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Effect of temperature and pressure on the extraction of strawberry receptacles with a mixture of supercritical carbon dioxide and entrainers

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ABSTRACT

Strawberries are one of the most popular fruits in Japan and they have a high antioxidant content. In this study, the contents in strawberry receptacles were extracted with supercritical carbon dioxide and entrainers. Using ethanol, acetone, and water as entrainers improved the antioxidant capacity and increased the amounts of total saccharides and cinnamic acid in the extract. In supercritical carbon dioxide + ethanol, the extract yield increased slightly with increasing pressure up to 20 MPa at 313 K whereas the extract yield was almost constant against temperature. The amounts of total saccharides and cinnamic acid increased with increasing temperature and pressure. The antioxidant capacity increased, and then decreased with increasing temperature at 20 MPa and increasing pressure at 313 K. The optimal conditions for antioxidant capacity were 313 K and 20 MPa.

1. Introduction

Strawberries are one of the most popular fruits in Japan and about 160,000 tons are produced each year. Strawberries have high antioxidant properties because they contain antioxidants such as phenolics, anthocyanins, and vitamin C [1–4]. Strawberry receptacle is red and main edible part of it. The main content of strawberry receptacles is moisture (90 wt%), and the amount of total phenolics is a few hundred micrograms per 100 g of sample. The extraction of useful fractions, such as antioxidant components from strawberries, is expected to be developed for new health foods and supplements.

Supercritical carbon dioxide (sc-CO_2) is a useful solvent for extracting natural products [5]. sc-CO_2 is an inert, non-toxic, and environment-friendly solvent and its critical temperature and pressure are 304.2 K and 7.4 MPa, respectively. The critical temperature and pressure are low enough to enable sc-CO_2 extraction at low temperatures and pressures, which suppresses the thermal denaturation of the extract that occurs at high temperatures and makes the construction of extraction systems easier. sc-CO_2 is rapidly taken up by osmosis into samples to achieve effective extraction because of its high diffusivity and low density, viscosity, and surface tension compared with those of liquid CO_2. Furthermore, the sc-CO_2 density changes greatly with temperature and pressure, which alters the solubility of compounds. sc-CO_2 dissolves lipophilic compounds because it is a nonpolar solvent. The addition of a polar entrainer, such as methanol, ethanol, or water, allows the extraction of polar components that are difficult to dissolve in pure sc-CO_2.

The extraction of berries with sc-CO_2 has been reported previously (Table 1). In these studies, the temperature was from 303 to 333 K and the pressure was from 8 to 45 MPa. These studies are classified as using an entrainer or not using an entrainer. Without the entrainer, the seed extracts of various northern berries, including strawberries, contain seed oil including fatty acids and tocopherols or tocotrienols [6]. The antioxidant activity of blueberry, cranberry, and raspberry extracts is as large as that of conventional solvent extracts, and the trends in the amounts of extract and phenolics against pressure depend on the type of berry [7]. For raspberries, the amount of extract increases with increasing pressure [8]. Entrainers can be used to improve the extraction. The mixture 90% CO_2 + 5% H_2O + 5% ethanol gives the highest amount of anthocyanins in the extraction of blueberries because anthocyanins are water-soluble [9]. In the extraction of sea buckthorn berries, both the antioxidant activity and the amount of carotene in the extract obtained with methanol as the entrainer are larger than those obtained with ethanol and 2-propanol [10]. In the extraction of elderberry pomace in the presence of ethanol and water, the solvent composition affects the extract yield and the properties of the extract, such as the total amount of phenolic compounds. These results indicate that ethanol and water play an important role in the extraction of anthocyanins [11]. Pretreatment with sc-CO_2 and sc-CO_2 + ethanol before extracting elderberries with ethanol or ethanol aqueous solution is effective for extracting polyphenols [12]. The extraction of blackberries was enhanced by ultrasonication [13] in sc-CO_2 + ethanol or water.

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Table 1
Summary of studies on the extraction of various berries with sc-CO_2.

Material	T [K]	P [MPa]	Entrainer	Reference
Wild bilberries, lingonberries, arctic cranberries, crowberries, cloudberries, wild sea buckhorn berries, cultivated raspberries, blackcurrants, redcurrants, snowball berries, European rowanberries, strawberries	323	35	None	[6]
Blueberries, cranberries, raspberries	333	8–30	None	[7]
Raspberries	303–333	10–45	None	[8]
Blueberries	313	15–25	Ethanol, water, mixtures	[9]
Sea buckthorn berries	308–328	15–35	Methanol, ethanol, 2-propanol	[10]
Elderberries	313	21	Ethanol, water, mixtures	[11]
Elderberries	313	15–30	Ethanol	[12]
Blackberries[a]	313–333	15–20	Ethanol, water	[13]
Strawberries	308–333	10–30	Ethanol, water, acetone	This study

[a] Assisted by ultrasound.

For strawberries, extraction has been performed on the seeds at only a single temperature and pressure. The extraction of strawberry receptacles has not been studied; therefore, the effect of solvent properties on the extraction is not known.

In this study, we examined the extraction of strawberry receptacles with sc-CO_2 and sc-CO_2 + entrainer. The extracts obtained with sc-CO_2 and sc-CO_2 + entrainer were compared to elucidate the effect of the entrainers. The dependence of the temperature and pressure on the extract yield, antioxidant capacity, and the amount of product were evaluated at 20 MPa and 313 K, respectively.

2. Experimental

Strawberry receptacles grown in Tochigi Prefecture in Japan were harvested and frozen. The moisture content of the strawberry receptacles was determined as 89.7 wt% by a moisture analyzer (MOC-120H, Shimadzu Corp.). The frozen strawberry receptacles were defrosted at room temperature and cut up. Ethanol (> 99.5%) and acetone (> 99.5%) were purchased from Wako Pure Chemical Industries Ltd. or Kanto Chemical Co., Inc. 1,1-Diphenyl-2-picrylhydrazyl (DPPH; > 97%) was purchased from Tokyo Chemical Industry Co., Ltd. Distilled water was obtained from a water purifier (WG-222, Yamato Co.). The purity of the CO_2 was 99.5%.

Fig. 1 shows the experimental apparatus. The extractor was made of stainless steel 316 (SUS 316) 1 in. port connectors (Swagelok) connected by a SUS 316 1 in. union (Swagelok). Both ends of the port connectors were connected to two SUS 316 0.5–1 in. reducing unions that had 0.125–0.5 in. reducers on both sides (Swagelok). A stainless steel wire mesh (pore size: 38 μm) was used to keep the sample in the extractor. The internal volume of the extractor was 32.8 cm^3. Initially, the prepared strawberry receptacles (15 g) were placed in the extractor and each end of the extractor was connected to the apparatus. The extractor was placed in a gas chromatography oven (GC-8A, Shimadzu Corp.).

The internal space of the extractor was purged with CO_2 and the temperature was increased to the extraction temperature. CO_2 and an entrainer were supplied to the extractor and the extraction started. CO_2 was supplied with a CO_2 delivery pump (SCF-Get, JASCO Corp.) and the entrainer was supplied with a high-performance liquid chromatography (HPLC) pump (PUS-3, GL Science Inc.). The typical CO_2 flow rate and the molar ratio of entrainer to CO_2 were 3.95×10^{-5} mol/s and 0.1, respectively. CO_2 and the entrainer were mixed, and then the mixture was preheated through the preheater and fed to the extractor. The extraction was conducted for a predetermined period of time, and then only CO_2 was supplied for about 30 min to recover the extract remaining in the pipes. The extract was recovered at the trap after reducing the pressure with a back pressure regulator (SCF-Bpg/M, JASCO Corp.). The extract was typically recovered after 120 min. To evaluate the time profile, the extract was recovered every 30 min by changing the trap. The CO_2 flow rate was measured with a rotary flow meter (DC-1, Shinagawa Co., Ltd.).

The extract was dried in an evaporator (Smart Evaporator, BioChromato, Inc.) and weighed. Ethanol was added to the dried extract for analysis. HPLC analysis was conducted by injecting a water and ethanol solution of the extract into an HPLC system (Prominence, Shimadzu Corp.) with an ODS column (Intersil ODS-4, GL Science Inc.) monitored at 254 nm. The peak for *trans*-3-phenylacrylic acid (cinnamic acid) was determined by fractionation followed by ^1H NMR analysis. The antioxidant capacity was evaluated by the DPPH radical-scavenging method [14]. 0.5 mM DPPH solution (3 mL) was added to an

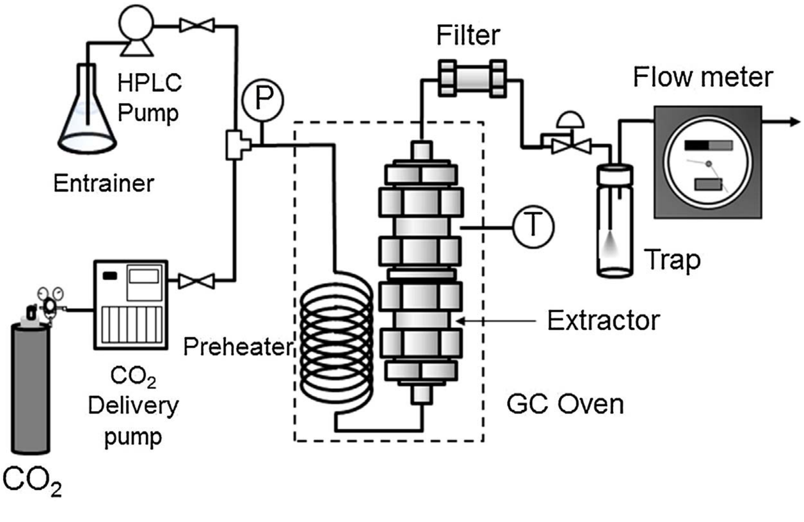

Fig. 1. Schematic of experimental apparatus.