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Supercritical CO_2 extraction of hemp (*Cannabis sativa* L.) seed oil

Krunoslav Aladić[a], Kristjan Jarni[b], Tina Barbir[c], Senka Vidović[d], Jelena Vladić[d,*], Mate Bilić[e], Stela Jokić[e]

[a] Croatian Veterinary Institute, Branch - Veterinary Institute Vinkovci, Josipa Kozarca 24, 32100 Vinkovci, Croatia
[b] Biotechnical Faculty, University of Ljubljana, Večna pot 83, 1000 Ljubljana, Slovenia
[c] Croatian Veterinary Institute Zagreb, Savska 143, 10000 Zagreb, Croatia
[d] University of Novi Sad, Faculty of Technology, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia
[e] Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Franje Kuhaca 20, 31000 Osijek, Croatia

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ABSTRACT

In this work, hemp (*Cannabis sativa* L.) seed oil was produced by extraction with supercritical CO_2 under different extraction conditions (temperature, pressure and time). The objective was to evaluate the influence of extraction conditions on concentration of tocopherols, fatty acids and pigments in hemp seed oil. The composition of hemp seed oil obtained with supercritical CO_2 was compared with the hemp oil extracted by *n*-hexane using Soxhlet method and with oil obtained by pressing using screw expeller. Using supercritical CO_2 extraction the extracts higher in concentration of tocopherol were produced. The amount of α- tocopherol in supercritical extracts ranged from 37.09 to 110.61 mg L^{-1}, depending on the applied process conditions, while γ-tocopherol content was significantly higher (2–3 times). The content of pigments in the hemp oil obtained by supercritical CO_2 had been changed significantly during the extraction time from 9.79 to 178.76 mg kg^{-1} for total chlorophyll content and 8.15 to 57.66 mg kg^{-1} for total carotene content. By selecting the relevant process conditions of supercritical extraction it is possible to obtain hemp seed oil with physical or nutritional properties of interest to the food industry.

1. Introduction

Hemp has been grown and has had a share in the industrial production in Croatia for a significant length of time. Hemp (*Cannabis sativa* L.) is fully usable in the production of a large range of products but its use has decreased in previous decades because of great similarities with Indian hemp (*Cannabis indica* L.). The main reason that hemp was banned in the past is the presence of psychoactive substance δ-9-tetrahydrocannabinol (THC) (Oomah et al., 2002). According to EU legislation production of hemp is permitted if the THC content is less than 0.2% and Kriese et al. (2004) found 51 varieties with levels less than 0.2%. Hemp is certainly a multifunctional plant and the most important products which can be produced from hemp are: oil, proteins, which can be used in food and feed production, and fibers used in the paper and textile industry (Callaway, 2004; Bertoli et al., 2010). Hemp seeds are high value with approximately 25–35% lipids, 20–25% proteins, 20–30% carbohydrate, 10–15% insoluble fibers and numerous of natural source minerals (Oomah et al., 2002; Deferne and Pate, 1996).

Supercritical carbon dioxide (CO_2) extraction is valuable technology in seeds, fruit and vegetable processing and preservation (Rawson et al., 2012). Supercritical CO_2 extraction as a green technology is certainly alternative method to replace or to complement conventional industrial process such as pressing and solvent extraction. Supercritical fluid extraction (SFE) technique has many advantages over traditional methods, especially in preservation of thermosensitive compounds using low temperatures, which results reduced energy consumption (Moslavac et al., 2014). Supercritical fluids, like supercritical CO_2, are characterized by high mass transfer rates, liquid like density, and variable selectivity (achievable by variation of temperature and pressure). By variation of supercritical CO_2 selectivity extracts of desirable content and concentration of certain compounds can be produced. One of very important characteristics of this extraction technique is production of extracts without residues of extraction solvent - "solvent free extracts".

Food industry is always looking for processes that can minimize the environmental impact, decrease toxic residues, use by-products more efficiently and also obtain high-quality products with good nutritional and organoleptic properties. SFE is still relatively new in the extraction of hemp oil and other edible oils mainly due to very high investment costs of SFE equipment (Jokić et al., 2014a).

* Corresponding author.
E-mail address: vladicjelena@gmail.com (J. Vladić).

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But nowadays, according to global trends, "green" products and technologies are needed to replace conventional ones.

Several authors used supercritical CO_2 extraction to obtain hemp oil. Da Porto et al. (2012a) followed fatty acid composition and oxidation stability of obtained oil at different process parameters. Also Da Porto et al. (2012b) optimized supercritical CO_2 extraction parameters in the production of hemp oil using response surface methodology. Kriese et al. (2004) investigated fatty acid composition and tocopherol content in 51 different genotypes of hemp. Tomita et al. (2013) followed different process parameters on solubility of hemp oil in supercritical CO_2 extraction. In this study, supercritical CO_2, was used as a solvent in the extraction of hemp seed oil at different process conditions of temperature, pressure and time in order to determine fatty acids, tocopherols and pigment content (chlorophyll *a* and *b* and total carotene) of extracted oil. According to our knowledge there are no previous reports on pigment content of hemp oil collected at different extraction period with supercritical CO_2. The composition of hemp seed oil obtained by supercritical CO_2 was compared with the hemp seed oil extracted by other extraction techniques.

2. Material and methods

2.1. Chemicals

The purity of CO_2 used for extraction was 99.97% (w/w) (Messer, Osijek, Croatia). For preparation of calibration curves standard compounds α-tocopherol (Dr. Ehrenstorfer Cat No. 1792430), β-tocopherol (Supelco Cat No. 46401-U), γ-tocopherol (Supelco Cat No. 47785) and δ-tocopherol (Supelco Cat No. 47784) were used. Standard compounds for determination of fatty acids were provided from Supelco™ 37. Component FAME Mix (Bellefonte, Pennsylvania, SAD) were used. Potassium hydroxide was supplied by Kemika (Zagreb, Croatia). *n*-hexane was provided from Merck KGaA (Darmstadt, Germany). All other chemicals and reagents were of analytical reagent grade.

2.2. Material

Hemp seeds (*Cannabis sativa* L.) genotype *Fedora 17* were obtained from family farm Organica Vita (Vraneševci, Croatia) in 2013. Moisture content of the hemp seeds was determined according to AOAC Official Method 925.40 (2000). Samples were cleaned from impurities and grounded using laboratory mill (IKA Basic A11, Germany).

2.3. Determination of particle size distribution of hemp seeds with sieving

Hemp seeds were sieved using a vertical vibratory sieve shaker (Labortechnik Gmbh, Ilmenau, Germany) for 20 min. About 200 g loading were used at each sieving. The raw material size distribution was determined using a nest of 9 sieves of aperture sizes 1.4, 0.8, 0.63, 0.5, 0.4, 0.315, 0.2, 0.1 and 0.05 mm. The mass of fragments remaining on each sieve after sieving was used to calculate the distribution of fragments, which was then normalized in respect of the total mass. For evaluation of sieve analysis results the Rosin-Rammler-Bennet (RRB) distribution (Allen, 1981) was chosen. The percentage by mass of particles (R) greater than screen size (d) is given as:

$$R = 100 \exp\left[-\left(\frac{d}{d_0}\right)^n\right] \quad (1)$$

where d_0 represents the particle size corresponding to the 36.8th percentile of the cumulative probability distribution (size constant), and n controls the shape of the distribution (uniformity coefficient). The function of the sum of sieve residue (R) was fitted to the experimental data by changing the representative particle size d_0 and the uniformity coefficient n, minimizing the sum of the mean square error using STATISTICA 8.0 software (Stat Soft Inc., USA).

2.4. Organic solvent extraction

The initial oil content in hemp seeds was measured by automatic extraction systems Soxterm by Gerdhart with *n*-hexane. 5 g of ground hemp seeds was extracted with 120 ml solvent, until totally depleted according to HRN ISO 6492 (2001). The whole process took 2 h and 45 min, at 180 °C. The measurement was done in triplicate.

2.5. Cold pressing

The cold pressed hemp oil was obtained by pressing the 1 kg of hemp seeds using following parameters: temperature of head presses of 60 °C, frequency of 20 Hz and using nozzle of ID 6 mm. The pressing of the seeds were performed in a screw expeller SPU 20 (Senta, Serbia) with capacity 20–25 kgh^{-1}. The measurement was done in duplicate.

2.6. Supercritical CO_2 extraction

The experiment was performed in SFE system explained in detail elsewhere (Jokić et al., 2014b; Moslavac et al., 2014). The grounded hemp seeds of 100 g were placed into extractor vessel. The extracts were collected in previously weighed glass tubes. The amount of extract obtained at regular intervals of time was established by weight using a balance with a precision of ±0.0001 g. Separator conditions were 15 bar and 25 °C.

The extraction was performed at different extraction conditions of pressure (300 and 400 bar) and temperature (40 and 60 °C) until the extraction yield became constant. During extraction process, every half hour, sample of extracted oil were collected for further analysis.CO_2 mass flow rate of 1.94 kg/h were kept constant. Each extraction experiment was conducted two times and the average value of two replication is given in Fig. 2.

2.7. Determination of chlorophyll a, chlorophyll b, and total carotene content

For determination of chlorophyll a and b, and total carotene content, the modified method of Dere et al. (1998) was used. The weighted sample, having been added diethyl ether (50 ml for each gram), was dissolved in an ultrasonic bath for one minute. It was then homogenized for 30 s with homogenizer, and again in the ultrasonic bath for one minute. The homogenate was centrifuged for 10 min at 3000 rpm. The supernatant was separated and the absorbances were measured at 400–700 nm in the UV spectrophotometer. Chlorophyll *a* showed the maximum absorbance at 660.0 nm, chlorophyll *b* at 642.5 nm, and total carotene at 470 nm. All analyses were repeated three times. The amount of these pigments was calculated according to the formulas given in our previous published paper (Aladić et al., 2014).

2.8. Determination of tocopherols

Preparation of samples for GC–MS analysis has been provided by saponification 0.5 g of sample in 50 ml of potassium hydroxide, and then by extraction unsaponifiable components using diethyl ether as extraction solvent.

For analysis of tocopherols Agilent 7890 A GC equipped with Agilent 5975 MSD has been used. For this analysis GC–MS was fitted