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A green separation process for recovery of healthy oil from pumpkin seed

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ABSTRACT

In this study, pumpkin seeds, called as "*Ürgüp Sivrisi*" and grown in Cappadocia region, were used as plant materials because of high aroma contents. In the supercritical fluid extraction of pumpkin seed oil, the effect of main process parameters as the particle size (250–2360 μm), the volumetric flow rate of supercritical solvent (0.06–0.30 L/h), the operating pressure (20–50 MPa), the operating temperature (40–70 °C), the type of entrainer (ethanol and *n*-hexane) and those concentrations (0–10 vol.%) on the extraction yield, the oil solubility and the initial extraction rate were investigated. A cross-over effect for the extraction of pumpkin seed oil using supercritical CO_2 was determined at the operating pressure of 20–30 MPa. The maximum extraction yield obtained with entrainer free was reached 0.50 g oil/g dry seed at 600–1180 μm, 0.12 L/h, 50 MPa and 70 °C for the operation time of 5 h. The maximum extraction yield obtained with ethanol as an entrainer in the experiments was reached 0.54 g oil/g dry seed at the conditions of 600–1180 μm, 0.12 L/h, 30 MPa, 40 °C and 8 vol.% for the operating time of 2 h. The oil compositions were determined by gas chromatography analysis and the results showed that the compositions of pumpkin seed oil which were obtained by means of organic solvent extraction and supercritical fluid extraction were similar. The average oil compositions determined as 9.3 (±0.43)% palmitic acid, 7.5 (±0.6)% stearic acid, 32.3 (±0.6)% oleic acid, 48.1 (±0.6)% linoleic acid and 0.7 (±0.3)% linolenic acid. The morphological changes in the seeds were determined by the scanning electron microscopy analysis.

1. Introduction

Pumpkin seed is a by-product of pumpkin fruit production. The amount of oil found in different kinds of pumpkin seeds varies from 40 to 60 wt.%. The majority of fatty acids in pumpkin seed oil consists of palmitic acid (C16:0, 9.5–14.5 wt.%), stearic acid (C18:0, 3.1–7.4 wt.%), linoleic acid (C18:2, 35.6–60.8 wt.%) and oleic acid (C18:1, 21.0–46.9 wt.%) [1]. Moreover, pumpkin seed is a rich source for medical applications and includes various bioactive compounds such as ω-3, -6 and -9 fatty acids, α- and γ-tocopherols, Δ5- and Δ7-sterols, β-carotene and lutein [1–4]. Pumpkin seed has received considerable attention in recent years because of the nutritional properties and the protective effects on health [1,2]. Nowadays, pumpkin seed oil is to be popular for future medical research due to its medical properties such as antidiabetic, antihypertensive, antitumor, immunomodulation, antibacterial, antihypercholesterolemia, intestinal antiparasitia, anti-inflammation, and antalgic [5].

The industrial production of pumpkin seed oil is produced by hydraulic cold pressing [4]. On the other hand, the oil production from plant seeds by hydraulic cold pressing has a major drawback since the level of residual oil left in the seed is high and varies from 5 to 15 wt.% [6]. Although *n*-hexane is a traditional solvent used commercially in solid–liquid extraction of oils from various plant materials, its usage is under greater scrutiny due to increasing governmental restrictions and consumer concerns regarding the safety of the use of organic solvents in food processing. The usage of hydraulic cold pressing is expanding in an effort to claim not processed with organic solvents in the product label. Consequently, supercritical CO_2 ($scCO_2$) has become a viable alternative to replace *n*-hexane and meet the growing consumer demand for natural products. Either $scCO_2$ or its supercritical solvent mixtures modified with ethanol are often promoted as a green solvent in green separation process [7]. Supercritical fluid extraction (SFE) is based on a very well-known high technology. In particular, $scCO_2$ has been used for extraction processes for many years and it has been developed for more and more applications. Supercritical fluids (SCFs) provide high solubility and improve mass transfer rate, also the extraction efficiency can be manipulated by changing the operating pressure or the operating temperature.

Several authors have studied the physical chemical characteristics of pumpkin seed oil obtained by $scCO_2$ extraction or by organic solvent extraction, each sample being obtained from different fruits originating from diverse plantations in several countries [8–11]. Wenli et al. [8] compared two pumpkin seed oils extracted from different pumpkin seed kinds using $scCO_2$. Optimal separation conditions were reported as the operating pressure range

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of 25–30 MPa, the operating temperature of 45 °C and the CO_2 mass flow rate range of 30–40 kg/h for the particle size range of 180–250 μm and the operating time of 2 h. In scientific literature of $scCO_2$ extraction of pumpkin seed oil, two statistical experiment designs based on the central composite non-factorial design method [9] and the central composite rotatable design method [10] were used to characterize the influence of process variables and arrive at optimal processing conditions. Bernardo-Gil et al. [9] selected the process variables as the operating pressure range of 18–20 MPa, the operating temperature range of 35–45 °C and the CO_2 superficial velocity range of 0.050–0.086 cm/s. They reported that the maximum oil recovery for 2 h was reached at 19 MPa, 35 °C and 0.060 cm/s. In the latter study, Mitra et al. [10] selected the process variables as the operating temperature range of 45–75 °C, the operating pressure range of 15.16–31.48 MPa and the operating time range of 30–120 min. They reported that the maximum extraction yield was 30.7 wt.% at 32.14 MPa, 68.1 °C, 94.6 min for a constant particle size of 500 μm and a constant volumetric flow rate of 0.25 L $scCO_2$/min. These studies also report that there is no significant difference in the fatty acid profiles of pumpkin seed oils obtained by $scCO_2$ extraction and by organic solvent extraction [8–10]. In a mathematical modeling study, the extraction yield for 7 h was reported as 0.49 g/g seed by Nagy et al. [11]. They obtained this result with only one operating condition of the operating pressure, the operating temperature, the particle size and the CO_2 mass flow rate being 45 MPa, 60 °C, 685 μm, 6.94 kg/h, respectively. Unfortunately, the knowledge about $scCO_2$ extraction of pumpkin seed oil in scientific literature was evaluated only after a few hours following extraction operation. To make an evaluation about the impact of process variables on the extraction yield, recovery and composition of the extracted oils, by using these literature data, is very difficult.

In this study, the effect of main process parameters as operating pressure, operating temperature, particle size, volumetric $scCO_2$ flow rate and entrainer concentration using both $scCO_2$ + ethanol mixture and $scCO_2$ + *n*-hexane mixture on the extraction yield of pumpkin seed oil was investigated in a semi-continue supercritical fluid extraction system. Even though there are numerous studies on $scCO_2$ extraction of pumpkin seed oil, the scientific literature is deficient in the particle size range, the entrainer types and its concentration on the extraction yield, the oil solubility and the initial extraction rate.

2. Materials and methods

2.1. Regents

Carbon dioxide with a purity of 99.995% was supplied by Hat-Grup Company (Kocaeli, Turkey). Ethanol (absolute) was provided from Riedel-de Haën (Seelze, Germany) and *n*-hexane (LiChrosol®) was provided from Merck (Darmstadt, Germany).

2.2. Raw material and pretreatment

Pumpkin seeds were obtained from *Cucurbita pepo* L. fruits which are grown in the Cappadocia region at Turkey. The seeds, called as Ürgüp Sivrisi, were provided by Simaksan Company (Nevşehir, Turkey). The shells of the seeds were separated manually. The deshelled seeds were used in all extraction experiments after grinding operation by means of a coffee grinder (Braun Coffee Grinder, KSM2 Model, Frankfurt, Germany). After grinding operations, ground pumpkin seeds were separated to four particle size ranges with sieve analysis (Retsch Type AS 200 Basic, Haan, Germany). The particle size ranges of fractions were determined such as 250–600 μm, 600–1180 μm, 1180–1700 μm and 1700–2360 μm. Samples were placed in the dark color bottles and were kept in the refrigerator at 4 °C up to use in the extraction experiments. All the above transactions took place in a nitrogen atmosphere.

2.3. Determination of moisture and oil content

The moisture content in pumpkin seeds was determined according to the AOCS methods Ca-2c-25 [12]. The moisture content of pumpkin seeds was found to be 6.96 (±0.03)% by mass. The total oil content was determined by Soxhlet apparatus using *n*-hexane for 18 h. The solvent was removed at 40 °C in a rotary vacuum evaporator (Büchi Rotavapor R-114 Model, Flawil, Switzerland) under a nitrogen stream. The oil content determined for 18 h was 47.2 (±0.1) wt.% by dry mass. Each value is the mean of the three repetitions.

2.4. Supercritical fluid extraction system

Supercritical fluid extraction of pumpkin seed oil was performed by a semi-continuous supercritical fluid extraction system (ISCO, SFX 220 Model, Lincoln, NE, USA). A schematic diagram of the system is shown in Fig. 1. The system consists basically of a supercritical fluid extractor (**1**), a controller unit (**2**), two syringe pumps (**3** and **4**), a restrictor temperature controller unit (**5**) and a coaxially heated adjustable restrictor (**6**). In each experiment, approximately 3.25 (±0.01) g of ground pumpkin seeds was put into a 10 mL 316-stainless steel extraction column (55.6 mm length × 15.2 mm i.d.) (**7**). Filter frits, are installed into the extraction column end caps before loading samples in the extraction column. The frit porosity was selected as 2 μm to prevent any carryover of ground pumpkin seeds. Thereafter, the extraction column was placed into the temperature-controlled chamber (**8**) of the supercritical fluid extractor. Operating temperature is controlled by a temperature controller which is located on the front panel of supercritical fluid extractor. Syringe pumps and supercritical fluid extractor are connected to the controller unit which controls all pumping and extraction operations. Supercritical fluid extractor is designed to prevent over-pressurization of chamber. Therefore, it incorporates six motor actuated valves which are controlled by controller unit. Supercritical fluid source for supercritical fluid extractor is supplied by syringe pumps. Each syringe pump consists of an external heating jacket which surrounds the pump cylinder and a pressure transducer which is located on the top of the pump cylinder. A heating fluid containing ethylene glycol solution at the operating temperature was pumped to the heating jacket by means of a circulator (**9**) (Thermo Electron Co., HAAKE Phoenix II P2-C25P circulator, Hampshire, UK). Liquid CO_2 container (**10**) and entrainer reservoir (**11**) are supercritical fluid sources. Supercritical fluid is introduced automatically into extraction column at a predetermined volumetric flow rate when both operating pressure and operating temperature reach to the desired conditions. Controller unit makes them easy to set up and monitor continuous flow and modifier addition systems. The direction of supercritical fluid in extraction column is downward. Extract phase pass through a coaxially heated adjustable restrictor which is controlled by restrictor temperature controller unit. The restrictor temperature was kept at 80 °C to reduce sample plugging in the restrictor. Extracted oil was collected in a collection vial (**11**) which contained glass wool under nitrogen atmosphere. A vent valve allows rapid depressurization of the chamber when the extraction operation is completed. The collection vial was replaced each 5 min for first 30 min, each 10 min for 90 min and each 15 min for 300 min with a new collection vial to obtain extraction profiles with operating time or solvent consumption. Water and entrainer contents co-extracted with pumpkin seed oil were removed by a vacuum oven (Memmert VO 300 Model,