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Application of a Supercritical CO_2 Extraction Procedure to Recover Volatile Compounds and Polyphenols from *Rosa damascena*

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A supercritical CO_2 (Sc-CO_2) extraction procedure to recover volatile compounds and polyphenols from *Rosa damascena* is investigated. It consists of two steps: the first by Sc-CO_2 at 16 MPa and 313.15 K and on-line fractionation using two separators (S1: 7 MPa/ 298.15 K; S2: 5 MPa/ 288.15 K) for volatile compounds, the second by Sc-CO_2 added with 10% ethanol-water mixture (57% v/v) at 8 MPa and 313.15 K for polyphenols. Sc-CO_2 extract obtained in S2 resulted of high quality compared with essential oil. Polyphenol yield by SC-CO_2 added with co-solvent resulted about 80 % of methanol extraction (3250 mg GAE/100 g dw).

Keywords supercritical CO_2; on-line fractionation; *Rosa damascena* var. 'Trigintipetala'; volatile compounds; polyphenols; HS-SPME/GC-MS

INTRODUCTION

Rosa damascena var. 'Trigintipetala' also known as Kazanlik is one of the most important Damask roses industrially cultivated for production of rose essential oil, rose water after water steam distillation, or rose concrete and rose absolute after solvent extraction. These products are not only applied in fine perfumery and cosmetic preparations [1, 2], but are also used in food as natural source of antioxidant and antibacterial activities [3, 4]. Besides this, in recent years medicinal properties of *R. damascena* have been also reported [5]. Traditionally, hydro or steam distillation are the techniques used to obtain the essential oil from rose, but they take at least several hours and require the application of heating, which can induce the degradation of thermo labile compounds present in the starting plant material. Due to its low content, rose essential oil is one of the most expensive in the world market. Rose oil consists of over 300 compounds [6], including monoterpene alcohols, as well as long-chain hydrocarbons and various minor constituents [7–9].

Recently, de-aromatised rose petals have been suggested as a rich source of polyphenols with antioxidant properties [10]. Different rose flower species have been evaluated as caffeine-free sources for preparing rose petal tea [11] .

Among new environmentally clean technologies, supercritical fluid extraction (SFE) with supercritical carbon dioxide (Sc-CO_2) which shows strong lypophilic selectivity, and Sc-CO_2 added with modifier for polar compounds is widely used for the extraction from natural products [12–17].

SFE represents an alternative to conventional extraction methods and offers several advantages over classical 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 [18]. Furthermore, the use of CO_2 is acceptable in the pharmaceutical and food industries.

To the best of our knowledge, there are no studies on the application of a supercritical CO_2 procedure to extract volatile compounds and then polyphenols from *R. damascena* flowers.

The aim of this work is the application of SFE in two steps: the first by supercritical CO_2 and on-line fractionation to recovery and isolate volatile compounds, and the second by supercritical CO_2 added with a modifier to extract polyphenols from the spent flowers (raffinate). The Sc-CO_2 extracts of volatile compounds are analyzed by HD-SPME-GC/MS and compared to flower and essential oil volatile composition. The overall extraction curves of polyphenols are reported and discussed and the total polyphenol contents compared with methanol extraction.

MATERIALS AND METHODS

Materials

Dried flowers of *Rosa damascena* var. 'Trigintipetala' were obtained from a cultivation carried out at Rovigo (Italy). Carbon dioxide (mass fraction purity 0.999 in the liquid phase) was supplied by Sapio s.r.l (Milan, Italy). Folin–Ciocalteau reagent and gallic acid were purchased from Sigma-Aldrich (Milan, Italy). Other reagents were of analytical grade or higher available purity.

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Hydrodistillation

An aliquot (150 g) of dried and ground (200-600 μm) flowers 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. Hydrodistillation was repeated three times. The yield of distillation was expressed as the percentage of the essential oil recovered from the plant material used.

Supercritical CO_2 Extraction

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, Milan, Italy). The flow sheet of SFE pilot plant is given in Figure 1.

The extractor was filled with 0.15 kg of flowers distributed in glass beads (0.005 m). The extractions were performed at pressure of 16 MPa and temperature of 313.15 K. On-line fractionation of the extract was accomplished maintaining S1 at 7 MPa and 298.15 K and S2 at 5 MPa and 288.15 K. CO_2 flow rate was set to 3 kg/h in both experiments. The sample recovered in S1 was solid and pasty. S_2 fraction was collected into a cold trap cooled with liquid nitrogen and had oily appearance. The fractions were weighted and kept under N_2 at -20°C in the dark until analysis.

After volatile compounds extraction and on-line fractionation, the spent flowers (raffinates) were extracted by Sc-CO_2 added with 15% water (W) or 10% ethanol-water mixture (57% v/v) (EtW) to recovery polyphenols [19, 20]. The carbon dioxide flow rate was fixed to 6 kg/h both with W and EtW as co-solvent, as well as the temperature to 313.15 K. Instead, the pressure was 10 MPa for Sc-CO_2 modified with 15% W, and 8 MPa for Sc-CO_2 modified with 10% EtW. The total extraction time was fixed at 250 min. The extractor was operated discontinuously, for intervals of about 60 min, to assess several data points for the overall extraction curves (OECs). The spent flowers extracts were collected during extractions in volumetric flask and the water or ethanol-water mixture was removed with rotary evaporator (Buchi, B465, Switzerland) at 318.15 K. After removal of solvents the extracts were weighted and analyzed. Extractions were carried out in triplicate.

Analytical Methods

HS-SPME Analysis Coupled to GC-MS

Head space solid-phase microextraction (HD-SPME) is a rapid, solventless sampling procedure which, combined with GC/MS analysis is a useful method for the analysis of volatile compounds [21]. In 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 [22].

Volatile compounds of *Rosa damascena* var. 'Trigintipetala' flowers, essential oil and Sc-CO_2

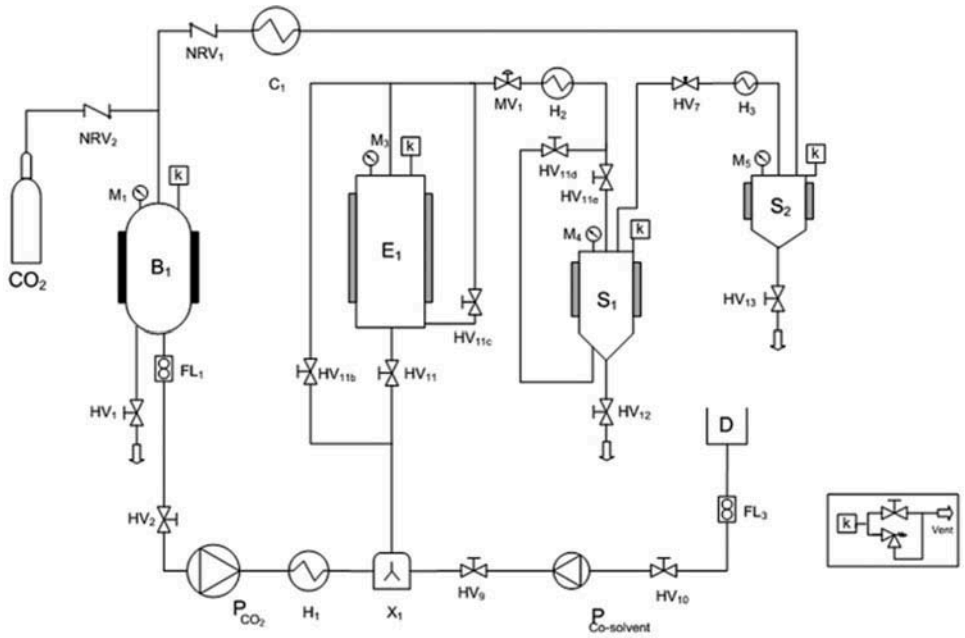

FIG. 1. SFE pilot plant flow sheet. (B_1) storage tank; (E_1) Extraction vessel; (S_1,S_2) Separators; (H#) Heater exchangers; (C_1) Condenser; (HV#) Hand valves; (MV_1) membrane valve; (NVR#) No return valves; (P) Diaphragm pumps; (F_1) Flowmeter; (M#) Manometers; (k) Safety devices; (FL_1) Coriolis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

fractions were isolated by solid-phase microextraction (SPME) using a 1 cm fiber coated with 50/30μm 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 minutes of adsorption [23, 24].

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 x 0.25mm 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 (C_7-C_{20}) with those reported by literature [25–27] and with spectra of the NIST and WILEY libraries coupled with the software of GC-MS and Adams' library [28]. The results are expressed as GC peak areas percent ± % RSD.

Methanol Extraction of Polyphenols

10 g of ground flowers was extracted with 100 mL methanol for 1 min using an Ultra Turrax mixer (24,000 rpm) and soaked overnight at room temperature as the combination of the methods used by Pizzale et al. [29] and Lu and Foo [30]. The extract was then filtered through Whatman No. 1 paper in a Buchner funnel. After filtration, the extract was concentrated by rotary evaporation under vacuum at 40 °C to get crude extract. Then this extract was used for the analysis.

Total Polyphenols

Total polyphenols were determined using the Folin–Ciocalteau reagent, according to Yu et al. [31]. Briefly, the reaction mixture contained 100 μL of extract or solvent, 500 μL of the Folin-Ciocateau agent, 1.5 mL of 20% sodium carbonate, and 1.5 mL of pure water. After 2 h of reaction at ambient temperature, absorbance was read at 765 nm using a UV–Vis spectrophotometer (Shimadzu UV 1650, Italy) to calculate TPC. Gallic acid was employed as the standard. A calibration curve was made with standard solutions of gallic acid in the range 0.2–10 mg mL^{-1} and measures were carried out at 765 nm (R^2=*0.99*). All analyses were performed in triplicate. Results were expressed as milligrams of equivalent gallic acid per gram of dried weight (mg GAE/g dw).

RESULTS AND DISCUSSION

The yield of volatile compounds, expressed as weight of extract divided by the weight of the starting material, were 0.022 % (± 4.7% RSD) for rose essential oil, 0.013 (± 7.3% RSD) for S1 and 0.007 (± 8.2% RSD) for S2. Compared with rose oil yields (0.04-0.032 % v/w) reported by Baydar and Baydar [32], the result obtained for rose oil yield was low. However, it is well known that the presence, yield and composition of secondary metabolites in plants are strongly influenced by environmental conditions, geographic variations, genetic factors as well as by cultivation conditions and time of harvest.

Table 1 reports the volatile composition of *Rosa damascena* var. 'Trigintipetala' flowers, essential oil (HD) and Sc-CO_2 fractions S1 and S2 analyzed by HS-SPME analysis coupled to GC-MS.

In flowers, the most abundant compounds were 2-phenylethanol (44.16%) and aliphatic hydrocarbons such as nonadecane (30.66%) and eneicosane (4.39%). Small quantities of monoterpenoids such as sabinene (2.52%), β-pinene (5.11%), myrcene (1.72%), linalool (1.89%), terpinen-4-ol (0.56%) and geraniol (1.18%) were detected. Some specific sesquiterpenes such as germacrene D (0.75%) and α-guaiene (0.34 %) were found to be in little amounts.

The most abundant compounds of the rose essential oil (HD) were aliphatic hydrocarbons such as nonadecane (59.79%) and heptadecane (8.19%). Hydrocarbons do not play an important role in determing the typical rose oil odors but are important for their "fixative" properties [33]. In comparison with flowers, the volatile composition of the essential oil was characterized by the absence of 2-phenylethanol which was lost in rose water during hydro-distillation, and the presence of farnesol (6.53%), an acyclic sesquiterpene alcohol. These results are in agreement with other published data reported in the literature [33].

The volatile profile of Sc-CO_2 fraction S1 resulted characterized by hydrocarbons and fatty acids which precipitated in S1, due to their lower solubility in supercritical CO_2. It is worth noting the volatile profile of Sc-CO_2 fraction S2 which included monoterpene alcohols, in particular linalool (9.48%) citronellol (0.49%), nerol (0.36%), geraniol (2.86%), β-damascone (0.22 %) and 2-phenylethanol (3.94%), as well as various minor constituents such as rose oxides. β-damascone, derived from the degradation of carotenoids, is considered an important contributor to the aroma of roses despite its relatively low concentration. The higher molecular weight compounds such as nonadecane, palmitic acid, stearolic acid, 1-docosene, tricosane, dotriacontane and 1-eicosanol were not found in S2 fraction. Therefore, on-line fractionation was a suitable technique to achieve the isolation of the finest rose volatiles in the second separator (S2).

Figure 2 shows a comparison of volatile compounds grouped into classes of chemical compounds (%) between flowers, essential oil and Sc-CO_2 fractions S1 and S2**.** As it can be observerd, the volatile profile of Sc-CO_2 fraction S2 was qualitatively similar to that of flower. This proves the superior quality of this extract in comparison with the essential oil.

TABLE 1
Comparative volatile profiles obtained by HD-SPME/GC-MS of *Rosa damascena* flowers, essential oil and $ScCO_2$ extracts S1 and S2

				Hydrodistillation	$ScCO_2$ extraction	
Compound	LRI[a]	LRI[b]	Flowers	HD	S1	S2
α-Pinene	931	935	–	5.30 ± 0.13	7.20 ± 0.05	2.59 ± 1.29
Sabinene	974	975	2.52 ± 0.13[c]	–	0.32 ± 1.74	2.28 ± 1.01
β-Pinene	979	979	5.11 ± 0.53	1.89 ± 0.14	–	1.44 ± 0.60
Myrcene	988	990	1.72 ± 1.54	0.77 ± 1.31	–	4.43 ± 1.03
Linabol	1099	1102	1.89 ± 0.55	0.77 ± 0.11	0.45 ± 1.07	9.48 ± 0.81
cis rose oxide	1112	1113	–	–	–	1.17 ± 0.83
Phenylethanol	1119	1120	44.16 ± 0.00	–	–	3.94 ± 0.88
trans rose oxide	1128	1130	–	–	–	0.31 ± 0.89
Terpinen-4-ol	1186	1184	0.56 ± 0.59	0.72 ± 4.15	–	0.37 ± 0.45
α-terpineol	1198	1198	–	–	–	0.50 ± 0.36
Citronellol	1240	1238	–	–	–	0.49 ± 0.47
Nerol	1241	1239	–	–	–	0.36 ± 0.48
Geraniol	1261	1263	1.18 ± 0.82	7.35 ± 0.03	–	2.86 ± 0.39
β-damascone	1381	1398	–	–	–	0.22 ± 0.15
Geranil acetate	1382	1384	–	4.32 ± 0.85	–	2.27 ± 1.08
Methyl eugenol	1407	1408	–	2.38 ± 1.42	–	0.36 ± 0.52
β-caryophyilene	1433	1435	–	–	–	0.38 ± 0.53
Germacrene D	1497	1496	0.75 ± 0.68	–	–	2.20 ± 0.14
Heptadecane	1697	1696	3.24 ± 0.79	8.19 ± 0.10	0.60 ± 1.31	13.00 ± 0.40
α-guaiene	1447	1447	0.34 ± 0.11	–	–	0.48 ± 0.26
Octadecane	1795	1796	–	–	–	0.67 ± 0.37
Nonadecene	1872	1874	2.69 ± 0.72	–	0.84 ± 0.67	0.45 ± 0.23
Farnesol	1728	1728	–	6.53 ± 0.72	2.72 ± 0.31	6.73 ± 0.59
Nonadecene	1898	1898	30.66 ± 0.16	59.79 ± 0.20	27.73 ± 0.31	–
Palmitic Acid			–	–	5.14 ± 0.21	–
Eicosane	1994	1995	0.75 ± 0.76	1.39 ± 1.45	1.87 ± .48	1.15 ± 0.06
Eneicosane	2106	2110	4.39 ± 0.07	0.59 ± 0.90	20.82 ± 0.17	0.98 ± 0.66
Steoarolic Acid			–	–	9.88 ± 0.32	–
1-docosene			–	–	3.34 ± 0.09	–
Tricosane			–	–	3.59 ± 0.14	–
Dotriacontane			–	–	3.15 ± 0.30	–
1-eicosanol			–	–	12.27 ± 0.06	–

[a]Calculated retention indexes.
[b]Pellari et al., 2012.
[c]GC peak area percentage ± % RSD. Results expressed as mean of three replications−, not detected.

In Figure 3 the overall Sc-CO_2 extraction curves (OECs) (total polyphenols content *vs*. time) were plotted to evaluate the effect of different operating conditions on polyphenols extraction.

The Sc-CO_2+15%W curve exhibited a constant-extraction rate period (CER) of 120 min, and a diffusion-controlled period (DC) followed. An intermediary falling extraction rate (FER) period was not observed [34]. The initial linear period corresponded about the 86% of the final extracted polyphenols (18.3 mg GAE/ g dw). The Sc-CO_2+10 % EtW curve exhibited a constant-extraction rate period (CER) of 180 min which corresponded about the 85% of the final extracted polyphenols (32.5 mg GAE/ g dw). It is worth noting that up to 60 min the slopes of both OECs were little different as they were related to the extract solubility, which only depends on pressure and temperature. However, beyond 60 min the extraction curves started to diverge. Herein there is a period where diffusion phenomena appear and the slopes depend on particle size and flow rate of the solvent used. Such trends corroborated the hypothesis of the broken plus intact cells model proposed by Sovová [35]. About the 15% of the final extracted phenols were deposited inside the flowers particles and phenols diffusion to the particle

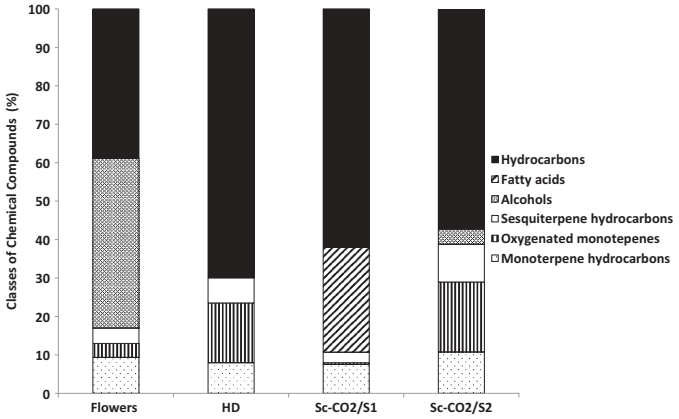

FIG. 2. Classes of chemical compounds (%) detected by HS-SPME/GC-MS of *Rosa damascena* flowers, essential oil (HD) and Sc-CO_2 extracts S1 and S2.

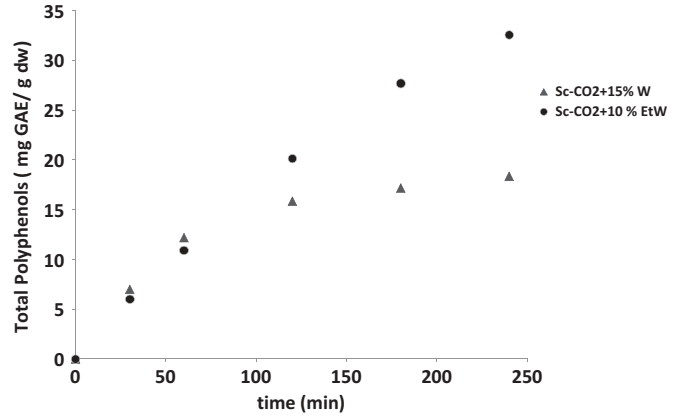

FIG. 3. Overall extraction curves (OECs) of total polyphenols obtained by Sc-CO_2 modified with 15% water or 10% ethanol-water mixture (57% v/v).

surface was slow, especially when water is added as co-solvent to Sc-CO_2.

By comparison of the results obtained by SC-CO_2+15% W extraction (18.3 mg GAE/ g dw) and SC-CO_2 + 10% EtW (32.5 mg GAE/ g dw), the phenols were much more effectively recovered when Sc-CO_2+10% EtW at 8 MPa was used. Chang et al. [36] reported methanol to be an effective solvent for extraction of compounds with antioxidative properties from spices. In this regard, it is interesting to note that phenols extracted by ScCO_2+10% EtW gave yield (3250 mg GAE/100 g dw) ranging about 80 % of methanol extraction yield (3832 mg GAE/100 g dw). These results are in agreement with data reported by Baydan et al [37] on *R. damascena* spent flowers.

CONCLUSIONS

The supercritical CO_2 extraction procedure studied to recovery volatile compounds and polyphenols from *Rosa damascena* was efficient. A two-steps separation procedure at 16 MPa and 313.15 K and on-line fractionation using two separators (S1: 7 MPa/ 298.15 K; S2: 5 MPa/ 288.15 K) produced in S2 an aromatic extract of superior quality containing 2-phenylethanol and β-damascone. This result is particularly noteworthy since 2-phenylethanol is water soluble and most of it is lost when hydrodistillation is used. Moreover, the following Sc-CO_2 + 10% ethanol-water mixture (57% v/v) extraction of polyphenols from spent flowers gave a good yield ranging about 80 % of methanol extraction yield.

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