Characteristics of hemp (*Cannabis sativa* L.) seed oil extracted with supercritical CO_2

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INTRODUCTION

Hemp cultivation (*Cannabis sativa* L.) of different cultivars (Felina, Finola, Uso 31, Chamaleon) characterized by a low concentration of δ-9-tetrahydrocannabinol (THC) (<0.2%), has been reintroduced in Carnia (Friuli Venezia Giulia) both for oil and fiber.
About 25–35% of the weight of hempseed is an edible oil. Hempseed oil is of high nutritional value because its 3:1 ratio of the two essential polyunsaturated fatty acids (EFAs), especially linoleic acid (C18:2ω6) and linolenic acid (C18:3ω3) which matches the balance required by the human body [1]. These fatty acids are important in relation to the pathogenesis (and prevention) of coronary heart disease and hypertension and during pregnancy and breastfeeding, besides showing a hypocholesterolemic effect when used as food supplements. The oil, because of its EFAs content and the presence of γ-linolenic acid (C18:3ω6), is also ideal as an ingredient for light body oils and lipid-enriched creams known for their high penetration into the skin. Among vegetable oils, hemp seed oil contains one of the highest levels of PUFAs (poly-unsaturated fatty acids) ≈ 80-81%) [2]. Antioxidant property of cold-pressed hemp seed oil has been investigated [3,4] and its effectiveness in inhibiting free radicals resulted higher than olive oil (extra-virgin). Antioxidants are well recognized for their potential in health promotion and prevention of aging-related diseases, including cancer and heart disease. The versatility of the hempseed lends itself to the development of numerous products for food, cosmetic, therapeutic, functional food and nutraceutical industries. Even though there are numerous reports on the SC-CO_2 extraction of oil from various seeds, there is little information on the SC-CO_2 extraction of oil from hempseed. The aim of this work was to establish a preliminary set of supercritical fluid extraction conditions through experimental design to obtain high quality hempseed oil.

MATERIALS AND METHODS

Characterization of the seeds

Hemp (*Cannabis sativa* L.) seed cultivar Felina, grown in Prato Carnico (Udine) in 2009, was obtained from experimental cultivation. Seed samples were natural dried in a cool, windy and dry location. Oilseeds were finely ground in a grinder and subsequently garbled to 1.50 mm particle size (Dp). The extraction process (8 h at 70 °C) of oil from ground hempseed samples was performed in a Soxhlet extractor using 30g of seed and 240 mL of *n*-hexane followed by solvent removal under vacuum at 60°C**.**

SC-CO_2 apparatus and procedure

The experiments were carried out using a high pressure pilot plant, (SCF 100 serie 2MP PLC, SEPARECO Italy) equipped with 1 L volume extractor vessel and two separators, the first one a gravimetric separator and the second one an high performance cyclonic separator. The high pressure vessel contains an extraction basket of 700 ml, closed with porous stainless

steel disks. The CO_2 is pressurized by a high pressure diaphragm pump (400 bar) with jacketed heads for cooling. Flow rate can be regulated between 2 and 10 kg/h. An auxiliary diaphragm pump is used to pressurize co-solvent or liquid matrix to be fractionate in the fractionation tower. Flow rate can be regulated between 0.2 and 1.5 L/h. A PID controlled chiller produces cold water for the CO_2 condenser, the CO_2 storage tank and the CO_2 pump head (max. T: 0° C). A PID controlled thermo regulator produces hot water for the evaporator, jacketed extractor and separators (max. T: 90° C), driving the operating process temperature. Cut off valves and regulating valves are used to control the pressure inside the extractor and the separators, while a coriolis mass flow meter (CO_2) and a volumetric flow meter (co-solvent/liquid) are placed just before the diaphragm pumps. Flow meter, pressure and temperature probes are connected to an electronic interface (PLC) indicates the pressures, the temperatures, the instantaneous flow rates, the mass of CO_2 passed through the extractor and the volume of co-solvent or liquid to be fractionate. The flow sheet of this plant is given in Figure 1.

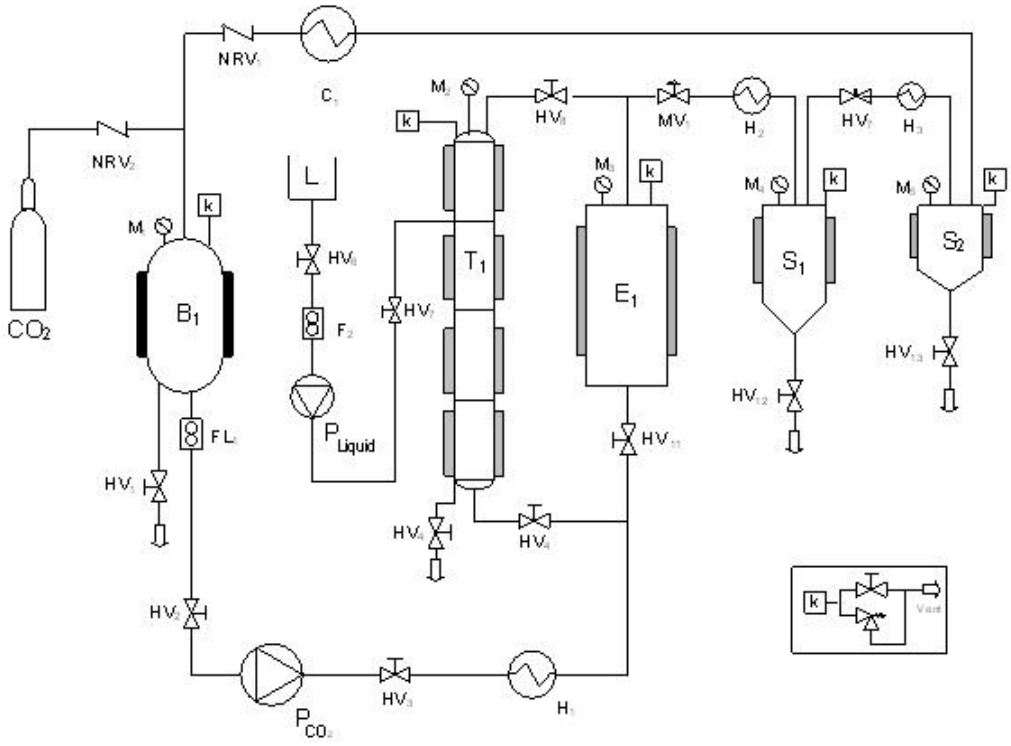

Figure 1. *Flowsheet of the pilot scale apparatus (SCF 100 serie 3 PLC-GR-DL-MP). B_1: storage tank; E_1 : extraction vessel ; $S_{\#}$: separators; $H_{\#}$: heat exchangers; C_1: condenser; $HV_{\#}$: hand valves; MV_1: membrane valve; $NVR_{\#}$: no return valves ; P: diaphragm pumps; F_1: flow meter; T: fractionation tower; $M_{\#}$: manometers; k: safety devices; FL_1: Coriolis mass flow meter; L: liquid storage tank*

About 3×10^{-1} kg of ground and garbled seeds (d=0.44 g/mL) were loaded into the extraction vessel for each experimental extraction. Flow rate was regulated at 10 kg/h. The independent variables were temperature (40, 60 and 80 °C), pressure (200, 300 and 400 bar) and solvent passed through the extractor, Q (30, 45 and 60 kg CO_2/kg feed). After extraction, the samples were centrifuged and the oil fraction separated.

GC analysis of Fatty Acids

The fatty acid methyl esters (FAME) were prepared by transesterification of oil with 2N 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 x 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°C to 250°C at 4°C/min. The identification of FAME was based on external standards using commercial reference compounds (Sigma). Each FAME sample was analysed three times.

Antioxidant Capacity Assay.

The antioxidant capacity of hempseed oil samples was evaluated by crocin kinetic competition test as described by Tubaro et al. [5]. The results were expressed as vitamin E mM equivalents

Statistical analysis

A factorial screening design at three experimental levels (3^3//9) was used to screen between process parameters of the supercritical CO_2 extraction of ground hempseed. Considered process variables were temperature (40, 60 and 80 °C), pressure (200, 300 and 400 bar) and solvent passed through the extractor, Q (30, 45 and 60 kg CO_2/kg feed). Experimental responses selected were oil yield, PUFAs content and antioxidant capacity

RESULTS

Moisture content of the original hempseed sample was 7.8±0.2%. Oil content of the seeds, determined by Soxhlet extraction, was 30.6 ± 0.04, antioxidant capacity expressed as vitamin E equivalents, resulted 0.17± 0.03.

The SC-CO_2 experimental extractions were carried out by following the arrangement of a Factorial Screening Design for three-level factors (a 3^3 //9) [6, 7] applied in the experimental domain reported in **Table 1**.

Table 1. Process factor, test levels and experimental responses

Experimental variables	Levels		
	1	2	3
X_T (Temperature, °C**)**	40 (**T1**)	60 (**T2**)	80 (**T3**)
X_P (Pressure, bar**)**	200 (**P1**)	300 (**P2**)	400 (**P3**)
X_Q (kg CO_2 / kg feed)	30 (**Q1**)	45 (**Q2**)	60 (**Q3**)
Y_1 **(Yield** %wt**)**			
Y_2 (**PUFAs** %**)**			
Y_3 **(Antioxidant capacity** mM Eq Vit E**)**			

Table 2 . Factorial Screening Design (3^3//9): Experimental plan and observed response values

Run	Random order	X_T	X_P	X_Q	T	P	Q	Oil yield	PUFAs	Antiox. capacity
					(°C)	(bar)	$kgCO_2$/kgfeed	(%)	(%)	(Eq.Vit E)
1	7	1	1	1	40	200	30	8.9	81.6	0.23
2	4	1	2	2	40	300	45	22.1	81.0	0.40
3	9	1	3	3	40	400	60	20.5	80.9	0.26
4	2	2	1	2	60	200	45	15.3	80.5	0.40
5	1	2	2	3	60	300	60	7.2	80.9	0.17
6	6	2	3	1	60	400	30	17.3	80.9	0.16
7	5	3	1	3	80	200	60	6.6	80.4	1.00
8	3	3	2	1	80	300	30	16.8	81.3	2.18
9	8	3	3	2	80	400	45	22.0	77.9	0.03

From the experimental results reported in Table 2 for each response the "weights" associated to the factor levels were estimated by means of the least square method (see Table 3). Since the upper level of each factor has been taken as the reference state for the factor itself, the weight associated to the upper levels are always equal to zero.
For a better interpretation of the influence of the different variable levels on the three analysed product characteristics, a graph-mode representation can be used (Fig. 2). This is a graphical representation of the effects listed in Table 3. this graphical procedure enables the visualisation and allows comparison of the effects of Y_1, Y_2 and Y_3. In this way, the factor levels that remarkably influence the final results are pointed out.

As regards to the effect of the levels on the variable Y1 **(Yield %wt**), to have a maximal effect a temperature of 40 and 60°C, a pressure of 400 and 600 bar, and finally a Q ($kgCO_2$/kgfeed) of 45, corresponding to an extraction time of 72 min, are necessary.
As for the effect of the levels on the variable Y2 **(PUFAs),** to have a maximal effect a temperature of 40 and 60°C, a pressure of 200 and 300 bar, and finally a Q ($kgCO_2$/kgfeed) of 30 and 60, corresponding to an extraction time of 48 and 96 min, are needed.
As for the effect of the levels on the variable Y3 **(Antiox. capacity),** to have a maximal effect a temperature of 80°C, a pressure of 200 and 300 bar, and finally a Q ($kgCO_2$/kgfeed) of 30, corresponding to an extraction time of 48 min, are required.

Table 3. Weight associated to each factor level estimated according to the upper level of each factor taken as a reference for Y_1, Y_2 and Y_3

		"Weight" for		
Factor	Level	**Y1**	**Y2**	**Y3**
Temperature (°C)	**T1 (40)**	2.03	1.30	-0.773
	T2 (60)	1.47	0.90	-0.827
	T3 (80)	0	0	0
Pressure (bar)	**P1 (200)**	-9.67	0.93	0.393
	P2 (300)	-1.23	1.17	0.767
	P3 (400)	0	0	0
$kgCO_2$/kgfeed	**Q1 (30)**	-0.43	0.53	0.380
	Q2 (45)	5.03	-0.93	-0.200
	Q3 (60)	0	0	0
	Constant	17.23	79.30	0.623

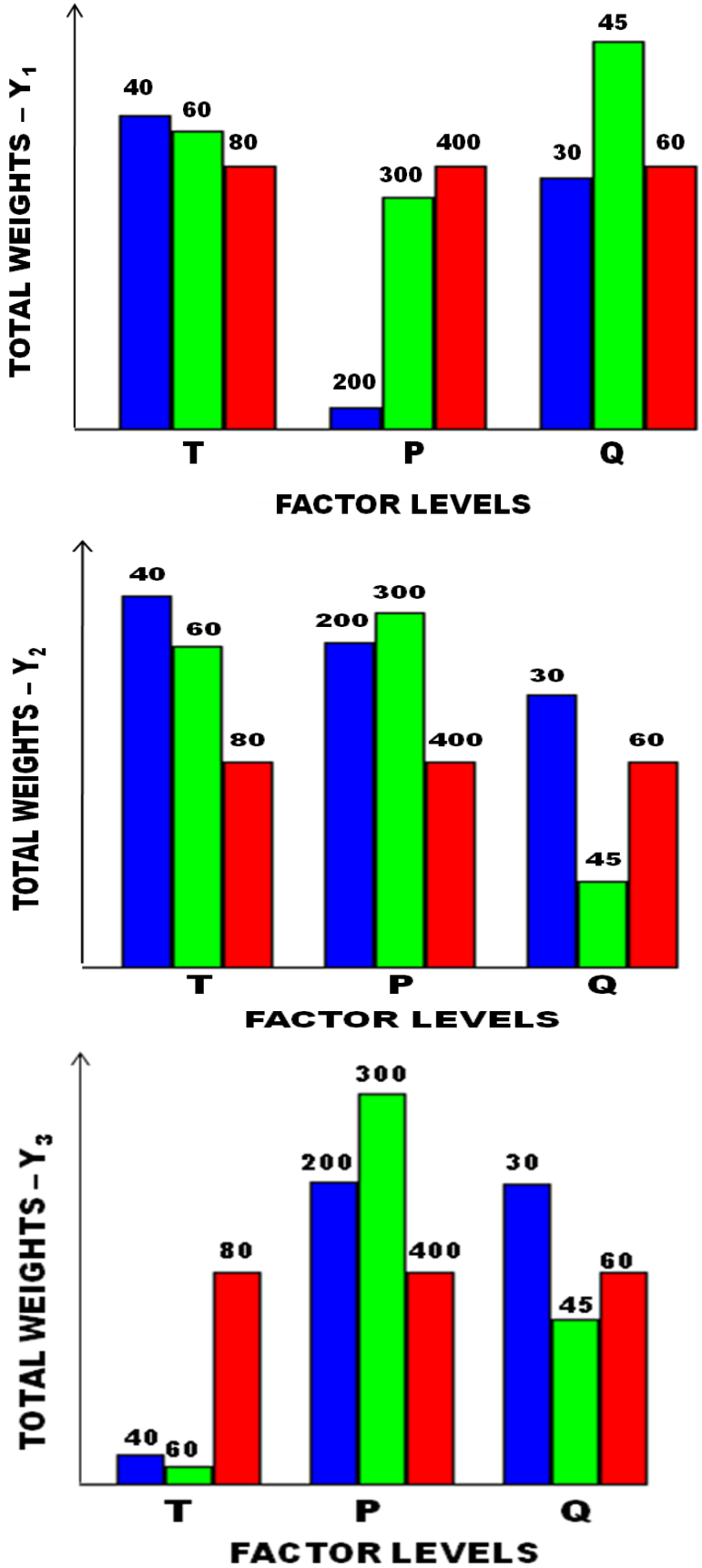

Figure 2. Graph mode representation of total weights associated to the factor levels.

In Table 2 the chemical composition of the hempseed oils obtained by SC-CO_2 extraction performed at T=80°C, P=300 bar and 30 kg CO_2/kg feed, corresponding to an extraction time of 48 min, and extracted by *n*-hexane in Soxhlet are compared.

Table 2. Chemical composition of hempseed oil, cultivar Felina, extracted by SC-CO_2 and by *n*-hexane.

	EXTRACTION	
	SC-CO_2	**Soxhlet**
Yield (g oil /100 g hempseed)	16.84 ± 0.07[a]	30.60 ± 0.04[a]
Fatty acid composition (%)		
Palmitic acid (C16:0)	5.19 ± 0.20	5.37 ± 0.13
Stearic acid (C18:0)	1.57 ± 0.04	1.56 ± 0.05
Oleic acid (C18:1)	10.99 ± 1.25	11.51 ± 1.05
Linoleic acid (C18:2ω6)	59.77 ± 0.85	59.16 ± 0.85
γ–Linolenic acid (C18:3ω6)	3.42 ± 0.14	3.48 ± 0.15
α–Linolenic acid (C18:3ω3)	18.15 ± 0.31	17.96 ± 0.23
Eicosenoic acid (C20:1)	0.78 ± 0.01	0.80 ± 0.01
Behenic acid (C22:0)	0.13 ± 0.05	0.18 ± 0.03
EFAs sum	77.92	77.12
ω-6/ω-3 ratio	3.29	3.29
PUFAs sum	81.35	80.60
Monounsaturated	11.12	11.66
Saturated	7.54	7.74
Polyunsaturated/ Saturated ratio	10.79	10.42
Antioxidant capacity (mM Eq Vit E)	2.18 ± 0.04	0.17 ± 0.07

[a] Each data represents the mean of three replicates ± standard deviation

No significant differences were found when the oils extracted by *n*-hexane in Soxhlet or by SFE were analysed by FAME analysis. The main component was linoleic acid followed by α-linolenic acid and oleic acid. The high amount of α-linolenic of hempseed oil makes it especially prone to oxidation, but may have favourable nutritional implications and beneficial effects in the prevention of coronary heart disease and cancer. The presence of γ-linolenic acid provides it with a high pharmaceutical value for neurodermic diseases. The oils were characterized by high polyunsaturated/ saturated (P/S) ratio which is regarded favourably for the reduction of serum cholesterol and arteriosclerosis and prevention of heart diseases. Similarly, the ratio of ϖ-6 to ϖ-3 ratio fatty acids resulted much higher than most vegetable oils.

Hempseed oil extracted by SC-CO_2 presented good antioxidant capacity (2.18 mM vitamin E equivalents) when compared with 0.17 mM vitamin E equivalents obtained for the oil extracted by *n*-hexane in Soxhlet. These results suggest that SC-CO_2 extraction of hemp seed oil is of particular interest because it permits to conserve substances (e.g. tocopherols, phenols and phospholipids) naturally present in oil, having antioxidant capacity and that are usually removed from oil at various stages of refining and processing. The preliminary finding of a good antioxidant capacity of hempseed oil extracted by SC-CO_2, in comparison with olive oil

(virgin oil ≈0.5 mM vitamin E equivalents) indicates that this oil is a potential source of bioactive compounds and may have a significant antioxidant capacity when ingested as part of a dietary regimen. In the light of this evidence, this oil could have extra-nutritional properties and might play a novel role in diet-disease relationships

CONCLUSION

A preliminary set of supercritical fluid extraction parameters has been established in order to obtain high quality hempseed oil which may serve as dietary source of EFA's and natural antioxidants for health promotion and disease prevention or may be used as ingredient for functional food or cosmetics.

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