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Integrated utilization of red radish seeds for the efficient production of seed oil and sulforaphene

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ABSTRACT

Supercritical CO_2 was used to obtain seed oil from red radish seeds. The influence of pressure, temperature, CO_2 flow rate and time on extraction yield of oil were investigated in detail. The maximum extraction yield of oil was 92.07 ± 0.76% at the optimal extraction conditions. The physicochemical properties and fatty acid composition of oil indicated that the seed oil can be used as a dietary oil. Meanwhile, the high purity sulforaphene (96.84 ± 0.17%) was separated by solvent extraction coupled with preparative high performance liquid chromatography from red radish seed meal. The initial pH, *R*, extraction temperature and extraction time for each cycle had a considerable influence both on the extraction yield and purity of sulforaphene of crude product. The extraction of oil was directly responsible for an increase of 18.32% in the yield of sulforaphene.

1. Introduction

Red radish (*Raphanus sativus* L.) is an important vegetable crops belonging to Cruciferae (or Brassicaceae) family in China. It has been used as a traditional Chinese herbal medicine for more than 1400 years, since being recorded in "Tang Materia Medica", the first Chinese pharmacopoeia (Duan et al., 2006). There is sufficient epidemiological evidences indicating that diet rich in cruciferous vegetables such as broccoli, cabbage, kale, cauliflower and radish, is associated with a reducing risk of developing many cancers and cardiovascular diseases as the presence of various phytochemicals, especially glucosinolates, polyphenols and flavonoids (Higdon, Delage, Williams, & Dashwood, 2007; Joseph et al., 2004; Lee & Lee, 2006; Lima, Rocha, Takaki, Ramos, & Ono, 2008; Truong, Baron-Dubourdieu, Rougier, & Guenel, 2010). Different parts of radish, including the roots, seeds, and leaves, have various medicinal properties. The radish seeds have been used to treat asthma and other chest complaints.

Surplus radish seeds after sowing of red radish remain unutilized because they lose viability with time. And the seeds contain from 30% to 50% of their weight of oil (Ahuja, Singh, Raheja, & Labana, 1987). Domings, Saad, Wilhelm, and Ramos (2008) reported that the seed oil was used to produce biodiesel and the condition for the ethanolysis of seed oil was optimized applying the response surface methodology. Ávila and Sodré (2012) compared physicochemical properties and thermal behavior between fodder radish crude oil and biodiesel. They found that fodder radish biodiesel can meet physicochemical property specifications, although its acid number requires attention. Ahuja et al. (1987) investigated the oil content and fatty acid composition seeds in seven genotypes of radish. However, the physicochemical properties of oil as well as the fatty acid composition have not been reported for red radish seeds. After oil extraction, the red radish seed meal is also often untapped for commercial and industrial use.

Several authors have studied the separation of isothiocyanates and sulforaphene (4-isothiocyanato-(1R)-(methylsulphinyl)-1-(E)-butene, $C_6H_9NOS_2$) from radish seeds due to these potential role in the prevention of cancer and other chronic and degenerative diseases. Especially, sulforaphene was strongly associated with cancer prevention (Papi, Orlandi, Bartolini, Barillari, & Iori, 2008), and 1.3–1.5 times more active than sulforaphane (1-isothiocyanato-(4R)-(methylsulphinyl)butane, $C_6H_{11}NOS_2$) (Shishu, 2009). In studies to date, solvent liquid–liquid extraction (Beevi, Mangamoori, Subathra, & Edula, 2010), normal-phase liquid chromatography (Brinker & Spencer, 1993), macroporous resin coupled with semi-preparative high performance liquid chromatography (Kuang, Song, Yuan, Lv, et al., 2013) and high-speed counter

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current chromatography (Kuang, Song, Yuan, Yi, et al., 2013) have been developed for separation and purification of sulforaphene, due to the large amounts of oil contaminants.

The aim of this present paper was to investigate the integrated utilization of red radish seeds for the efficient production of seed oil and sulforaphene. The extraction conditions of seed oil from red radish seeds by supercritical CO_2 was optimized. The physicochemical properties and fatty acid composition of oil were evaluated in detail. After oil extraction, the meal of red radish seeds was used to produce high purity sulforaphene by solvent extraction coupled with preparative high performance liquid chromatography (HPLC). The influence of the ratio of organic phase to aqueous phase, initial pH, extraction temperature and extraction time on the solvent extraction of sulforaphene were also studied.

2. Materials and methods

2.1. Materials

Red radish seeds were provided by Haiju Agriculture Development Co. Ltd., Chongqing, China. These seeds were dried at 30 ± 5 °C for 8 h using a floor drier and ground in a blender to uniform particle size of approximately 250 μm. The particle size was determined using standard I.S. sieve. The ground seeds were kept in the dark color glass bottles with an air-tight seal which were stored in the refrigerator at stored at −20 °C till experiment begins. Carbon dioxide having purity 99.99% was supplied by Jinsheng Gas Supplier, Chongqing, China. Sulforaphene standard was purchased from Enzo Life Science, New York, American. SP 700 and SP 850 resins were purchased from Green-herbs Science and Technology Development Co. Ltd., Beijing, China. Methanol used for HPLC was of chromatography grade and purchased from Titan chemical, Kuala Lumpur, Malaysia. All other reagents were of analytical grade and used without further purification.

2.2. Experimental methods

2.2.1. Determination of moisture and oil content

The moisture content of red radish seeds was determined according to AOCS standard method (2005). The oil content was determined using the method of AOAC (2008). Approximately 10 g of ground seeds was taken in cellulose thimble and it was kept in Soxhlet apparatus. The oil was then extracted using hexane for 12 h. After the extraction, the solvent was removed at 40 °C in a rotary evaporator, cooled and then the residual oil was weighed. All experimental results reported are average values from three repeated independent experimental runs.

2.2.2. Supercritical CO_2 extraction of red radish seed oil

Supercritical CO_2 extraction (SC-CO_2) of red radish seed oil was performed by a supercritical fluid extraction system (HA 221-50-06 Model, NTHA, Jiangsu, China). About 200 g of ground seeds were loaded into the extraction vessel of 1 L capacity. The extraction was carried out at pressure of 10–50 MPa, temperature of 20–60 °C, CO_2 flow rate of 0.10–0.60 kg $min^{-1}$and time of 30–100 min. The extract (oil) and seed meal were collected after extraction. The oil was stored under a stream of N_2 at room temperature (20–25 °C). The extraction yield was determined by comparing the oil content obtained by SC-CO_2 to the oil content obtained by Soxhlet extraction.

2.2.3. Optimization of red radish seed oil extraction

An orthogonal $L_9(3^4)$ test design was used to investigate the optimal extraction condition of red radish seed oil. The extraction experiment was carried out with four factors and three levels, namely extraction pressure (30, 35, 40 MPa), extraction temperature (40, 45, 50 °C), CO_2 flow rate (0.40, 0.45, 0.50 kg min^{-1}) and extraction time (70, 80, 90 min). The range of each factor level was based on the results of preliminary experiments. The extraction yield of seed oil was the dependent variable.

2.2.4. Physical and chemical analysis of red radish seed oil

The refractive index of red radish seed oil was determined using a WYA-2W refractometer (INESA Instrument, Shanghai, China). The acid value, peroxide value, saponification value and iodine value of oil were determined according to standards ISO (1996, 2001, 2002, 2009), respectively.

2.2.5. Determination of the fatty acid composition

The preparation of fatty acid methyl esters and fatty acid composition of red radish seed oil extracted by SC-CO_2 were studied as described by Nehdi (2011). And the procedure was used without any modification.

2.2.6. Preparation of crude product of sulforaphene

The obtained red radish seed meal was added into citrate buffer solution (0.1 M, pH 4.5), and the ratio of liquor to material was 12:1. The spontaneous hydrolysis of the glucosinolates occurred by endogenous myrosinase enzyme at temperature of 35 °C for 7 h to produce sulforaphene. Then the pH value of the mixture was adjusted to 1.0 with hydrochloric acid (6 mol L^{-1}) and centrifuged with a high speed centrifuge (18,000 rpm) to remove plenty of proteins.

And the clarified solution was processed further by two separation methods to prepare crude product of sulforaphene. One was solvent extraction, and ethyl acetate, dichloromethane and hexane were used individually for extracting sulforaphene. The extraction conditions were as follow: the ratio of organic phase to aqueous phase (R, v/v), 1.0, the initial pH value of the clarified solution, 2.0, temperature, 25 °C. The extraction was conducted three cycles and performed for 30 min in each cycle. The extracts were dried at 40 °C in a rotary evaporator, the crude product of sulforaphene was obtained. The other was to use macroporous resins (SP 700 and SP 850) to concentrate sulforaphene. The resins needed to be pretreated prior to use. The resin was soaked in ethanol for 24 h and then washed with ethanol until there was no turbidity when a threefold volume of water was added into the eluent. The resins were subsequently washed with deionized water until the ethanol was thoroughly replaced. The pretreated resin (10 g) was dispersed in the clarified solution (150 mL). The mixture was shaken 60 rpm at 25 °C for adsorption and the concentration of sulforaphene was determined by an analytical HPLC system (see Section 2.2.9 for full details). Then the resin loaded sulforaphene was desorbed with 80% ethanol solution at 25 °C. The concentration of sulforaphene was determined when the desorption equilibrium was reached. The ethanol and water of the desorption solution were removed by a rotary evaporator at 40 °C, and the crude product of sulforaphene was obtained accordingly.

2.2.7. Optimization of sulforaphene extraction

Based on preliminary experiments, the extraction was conducted two cycles with ethyl acetate at room temperature (20–25 °C). A $L_4(2^3)$ orthogonal matrix with three factors, each factor containing two levels was selected to arrange the experiments. R were 0.5 and 1.0, the initial pH value were 1.0 and 2.0, and extraction time for each cycle were 20 and 30 min. The purity of sulforaphene was selected as the dependent variable.

2.2.8. Preparation of high purity sulforaphene

In order to obtain high purity sulforaphene product, the preparative HPLC purification method was used. The preparative HPLC