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Response surface optimization of hemp seed (*Cannabis sativa* L.) oil yield and oxidation stability by supercritical carbon dioxide extraction

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ABSTRACT

Hemp seed oil is considered one of the best nutritional oil for health. The present work is focused on the optimization of the hemp seed oil extractive process at laboratory level using supercritical carbon dioxide (SC-CO_2) as solvent. Response surface methodology (RSM) was used to optimize hemp seed oil extraction yield and oxidation stability. Independent variables were operating temperature (40, 50 and 60 °C), pressure (250, 300 and 350 bar) and particle diameter (0.59, 0.71 and 0.83 mm). A second-order polynomial equation was used to express both the oil yield and the oil oxidation stability as a function of independent variables. The responses and variables were fitted well to each other by multiple regressions. The maximum oil yield, 21.50% w/w, was obtained when SC-CO_2 extraction was carried out at 40 °C, 300 bar and 0.71 mm of particle size. The maximum oil oxidation stability, 2.35 Eq α toc/ml oil, was obtained at 60 °C, 250 bar and 0.83 mm of particle size. A comparison between hemp seed oil composition extracted by SC-CO_2 under the optimum operating conditions determined by RSM for oil yield and by organic solvent was reported.

1. Introduction

In hemp (*Cannabis sativa* L.) seed, the amount of oil is about 30% (w/w) depending on the variety, the year of cultivation, the climatic conditions and the location [1]. The fatty acid composition of hemp seed oil shows that it contains 70–80% polyunsaturated fatty acids (PUFA) with ~10% saturated fatty acids. The two polyunsaturated essential fatty acids (EFAs), linoleic acid (LA, 18:2*n*−6) and α-linolenic acid (ALA, 18:3*n*−3), usually account for approximately 50–70% and 15–25% respectively, of the total seed fatty acid content. The ratio between *n*−6 and *n*−3 fatty acids is 3:1 [2–5]. Such balance has been claimed optimal for human nutrition and is apparently unique among the common plant oils [6]. The potential health benefits of these two polyunsaturated fatty acids (PUFA) are interesting owing to their anti-inflammatory, antithrombotic, antiarrhythmic and hypolipidemic properties [7].

Unlike most vegetable oils, hemp seed oil contains significant amounts of γ-linolenic acid (GLA,18:3*n*−6) typically about 4%. Higher amounts of this fatty acid are synthesized in evening primrose (*Oenothera macrocarpa*) (~8%) and borage (*Borago officinalis*) (~20%) [8,9]. Positive effects of γ-linolenic acid have been observed on patients with rheumatoid arthritis, atopic dermatitis and allergies [10–12].

Due to the high content of polyunsaturated fatty acids, hemp seed oil is very susceptible to oxidative degradation. The oxidation stability of an oil is determined by its fatty acid composition in addition to several minor components that have antioxidant properties. In vegetable oils, tocopherols are the most important natural antioxidants present [13]. Oomah et al. [14] found in hemp seed about 800 mg/kg of oil tocopherols, mostly in the form of γ-tocopherol (about 85%). γ-Tocopherol has higher antioxidant activity than the form α and β and less than δ [15].

Currently hemp seed oil is obtained by solvent extraction or by cold-pressing. Solvent extraction is efficient and relatively inexpensive, however, it involves longer extraction time and the chemicals employed are generally hazardous to both workers and the environment. Additionally, the oil obtained by solvent extraction generally requires significant refining and solvent residues can remain in the final product. Screw pressing is relatively inexpensive, although it is not as efficient as solvent extraction. Matthäus and Brühl [5] reported that 60–80% of available oil can be extracted by a screw press from the hemp seeds, depending on the settings of the screw press. Latif and Anwar [16] reported that the oil contents (28.4–32.8%) obtained by enzyme-assisted cold-pressing of hemp seeds were found to be significantly higher than that determined for the control (26.7%). The problem of hemp seeds cold-processing is the high amounts of chlorophyll coextracted with the oil. This is due to the unripe seeds which are very present, since the most

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hemp cultivars are developed for the production of straw and fibre and are not optimized for the production of oil. Chlorophyll content requires to be reduced, however, this adds an additional step and cost. Moreover, unripe seeds contain higher amounts of moisture, which influences the moisture content of the whole material stored together and at least the taste and smell of the resulting oil [5].

Supercritical fluid extraction (SFE) has been widely employed as alternative of organic solvent extraction for vegetable oils extraction. The application of SFE has grown continuously because it showed several advantages over classical extraction processes with organic solvents. CO_2 is probably the most widely used supercritical fluid because it is non-toxic, recyclable, cheap, relatively inert, non-flammable and easily separated from the extract Due to low critical temperature (31.1 °C), CO_2 is applied in SFE processes at near-environmental temperatures thus minimizing heat requirement and thermal damage to bioactive compounds [17–19].

Although SC-CO_2 extraction of vegetable oils has been extensively studied in the laboratory, no studies have been reported on total oil yield and oxidation stability of hemp seed oil in SC-CO_2 by response surface methodology to our knowledge. In a previous paper we established a preliminary set of supercritical CO_2 extraction conditions to obtain high quality hemp seed oil [20]. Thus, the aim of this research was to ascertain how pressure, temperature and particle size influenced oil yield and quality of the extract (expressed here in terms of oxidation stability).

2. Materials and methods

2.1. Materials

Hemp seeds were collected from experimental cultivation of hemp Felina cultivar (THC ≤ 0.2%) (Regulation EC n. 2860/2000) carried out at Prato Carnico (Udine, Italy). They were harvested during September 2011. Subsequently after drying, hemp seeds were stored at 4 °C prior to extractions. The finale moisture content of the seeds was 9.8 ± 0.3%.

2.2. Solvents and reagents

The carbon dioxide used (purity > 99.99%) was supplied by Rivoira Spa (Udine, Italy). All other solvents and reagents used in analytical determinations were Sigma–Aldrich Co. (Milan, Italy), pro analysis type. The chemicals used were of analytical reagent grade that include 1,1-diphenyl-2-picrylhydrazyl (DPPH – 90% purity, Sigma–Aldrich Co., Milano, Italy) and (+)-α-tocopherol (Sigma–Aldrich Co., Milano, Italy).

2.3. Methods

2.3.1. Soxhlet extraction

Thirty grams of hemp seeds were ground in a stainless steel blender, transferred into a filter paper extraction thimble and extracted with 240 ml *n*-hexane for 8 h at a maximum temperature of 70 °C in a Soxhlet apparatus. After extraction was completed, *n*-hexane was removed at 50 °C under reduced pressure using a rotary evaporator (Rotavapor R210, Buchi, Flawil, Switzerland). Subsequently, the flask was placed into a desiccator chamber for 1 h. The oil obtained was weighed and the yield was calculated. Determination was done in triplicate.

2.3.2. Supercritical CO_2 extraction

Supercritical CO_2 extractions were performed using a Lab Scale supercritical fluid system (M-LAB-SFE100; Tecnoprocess srl, Roma, Italy) equipped with a 100 cm^3 extraction vessel (Fig. 1). For SC-CO_2 extractions hemp seeds were ground in a stainless steel blender for 10, 30 and 60 s. The amount of ground hemp seeds placed in the extractor was 15 g. Glass beads were placed on the bottom of the extractor, the ground hemp seeds were placed above them and another layer of glass beads was put at the top. After the extraction vessel was tightly sealed, the desired extraction temperature was set. Pressure within the extraction vessel was built up with a constant dioxide flow rate at 8×10^{-5} kg/s. The SFE extraction was initiated after the desirable temperature and pressure were achieved. The entire extraction process lasted 60 min and the collected oil was weighted. After extraction the exhausted matrix was characterized by size classification in a standard sifter with several mesh sizes (<0.25, 0.25–0.5, 0.8–1.0, 1.0–1.25, 1.25–1.50, 1.50–1.75, 1.75–2.0, >2.0 mm) [21]. Corresponding to the different grinding times 10, 30 and 60 s, the average particle diameters resulted of 0.59, 0.71 and 0.83 mm. These values were calculated by Sauter's equation [22] applied to each set of fractions, within the previous mesh sized:

$$d_p = \frac{m_t}{\sum_{i=1}^{k} m_i / d_{pi}}$$

where m_i is the mass of particles retained below mesh size d_{pi}, m_t is the total mass of milled seeds and k is the number of mesh sized.

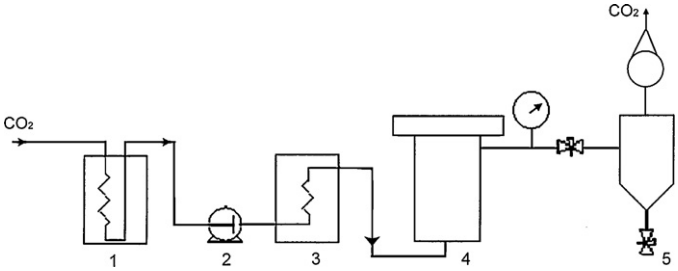

Fig. 1. Scheme of the SC-CO_2 laboratory unit for supercritical fluid extraction: (1) solvent cooler; (2) pump; (3) heater; (4) extractor; (5) separator.

Table 1
Range and variables used for the experimental designs.

Experimental variables	Experimental levels		
Particle diameter, X_1 (mm)	0.59	0.71	0.83
Pressure, X_2 (bar)	250	300	350
Temperature, X_3 (°C)	40	50	60

2.3.3. GC analysis of fatty acids

The fatty acid methyl esters (FAME) were prepared by transesterification of oil with 2 N KOH in methanol and *n*-hexane. Gas chromatographic (GC) analysis of FAME were performed in a Varian 3400 gas chromatograph equipped with a SP-2380 fused-silica column (Supelco, Bellafonte, PA) (30 m × 0.32 mm i.d., film thickness 0.20 μm), a split injector at 250 °C; flame ionization detector at 260 °C. Helium was used as carrier gas and the split ratio was used 1:50. The programmed temperature was: 2 min at 50 °C, 50–250 °C at 4 °C/min. The identification of FAME was based on external standards using commercial reference compounds (Sigma Aldrich, Milan, Italy). Each result presents the mean and the standard deviation for a minimum of three analyses.

2.3.4. Oxidation stability

The oxidation stability of the hemp seed oil samples was evaluated by the total free radical scavenger capacity (RSC) following the methodology described by Espín et al. [23] with slight modification. In brief, 10 μl of ethyl acetate sample solution at different concentrations was added with 1990 μl of fresh ethyl acetate DPPH solution (93 μM). Then the mixture was shaken vigorously and left in darkness for 60 min. Finally, the absorbance of the mixture was measured against pure ethyl acetate (blank) at 515 nm using