International Journal of Secondary Metabolite
2025, Vol. 12, No. 3, 593–603
https://doi.org/10.21448/ijsm.1509230
journal homepage: https://dergipark.org.tr/en/pub/ijsm
Research Article

Comparison of volatile oil compositions of five *Narcissus* (*Narcissus tazetta* L.) varieties extracted

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ARTICLE HISTORY
Received: July 2, 2024
Accepted: Nov. 16, 2024

KEYWORDS
Benzyl acetate,
Enfleurage,
Narcissus,
Trans-β-ocimene,
Volatile oil.

Abstract: *Narcissus* is one of the most important ornamental and medicinal plants, whose oil is widely used in the cosmetic, sanitary, and perfume industries. Khuzestan province, especially Behbahan district, is one of the most important *Narcissus* production centers in Iran. This research was conducted to evaluate and compare the volatile oil components of five *Narcissus* varieties (Shahla, Meskinack, Panjegorbai, Porpar, and Golsefied) which are commercially grown in Khuzestan Province. Enfleurage and head space solid-phase micro extraction methods were used for extraction. The fresh flowers of *Narcissus* were harvested and immediately transferred to the laboratory; the volatile oil was extracted by the enfleurage method. The components were analyzed by GC-MS and then the results were also compared against the headspace solid-phase micro extraction method. *Trans-β*-ocimene, benzyl acetate, linalool, 1.8-cineole, and 2-Phenyl ethyl acetate were the most common constituents which were identified in different varieties of *Narcissus* from both methods of extraction. According to the results of GC-MS, *trans-β*-ocimene was the dominant compound of the HS-SPME method while benzyl acetate was the dominant compound of the enfleurage method. The diverse varieties of *Narcissus* contain different components, therefore more research studies are recommended.

1. INTRODUCTION

The Amaryllidaceae family is well known for its fragrant, attractive flowers, and has long been used for ornamental purposes. *Narcissus* species are extensively cultivated and exported for ornamental use in most parts of the world. The genus of *Narcissus* comprises more than one hundred wild species and is mainly distributed around the Mediterranean Sea, Southwestern Europe, North Africa, Italy, and the Balkans (Claveria *et al*., 2017). *Narcissus tazetta* L. subsp. *tazetta* is a wild plant that has a pleasant aroma and has been mainly cultivated for ornamental purpose (Melliou *et al*., 2007). Many flowers' volatiles are pleasant to human sensory system and have potential application in perfume industries (Li *et al*., 2006).

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e-ISSN: 2148-6905

Volatile oils are extensively used for many different purposes and in different ways. They have a deep effect on the central nervous system, relieving depression, anxiety, reducing stress and inducing relaxation. Further, volatile oil is utilized in aromatherapy which is a form of medicine (Hesham *et al*., 2016; Padumanonda *et al*., 2007). The pharmacological studies on *Narcissus tazetta* have demonstrated that it has antibacterial and antifungal, antiviral, antimalarial, anticancer, antioxidant, dermatological, cardiovascular, immunomodulatory, and acetylcholinesterase inhibitory effects (Al-Snafi, 2020). In terms of smell, the fragrances of *Narcissus* flowers vary depending on the species and cultivar. Some varieties of *Narcissus* have a sweet, floral scent, while others may have a more intense or musky fragrance. The smell of *Narcissus* is often described as fresh, aromatic, and reminiscent of springtime (Dobson *et al*., 1997).

The analysis of organic compounds in plants or released by plants has gained much interest in recent years. Most of the plants can emit volatile organic compounds from different organs, such as fruits, flowers, leaves, stems, and roots (Zhu *et al*., 2013). Different methods have been used for the extraction of volatile oils. However, since volatile profile may significantly change in the distillation method due to the sensitivity to high temperature (Li *et al*., 2006), headspace solid phase micro extraction analysis seems to be the best one for analyzing the volatile compositions of delicate and odoriferous flowers. In HS- SPME method, no organic solvent are used and is very simple to operate. This method includes extraction, concentration, and sampling in a single step (Prakash *et al*., 2012).

Furthermore, some flowers, such as jasmine, daffodil, and tuberose have a low content of volatile oil which is sensitive to high temperature and would damage the flowers before releasing the volatile oils. Recently, it was confirmed that applying the HS-SPME method provides a more natural way for extraction of plant volatile oils and detect the volatile constituents emitted by fresh flowers in a fast and easy way (Drew *et al*., 2012; Flamini *et al*., 2003). In addition, the volatile compounds which are present in the headspace of odoriferous flowers may not be detectable by conventional methods, such as solvent extractions and hydrodistillation (Li *et al*., 2006).

Enfleurage method is mainly used for oil extraction of rose flowers, tuberose, and sometimes for orange flowers and *Narcissus*. This method was designed for high amounts of plant materials as well as for plants which comprise low volatile oil or when the volatile oil is susceptible to high temperature. However, this method is very labor-intensive and costly (Handa *et al*., 2008). There are some reports on daffodil oil‘s components. *Trans-β*-ocimene (61.12%) in *N. tazetta* and benzyl acetate (19.36%) in *N. serotinus* was reported by Milliou *et al*., (2007). Similarly, (Chen *et al*., 2013) reported that main oil components of *N. tazetta* are *β*-ocimene (62.73%), benzyl acetate (25.02%), 1.8 cineol (1.49%), linalool (1.14%), and 2-phenyl ethyl acetate (1.12%). Similar oil compositions were reported by Ruíz-Ramón *et al*. (2014) and Li *et al*., (2018) in *Narcissus* species. However, there is no information about oil components of Iranian *Narcissus*; therefore, this research was conducted to evaluate the volatile components of five different *Narcissus* varieties which are grown commercially in Khuzestan province of Iran and using two methods of volatile oil extraction.

2. MATERIAL and METHODS

2.1. Plant Materials

Narcissus is known for its showy, trumpet-shaped flowers and long, narrow leaves. The flowers of *Narcissus* vary in color, including white, yellow, orange, and pink. The vegetation period of *Narcissus* plants typically begins in the fall, with flowers blooming in late winter or early spring. The leaves of *Narcissus* are long and slender, often growing in clumps or tufts from the base of the plant. The flowers of *Narcissus* are characterized by a central corona surrounded by six petals. It blooms in late winter or early spring, with clusters of flowers on each stem for a short period of a few weeks. After flowering, the plant enters a dormancy phase during the summer, with leaves eventually wilting and dying back (Chehrazi *et al*., 2007; Demir *et al*., 2021).

Behbahan district is located in the southeastern part of Khuzestan Province in Iran and situated at an altitude of 280 meters above sea level, with an average annual temperature of 24 degrees Celsius and an average annual rainfall of 370 millimeters. The central area of Behbahan district is situated in a dry and saline plain. Due to its natural characteristics and location in a tropical region, the climate in this region can be described as semi-arid, marked by intense hot summers and brief, cold winters, along with limited precipitation. *Narcissus* growth area in this district features a variety of soils influenced by the geographical and climatic characteristics, as well as the type of vegetation in the region. The soils in this area are typically loamy and clay, which have a suitable structure for cultivating *Narcissus*. These soils can retain moisture effectively and are very suitable for the growth of the plant. The soil pH in this region usually ranges from neutral to slightly alkaline, approximately around 7.6. The soil in this area is rich in nutrients such as nitrogen, phosphorus, and potassium, which is vital for the growth of *Narcissus* and other plants (Dourr *et al*., 2020).

The flowers of five varieties of *Narcissus* (Shahla, Meskinack, Porpar, Panjegorbai and Golsefied as displayed in (Figure 1, Table 1) were collected from a commercial farm in an area of about 20 hectares located in Behbahan district (Khuzestan, Iran). After harvesting, the plant materials wrapped with paper envelopes, and then immediately transferred to the medicinal plants laboratory of agriculture faculty, Shahid Chamran University of Ahvaz. The volatile oils were extracted from the fresh flowers of *Narcissus* using two methods of extraction: enfleurage and headspace-solid phase micro extraction.

Table 1. Morphological characteristics of *Narcissus* varieties.

Characteristics	*Narcissus* varieties				
	Shahla	Meskinack	Porpar	Panjegorbai	Golsefied
Crown color	Yellow	Yellow	Yellow	Yellow	White
Shape	Round	Elongated	Spoon Shaped	Spoon Shaped	Elongated
Color	Cream	Cream	Cream	Cream	White
Petals and Sepals form	Compact	Compact	Full Feathered	Full Feathered	Compact

Figure 1. The flower of five *Narcissus* varieties.

2.2. Enfleurage Method

In this method, 200 g of the palm lard was spread on glass plates (50×50×4 cm) containing wooden frame and then 300 g of *Narcissus* petals were placed on fat and left for about 48 hours. Thereafter, the old flowers were replaced by fresh ones. This action was repeated four times, and then the saturated fat or (pomade) was scraped out from chassis with spatula and dissolved in ethanol for isolation of volatile oil for six days while being stirred daily and then filtered. The solvent was evaporated by the rotary apparatus at 45°C after which the volatile oil was kept in glass vials at 4°C in refrigerator. Finally, the volatile oil compositions were detected by GC/MS as the same program and condition like below.

2.3. Headspace Solid – Phase Micro Extraction Method

In order to compare the *Narcissus* volatile oil compositions, the HS-SPME method was used. Firstly, 20 g of fresh flower from every variety was placed in glass bottles comprising special septum and kept for 1 h under room conditions. Extraction fiber was inserted through septum into bottles and held for another 1 h, and then the fiber was injected to GC/MS Agilent Hewlett-Packed gas chromatograph equipped with an HP-5MS column (30m, 0.25mm diameter and with 0.25µm film thickness), with desorption continued for 10 min. The analyses were operated under the following condition; the initial temperature of the column was 60°C with increasing 5°C/ min and held for 3 min till 325 °C as final temperature. The injector and detector temperatures were 250 °C and 340°C. Helium with a constant flow of 1.5 ml/ min was used as a carrier gas. EOs constituents were identified based on the retention indices relative to C5-C28 n-alkanes obtained in the same conditions, and by comparing their mass spectra with those recorded in the Wiley 7 n.L and those reported in the literature (Adams, 2017).

2.4. Statistical analysis

The yield of volatile oil was determined in triplicate. The percentage of the volatile oil yield was calculated as the weight of volatile oil divided by the weight of fresh flower using below formula.

$$\text{Percentage of volatile oil} = \frac{\text{weight of volatile oil}}{\text{weight of fresh flower}} \times 100$$

3. RESULTS

3.1. Volatile Oil Yield

The comparison of volatile oil yield of *Narcissus* extracted by enfleurage method is very different among the *Narcissus* varieties (Figure 2). The maximum percentage of volatile oil (0.16%) belongs to Shahla variety while the minimum oil content (0.11%) was recorded in Porpar. However, the volatile oil yields of Golsefied, Meskinack, and Panjegorbai were 0.14%, 0.12%, and 0.12%, respectively. These results are in agreement with Paibon *et al*. (2011) experiment which was studied on rose flower, since the method of extraction and type plant material were similar. There is no report in the world about the *Narcissus* volatile oil yield in the literature and it was the first time that the *Narcissus* volatile oil was extracted by enfleurage method.

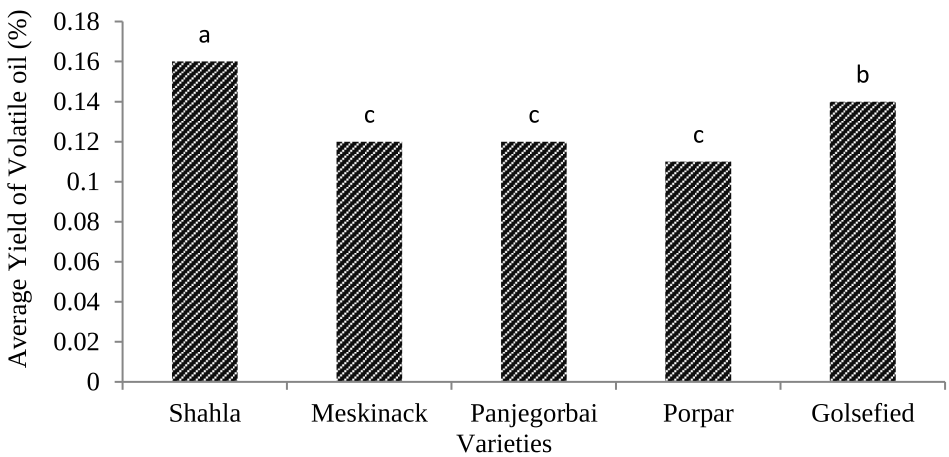

Figure 2. Percentage of volatile oil of five *Narcissus* varieties (The letters indicate a statistically significant difference at the level of 5%).

3.2. Volatile Oil Components of Enfleurage Method

Volatile components of five *Narcissus* varieties which are extracted by the enfleurage method are reported (see Table 2). According to the results, 21 components of volatile oil were detected by GC/MS in Shahla, 20 components in Meskinack, 19 components in Porpar, 19 components in Panjegorbai, and 7 components in Golsefied. The predominant compounds identified in this method across all varieties were benzyl acetate, linalool, 1, 8-cineole, 2-Phenyl ethyl acetate, and trans-*β*-ocimene which are categorized as monoterpenes, sesquiterpenes, alcohols, amines, esters, acids, and aromatic compounds.

Results indicated that there are significant differences between volatile components of *Narcissus* varieties. Benzyl acetate was the dominant component of all varieties, and its relative amount was 50.37%, 40.04%, 38.16%, 34.3%, and 31.5% in Golsefied, Porpar, Panjegorbai, Meskinack, and Shahla, respectively. The amount of this compound has been reported to be 37.34% and 19.34% in simple and double flowers floral type *Narcissus* by Ruíz-Ramón *et al*., (2014). Additionally, 1.8-cineole was identified in all varieties and its amount was 12.18%, 7.78%, 5.07%, 2.51%, and 2.1% in Golsefied, Porpar, Meskinack, Shahla, and Panjegorbai, respectively. These results were higher than what have been reported by Chen *et al*., (2013).

Table 2. The volatile components of five varieties of *Narcissus* identified by enfleurage.

Volatile compounds	RT	RI_c	RI_r	Shahla	Meskinack	Porpar	Panjegorbai	Golsefied
Ethanol-2-butoxy	4.10	889	891	0.18	0.75	0.27	1.43	3.03
Hexanoic acid	5.32	967	975	0.33	0.14	0	0.71	0
1,8-Cineole	6.71	1025	1026	2.51	5.07	7.78	2.1	12.18
trans-β-Ocimene	7.04	1041	1044	0.13	0.26	3.17	0	4.37
1,2,3-Triethoxypropane	7.71	1071	1075	0.48	0	0.27	1.75	0
2-Butoxyethyl acetate	8.03	1086	1089	0.45	1.95	0.74	2.12	0
Linalool	8.32	1094	1095	22.06	25.5	8.82	9.56	0
Ethyl hexanoic acid	8.70	1121	1123	0.62	1.69	0.61	2.22	0
Benzyl acetate	9.99	1156	1157	31.5	34.3	40.04	39.16	50.39
α-Terpineol	10.70	1184	1186	1.49	2.14	1.33	2.95	0
Octanoic acid	10.78	1188	1191	0.65	0.14	0.26	0.43	0
Hydrocinnamyl alcohol	11.67	1225	1227	0.31	0.26	0.32	1.08	0
Phenyl ethyl acetate <2->	12.40	1251	1254	6.13	11.96	3.99	4.02	0
Indol	13.40	1287	1290	3.73	3.74	3.29	4.7	0
2,4-Decadienal, (E,E)	13.91	1314	1316	0.15	0.78	0	1.42	0
Hydrocinnamyl acetate	15.31	1368	1370	6	1	3.83	7	0
Ethyl decanoate	15.92	1391	1395	0.58	0.19	0.23	1.92	0
Cinnamyl acetate	17.16	1445	1446	0.24	0.25	0.35	0.56	0
Butylated hydroxy anisole	17.60	1487	1489	3.68	2.19	6.44	4.84	5.96
lauric acid	20.72	1561	1566	13.44	0.36	6.75	0	4.99
Palmitic acid	28.37	1962	1964	0.77	1.11	5.31	4.68	12.98
Total				95.43	94.17	93.8	93.21	93.9

RT = Retention Time
RI = Retention Index

Trans-β-ocimene was not detected in Panjegorbai. However, the amount of this component in Shahla and Meskinack varieties was lower than 1%, while Golsefied and Porpar oils contained 4.37% and 3.17%, respectively. Linalool was identified in varieties of Meskinack, Shahla, Panjegorbai, and Porpar with amount 25.5%, 22.06%, 9.56%, and 8.82%, respectively, while it was not detected in Golsefied in both experiments.

2-Phenyl ethyl acetate was not detected in Golsefied, while it is founded in other varieties ranged from 3.99% to 11.96% being in accordance with Melliuo *et al*., (2007). Indole was registered in Panjegorbai, Meskinack, Shahla, and Porpar and its amount was higher than the results obtained in HS-SPME. Probably, this component is extracted better with fat. Hydrocinnamic was recorded in Panjegorbai (7%), Shahla (6%), Porpar (3.83%), and

Meskinack (1%), but this component was not detected in Golsefeid. These results were in contrast with Ruíz-Ramón *et al*., (2014) which might be due to differences in *Narcissus* species, stage of growth, method of extraction, flowering season, and time of flower harvest.

Butylated hydroxy anisole was not detected from variety of Meskinack in HS-SPME method, but was identified in enfleurage method and this component has not been reported in any literature. Lauric acid did not exist in the variety of Panjegorbai, but it was detected in varieties of Shahla, Porpar, Golsefied, and Meskinack 13.44%, 6.57%, 4.99%, and 0.36% respectively. Palmitic acid was identified in all varieties of *Narcissus*. However, Golsefied had the highest amount (12.98%) among the varieties while in contrast the lowest amount (0.77%) was observed in Shahla

3.3. Volatile Oil Components of HS-SPME Method

The volatile components of five *Narcissus* varieties extracted by HS–SPME method are illustrated (see Table 3). Approximately, 15 components of volatile oil were found in Shahla, 11 components in Meskinack, 13 components in Porpar, 13 components in Panjegorbai, and 10 components in Golsefied. The most dominant compounds observed in this method in all varieties were *trans-β*-ocimene, benzyl acetate, linalool, 1, 8-cineole, and 2-Phenyl ethyl acetate, which are categorized as monoterpenes, alcohols, amines, esters, acids, and aromatic compounds.

Table 3. The volatile components of five varieties of *Narcissus* identified by HS-SPME.

Volatile compounds	RT	RI_c	RI_r	Shahla	Meskinack	Porpar	Panjegorbai	Golsefeid
Buten-1-ol <3-methyl-2->	3.73	768	773	0.55	0	1.36	1.24	0
α-Pinene	4.74	932	932	0.29	0.88	0.48	0.33	0
Myrcene	5.8	986	988	0.31	1.59	1.03	1.23	0.35
Anisole <ρ-methyl->	6.44	1012	1015	0	0	0	0	0.42
1,8-Cineole	6.75	1025	1026	2.93	13.92	8.11	4.93	0.68
trans-β-Ocimene	7.32	1041	1044	45.25	39.47	53.75	61.06	68.76
α-Terpinolene	8.11	1082	1086	0.05	0	0		0
Linalool	8.43	1094	1095	6.21	9.31	1.68	3.32	0
Ocimene	9.12	1126	1128	1.47	0.64	0.98	1.56	0.68
Benzeneacetonitrile	9.42	1133	1134	0.72	0	0	0	0
Benzyl acetate	10.32	1156	1157	31.71	20.04	20.75	14.84	23.62
α-Terpineol	10.77	1184	1186	0.36	0.85	0.37	0.4	0
2-Phenyl ethyl acetate	12.49	1251	1254	4.19	12.46	1.45	1.35	0.42
Indole	13.38	1287	1290	0.6	0.28	0.35	0.53	1.12
Hydrocinnamyl acetate	15.37	1368	1370	2.13	0.55	3.7	4.37	1.82
trans-cinnamyl acetate	17.16	1445	1446	0.13	0	0	0	0
Butylated hydroxy anisole	17.64	1487	1489	1.97	0	5.55	4.17	2.13
Total				98.87	99.99	99.56	99.33	100

RT = Retention Time
RI = Retention Index

Among the oils components identified by GC/MS, *trans-β*-ocimene was the main compound detected in all varieties with 68.76%, 61.06%, 53.75%, 45.25%, and 39.47%, in Golsefeid, Panjegorbai, Porpar, Shahla, and Meskinack, respectively as also found by Chen *et al*., (2013) and Melliou *et al*., (2007). Further, benzyl acetate was identified as the second most abundant component whose amount was 31.71%, 23.62%, 20.75%, 20.04%, and 14.48% in Shahla, Golsefeid, Porpar, Meskinack and Panjegorbai, respectively. This finding was in agreement with results reported by Chen *et al*., (2013). Meanwhile, in the current research, linalool was not detected in Golsefeid, while it was recorded in other varieties. For example, the amount of this component ranged from highest to lowest (9.31%) and (1.68%) in Meskinack and Porpar, respectively. Additionally, Ruíz-Ramón *et al*., (2014) reported the amount of this compound as about 5.98% in double flower cultivar of *Narcissus*. However, this contradiction might be due to environmental conditions, harvest time, and as well as flowers age. 2-Phenyl ethyl acetate was found in five varieties. However, Meskinack with 12.48% 2-Phenyl ethyl acetate had the highest content of this component. In addition 1, 8-cineole was recorded in all varieties. However, the highest amount (13.92%) and the lowest amount (0.68%) were found in Meskinack and Golsefeid, respectively. Although α-pinene was identified less than 1% in four varieties, and was not observed in Golsefeid. These contradictory findings might be due to genetic factors and morphological differences.

4. DISCUSSION

Narcissus produces volatile small molecular compounds in its flowers. The amount and aromatic properties of volatile compounds determine the aromatic characteristics of *Narcissus* varieties. According to the results of experiments, there is an obvious difference in volatile oil content of *Narcissus* varieties and mainly various parameters such as genotype, size of flower petals and sepals, harvesting, development stage, phenological stages as well as daily rhythms influenced the oil content (koksal *et al*., 2015; Daghbouche *et al*., 2020). The flower odor of *Narcissus* is very popular in the fragrance industry. The most valued species for the perfume industry are most importantly *Narcissus tazetta*, *Narcissus poeticus*, and *Narcissus jonquilla* which are very odorous and mostly used for extraction of perfume (Ferri *et al*., 2009; Baranauskien & Venskutonis, 2022).

As the results demonstrate, benzyl acetate was the dominant volatile component in five varieties of *Narcissus* in enfleurage method, while in HS–SPME method, *trans-β*-ocimene was the dominant compound with different percentages. Commonly, the main components of *Narcissus* are benzyl acetate, *trans-β*-ocimene, linalool, and indole explaining the fragrance of *Narcissus* flowers (Chen *et al*., 2013). Benzyl acetate and *trans-β*-ocimene are also the main component of the *Narcissus* volatile oil and was reported as the major volatile component in several studies (Zarifikhoroshahi *et al*., 2021; Ruíz-Ramón *et al*., 2014; Song *et al*., 2007). The third compound with a high percentage is linalool which was detected in four varieties. In contrast, it was not found in Golsefied in both methods of extraction, and may not exist in this one. Linalool plays a major role in floral fragrance of daffodil flowers and has a pleasant odor; consequently, it is responsible for the reduction of low mood and anxiety in human (Sharmeen *et al*., 2021). The floral scent of *Narcissus* species is predominantly composed of *trans-β*-ocimene, benzyl acetate, and linalool, as identified in numerous studies (Losch *et al*., 2023). Another important component of Narcissus volatile oil is 1, 8 cineole, which was identified in all varieties and most studies have reported it from *Narcissus* volatile oil. 1, 8-cineole is known for its pleasant, refreshing aroma and is often used in aromatherapy and traditional medicine. 2-Phenyl ethyl acetate was consistently detected in all varieties of *Narcissus* with varying percentages, a finding that is supported by the research of Melliou *et al*., (2007). Palmitic acid was present in all varieties, particularly in high concentrations in the enfleurage extraction method. This compound has been previously reported in species such as *Narcissus trevithian* and *Narcissus geranium* by Van Dort *et al*., (1993). Indole, another prominent compound in *Narcissus* volatile oil, was identified in all varieties regardless of the extraction method. Indole is a common constituent of various natural sources known for its distinct odor and biological activities. Terry *et al*., (2021) also reported the presence of indole in *Narcissus*. The remaining components detected in the volatile oil varied among the different *Narcissus* varieties.

The variation in the number and percentage of detected compounds among different varieties of *Narcissus* is likely attributed to their genetic characteristics and the specific timing of their blooming. This variability underscores the complex interplay of genetic factors and environmental influences on the chemical composition of *Narcissus* varieties. The distinct genetic properties of each variety may contribute to the unique profile of volatile compounds present in their flowers, while the timing of blooming can also impact the development and abundance of specific chemical constituents. Thus, understanding the genetic and phenological factors that influence the chemical composition of *Narcissus* varieties is important for elucidating their aromatic properties and potential applications in various industries.

Volatile oils extracted from *Narcissus* plants have significant chemophenetic and taxonomic importance. These oils contain a complex mixture of volatile compounds that contribute to the unique fragrance of the flowers. The composition of volatile oils in *Narcissus* can vary among different species and varieties, providing valuable chemical markers for distinguishing between closely related species and varieties. Studies have shown that the volatile oils of *Narcissus* species contain a variety of compounds such as monoterpenes, sesquiterpenes, phenylpropanoids, and benzenoids, which contribute to the characteristic fragrance of the flowers. These chemical constituents play a crucial role in attracting pollinators and repelling herbivores, highlighting their ecological significance in the plant kingdom. Furthermore, the presence of specific compounds in the volatile oils can serve as diagnostic markers for taxonomic classification and species differentiation within the genus of *Narcissus* (Diaz-Maroto *et al*., 2006). The unique scent of *Narcissus tazetta* flowers is due to various volatile compounds. These compounds not only enhance the plant's sensory attraction but may also positively influence mood and psychological well-being (Mishra *et al*., 2023).

There is a very high diversity among volatile oil components of different *Narcissus* varieties, and this diversity depends on flowering, time of harvesting, and environmental conditions. In addition, volatile oil components of *Narcissus* are affected by the flower age. An experiment was conducted by Song *et al*., (2007) on four days and fresh flowers of *Narcissus*, simple and double flower cultivars. Hence, about 58% reduction in volatile oil of double flowers and 37% decline in volatile oil of simple flower were observed. Similarly, diversity in volatile oil components of *Narcissus* varieties depends on ecological differences such as latitude and longitude, height, temperature, humidity, climate, environmental stress, and soil (koksal *et al*., 2015).

The chemical analyses of volatile oils extracted from five different varieties of *Narcissus* using two methods revealed significant differences in the number and percentages of components. Despite similar conditions during harvesting, extraction, and GC/MS analysis, the variations observed are likely attributed to genetic factors and the extraction method employed. *Trans-β*-ocimene emerged as the predominant compound in all varieties when analyzed using the HS–SPME method, whereas benzyl acetate was identified as the dominant component in the enfleurage method. The preference for benzyl acetate extraction in enfleurage may be due to its higher molecular weight, which is more efficiently extracted by fat. In contrast, *trans-β*-ocimene, with a lower molecular weight, may be prone to vaporization at high temperatures during rotary processing. Thus, the HS–SPME method appears to be more suitable for identifying sensitive components in volatile oils.

The results of this study revealed significant variations in the volatile oil compositions of different *Narcissus* varieties. This information can be valuable for cosmetic formulators looking to create unique and distinctive fragrances for their products. The comparison of enfleurage and HS-SPME methods also provides insights into the most effective extraction technique for capturing the desired fragrance compounds from *Narcissus* flowers and other flowers. Several compounds were found in the enfleurage extraction methods that were not detected by the HS-SPME method. Notably, linalool was the only compound present in four varieties in both extraction methods, while it was not identified in the Golsefied variety using either method. This suggests that linalool may not be present in this particular variety.

5. CONCLUSION

There are significant differences between *Narcissus* varieties oil content and composition by two extraction methods. The maximum volatile oil yield (0.16%) belongs to the variety of Shahla while the minimum yield (0.12%) was observed in Porpar. *Trans-β*-ocimene, benzyl acetate, linalool, 1, 8-cineol, 2-phenyl ethyl acetate, and indole were the most important components detected by HS-SPME. Among them, *trans-β*-ocimene was identified as the dominant component and its recorded amount was 68.76%, 61.06%, 53.75%, 45.25%, and 39.47%, in Golsefeid, Panjegorbai, Porpar, Shahla, and Meskinack, respectively. Similarly, the same compounds with different percentage were detected in enfleurage. However, benzyl acetate was the dominant component of all varieties in enfleurage method, and its relative amount was 50.37%, 40.04%, 38.16%, 34.3%, and 31.5% in Golsefied, Porpar, Panjegorbai, Meskinack, and Shahla, respectively.

One notable similarity between the two extraction methods was the absence of linalool in the Golsefied variety of *Narcissus*. This suggests that linalool may not be present in this specific variety, as it was not detected in the volatile oil samples obtained through either enfleurage or HS-SPME. This finding highlights the variability in chemical composition among different *Narcissus* varieties and underscores the importance of considering such differences in botanical studies. Generally, according to the current results, enfleurage method can extract the main components of *Narcissus* and is a suitable method for extraction of volatile oil from the flowers. However, further research is recommended to optimize enfleurage method and obtained more volatile oil as well as similar components which are emitted by natural *Narcissus* flowers.

Acknowledgments

The authors of this research are thankful to Mr. Hasan Tajalli for supplying the *Narcissus* flowers and facility encouragement. We are grateful to the research council of Shahid Chamran University of Ahvaz for financial support with grant number (SCU.AH1400.775).

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Mahem, M.Y: Conceptualization; Methodology; Investigation; Resources; Data curation; Writing - Original draft; Visualization. **Mahmoodi Sourestani, M:** Conceptualization; Investigation; Methodology; Project administration; Review and Editing. **Motamedi, H:** Investigation; Methodology; Validation; Review and Editing. **Syyed Nejad, S.M:** Investigation; Validation; Supervision; Review and Editing. All the authors approved the submitted version.

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