.

Result and discussion

Comparison of the essential oil yield obtained by SC-CO_2 and other conventional methods

Figure 1 shows the yield of kabosu essential oil obtained by various extraction methods. The yield by steam distillation methods was the highest, reaching 2.32%. However, this method is not normally applied to extraction of citrus oils except for lime, because some aliphatic aldehydes, which give unpleasant odor to the oil, are also being extracted under this hydrothermal condition [25]. The yield obtained by steam distillation was used as an indication of the total amount of kabosu essential oil in the sample, and was taken as a basis for the calculation of recovery for the other methods employed.

The highest yield of SC-CO_2 extraction reached 1.55% (by weight of wet sample), which was obtained at a temperature of 353 K and pressure of 20 MPa for 300 min. This corresponds to 66.7% recovery based on the total amount of essential oil. This yield was over 13 times higher than that obtain by cold press method, which was the lowest. Based on the results, in terms of yield or recovery of essential oil, SC-CO_2 extraction method was considered to be better compared to that of the conventional cold press method. Pioana *et al.* [13] have also compared the yields obtained between wet and dry materials. They reported that the essential oil obtained was about 1.9% v/w from the fresh peel and about 8.4% v/w when dried. In this case, water content of peel decreased from 80% to 19%.

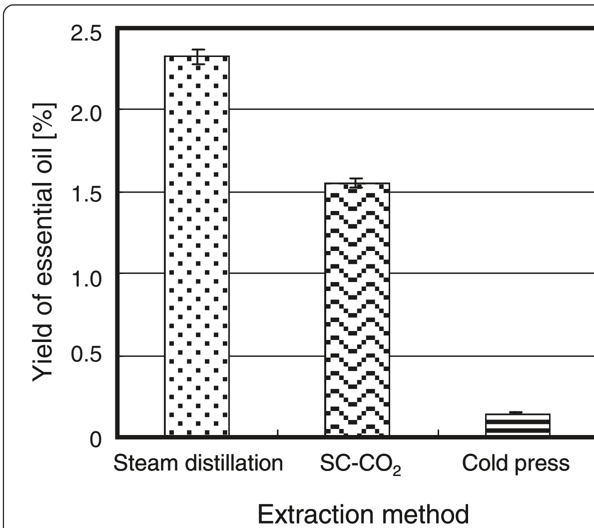

Figure 1 Comparison of kabosu essential oil yield extracted using various methods. (Error bars are standard deviations.)

GC/MS analysis of SC-CO_2 extract

All obtained extract samples were directly injected into GC/MS for component analyses. Figure 2 shows a typical GC/MS chromatogram of SC-CO_2 extract. Monoterpene hydrocarbons such as limonene and myrcene were detected as major compounds. In addition, oxygenated aldehydes such as decanal, alcohols such as nerol, esters such as neryl acetate, and sesquiterpenes such as β-farnesen were detected

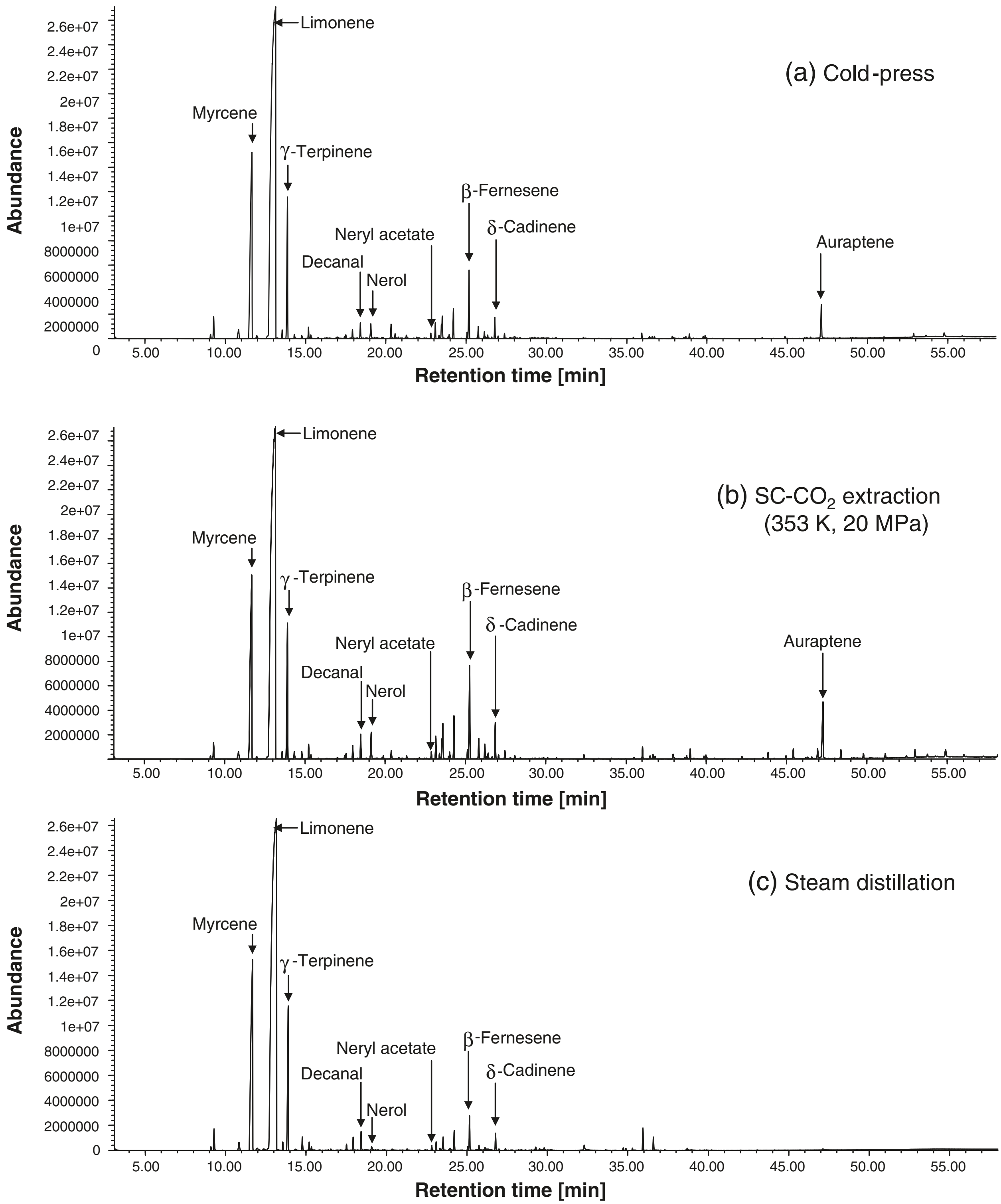

Figure 2 GC/MS chromatogram of kabosu peel extracts. (**a**) cold pressed extract, (**b**) steam distillated extract, (**c**) SC-CO_2 extract.

as the temperatures of GC oven increased. Volatile components of kabosu essential oil were previously reported in detail [7,8]. The result obtained in this study well matched those reported in above mentioned literatures. Other than the volatile components, trace amount of non-volatile compounds such as free fatty acids and their esters were detected. Coumarins, mainly auraptene, were also detected at higher temperatures.

Effect of pressure and temperature in SC-CO_2 extraction

The effects of pressure and temperature on the yield of total oil extract were studied under the following conditions: pressures at 10–30 MPa; temperatures at 313–353 K. Figure 3(a) shows the effect of pressure on the yield of essential oil extract. At pressures of 20 and 30 MPa, the yields of oil extract were higher compared to 10 MPa. At 353 K, under a pressure of 10 MPa, the density of CO_2 is 0.222 g/cm^3, which is close to that of a gas. In general, it is thought that increasing pressure at a constant temperature increases extraction rate due to apparent increase in SC-CO_2 density [24]. However, on the contrary, results in Figure 3(b) show that the yield of oil extract was obviously increasing with decreasing SC-CO_2 density. For example, at temperatures of 313, 333, and 353 K, the density of SC-CO_2 is 0.910, 0.830, and 0.746 g/cm^3, respectively, under a pressure of 30 MPa. If the density of SC-CO_2 is sufficient enough to dissolve the target compounds such as terpenes, then the temperature should also have an effect on the yield. These results indicate that solubility of essential oil in SC-CO_2 depends on both density and temperature.

Other than the solubility of essential oil in SC-CO2, its vapor pressure should also be considered an important factor for extraction. Higher vapor pressure of the oil at higher temperature allows its easy dissipation through the sample matrices, while higher diffusivity of SC-CO2 and lower surface tension also aid in the transport of the target compounds through the matrix and into the solvent resulting into higher extraction efficiency.

Figure 4 shows the physical appearances of the oil extracts. The extracted oils were yellow in color at lower pressure of 10 MPa, then gradually changed to green with increasing pressure at all extraction temperatures. In other words, the amount of extracted non-volatile compound such as pigment increases with increasing SC-CO_2 pressure due to increasing SC-CO_2 density. Therefore, it is expected that essential oils with different quality can be recovered by changing operating conditions especially that of pressure.

Optimum condition of kabosu peel extract

In order to determine the optimum condition in the range of experimental conditions investigated in this study, the yield of total extract from kabosu peel was

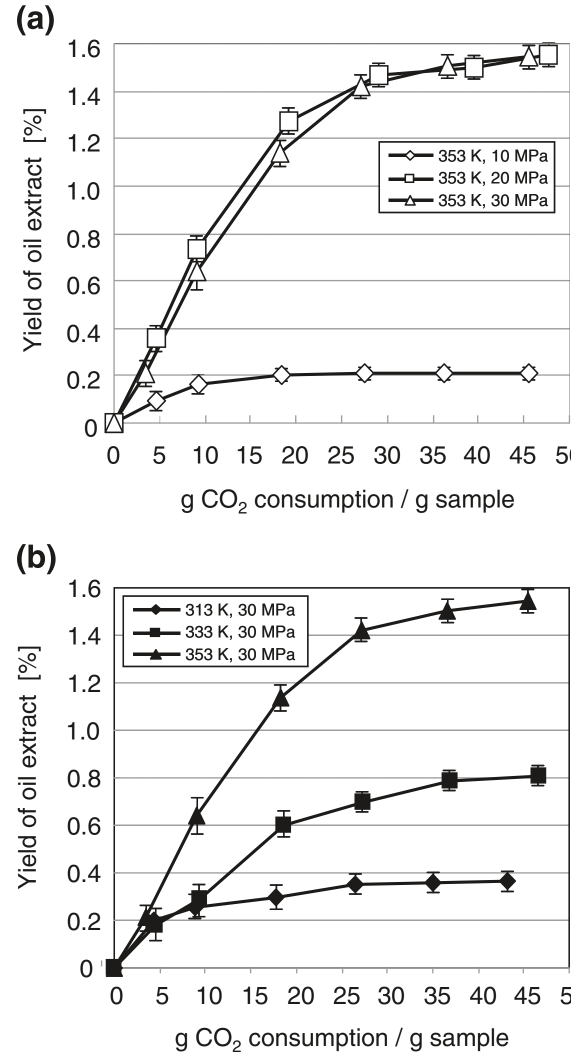

Figure 3 Effect of pressure and temperature on the yield of essential oil extract. (**a**) Extraction behavior under the constant temperature condition, (**b**) extraction behavior under the constant pressure condition. (Error bars are standard deviations.)

plotted against temperature and pressure. Figure 5 shows the yield of total extracted oil of kabosu peel at various temperatures and pressures obtained in 300 min. At a higher temperature like 353 K, the yield of essential oil increased as the pressure of SC-CO_2 increased (along with increasing density). The maximum yield of 1.55% was obtained at a pressure of 20 MPa. Similarly, the yield of essential oil increased with an increase of SC-CO_2 density at the lowest pressure of 10 MPa. However, the yield decreased when the extraction was carried out at a lower temperature of 313 K and a higher pressure of 30 MPa, even though the SC-CO_2 density was at the highest. As explained in the previous section, the yield is not only dependent on the SC-CO_2 density but also on the extraction temperature. Another likely reason for

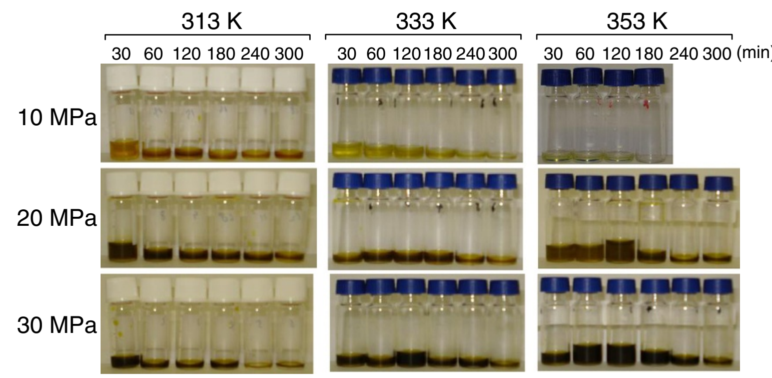

Figure 4 Physical appearances of kabosu essential oils extracted under various conditions of pressures, temperatures, and times.

this decrease in yield was the amount of water in the extracts. Water content in the extract was determined by measuring the weight of the aqueous phase. Water might have an influence on extraction process of essential oil. In most of the extracts, the water content was about 30% by weight. Exceptionally, at 313 K and 30 MPa, the water content was only 10%. Moreover, it is interesting that the SC-CO_2 density had less influence on extraction yield at 333 K. The possible reason for the influence of pressure may be the result of contradicting effects of solubility increase with fluid density, and a decrease in mass transfer rate with high pressure. The optimum condition of SC-CO_2 extraction was at the highest temperature of 353 K and at higher pressures, 20–30 MPa, having enough vapor pressure of essential oil and SC-CO_2 density.

Chemical composition of essential oils

Forty-nine compounds in kabosu oil were identified by GC/MS analyses. Figure 6 shows chemical composition of major compound groups in the essential oil extracted at various conditions classified into monoterpenes, aldehydes, alcohols, esters, sesquiterpenes, auraptene, and others. SC-CO_2 extracts have low content of monoterpenes compared to the other methods. Several researchers have compared the volatile compounds extracted by SC-CO_2 with the corresponding essential oil collected by steam distillation, the latter being the reference oil. In citrus fruit, Poiana *et al.* [13,26] reported that monoterpene hydrocarbons decreased in SC-CO_2 extracted essential oil with respect to their quantity in obtained by hydrodistillation. Correspondingly, they obtained higher percentage of sesquiterpene, aldehyde, alcohol, and ester in SC-CO_2 extracts based on GC peak areas. Using SC-CO_2 at 353 K and 30 MPa, the percentage of oxygenated compounds such as alcohol, aldehyde, and ester were a little higher compared to that of other methods. Other than the compounds detected by GC-MS analyses in this work, four oxygenated compounds, citronellal, neral, linalyl acetate, and octyl acetate, which have characteristic kabosu-like, odor were also reported [8]. Because these compounds cannot be identified in the GC/MS due to very low content, they employed sniffing test a combination of gas chromatography, GC/O analysis, to identify these compounds. From their report, these four components have a strong flavor activity despite low content. These key components were also not detected by GC/MS analysis and procedures employed in this work. However, it is expected that SC-CO_2 extract contained these four oxygenated compounds since the analysis of the extract showed to have more oxygenated compounds than that obtained from other methods as shown in Figure 6. More accurate identification procedures similar to the one employed in reported literature might confirm the presence of these compounds in the extracts. Also, other components such as sesquiterpenes and coumarins had a higher percentage compared to the other methods. Specifically, auraptene, a valuable compound, was detected and quantitated in SC-CO_2 extract. A higher SC-CO_2 density favored extraction of

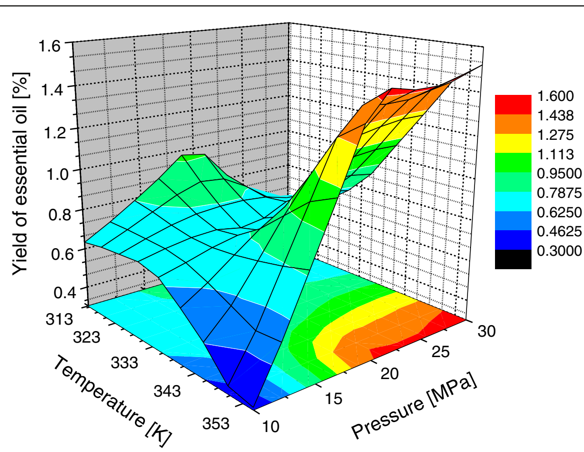

Figure 5 Pressure and temperature relations of extraction yield of kabosu peel extracts.

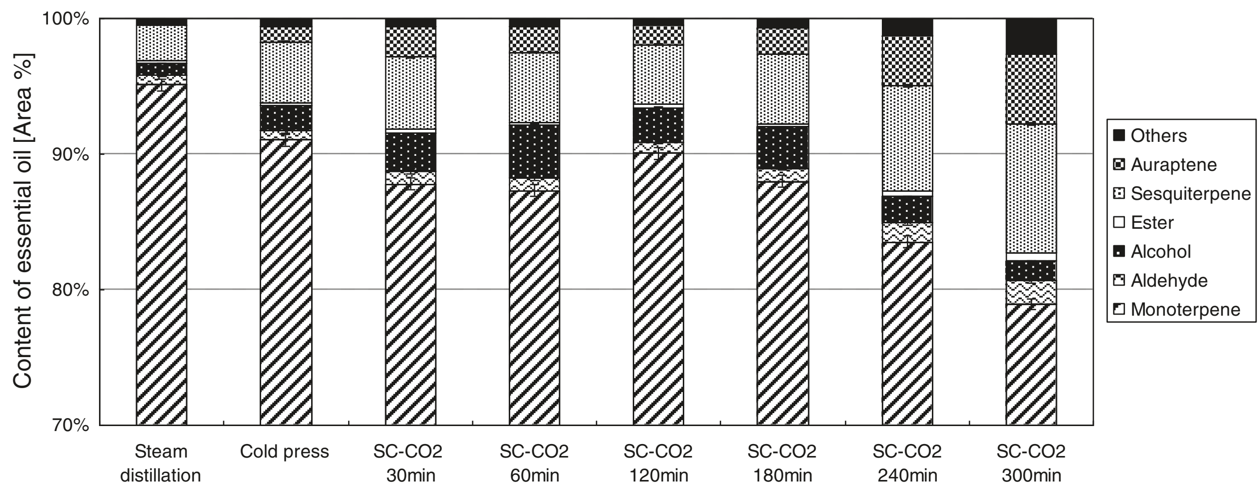

Figure 6 Comparison of chemical composition of essential oils extracted under various conditions.

non-volatile compounds such as pigments, free fatty acids, and coumarins. Not all coumarin compounds are valuable, others are phototoxic and cause allergenic effects on human skin. Martin *et al.* [27] and *Fang et al.* [28] have succeeded in separating these coumarins from oxygenated compounds by the combined method of SC-CO_2 and vacuum distillation, confirming the solubility of these kinds of organic compounds in SC-CO_2. The technique can also be applied to obtain high quality essential oil containing valuable compounds such as auraptene.

Conclusions

Essential oil was extracted from kabosu peel by employing SC-CO_2 at various temperatures and pressures. The process was optimized, and based on the results it was suggested that the solubility of essential oil from kabosu peel in SC-CO_2 was controlled by the balance between the solvent power of SC-CO_2 and the vaporization power of essential oil, which are responsible for the solubility and transport properties of essential oil with SC-CO2, respectively. As a result, the maximum yield of SC-CO_2 extraction was obtained at a temperature of 353 K and pressure of 20 to 30 MPa, at which the vapor pressure of essential oil and SC-CO_2 density were relatively high compared to other conditions. In addition, the obtained oil contained higher amounts of volatile compounds which contribute to the specific aroma, and valuable bioactive compounds such as auraptene compared to the conventional cold-press and steam distillation methods.

Methods

Materials and chemicals

Samples of kabosu fruits were harvested in October 2010 in Oita prefecture. Its peel was scraped, placed inside an air-tight wrapping plastic bag, and then stored in a freezer at 253 K until extraction experiments were performed. No pre-drying treatment was carried out on

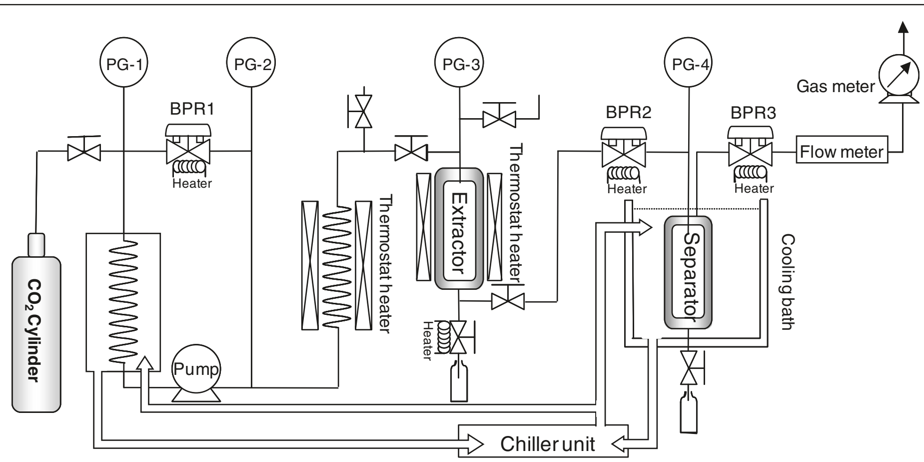

Figure 7 Schematic diagram of SC-CO_2 extraction apparatus.

the sample. The feed material contained water of approximately 80 wt%. 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 20 g of scraped citrus peel was minced with 500 mL of distilled water in a food processor, and placed into a 1 L round flask. The flask was then connected to a distilling receiver with Liebig condenser. Distillation was carried out at a temperature from 403 to 423 K for 24 h at atmospheric pressure. The essential oil evaporated with steam during the distillation process, and was separated from the condensates and collected in the distillate receiver. After reaching a certain level, the water was refluxed to distiller, and the distillation process continued.

Cold-pressed oil extraction

Approximately 50 g of scraped citrus peel was minced in 100 mL of distilled water and dispensed into 50 mL centrifuge tubes. These samples were centrifuged at 10,000 G for 15 min and frozen in a freezer at 253 K. The frozen samples were gently thawed to room temperature. These steps of centrifugation, freezing, and thawing were repeated three times. After these preparation procedures, the essential oil and wax layer were separated from the water layer. The essential oil was collected carefully to avoid mixing with the wax.

SC-CO_2 extraction

A semi-continuous flow SC-CO_2 extraction apparatus (AKICO Co., Ltd., Tokyo, Japan) shown in schematic diagram in Figure 7, 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 273 K, 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 273 K 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 and weighed right after collection, then stored at 253 K prior to component analysis. Extracts were collected every 30 min for the first 60 min, and thereafter, collected every 60 min for a total extraction time of 300 min. Extraction experiments were carried out at temperatures of 313, 333, and 353 K, pressures of 10, 20, and 30 MPa, with CO_2 flow rate of 0.19-0.21 g/s.

Yield of extracted oil

The yield was defined as the weight of extracted oils per 100 g of wet feed material.

GC/MS analysis

Qualitative analysis of the extracted essential oil was carried out using GC/MS (Hewlett-Packard - 5890 series, Palo Alto, CA, USA), coupled with a mass selective detector (HP 5972). The GC conditions were as follows: The oven temperature was initially at 313 K for 3 min, then allowed to ramp up to 553 K at a rate of 278 K min^{-1}, then to 593 K for 3 min. The injector and detector temperatures were set at 523 K. The split ratio was 12:1; with a total carrier gas (helium) flow rate of 24 mL/min; and ionizing energy of 70 eV. The injection volume was 0.02 μL. For the identification of the peaks in the chromatograms, the probability-based matching algorithm was employed for finding the most probable match in the reference library (NIST library of mass spectra and subsets, HPG 1033A). The relative composition of the components present in essential oil was calculated from the GC peak area.

Statistical analysis

All extraction experiments were duplicated. GC/MS analysis of each fraction obtained in each run was carried out in duplicate. Analysis of variance (ANOVA) was carried out using Excel Statistics 2004 to analyze the effect of temperature and pressure on the total yield of extract and composition of essential oil extract. The significance level was stated 95% with P value <0.05.

Abbreviations

ANOVA: Analysis of variance; BPR: Back-pressure regulator; GC/O: Gas chromatography/olfactometry; SC-CO_2: Supercritical carbon dioxide.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

TS carried out the experiment, analyzed component composition of essential oil, and drafted the manuscript. HI analyzed component composition of essential oil. TM, YK, CS, and YS were responsible for the experiment assistance of TS. MT, MH, JS, and MG conceived the idea of the study and designed the experiment. MT, ATQ, and MS conducted the study. All authors read and approved the final manuscript.

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Author details

[1]Graduate School of Science and Technology, Kumamoto University, 2-39-1 Kurokami, Chuo-ku, Kumamoto 860-8555, Japan. [2]Maruboshi Vinegar Co., Ltd., 2425 Tabara, Kawasaki-machi, Tagawa-Gun, Fukuoka 827-0004, Japan. [3]ASCII Co., Ltd., Kawasaki 3086-9, Kawasaki-machiTagawa-Gun, Fukuoka 827-0003, Japan. [4]Department of Bioscience and Bioinformatics, Kyushu Institute of Technology, Kawazu 680-4, Iizuka, Fukuoka-ken 820-8502, Japan.

[5]Department of Chemical Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan.

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