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Antioxidant and antimicrobial activity of oils obtained from a mixture of citrus by-products using a modified supercritical carbon dioxide

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ABSTRACT

This study investigated the impact of combining citrus seeds and citrus peels on the bioactivity of the resulting oil obtained using a modified supercritical carbon dioxide. The total phenolic and total flavonoid contents were determined, and the citrus-peel and citrus-seed oils exhibited high content. In addition, antioxidant activity was determined, and the oil extracted using SC-CO_2 + ethanol at 200 bar exhibited high IC_{50} values of 0.52 and 0.53 mg/ml for citrus-peel alone and mixture oils, respectively, for the DPPH assay. Oil from the mixture exhibited high antimicrobial activity, and the oils were more susceptible for gram-positive than gram-negative bacteria.

Introduction

Consumption of citrus fruits, either as fresh produce or in the juice form, is common due to their dietary benefits and particular flavor. These fruits are broadly grown around the world with a yearly production of about 102 million tons [1]. The production of juice and other products from citrus fruits results in the generation of large amounts of citrus by-products every year. This not only wastes useful materials but also may pose some pollution, disposal, and other related environmental problems due to microbial spoilage [2]. These citrus by-products can be valorized since they contain a wide range of healthy bioactive compounds [3,4].

Citrus peels have a high proportion of natural flavonoids and are among the rich sources of phenolic compounds [5]. In addition, several compounds including flavanone glycosides, polymethoxylated flavones, and flavanones that are unique to citrus have been found to be comparatively rare in other plants [6,7]. It has been reported that the citrus peel extracts demonstrate high antioxidant activity [5] and exert antimicrobial effects against food-borne pathogens [7,8] due to the present quinones, terpenoids, polyphenols, phenolic acids, and tannins [9–11]. Citrus seeds are other by-products of citrus fruit processing. Although many researchers have paid much attention to citrus peels, the importance of citrus seeds has been also studied owing to the present diverse compounds including polyphenols, tocopherols, phytosterols, and high amount of unsaturated fatty acids that can be useful for adding value to many products [4,12,13].

The different techniques utilized to obtain extracts from plant matrices include solvent extraction and distillation among others [14]. However, those techniques have some drawbacks such as long extraction time, volatile compound loss, residues of toxic substances, and unsaturated compound degradation due to high temperature [15,16]. The supercritical carbon dioxide (SC-CO_2) extraction of natural products has recently drawn attention by many researchers. This type of extraction is not only eco-friendly but also affords the minimum degradation of bioactive compounds (since CO_2 has a close-room critical temperature of 31 °C), and the prospect of getting solvent-free products [17] has made this a promising technique. In SC-CO_2 extraction, the solvating power of SC-CO_2 fluid can be increased or decreased by manipulating pressure and/or temperature, resulting in high selectivity. Moreover, the separation of dissolved solutes and SC-CO_2 could be simply performed via depressurization [18]. However, the limitation of CO_2 for extraction of polar compounds due to its non-polar characteristic has been a challenge for extracting polyphenols and other polar compounds. Nonetheless, SC-CO_2 polarity can be improved by incorporating modifiers such as ethanol, methanol, and water [14,19]. Therefore, the use of SC-CO_2 extraction with ethanol as a modifier can not only afford a high bio-potentiality extract but also might help in eliminating or notably decreasing the need for eco unfriendly organic solvents [20].

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Although many studies have been dedicated towards studying citrus by-products [21,22], to the best of our knowledge, no study on the bioactive compounds and the antioxidant and antimicrobial activities of oils resulting from a combination of citrus peels and citrus seeds either by using neat SC-CO_2 or modified SC-CO_2 extraction has been conducted. The combination of citrus peels and citrus seeds may cause increase in bioactivity of the resulting oils owing to the synergistic effect of the compounds they contain and may enhance the bioavailability of some active compounds [23,24]. Therefore, this study was designed to study the effect of combining the citrus peels and citrus seeds on the bioactive compounds and the antioxidant and antimicrobial activities of the resulting oils to assess if their bioactivity can make them applicable in many fields.

The purpose of the present study was threefold: First, to extract the oils from citrus seeds, citrus peels, and the mixture of citrus seeds and peels using neat SC-CO_2 or SC-CO_2 with ethanol as a modifier. Second, to determine the total phenolic, total flavonoid, tocopherol, and phytosterol contents of the extracted oils. Third, to study the antioxidant and antimicrobial activities of the extracted oils and assess whether these oils exhibit potential bioactivity, so they can be used for different applications.

Materials and methods

Chemicals

Folin–Ciocalteu's reagent, gallic acid, quercetin, tocopherol standards (α-, β-, γ- and δ-tocopherol), sterol standards (brassicasterol, campesterol, stigmasterol, sitosterol and Δ-avenasterol), 1,1 diphenyl-2-picryl-hydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), Tryptone Soy Broth (TSB), and Mueller–Hinton agar (MHA) were purchased from Sigma–Aldrich (St. Louis, USA). Ethanol, methanol, and 2, 3, 5-triphenyltetrazolium chloride (TTC) were purchased from Samchun Company (Busan, South Korea). Carbon dioxide (99.99%) was obtained from KOSEM Company (Busan, South Korea). All the chemicals and reagents were of HPLC or analytical grade.

Sample collection and preparation

The citrus fruits (*Citrus junos*), common name: Yuzu, provenance: Namhae-gu, Gyeongsangnam-do Province (Busan, South Korea) provided given by Y.G., Co. Citrus fruits were cleaned and peeled, and the peels and seeds were collected. The citrus peels were freeze dried (−50 °C for 4 days) while the citrus seeds were oven dried at 103 °C and then crushed and sieved (using a 710-μm metal sieve) to obtain the powder for extraction.

Extraction procedure

The SC-CO_2 extraction diagram used in this study is shown in Fig. 1. For neat SC-CO_2 (without ethanol) extraction, the extraction setup and procedures were the same as those reported in our previous work [25]. For modified SC-CO_2 (with ethanol) extraction, a second pump was connected to the extraction line, which supplied ethanol (ca. 1 ml/min: flow rate) and then mixed with CO_2. The CO_2 + ethanol passed through a heat exchanger and then flowed through the sample in the extraction vessel. The ethanol-oil mixture was received in a vial, whereas ethanol-saturated CO_2 left through the flow meter. The residual ethanol was removed using a rotary evaporator (Model N-1100, Eyela, Japan).

The extraction (either neat or modified SC-CO_2) was performed at the temperature of 45 °C, the pressure of 200 and 300 bar, the extraction time was 2 h, and the CO_2 flow rate was 27 g/min.

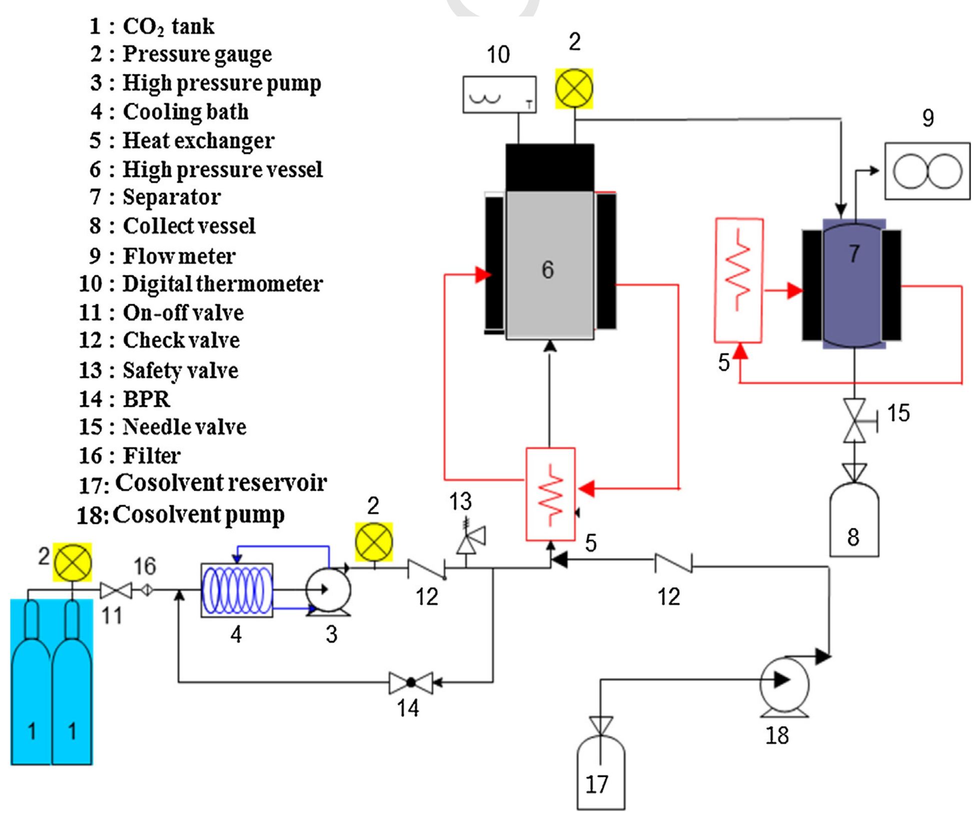

Fig. 1. Schematic diagram of SC-CO_2 and co-solvent extraction process.

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