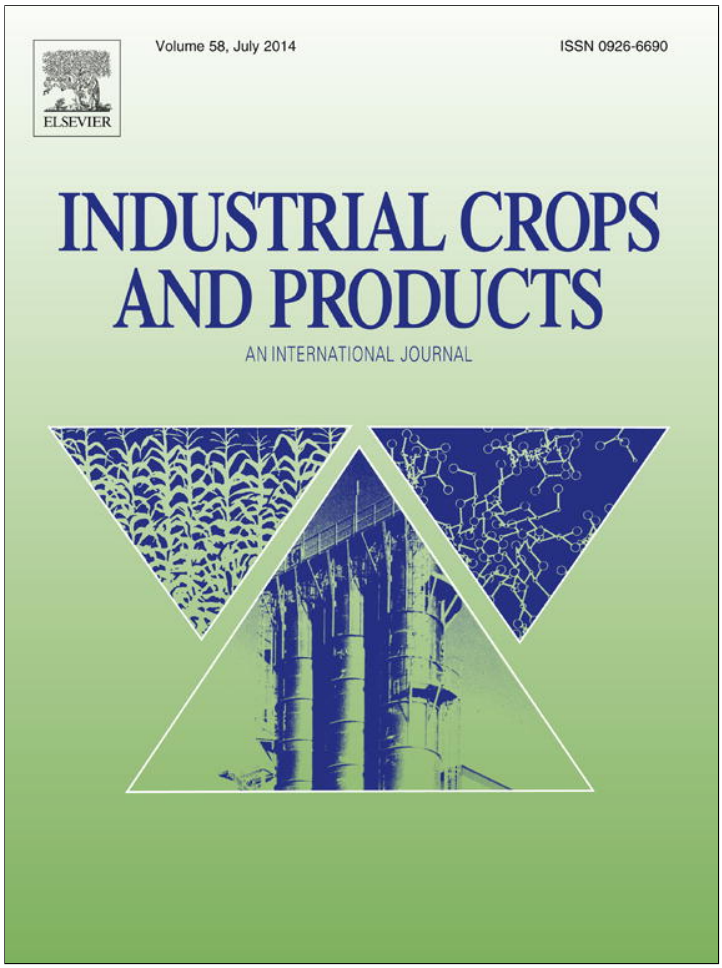

Industrial Crops and Products 58 (2014) 99–103

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Separation of aroma compounds from industrial hemp inflorescences (*Cannabis sativa* L.) by supercritical CO_2 extraction and on-line fractionation

Carla Da Porto*, Deborha Decorti, Andrea Natolino

Department of Food Science, University of Udine, via Sondrio 2/A, 33100 Udine, Italy

ARTICLE INFO

Article history:
Received 24 December 2013
Received in revised form 18 March 2014
Accepted 31 March 2014
Available online 4 May 2014

Keywords:
Supercritical CO_2 extraction
On-line fractionation, *Cannabis sativa* L.
GC–MS
HS-SPME/GC–MS

ABSTRACT

The use of supercritical carbon dioxide (Sc-CO_2) extraction at 10 and 14 MPa and 40 °C and on-line fractionation using two separators (Sep 1: 7 MPa/25 °C; Sep2: 5 MPa/15 °C) to recovery volatile compounds from the inflorescences of fiber type *Cannabis sativa* L. was investigated by HS-SPME/GC–MS and direct GC–MS and compared with hydrodistillation. The best results were obtained by Sc-CO_2 extraction carried out at 10 MPa and 40 °C. Under these operating conditions, cuticular waxes covering the surface of flowers were collected in the first separator and volatile compounds (100%) in the second. The superior quality of this last extract was proved by the perfect overlapping of its HS-SPME/GC–MS volatile profile to that of inflorescences. The recovery of fractions with different composition and biological properties, made the inflorescences of fiber type *Cannabis sativa* L suitable for cosmetic and/or food industry.

1. Introduction

Industrial hemp is a number of varieties of *Cannabis sativa* L. cultivated for fiber and/or seed production. Only varieties of industrial hemp published by EU (Regulation (EC) No 1251/99 and subsequent amendments) are approved for planting in Europe. These varieties are eligible for cultivation only after the verification of their δ-9-tetrahydrocannabinol (THC) content, the principal psychoactive constituent of the cannabis plant, which must be less than 0.2% w/w (Regulation EC No. 1124/2008-12 November 2008). Inflorescences of fiber type *Cannabis sativa* L. cultivars are generally considered waste parts for fiber industry, although the inflorescences' volatiles are pleasant to the human sensory system and could be used as flavorings for beverages (food industry) or ingredients for body care products (cosmetic industry). Cannabis scent does not originate from the terpenophenolic cannabinoids, *produced* by glandular trichomes that occur on most aerial surfaces of the plant (Dayanandan and Kaufman, 1976; Turner et al., 1978), but from the more volatile monoterpenes and sesquiterpenes (Turner et al., 1980).

Traditionally, the recovery of floral fragrances from plants is by hydrodistillation or steam distillation to produce essential oils. However, these techniques take at least several hours and require the application of heating, which can produce the degradation of thermo labile compounds present in the starting plant material.

Among innovative process technologies, supercritical CO_2 (Sc-CO_2) extraction and fractionation can be applied as alternative method to extract and isolate compounds from plant material (Reverchon and De Marco, 2006; Pourmortazavi and Hajimirsadeghi, 2007). Carbon dioxide is economical, safe, non-toxic (it does not leave residues in extract) and reaches supercritical conditions easily (32 °C and 7.38 MPa). Furthermore, the use of CO_2 is acceptable in the food and pharmaceutical industries.

To the best of our knowledge, there are no studies on the separation of volatile compounds extracted by supercritical CO_2 from the inflorescences of *Cannabis sativa* L.

The aim of this work was to apply supercritical CO_2 extraction and on-line fractionation process to separate hemp volatile compounds. The Sc-CO_2 extracts were compared to the essential oil obtained by hydrodistillation.

2. Materials and methods

2.1. Plant material

Fresh inflorescences of *Cannabis sativa* L. cv. Felina (THC < 0.2%) were obtained from experimental trials carried out in Carnia (FriuliVenezia-Giulia region-Italy). On August 2013, from at least thirty plants of hemp the inflorescences were selected randomly

* Corresponding author. Tel.: +39 0432 558141; fax: +39 0432 558130.
E-mail address: carla.daporto@uniud.it (C. Da Porto).

http://dx.doi.org/10.1016/j.indcrop.2014.03.042
0926-6690/

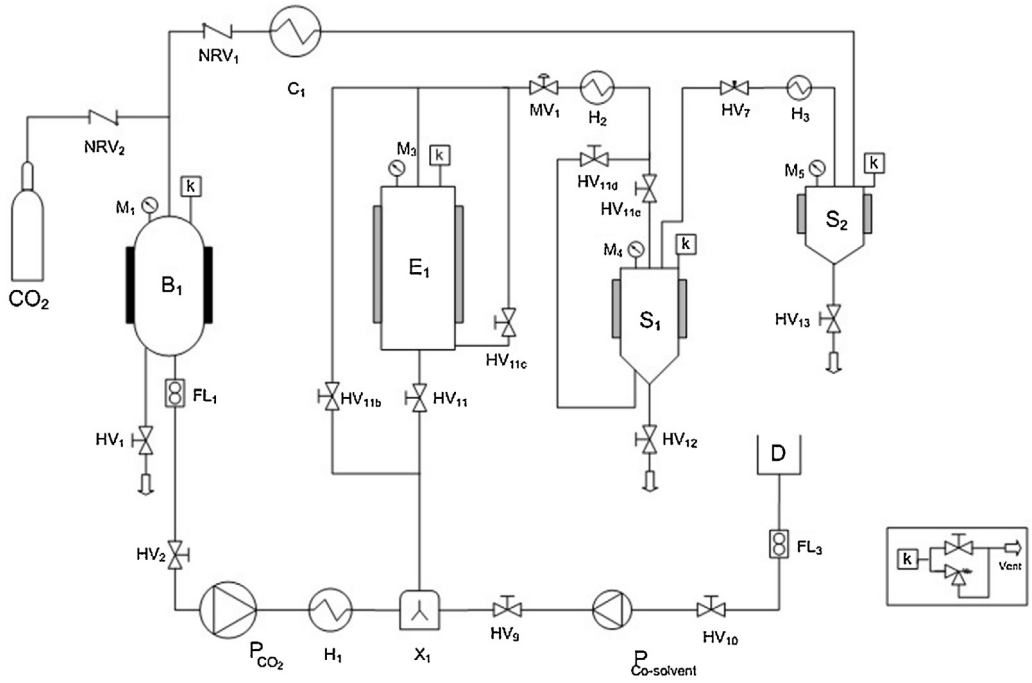

Fig. 1. SFE pilot plant flow sheet. (B_1) storage tank; (E_1) Extraction vessel; (S_1, S_2) Separators; (H#) Heater exchangers; (C_1) Condenser; (HV#) Hand valves; (MV_1) membrane valve; (NVR#) No return valves; (P) Diaphragm pumps; (F_1) Flowmeter; (M#) Manometers; (k) Safety devices; (FL_1) Coriolis mass flowmeter; (D) Co-solvent storage tank and (X#) Mixer.

from the cultivation area, handpicked and dried in the shade (moisture content 9.60% w/w, ±1.1).

2.2. Hydrodistillation apparatus and procedure

A kitchen-type knife mill was employed to carry out grinding of the inflorescences. The particle size distribution was determined with a vibratory sieve shaker. Particle size obtained was in the range of 200–600 μm.

An aliquot (150 g) of dried and ground inflorescences was submitted to hydrodistillation with a Clevenger type apparatus for 3 h. At the end of the distillation process the essential oil was collected, dried over anhydrous sodium sulphate and stored at −18 °C until use. The procedure was repeated three times. The yield of distillation was expressed as the percentage of the essential oil recovered from the plant material used.

2.3. Supercritical CO_2 extraction and on-line fractionation

SFE pilot-plant (SCF100 model 3 PLC-GR-DLMP, Separeco S.r.l, Pinerolo, Italy)equipped with 1 L extraction vessel (E_1), two 0.3 L separators in series (S_1, S_2), and a tank (B_1) where CO_2 is stored and recycled was used. The solvent used was carbon dioxide (Sapio s.r.l,Udine, Italy). The flow sheet of SFE pilot plant is given in Fig. 1.

The extractor was filled with 0.15 kg of inflorescences distributed in glass beads (0.005 m). The extractions were performed at pressure of 10 and 14 MPa and temperature of 40 °C. On-line fractionation of the extracts was accomplished maintaining S1 at 7 MPa and 25 °C and S2 at 5 MPa and 15 °C in both experimental assays. CO_2 flow rate was set to 3 kg/h in both experiments (CO_2/inflorescences = 80 kg/kg). Extractions were carried out by duplicate. The samples recovered in S1 were solid and pasty. S_2 fractions were collected into a cold trap *cooled* with *liquid nitrogen* and had oily appearance. The fractions obtained in S1 and S2 were recuperated and placed in vials. They were weighted and kept under N_2 at −20 °C in the dark until analysis.

2.4. Static HS-SPME analysis coupled to GC–MS

Head space solid-phase microextraction (SPME) is a rapid, solventless sampling procedure which, combined with GC/MS analysis is a useful method for the analysis of volatile compounds (Zhang and Pawlisyn, 1993). In Head Space SPME (HS-SPME) mode, a polymeric film is exposed to the gas phase that lies immediately over the solid or liquid sample. This operation strategy has an advantage of being a non-destructive technique and allows the evaluation of the samples at different experimental conditions (Pawliszyn, 1999).

Volatile compounds of *Cannabis sativa* L. inflorescences, essential oil and Sc-CO_2 fractions were isolated by solid-phase microextraction (SPME) using a 1 cm fiber coated with 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane phase (DVB/CAR/PDMS) (Supelco, Milan, Italy) and analyzed by GC–MS. The extraction temperature chosen was 30 °C in order to give a better estimation of the volatile profile as perceived by the human nose. The equilibrium of aroma compounds between the SPME coating fiber and headspace of each sample was considered achieved after 50 min of adsorption (Da Porto and Decorti, 2012; Da Porto et al., 2013).

GC–MS analysis of the volatile compounds was performed using a Shimadzu gas chromatograph (model GC-17A) coupled to a Shimadzu mass spectrometer (model QP-5000). The fused silica column was a DB-5 fused-silica column (Supelco, Bellafonte, PA) (30 m × 0.25 mm i.d., film thickness 0.25 μm). Working conditions were: injector 250 °C, transfer line to MS 250 °C, oven temperature: start 45 °C, hold 3 min; programmed from 45 to 190 °C at 3 °C min^{-1}, hold 5 min, then further increase to 250 °C at 20 °C min^{-1}, hold for 5 min; carrier gas helium at flow rate 2.0 ml min^{-1};ionization: EI 70 eV; acquisition parameters: scanned m/z: 35–700. Splitting was set in the splitless mode for inflorescences and the split ratio was 1/40 (v/v) for essential oil and Sc-CO_2 fractions.

Identification of the volatile compounds was carried out by comparing the Kovats retention indices determined by inserting a solution containing the homologous series of normal alkanes

Table 1
HS-SPME/GC–MS analysis of natural aroma compounds released by inflorescences of *Cannabis sativa* L.

Compound	LRI[a]	Mean[b] ± RSD (%)
α-Pinene	**945**	**12.39 ± 0.69**
Camphene	958	0.13 ± 3.85
β-Pinene	**982**	**4.04 ± 0.07**
Myrcene	**991**	**23.67 ± 0.87**
Limonene	1033	0.86 ± 5.46
1,8-Cineol	1039	0.47 ± 0.52
(Z)-ocimene	1041	0.24 ± 3.48
(E)-ocimene	1051	1.08 ± 4.64
γ-terpinene	1059	0.13 ± 8.17
Terpinolene	**1089**	**10.17 ± 0.93**
Linalool	1101	1.75 ± 0.48
Caryophyllene	**1418**	**29.66 ± 0.47**
(E)-β-farnesene	1455	0.59 ± 5.63
α-Humulene	**1461**	**6.72 ± 2.24**
Caryophyllene oxide	**1587**	**4.70 ± 3.57**
β-Eudesmol	1657	1.36 ± 1.72
β-Bisabolol	1677	0.89 ± 1.57
α-Bisabolol	1686	1.13 ± 4.86
Monoterpene hydrocarbons		52.73 ± 3.12
Oxygenated monoterpenes		2.22 ± 0.50
Sesquiterpene hydrocarbons		36.96 ± 2.78
Oxygenated sesquiterpenes		8.08 ± 2.93

Bold values are referred to the main constituents.
[a] LRI = Linear retention indeces on DB5-column.
[b] GC peak area percentage. Results expressed as mean of three replications.

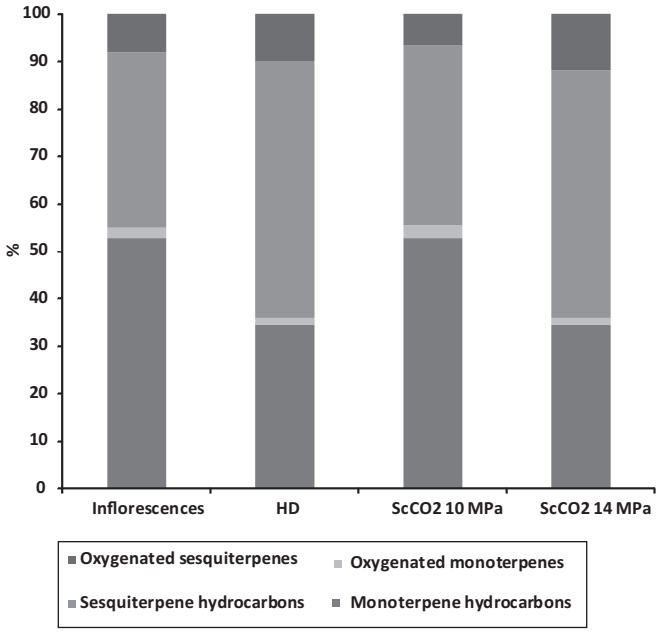

Fig. 2. Comparison of HS-SPME/GC–MS analysis performed on inflorescences, essential oil (HD) and S2 fraction from Sc-CO_2 extraction at pressure of 10 and 14 MPa and temperature of 40 °C.

(C_7–C_{20}) with those reported by literature (Bertoli et al., 2010) and with spectra of the NIST and WILEY libraries coupled with the software of GC–MS and Adams' library (Adams, 2001). The results are expressed as GC peak areas percent.

2.5. Direct GC–MS analysis

The volatile composition of essential oil and ScCO_2 fractions were determined by direct GC–MS analysis. GC–MS analysis was performed using a Shimadzu gas chromatograph (model GC-17A) coupled to a Shimadzu mass spectrometer (model QP-5000). The fused silica column was a DB-5 GC column (Supelco, Bellafonte, PA, USA) (30 m × 0.25 mm i.d., film thickness 0.25 μm). GC–MS data were obtained using the following conditions: carrier gas helium (He 99.9995%); flow rate 2.0 ml min^{-1}; split ratio 1/40 (v/v).

An aliquot of 50 mg of distilled oil and Sc-CO_2 fractions were diluted with 25 ml *n*-hexane and 1.0 μl was injected into the GC–MS system. The oven temperature program was: 45 °C for 3 min, from 45 °C to 250 °C at 3 °C min^{-1} and holding 250 °C for 5 min. The injector and transfer line temperatures were 250 °C. The electron impact (70 eV) spectra were recorded at 1 s/scan with a filament emission current of 10 μA.

Identification of the volatile compounds was carried out as previously reported for HS-SPME analysis. The results are expressed as GC peak areas percent ± RSD (%).

3. Results and discussion

A preliminary screening of the headspace (HD) by SPME analysis of inflorescences was carried out to define the original volatile composition that produces the natural fragrance. Table 1 presents the volatile compounds identified according to the GC–MS analysis. As can be deduced from table, the main (more abundant) compounds identified in inflorescences were α-pinene (12.39%), β-pinene (4.04%) myrcene (23.67%), terpinolene (10.17%), caryophyllene (29.66%), α-humulene (6.72%) and caryophyllene oxide (4.70%), in accordance with the literature (Bertoli et al., 2010).

GC peaks were identified as hydrocarbon monoterpenes (52.73%) and oxygenated monoterpenes (2.22%), sesquiterpenes (36.96%), and oxygenated sesquiterpenes (8.07%).

The volatile composition of the essential oil (HD) and the different fractions (S1 and S2 samples) obtained by supercritical CO_2 extraction were analyzed by direct GC–MS analysis (Table 2).

The main constituents of the essential oil were α-pinene (11.08%), β-pinene (3.75%) myrcene (10.83%), terpinolene (5.83%), caryophyllene (41.14%), α-humulene (9.85%) and caryophyllene oxide (5.27%). The essential oil composition showed significant quantitative differences in comparison with the essential oils from different fiber hemp inflorescences reported by Bertoli et al. (2010), but these main constituents were confirmed. In the essential oil, sesquiterpenes (52.63%), and related oxygenated compounds (11.61%) were present in high percentage in comparison with hydrocarbon monoterpenes (34.31%) and oxygenated monoterpenes (1.44%).

Supercritical fluid extraction (SFE) with supercritical carbon dioxide (Sc-CO_2) has been widely used for the extraction from natural products. SFE is an environment-friendly technology that represents an alternative to conventional extraction methods and offers several advantages over classical solvent extraction methods. CO_2 is the most commonly used solvent in SFE because it is cheap, inert, non-toxic, and allows extraction at lower temperature and relatively low pressure. (Brunner, 1994).

Supercritical CO_2 extraction on hemp inflorescences were performed at pressure of 10 and 14 MPa and temperature of 40 °C (CO_2 density higher than about 600 kg/m^3). On-line fractionation of the extracts was achieved by decreasing pressure and temperature in the two separators S1 and S2, with respect to the operating conditions used during supercritical extractions. In the first separator S1, pressure was lowered to 7 MPa and temperature to 25 °C, in the second separator S2, pressure was lowered to 5 MPa and temperature to 15 °C. Under these conditions of pressure and temperature, CO_2 density was lower than 600 kg/m^3 and this allowed to exclude all but one of the nonvolatile compounds families from the extract. The only exception was represented by paraffins constituting the cuticular waxes (Reverchon et al., 1995). Fig. 2 shows that the extraction

Table 2
Direct GC–MS analysis of volatile compounds of essential oil (HD) and $ScCO_2$ extracts (10, 14 MPa, 40 °C)) of *Cannabis sativa* inflorescences.

Compound	Hydrodistillation	Sc-CO_2 extraction			
	HD	10 MPa		14 MPa	
		S1	S2	S1	S2
α-Pinene	**11.08 ± 0.12**[a]	**–**	**13.78 ± 0.10**	**–**	**9.21 ± 0.29**
Camphene	0.56 ± 1.35	–	0.47 ± 0.60	–	0.53 ± 0.78
β-Pinene	**3.75 ± 0.20**	**–**	**4.23 ± 0.15**	**–**	**4.06 ± 0.14**
Myrcene	**10.83 ± 0.80**	**–**	**22.65 ± 0.08**	**–**	**12.58 ± 0.25**
Limonene	0.36 ± 3.11	–	0.87 ± 0.45	–	0.47 ± 0.26
1,8-cineol	0.26 ± 0.47	–	0.80 ± 1.27	–	0.36 ± 1.57
(Z)-ocimene	0.30 ± 2.52	–	0.52 ± 2.19	–	0.41 ± 0.28
(E)-ocimene	1.01 ± 2.62	–	1.03 ± 0.57	–	1.47 ± 0.29
γ-terpinene	0.58 ± 0.61	–	0.62 ± 0.05	–	0.57 ± 0.30
Terpinolene	**5.83 ± 0.20**	**–**	**7.55 ± 0.20**	**–**	**5.35 ± 0.84**
Linalool	1.18 ± 0.89	–	1.91 ± 0.00	–	1.08 ± 0.32
Caryophyllene	**41.14 ± 0.38**	**–**	**30.80 ± 0.06**	**–**	**39.6 ± 0.06**
(E)-b-farnesene	1.63 ± 1.64	–	1.15 ± 0.66	–	1.77 ± 0.34
α-Humulene	**9.85 ± 0.17**	**–**	**7.15 ± 0.18**	**–**	**9.52 ± 1.24**
Caryophyllene oxide	**5.27 ± 0.04**	**–**	**2.33 ± 0.13**	**–**	**6.11 ± 0.57**
β-Eudesmol	2.20 ± 2.12	–	1.32 ± 0.51	–	2.39 ± 1.02
β-Bisabolol	1.70 ± 1.72	–	1.35 ± 0.16	–	2.80 ± 1.39
α-Bisabolol	2.44 ± 2.09	–	1.47 ± 1.86	–	1.41 ± 2.39
Monoterpene hydrocarbons	34.31 ± 1.28		51.73 ± 0.49		34.67 ± 1.90
Oxygenated monoterpenes	1.44 ± 0.68		2.70 ± 0.64		1.44 ± 0.51
Sesquiterpene hydrocarbons	52.63 ± 0.73		39.10 ± 0.30		51.16 ± 0.56
Oxygenated sesquiterpenes	11.61 ± 1.49		6.46 ± 0.67		12.73 ± 1.44

Bold values are referred to the main constituents.
[a] GC peak area percentage ± RSD (%).

yield (mass extracted/mass loaded in the extractor × 100) was significantly higher in S1 than in S2 for both extractions. It is apparent that cuticular waxes precipitated in S1, due to their lower solubility in supercritical CO_2 in comparison to terpenes and their derivatives (Stahl and Gerard, 1985). The extraction yield obtained in the separator S1 for inflorescences processed at 14 MPa (1.39% w/w, ±0.58) was significantly higher than in S1 for inflorescences processed at 10 MPa (1.03% w/w, ±0.73) because of the higher extraction pressure employed (Simandi et al., 1999). Instead, lower extraction yields were achieved in the separator S2 for inflorescences processed, respectively at 10 MPa (0.67% w/w, ±0.18) and 14 MPa (0.34% w/w, ±0.11). However, both the extraction yields obtained in S2 fractions resulted higher than essential oil (HD) yield (0.24% w/w, ±0.13).

The SFE energy consumption was about 4.5 kWh per kilo of plant matter, taking into account the mechanical energy required by the pump to increase the fluid pressure (1.2 kWh) and the heating energy to increase the fluid temperature and the cooling energy to condense the fluid vapour (3.3 kWh). Instead, the hydro-distillation of one kilo of plant matter consumed about 9.6 kWh, due to the high heat of vaporization of water. It is to be noted that extraction by supercritical CO_2 is particularly advantageous in terms of energy consumption because of the small volume of solvent introduced, the separation of the extract by decompression, plus the fact that it is possible to recuperate the calories produced by the cold group (passage from gas form to liquid form) to feed the heating system (passage from liquid form to supercritical state).

Pereira et al. (2010) reported that the COM (manufacturing cost) for SFE process is generally lower than the COM of conventional processes as well as the CUT (utilities cost) share (usually below 1%). SFE is economically feasible after appropriately optimization of the process.

As can be observed in Table 2, the direct GC–MS analysis of the different fractions collected (S1 and S2 samples) indicates that almost all volatile compounds were recovered in S2 fraction. That is, on-line fractionation was a suitable technique to achieve the isolation of hemp volatiles in the second separator. It is interesting to note the volatile composition of the different S2 fractions in terms of the percentage of terpenes, with respect to the volatile composition of essential oil. For inflorescences processed at 10 MPa and 40 °C, the higher molecular weight compounds, namely hydrocarbon sesquiterpenes (caryophyllene, β-farnesene, α-humulene) and oxygenated sesquiterpens (caryophyllene oxide, β-eudesmol, β-bisabolol and α-bisabolol) were found in lower percentage (45.56%) than at 14 MPa and 313.15 K (63.89%). This could be attributed to the fact that at constant temperature, the increase of pressure enhances the CO_2 density and, consequently its solvation power and the solubility of these compounds in Sc-CO_2. The S2 fraction obtained for inflorescences processed at 14 MPa and 40 °C had a chemical profile similar to that obtained by hydrodistillation (HD).

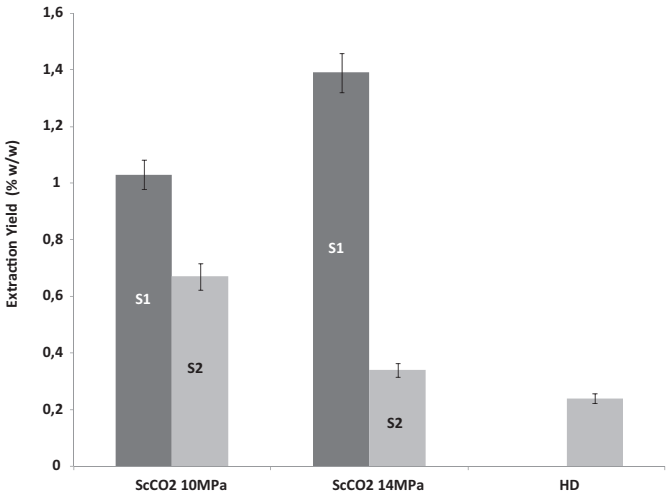

Fig. 3. Extraction yield (% w/w) obtained by Sc-CO_2 extraction (10, 14 MPa and 40 °C) in the separators S1 and S2, and by hydrodistillation (HD).

A comparison of the results obtained by HS-SPME/GC–MS analysis performed on inflorescences, essential oil (HD) and S2 fractions collected is shown in Fig. 3. As can be observed, there is a perfect

overlapping between the fraction collected in the separator S2 for inflorescences processed by Sc-CO_2 extraction at 10 MPa and 40 °C and inflorescences in terms of the percentage of terpenes. This proves the superior quality of this extract in comparison with the other one.

4. Conclusions

Supercritical CO_2 extraction carried out at 10 MPa and 40 °C on-line fractionation of the extract of *Cannabis sativa* inflorescences allowed the recovery of fractions with different composition and biological properties, suitable for cosmetic and/or food industry. The low processing temperature resulted in non-damaged volatile compounds, giving to the aromatic extract superior quality.

The supercritical CO_2 extraction process of hemp inflorescences resulted particularly advantageous in terms of energy consumption in comparison with hydrodistillation.

References

Adams, R.P., 2001. Quadrupole Mass Spectra of Compounds Listed in Order of Their Retention Time on DB-5. Identification of Essential Oils Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured Publishing, Carol Stream, IL, USA.

Bertoli, A., Tozzi, S., Pistelli, L., Angelini, L.G., 2010. Fibre hemp inflorescences: from crop-residues to essential oil production. Ind. Crops Prod. 32, 329–337.

Brunner, G., 1994. Gas Extraction: An Introduction to Fundamentals of Supercritical Fluids and The Application to Separation Processes. Darmstadt: Steinkopff, Springer, New York.

Da Porto, C., Decorti, D., 2012. Analysis of the volatile compounds of aerial parts and essential oil from *Thymus serpyllum* L, cultivated in North East Italy by HS-SPME/GC–MS and evaluation of its flavouring effect on ricotta cheese. J. Ess. Oil Bear. Plants 15, 561–571.

Da Porto, C., Decorti, D., Natolino, A., 2013. Ultrasound-assisted extraction of volatile compounds from industrial *Cannabis sativa* L. inflorescences. Int. J. Appl. Res. Nat. Prod. 7, 1–14.

Dayanandan, P., Kaufman, B., 1976. Trichomes of *Cannabis sativa* L. (*Cannabaceae*). Am. J. Bot. 63, 578–591.

Pawliszyn, J., 1999. Applications of Solid Phase Microextraction. Royal Society of Chemistry, Cambridge.

Pereira, C.G., Prado, J.M., Meireles, M.A.A., 2010. Economic evaluation of natural product extraction processes. In: Rostagno, M.A., Prado, J.M. (Eds.), Natural Product Extraction Principles and Applications. RSC Publishing, United Kingdom, pp. 442–469.

Pourmortazavi, S.M., Hajimirsadeghi, S.S., 2007. Supercritical fluid extraction in plantessential and volatile oil analysis. J. Chromatogr. A 1163, 2–24.

Reverchon, E., Della Porta, G., Senatore, F., 1995. Supercritical CO_2 extraction and fractionation of lavender essential oil and waxes. J. Agric. Food Chem. 43, 1654–1658.

Reverchon, E., De Marco, I., 2006. Supercritical fluid extraction and fractionation of natural matter. J. Supercrit. Fluids 38, 146–166.

Simandi, B., Oszagyan, M., Lemberkovics, E., Kéry, A., Kaszacs, J., Thyrion, F., Matyas, T., 1999. Supercritical carbon dioxide extraction and fractionation of oregano oleoresin. Food Res. Int. 31, 723–728.

Stahl, E., Gerard, D., 1985. Solubility behavior and fractionation of essential oils in dense carbon dioxide. Perf. Flav. 10, 29–33.

Turner, C.E., Hemphill, P., Mahlberg, G., 1978. Quantitative determination of cannabinoids in individual glandular trichomes of *Cannabis sativa* L. (*Cannabaceae*). Am. J. Bot. 65, 1103–1106.

Turner, C.E., Elsohly, M.A., Boeren, E.G., 1980. Constituents of *Cannabis sativa* L. XVII. A review of the natural constituents. J. Nat. Prod. 43, 169–234.

Zhang, Z., Pawlisyn, J., 1993. Headspace solid-phase microextraction. Anal. Chem. 65, 1843–1852.