Traditional and Supercritical CO_2 Extraction of the Volatiles from *Narcissus poeticus* L.

Quantitative and qualitative comparison of yield from the two extraction techniques

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Narcissus is a monocotyledon belonging to the Amaryllidaceas family. In Italy, a few of its species are widely distributed: *N. pseudonarcissus* L. variant: *N. incomparabilis* Miller, *N. odorus* L., *N. jonquilla* L.; *N. radiiflorus* Salisb.; *N. serotinus* L.; *N. tazetta* L. variant *N. biflorus* Curtis; *N. poeticus* L.[1] The narcissus volatiles are extracted from three species of *Narcissus*: *N. poeticus*, *N. tazetta* and *N. joquilla.* However, in perfumery, *N. poeticus* is the most important of the three due to its floral and pungent smell.[2]

Narcissus poeticus is a bulbous herbaceous plant of about 20–30 cm in height. The flowers are very fragrant and usually there is only one flower per stem. The perianth is pure white and has six tepals that are fused at the base forming a green floral tube 5 mm in diameter and 3 cm long.[3] The corona, or floral cup (1.3 cm in diameter and 2–4 mm long), is yellow with touches of red. The fruit of *N. poeticus* is capsular, oblong and divided into three compartments that contain black seeds.[3] The plant blooms between April and June at an altitude between 300 m and 2,300 m; it appears in large colonies in humid zones (in mountain meadows and alongside streams and rivers), in calcareous soils with full sunlight or partial shade.[3] *Narcissus poeticus* is thought to originate from the Middle East or Eastern Mediterranean, but now it is found all over Europe, particularly in France, Spain and Greece.[3] Within Italy, its geographical distribution is predominantly central and includes the Lazio and Abruzzo regions.

Narcissus poeticus of Rocca di Mezzo blooms spontaneously in May in the grass pastures of Terranera at an altitude of 1,300–1,400 m. The area is subject to snowfall in winter and temporary flooding in spring due to melting snow. This region's climate is humid and very sunny, and has karst soil. Thanks to its strong, pungent, green, woody and floral smell, *N. poeticus,* is frequently used in compositions of modern perfumes, mostly as a heart note, providing exquisite tonalities. Its fragrance blends well with many floral absolutes, such as clove bud, carnation, jasmine, neroli, ylang ylang, mimosa, karo karoundè and mate.[3] The price of *N. poeticus* reflects its importance in perfumery: 28 g (~1 oz) of absolute costs about $1,100.[3]

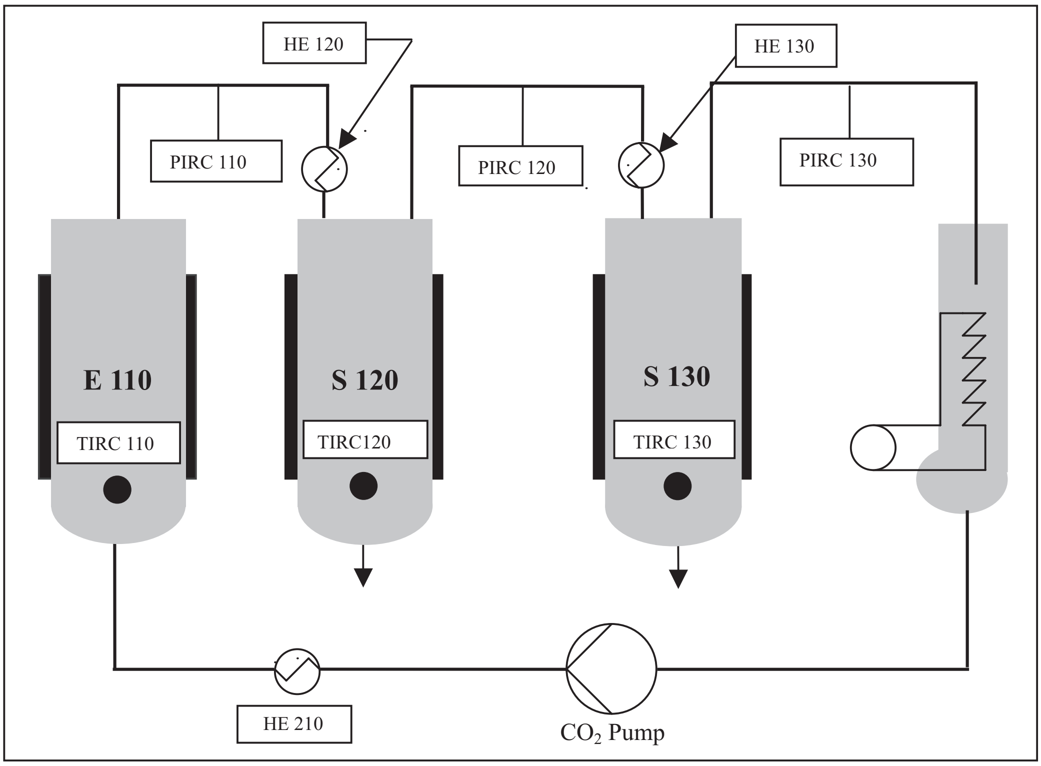
Essential oils from fresh blooms are traditionally produced by steam distillation, while extracts are produced by solvent extraction. Steam distillation produces an oil without waxes, but the yield is very low and the process induces thermal degradation of many fragrant compounds; therefore, extraction of the volatiles with a low boiling solvent is preferred. Solvent extraction (such as hexane or petroleum ether) produces a concrete (absolute and waxes). To eliminate the waxes, the concrete is further treated with ethanol producing an absolute. Solvent extraction is very efficient, but solvent elimination after extraction poses a major problem: the possible thermal degradation of the oil and the solvent contamination of the extracts are the drawbacks of this process. Supercritical CO_2 extraction is a promising technique for the production of flavors and fragrances from a vegetable matrix, as it offers several advantages such as extraction near room temperature (to avoid thermal degradation of the compounds) and ease of solvent removal after extraction (leaving no trace in the extract). The extraction is also environmentally safe (not harmful or toxic). These advantages are, however, partially offset by the high costs necessary for the extraction equipment; hence, supercritical CO_2 technique is generally considered convenient in producing just small amounts of high quality products. In order to enhance the affinity of this solvent toward polar substances (which have a very low solubility in supercritical CO_2), a small amount of polar liquid cosolvent can be added to supercritical fluid.

Supercritical CO_2 extraction of volatiles has been one of the early and most studied applications in the supercritical fluid literature.[4–6] An advantage offered by supercritical CO_2 technology applied to volatile extraction is the potential to fractionate the extract and separate the volatiles from the nonvolatiles and waxes. At low temperature (in the range between 0°C and -15°C in function of vegetable matrix

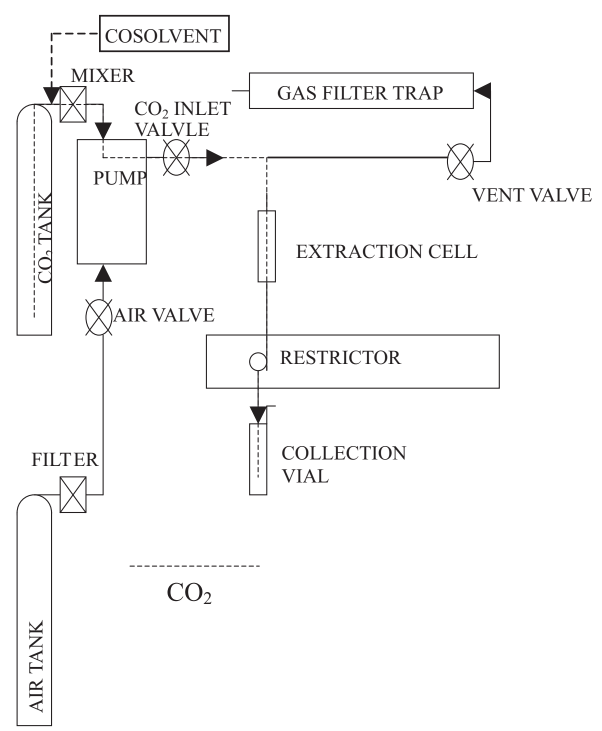
At a Glance

Narcissus poeticus L. is an important floral note that is widely used and appreciated, especially in sophisticated modern perfume. The work presented here focuses on extraction of *N. poeticus* flowers harvested in Rocca di Mezzo, Italy, with the conventional technique of hexane extraction, and detection and quantification of the main compounds of the extract by GC/MS.

Simplified scheme of pilot plant apparatus with two separation steps: E110 extractor, S120 first separator, S130 second separator **F-1**



Simplified scheme of the Dionex 703-SFE extractor **F-2**



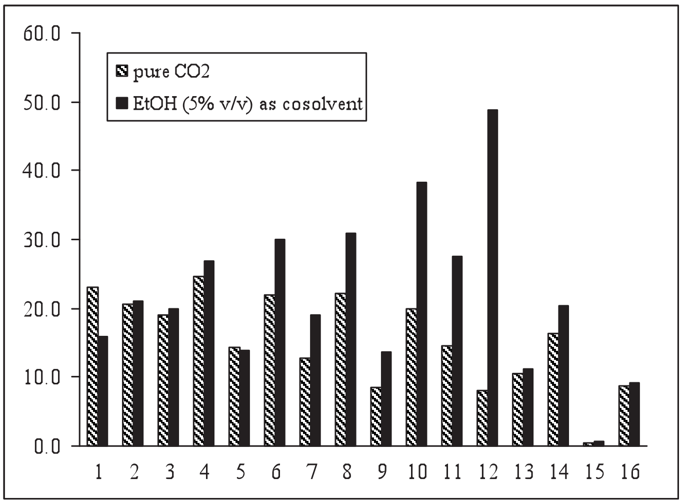
tested) the solubility of waxes is close to zero, whereas volatiles are completely soluble in CO_2. Therefore, it is possible to fractionate the extract by operating two separations in series, and setting the separators at different pressure and temperature conditions to precipitate, selectively, waxes in the first separator (maintained at lower temperature), and volatile concentrate in the second separator, where CO_2 returns to gaseous state.[4,5,7–10] A scheme of a pilot plant with two separation steps is shown in **F-1**.

In this work *N. poeticus* flowers, picked in Rocca di Mezzo, were extracted with a conventional technique of hexane extraction, at room temperature, and with supercritical CO_2. The aim of the present study was twofold: determining composition of the extract from the narcissus of Rocca di Mezzo in terms of main compounds found and of their abundance, and comparing traditional hexane extraction with supercritical CO_2 technology in terms of extraction of main compounds.

Materials and Methods

Chemicals: CO_2N_{45} (at 99.995% purity), pressurized with helium at 110 bar, was purchased from Air Liquide (Paris). N-hexane HPLC grade and ethanol RG were supplied by Sigma-Aldrich Chemie (Steinheim, Germany). Standard solutions of cinnamyl alcohol, methyl isoeugenol, isoeugenol, methyl eugenol, α-terpineol, eugenol, benzyl alcohol, methyl benzoate, and benzyl salicylate were purchased by Sigma-Aldrich Chemie.

Comparison between supercritical extractions with pure CO_2 and with CO_2 + EtOH (5% v/v) as co-solvent **F-3**



1: benzyl alcohol; 2: methyl benzoate; 3: α-terpineol; 4: phenylpropyl alcohol; 5: cinnamyl aldehyde; 6: cinnamyl alcohol; 7: eugenol; 8: methyl eugenol; 9: isoeugenol; 10: methyl isoeugenol; 11: benzyl benzoate; 12: benzyl salicylate; 13: p-methyl anisole; 14: p-cresol; 15: nonanal; 16: (Z)-β-ocimene.

Harvest of flowers: The harvest of flowers was carried out in Rocca di Mezzo (L'Aquila), Italy in May 2008. The flowers were picked in the morning—manually, with scissors—with about 20 cm of stem. Only full open blooms were picked. The number of flowers harvested by a person in 1.5 hr was about 1,000. The pickers placed the blooms in straw baskets, grouped in bunches of about 50 flowers. After harvesting, flowers were separated from the stem with scissors. The weight of about 100 flowers without stem was ~265 g.

Some fresh flowers were used the day after the harvesting for hexane extraction, while the rest were ground with liquid nitrogen and then stored at -18°C, to preserve them for successive supercritical CO_2 extractions.

Apparatus and procedure: Hexane extractions were performed leaching the flowers in hexane HPLC grade for 3 hr at room temperature, while stirring. Eighty-five grams of fresh flowers were extracted in 850 mL of hexane; the extracted solution was analyzed. The hexane was then evaporated in air and total yield was determined. Moreover, to obtain absolute percentage, ethanol was added to hexane extract (concrete) and submitted to ultrasound to dissolve the extract; then the solution was cooled at -18°C for 24 hr to precipitate the waxes and centrifuged to separate ethanol solution containing the absolute. Finally, ethanol solution was removed, waxes were weighed, and the absolute amount was determined as the difference between total extract (concrete) weight and waxes weight.

Supercritical CO_2 extractions were carried out on a Dionex 703-SFE extractor (Dionex; Bannockburn, Illinois) with an extraction cell of 32 mL (**see F-2**). The experimental procedure started by heating the oven to the desired temperature and filling the cell with the flowers evenly distributed in inert glass beads. Then, the cell was connected in the oven cavity and, when the desired temperature was reached, the CO_2 was compressed by a high pressure pump (up to the desired extraction pressure) and passed through the extraction cell, starting the extraction. The extract was accumulated in a collection vial filled with hexane to enhance extract recovery; a second cooled vial, filled with hexane, was connected to the output of the equipment to avoid loss of extract with CO_2 waste. The equipment did not allow for the performance of two separation steps; therefore, the volatiles and waxes were collected together. Supercritical CO_2 extraction was carried out at extraction conditions of 120 atm and 40°C for 1 hr. Flow rate was set to about 1 L/min, and the extraction cell was loaded with 10 g of flowers previously ground with liquid nitrogen and then stored at -18°C, after thawing. Furthermore, the extraction was repeated using ethanol (5% v/v) as a modifier to enhance the solubility of *N. poeticus* characteristic compounds in supercritical CO_2. Each extraction was repeated three times.

Percentage of concrete and absolute of hexane extraction of *Narcissus poeticus* flowers **T-1**

	Total yield (concrete) (% w/w of fresh flowers)	Absolute (% w/w of concrete)	Absolute (% w/w of fresh flowers)
Hexane extraction	0.41	28.65	0.116

Main compounds in *Narcissus* hexane extract **T-2**

Main compounds contained in hexane extract from *N. poeticus* of Rocca di Mezzo	
benzyl benzoate	benzyl alcohol
cinnamyl alcohol	phenylpropyl alcohol
methyl isoeugenol*	p-cresol
(Z)-β-ocimene	nonanal
isoeugenol*	cinnamyl aldehyde
methyl eugenol	benzyl salicylate
α-terpineol	p-methyl anisole
eugenol	p-propenyl anisole
methyl benzoate	cinnamyl acetate

*correct isomer not identified

Concentrations of main compounds in hexane extract of *Narcissus poeticus* flowers **T-3**

Compounds	Concentrations in hexane extract (µg/g of fresh flowers)
cinnamyl alcohol	29.91
methyl isoeugenol*	28.07
isoeugenol*	23.12
methyl eugenol	20.72
α-terpineol	20.31
eugenol	16.17
benzyl alcohol	8.45
methyl benzoate	5.84
benzyl salicylate	2.80

*correct isomer not identified

Analytical method: The analyses were performed by gas chromatography with mass selective detector (GC/MS) using a Thermo Trace GC 2000 gas chromatograph coupled to a Trace MS detector, split-splitless injector and AS 2000 auto sampler (all from Thermo Electron Corp.; Waltham, Massachusetts). A fused silica capillary column DB-5 MS (Agilent Technologies; Santa Clara, California), 60 m x 0.25 mm, film thickness 0.25 µm, was used. The injector and interface temperatures were 250°C and 280°C, respectively. The carrier gas was helium at a flow of 1 mL/min. One µL of the sample was injected in splitless mode (60 s); the oven temperature was programmed as follows: 60°C for 1 min, 60–100°C at 30°C/min, 100°C for 1 min, 100–300°C at 3°C/min. The mass spectrometer was operated in scansion mode. The analyzed compounds were detected by comparison of their mass spectra with spectra from a NIST library. Some compounds were detected and quantified by comparison with retention time and mass spectra of reference compounds.

Results

Total yield (concrete) and percentage of absolute of hexane extraction are shown in **T-1**. Total yield was found to be 0.41% from fresh flowers: this value is higher than the

Yields of main compounds of SC-CO_2 extraction of *Narcissus* flowers expressed in percentage, referred to hexane extract (= 100%); each result is the media of three trials **T-4**

Compounds	SC-CO_2 extract 120 atm, 40°C	SC-SO_2 extract 120 atm, 40°C EtOH 5% v/v	Hexane extract
benzyl alcohol	23.1	15.9	100
methyl benzoate	20.5	21.0	100
α-terpineol	19.0	19.9	100
phenylpropyl alcohol	24.5	26.9	100
cinnamyl aldehyde	14.3	14.0	100
cinnamyl alcohol	21.9	30.0	100
eugenol	12.8	18.9	100
methyl eugenol	22.3	30.9	100
isoeugenol*	8.6	13.6	100
methyl isoeugenol*	19.8	38.3	100
benzyl benzoate	14.6	27.5	100
benzyl salicylate	8.2	48.8	100
p-methyl anisole	10.6	11.2	100
p-cresol	16.3	20.5	100
nonanal	0.5	0.7	100
(Z)-β-ocimene	8.6	9.2	100

* correct isomer not identified

values of literature for *N. poeticus*: Anonis and Harris reported a total yield ranging between 0.2% and 0.26% on fresh flowers (the Anonis data referred to petroleum ether as solvent, while Harris' doesn't specify the solvent).[2,3] Ehret et al. reported a percentage of concrete obtained with hexane extraction of 0.2–0.3% on fresh flowers. [11]

Percentage of absolute in the concrete extracted by means of hexane was found to be 28.65%, in agreement with literature data; Anonis reported a percentage of absolute ranging between 27% and 37%, Harris reported a percentage of 27–32%, and Ehret et al. reported a percentage of absolute in the extract ranging between 20% and 30%.[2,3,11] Harris also reported extraction data of Laboratoire Monique Remy with hexane as solvent to be: total yield 0.22% from fresh flowers, while the percentage of absolute is 35% from concrete.

T-2 shows the main compounds (listed in order of abundance) individuated in hexane extract by comparison of their mass spectra with spectra from an NIST library. **T-3** reports yields of some of the characteristic components for hexane extract, detected and quantified by comparison with retention time and mass spectra of reference compounds; concentrations are expressed as compound to fresh flowers mass ratio (µg/g).

Yields of main compounds obtained by supercritical CO_2 extraction are shown in **T-4** and are expressed as percentage to hexane extract (assumed equal to 100%); each result is the media of three trials. From the data it can be seen that supercritical CO_2 extract contains all the main components found in hexane extract. The results also indicate that, generally, addition of ethanol to supercritical CO_2 enhances yields of characteristic compounds (see **T-4**, **F-3**). From the data it is clear that the supercritical CO_2 technique gives yields of characteristic compounds lower than hexane extraction, even if ethanol (5% v/v) is added to supercritical fluid as cosolvent. Nevertheless it must be noted that conventional technique, by means of hexane, needs the successive step of solvent removal from the extract (usually carried out under vacuum conditions), which causes a loss of characteristic compounds, particularly of more volatile substances.

Moreover, it should be noted that supercritical CO_2 extraction is a renowned clean process, which allows substitution of organic solvents with the nontoxic CO_2 and produces an extract without solvent traces. Finally, this technology enables the separation of the waxes from the volatile concentrate by operating two separation steps in series and taking advantage of the fact that at low temperature (between 0°C and -15°C) waxes are practically insoluble in CO_2, whereas the volatiles are highly soluble.

Conclusion

In this work, the flowers of *N. poeticus* of Rocca di Mezzo, Italy were extracted with hexane, the main components of the extract were determined and some of them were quantified by means of GC/MS. Supercritical CO_2 technology was also applied to the extraction of volatiles from *N. poeticus*; the use of ethanol as a cosolvent was also tested. Then traditional (hexane) and innovative (supercritical CO_2) extraction technologies were compared in terms of characteristic compounds extracted. The experiments have demonstrated that supercritical CO_2 extract contains all the main compounds found in hexane extract, but hexane extraction gives higher yields. Nevertheless, supercritical CO_2 extraction allows to carry out a process without the use of an organic solvent and an extract without cuticular waxes, if a separator, working at low temperature, is used.

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