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COMPARATIVE GC/MS ANALYSIS OF ROSE FLOWER AND DISTILLED OIL VOLATILES OF THE OIL BEARING ROSE *ROSA DAMASCENA*

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ABSTRACT

The accelerated and successful oil bearing rose breeding requires routine application of efficient procedure for analysis of flower volatiles, with capacity to extrapolate the obtained flower data to the volatile composition of the distilled rose oil. In the current study a procedure for solvent extraction and GC/MS analysis of rose flower and rose oil volatiles from oil bearing roses including Rosa damascena is presented. The procedure allows reliable identification of 68 volatiles in the rose flowers which are also detected in the distilled rose oil. The described procedure was further applied for comparative analysis of the flower and distilled rose oil volatiles from eight different genotypes of oil bearing roses. A data set consisting of ratios of the relative abundance of given volatile in the flower spectra to the relative abundance of the same volatile in the distilled rose oil spectra was generated. ANOVA test for a data subset of 27 volatiles detected in the flowers and rose oils of all analyzed oil bearing rose genotypes showed no significant influence of the genotype on the ratio of relative abundances of flower to rose oil volatiles. The average and relative standard deviation values of the obtained ratios between relative abundances of flower and rose oil volatiles for the analyzed genotypes were calculated for each identified flower compound. The results demonstrate that the described flower solvent extraction and GC/MS analysis procedure could be reliably applied for prediction of the volatile composition of distilled rose oils from wide range of oil bearing rose genotypes based on the extrapolation of GC/MS analysis data from single or few flowers from each studied plant. The possibilities for incorporation of the described procedure into oil bearing rose breeding and genetic resources characterization are discussed.

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Keywords: oil bearing rose, *Rosa damascena*, volatiles, flower, solvent extraction, rose oil, GC/MS

Introduction

In the past several years it has been demonstrated by several authors that the world production of rose oil is based on a single or a very few genotypes of the world famous oil bearing rose *Rosa damascena* (1, 2, 4, 11, 14). The whole production of rose oil in Bulgaria and Turkey, the two main world producers of rose oil, is based on a single main genotype which has been vegetatively propagated for centuries (1, 4, 14). The last points out the necessity to extend the oil bearing rose genetic resources and genetic background for their industrial cultivation. The possibilities for improvement of the oil rose have been recently reviewed in two papers (12, 13). Until now the *R. damascena* improvement in Bulgaria has been based only on clonal selection. The cross-breeding was avoided in order to preserve the traditional *R. damascena* odor and rose oil composition. During the past decade a solid ground has been established for application of molecular breeding for oil rose improvement (12, 13). However, the introduction of desired traits in *R. damascena* through intra- or inter- specific hybridizations should be done with extreme care on the changes of the rose flower volatiles since the composition of the distilled rose oil is under the control of an international standard (7). That's why, *R. damascena* cross-breeding requires application of efficient and high throughput procedure for routine assessment of the composition of the rose oils, separately distillated from flowers of large number of individual breeding lines and accessions of natural and segregating oil bearing rose populations.

The industrial production of rose oil is based on steam distillation of bulk of flowers harvested from the rose fields, where 3500 to 4000 kilograms of rose flowers are necessary to produce 1 kg of rose oil. The present industrial facilities generally require large amounts of rose flowers for single distillation. In laboratory conditions rose oil could be obtained through Clevenger distillation where approximately 200-1000 g (depending on the used apparatus) of fresh flowers are necessary to obtain small drops of distilled rose oil (3, 8, 9, 10). Thus, even the application of Clevenger microdistillation would require obtaining a significant number of flowers from each studied rose accession in order to evaluate its rose oil quality and industrial potential. This could be substantial time-limiting factor since within the first two years of rose growing the young plants produce only few flowers. Moreover, the distillation process could potentially bring additional variation and errors in the evaluation of the rose accessions since it adds additional technological step between the rose flower collection and the finally analyzed rose oil product which could be influenced by different factors.

A much faster and straightforward way to avoid the multiple microdistillations would be to evaluate the potential for rose oil production and oil composition directly by analyzing the fresh rose flowers through organic solvent extraction and GC/MS analysis of the flower volatiles. Organic solvents extraction of whole rose flowers and organs has been utilized for analysis of volatile compounds in hybrid-tea and Damask roses (5, 6), but no comparison with the volatiles of distilled rose oil was performed. Here we present a procedure for solvent extraction of rose flowers and comparative GC/MS analysis of the rose flowers and distilled rose oil volatiles.

Materials and Methods

Plant material harvest and storage

Rose flowers from eight accessions from the oil bearing rose collection of the Institute of Roses, Essential and Medicinal Cultures (IREMC) were collected according to the traditional rose flower collection practice during the 2010 rose harvest season, (early morning hours on the 3th of June 2010). The accessions from three groups of oil-bearing roses included: (a) *Rosa damascena*: cv. Svezhen, cv. Iskra, cv. Janina, cv. Elejna and a plant from Population 5 (population of *R. damascena* clones used for industrial cultivation); (b) the *R. damascena* hybrids: 836/61 [(*R. gallica L. Subsp. Eriostyla Kell. Var. Austriaca Crants f. Panonica Br.* X *R. damascena*) X *R. damascena*] and IV/11 (*R. damascena* x *R. gallica*); (c) accession of oil bearing *Rosa alba*. Following the collection, part of the flowers were immediately frozen in liquid nitrogen and stored in closed containers at -80°C for further analysis. The rest of the flowers, 0.6 kg of fresh flowers from each accession were used immediately for Clevenger distillation of rose oil.

Clevenger distillation of rose oil

Rose oil from each accession was obtained through Clevenger microdistillation in the essential oil distillation facility of IREMC. Two hundred grams of the fresh rose flowers were placed in 800 ml water and hydrodistilled for a period of 2.5 hours. The obtained essential oil samples were stored in closed glass vials at 4°C until GC/MS analysis was performed.

Flower metabolite extraction and sample preparation for GC/MS analysis

The flower material stored at -80°C was ground to powder and homogenized for 2 min at 30 Hz using liquid nitrogen pre-frozen Teflon jars and the Qiagen Tissue Lyser II Mill (Qiagen). Two hundred milligrams of the frozen ground material was transferred to pre-frozen 2ml screw top glass vial (Agilent). Extraction was carried out by addition of 400 µl of hexane containing 2 µg/ml 2-nonadecanone (Sigma-Aldrich) as internal standard for GC/MS analysis and immediate vortexing (Vortex Genius 3, VWR) at 2000 rpm, at room temperature for four hours. Remaining water in the sample was removed by addition of 100 mg anhydrous sodium sulfate at the end of the extraction and further vortexing for additional 20 min. The vials containing the extracts were centrifuged at 3500 rpm for 15 min. The clear supernatant was transferred to 400 µl microvolume insert (Agilent) placed in a new 2ml screw top vial and proceeded for GC/MS analysis. The preparation of Clevenger distilled rose oil samples for GC/MS analysis was done by dilution of 2 µl of distilled rose oils in 500 µl hexane containing 2 µg/ml 2-nonadecanone (Sigma-Aldrich) as internal standard.

GC/MS analysis

The GC/MS analysis of the prepared flower extracts and diluted rose oil samples was carried out on Agilent 7890A/5975C GC/MS system equipped with HP-5MS non-polar column using helium 5.0 as a carrier gas at a septum purge flow of 3 ml/min, splitless injection of 1 µl of the sample and the following acquisition parameters: injector temperature 250°C; Oven Program: 40°C for 3 min then 5°C/min to 300°C for 5 min; Run Time 60 min.

GC/MS Data and statistical analysis

Individual chemical compounds were identified by deconvolution using the AMDIS ver. 2.69 software (National Institute of Standards and Technology (NIST), USA) and the NIST 2008 mass spectral library searched by NIST MS Search v2.0 software. In order to minimize scoring of false positive signals the deconvolution parameters in AMDIS were set to resolution (low), sensitivity (very low) and shape requirements (low). The compounds related to NIST library hit with a match score of 800 or greater were further used for building a custom library. Retention index was calculated for each component using C10-C40 n-alkane mixture (Sigma-Aldrich). The generated custom built AMDIS library of compounds identified in the rose oil samples, was used as a target library for screening of all flower extracts and distilled rose oils. Data analysis and single factor ANOVA testing was performed using Microsoft Excel (Microsoft Corporation).

Results and Discussion

Flowers from eight different accessions of oil bearing roses from the collection of IREMC were subjected to comparative GC/MS analysis of rose flower and distilled oil volatiles. Rose oil samples from the studied oil bearing rose accessions were obtained following Clevenger distillation from collected rose flowers. The volatiles of hexane diluted rose oil samples were analyzed by GC/MS. Custom built AMDIS MS library consisting of 151 individual compounds identified in the different rose oil samples was constructed. The total number of library hits for each individual rose oil sample was: 98 for *R. damascena* cv. Svezhen, 99 for *R. damascena* cv. Iskra, 110 for *R. damascena* cv. Janina, 98 for *R. damascena* cv. Elejna, 110 for *R. damascena* population 5, 115 for *Rosa alba*, 96 for hybrid 836/61 and 104 for hybrid IV/11. The obtained library was further used as a target library for screening of the GC/MS spectra of solid-liquid solvent extracts of oil bearing rose flowers.

Several solvents were tested for their ability to extract metabolites from the oil bearing rose flowers including

chloroform, ethyl acetate, dichloromethane, hexane and methanol. All tested solvents with exception of methanol produced extracts with very similar spectra (data not shown). The methanol extract produced only a few essential peaks and a high signal background. The best results in terms of peak quality and clarity of the extracts were obtained with hexane which was chosen for further analysis. Different solvent extraction times (1, 2, 4, 6 and 17 hours) were also tested, (**Fig. 1**). The comparison of the obtained GC/MS data revealed no significant changes in the spectra (in terms of total number of peaks and abundance of the compounds) for extraction longer than 4 hours. Therefore 4 hours of extraction time was chosen for all subsequent analyses. No significant changes in the volatile spectra were observed also after ten times scale up of the extraction mixture (2 g of flower powder with 4 ml hexan) and extraction in 20 ml glass vial (data not shown). The smaller extraction volume procedure was chosen, due to its increased throughput and usage of less flower material. **Fig. 2** shows typical total ion chromatograms (TIC) of hexane extract of *R. damascena* flowers and hexane diluted Clevenger distilled rose oil from the same flower sample. In order to evaluate the reliability of the procedure of solvent extraction, three flower samples were prepared in parallel and GC/MS analyzed. The relative standard deviation (RSD) of the abundances for each of the detected compounds, relative to the abundance of the internal standard 2-nonadecanone, was calculated based on the data from the five *R. damascena* samples. As can be seen from **Table 1** the RSD for each compound is below 15% (with exception to Caryophilen oxide- 24%) which reveals a very good reproducibility and reliability of the procedure.

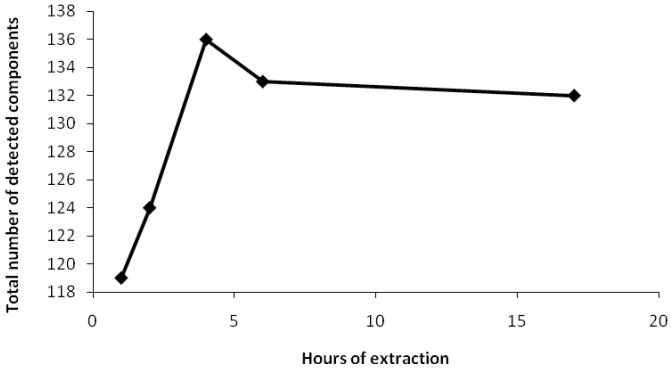

Fig. 1. Influence of the extraction duration on the total number of compounds detected by the AMDIS deconvolution algorithm for a *R. damascena* sample

A total of 68 compounds from the target library, identified positively in the analyzed Clevenger distilled rose oils, were also detected in the rose flowers extracts of the five different *R. damascena* accessions analyzed in the present study (**Table 1**). All compounds part of the international rose oil standard (7), with exception of nerol and ethanol, were reliably detected in the rose flower extracts including geraniol, citronellol, 2-phenylethanol, heptadecane, nonadecane and heneicosane. Nerol was not detected due to the use of the non-polar column HP-5MS where nerol and citronellol have similar retention times and were not chromatographically separated. In additional analysis of the same flower extracts, nerol was readily separated from citronellol and both compounds were quantified by using the polar GC column DB-WAXetr-Agilent, (data not presented). However, the use of non-polar column produced a much richer spectrum in terms of number of compounds and was preferred in the current study.

The main compound in the analyzed *R. damascena* flower extracts was Phenylethyl Alcohol 7.99-8.44% (abundance of the deconvoluted compound relative to the integrated total ion count as reported by the NIST AMDIS software), followed by Nonadecane 6.63-7.32%, Heneicosane 3.92-4.38%, 9-Nonadecene 3.01-3.74%, Heptacosane 2.84-3.46%, Tricosane 2.33-2.73%, Nonacosane 1.98-2.36%, beta-Citronellol+Nerol 1.91-2.43%, trans-Geraniol 1.51-2.25%, n-Heptadecane 1.46-1.75%, Pentacosane 1.38-1.60%, etc. The main compounds in the corresponding *R. damascena* rose oils were beta-Citronellol+Nerol 10.20-13.20%, Nonadecane 8.16-9.11%, 9-Nonadecene 6.36-7.29%, Heneicosane 5.48-6.68%, trans-Geraniol 4.97-6.53%, n-Heptadecane 3.49-4.45%, Eicosane 1.41-1.82%, Tricosane 1.57-2.15%, trans-Farnesol 1.24-1.85%, Geraniol acetate 1.00-1.69%, etc. The comparison of the ratio of the relative abundances of the flower compounds that were determined for the flower extract and for the distilled rose oil showed large variations between the compounds, due to their different rate of recovery within rose oil distillation. For example the relative abundance of the main flower compounds Phenylethyl Alcohol and Nonacosane was reduced above 15 times in the distilled oil, whereas the relative abundance of trace flower compound like Geraniol acetate was increased above 50 times in the rose oil. The substantial increase in the relative abundances of large number of the flower volatiles during the rose oil distillation is the main reason why the GC/MS analysis of distilled oil readily detects 98-110 different compounds in rose oil from the different *R. damascena* accessions, but only up to 68 of these compounds were detected in the flower extracts after using the same parameters for GC/MS and data analysis.

The main goal of development and application of procedure for GC/MS analysis of volatiles in rose flower extracts rather than analysis of the final product, flower distilled rose oil, is to use such procedure for acceleration and throughput increase of oil bearing rose breeding. Indeed, the GC/MS analysis of extract from single or few flowers derived from young oil rose plant will make possible to reduce by one to two years the time for first evaluation of newly developed rose breeding lines and segregating populations. Whereas the first flowers of *R. damascena* could be collected eventually at the second year after planting, one to two more years will be necessary for collection of enough flowers from single plant for running of Clevenger micro distillation. The direct volatile analysis of flower extracts will also boost the capacity and throughput for metabolite screening of large number of rose breeding lines, which is another limitation factor in oil rose breeding considering the short flowering period of oil bearing roses and restricted capacity of rose oil distillation facilities. In general,

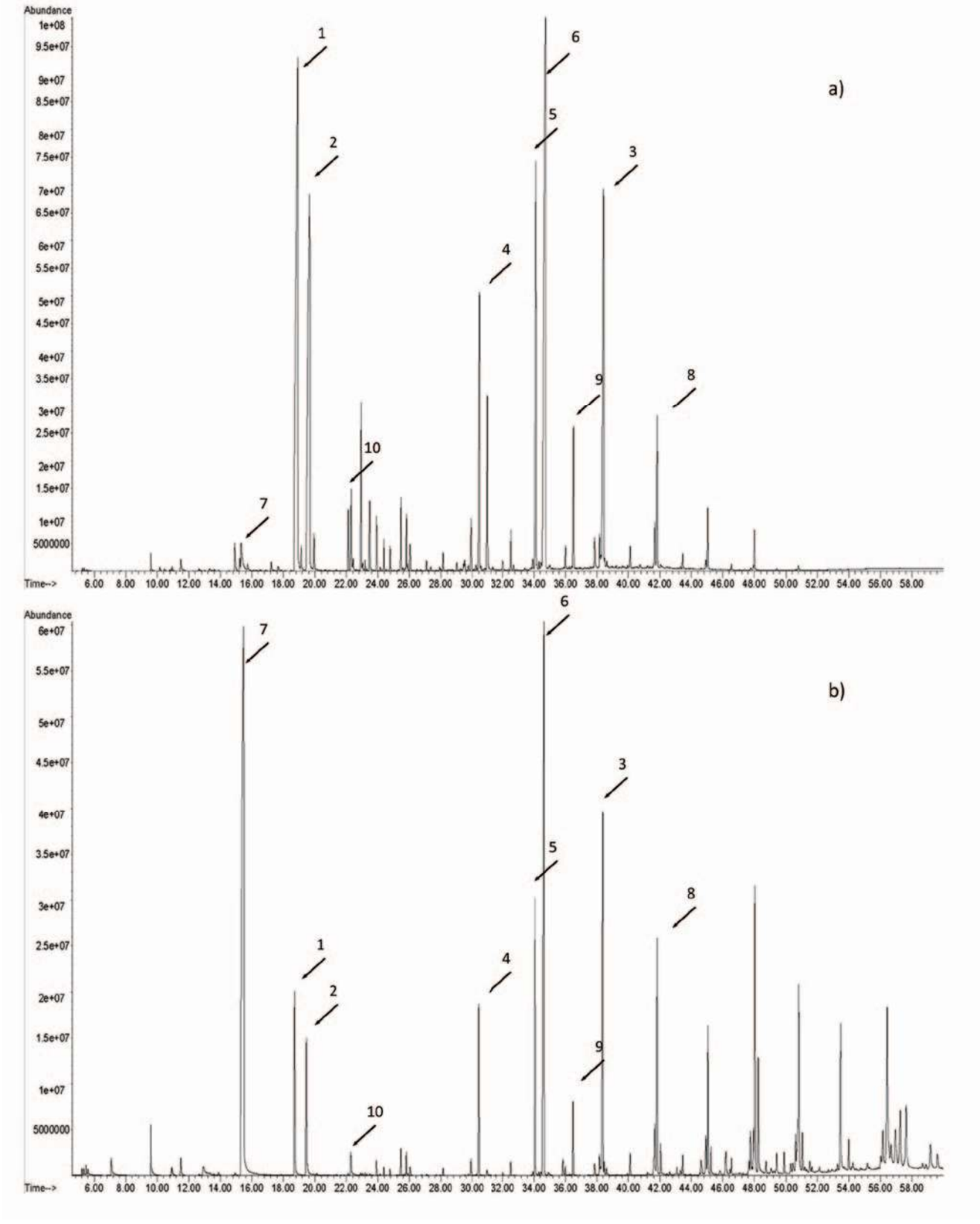

Fig. 2. GC/MS chromatograms of a) rose oil from cv. Svezhen and b) hexane flower extract from cv. Svezhen

Arrows show chromatogram peaks corresponding to identical compounds found in both chromatograms; (1) Citronellol+Nerol; (2) Geraniol; (3) Heneicosan; (4) Heptadecane; (5) 9-nonadecene; (6) Nonadecane; (7) 2-Phenylethyl Alcohol; (8) Tricosane; (9) Eicosane

TABLE 1
Results from the screening of the flower extracts spectra with the custom built AMDIS MS library

Compound	RSD *R.damascena* AFE	Svezhen	Iskra	Janina	Elejna	Pop5	Alba	836/61	IV/11	Svezhen	Iskra	Janina	Elejna	Pop 5	Alba	836/61	IV/11	Average AFE/ ARO	RSD AFE/ ARO
		AFE in %								AFE/ARO									
(-)-beta-Pinene	9	0.07	0.05	0.04	0.02	0.04	ND	0.02	0.06	0.88	0.31	0.30	0.27	0.33	ND	0.40	0.38	0.41	51.17
10-Heneicosene (c,t)	5	0.12	0.16	0.17	0.16	0.15	0.13	0.05	0.07	0.20	0.11	0.12	0.12	0.12	0.15	0.14	0.19	0.14	22.74
1-Docosene #1	8	0.04	0.03	0.04	0.04	0.04	0.08	ND	ND	1.00	0.25	0.60	0.54	0.33	0.40	ND	ND	0.52	51.44
1-Heneicosyl formate	6	0.21	0.18	0.21	0.21	0.18	0.58	0.08	ND	0.32	0.11	0.14	0.15	0.11	0.16	ND	ND	0.16	47.9
1-Heptacosanol	3	0.43	0.38	0.41	0.37	0.43	0.66	0.17	0.36	5.38	1.36	2.05	1.68	1.34	1.65	ND	3.38	2.40	61.7
1R-alfa-Pinene	11	0.61	0.39	0.32	0.14	0.38	0.01	0.17	0.41	1.69	0.46	0.53	0.35	0.86	ND	0.68	0.55	0.73	61.98
2-Methyl-7-nonadecene	6	0.08	0.09	0.10	0.10	0.09	0.20	ND	ND	0.19	0.09	0.12	0.11	0.09	0.16	ND	ND	0.13	31.53
5-Nonadecen-1-ol	7	0.03	0.05	0.05	0.04	0.04	0.07	0.03	0.03	0.15	0.11	0.14	0.09	0.10	0.13	0.20	0.14	0.13	25.6
8-Heptadecene	7	0.19	0.23	0.27	0.23	0.24	0.29	1.40	0.77	0.17	0.08	0.11	0.08	0.11	0.08	0.16	0.13	0.12	29.04
9-Nonadecene	6	3.01	3.23	3.74	3.54	3.40	5.11	2.00	1.58	0.24	0.12	0.15	0.13	0.13	0.15	0.17	0.12	0.15	25.3
alfa-Guaiene	6	0.08	0.05	0.04	0.02	0.05	ND	ND	0.03	0.14	0.03	0.04	0.05	0.05	ND	ND	0.05	0.06	67.99
alfa-Humulene	7	0.06	0.04	0.03	0.01	0.04	0.03	ND	ND	0.13	0.03	0.04	0.02	0.04	0.03	ND	ND	0.05	80.3
beta-Bourbonene	14	0.01	0.01	ND	ND	0.01	ND	ND	ND	0.10	0.04	ND	ND	0.05	ND	ND	ND	0.06	54.53
beta-Caryophyllen	7	0.16	0.10	0.07	0.03	0.11	0.61	ND	0.11	0.15	0.04	0.04	0.04	0.05	0.05	ND	0.05	0.06	69.26
beta-Citronellol + Nerol	7	2.04	2.03	2.43	1.91	2.27	0.25	1.08	1.02	0.08	0.04	0.07	0.04	0.04	0.01	0.06	0.03	0.05	46.12
beta-Cubebene #1	14	0.01	0.01	0.01	ND	0.01	0.02	ND	0.00	0.17	0.05	0.08	ND	0.08	0.03	ND	0.00	0.07	85.99
beta-Cubebene #2	6	0.27	0.18	0.14	0.06	0.17	0.07	0.03	0.09	0.19	0.04	0.05	0.05	0.06	0.17	0.05	0.06	0.09	69.87
beta-Myrcene	7	0.21	0.11	0.09	0.03	0.10	ND	0.03	0.17	0.75	0.20	0.19	0.12	0.28	ND	0.12	0.27	0.27	79.84
beta-Phenethyl acetate	16	0.05	0.09	0.08	0.07	0.08	ND	0.06	0.07	0.21	ND	0.30	0.32	0.17	ND	0.08	0.09	0.19	53.49
Citronellol acetate	9	ND	0.03	0.02	0.03	0.03	ND	ND	ND	ND	0.02	0.02	0.02	0.02	ND	ND	ND	0.02	16.85
Citronellyl propionate	5	0.31	0.46	0.46	0.39	0.43	ND	0.04	0.51	7.75	3.83	4.60	3.54	2.15	ND	ND	4.78	4.44	42.12
Docosane	6	0.20	0.20	0.21	0.23	0.20	0.24	0.30	0.25	0.53	0.23	0.25	0.27	0.23	0.12	0.19	0.16	0.25	49.64
Dodecane	ND	0.01	ND	ND	ND	0.01	0.01	ND	ND	0.63	ND	ND	ND	0.25	0.15	ND	ND	0.34	73.31
Dodecane, 2,7,10-trimethyl-	5	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.50	0.25	0.30	0.27	ND	ND	0.20	0.19	0.29	39.86
E-7-Octadecene	6	0.03	0.03	0.04	0.03	0.04	ND	0.04	0.05	0.17	0.07	0.10	0.07	0.10	ND	0.13	0.13	0.11	33.25
Eicosane	6	0.70	0.61	0.67	0.69	0.64	0.69	0.65	0.59	0.25	0.09	0.11	0.11	0.09	0.08	0.12	0.09	0.12	46.08
Eugenol	5	0.45	0.58	0.59	0.53	0.57	ND	ND	ND	0.26	0.24	0.35	0.20	0.16	ND	ND	ND	0.24	29.69
Geraniol acetate	12	0.02	0.02	0.03	0.03	0.05	0.04	0.04	0.03	0.01	0.00	0.01	0.01	0.01	0.00	0.01	0.00	0.01	35.35
Heneicosane	11	4.31	3.92	4.25	4.38	3.93	5.63	6.61	4.79	0.39	0.15	0.21	0.21	0.15	0.14	0.20	0.15	0.20	41.71
Heptacosane	4	3.13	3.39	3.21	3.46	2.84	5.04	2.39	3.66	4.35	1.32	1.61	1.55	1.13	0.99	1.23	1.63	1.73	62.84
Hexacosane	5	0.15	0.16	0.15	0.15	0.14	0.24	0.14	0.20	1.50	0.57	0.64	0.58	0.58	0.40	0.16	0.47	0.61	63.64
Hexadecane	6	0.06	0.06	0.06	0.06	0.07	0.02	0.05	0.03	0.20	0.08	0.09	0.08	0.12	0.06	0.10	0.06	0.10	45.92
Methyleugenol	8	0.02	0.09	0.03	0.08	0.04	ND	ND	ND	0.01	0.08	0.03	0.07	0.01	ND	ND	ND	0.04	75.5
Muscalure	5	0.45	0.39	0.49	0.49	0.40	0.90	0.23	0.14	0.58	0.18	0.27	0.26	0.17	0.21	0.33	0.22	0.28	47.83
n-Heptadecane	7	1.67	1.46	1.60	1.58	1.75	0.14	3.05	0.95	0.24	0.09	0.11	0.10	0.12	0.04	0.16	0.09	0.12	49.23
Nonacosane	3	2.05	2.36	2.22	2.13	1.98	2.59	1.38	1.28	20.50	4.92	6.67	5.28	4.76	3.24	1.10	6.00	6.56	89.85
Nonadecane	6	7.32	6.63	6.98	7.01	7.12	5.98	12.03	9.72	0.40	0.19	0.26	0.23	0.20	0.14	0.22	0.21	0.23	33.69
Octacosane	0	0.20	0.22	0.21	0.20	0.20	0.41	0.14	0.19	5.00	1.83	3.15	1.82	2.50	1.54	0.04	1.19	2.13	69.14

Octadecane	6	0.14	0.11	0.12	0.12	0.13	0.04	0.12	0.08	0.19	0.07	0.08	0.08	0.09	0.04	0.09	0.05	0.08	57.78
Pentacosane	4	1.44	1.55	1.50	1.60	1.38	1.70	1.71	1.49	1.29	0.44	0.54	0.53	0.40	0.33	0.43	0.51	0.56	54.34
Pentadecane	8	0.21	0.15	0.20	0.17	0.19	ND	0.23	0.05	0.21	0.06	0.09	0.07	0.11	ND	0.12	0.08	0.11	50.65
Phenylethyl Alcohol	4	7.99	8.00	8.30	8.47	8.42	5.04	8.73	8.05	7.54	7.41	13.85	7.96	5.01	9.45	2.18	4.08	7.18	49.81
Sabinen	6	0.08	0.04	0.04	0.01	0.04	ND	0.01	0.07	2.00	0.33	0.40	0.27	0.50	ND	ND	0.66	0.69	94.26
Tetracosane	6	0.18	0.19	0.19	0.09	0.18	0.25	0.24	0.20	0.69	0.28	0.32	0.14	0.28	0.19	0.80	0.20	0.36	67.7
Tetradecane	3	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.50	0.13	0.15	0.14	0.25	0.30	0.10	0.06	0.20	70.53
trans-Farnesol	6	0.07	0.06	0.09	0.08	0.09	0.08	ND	ND	0.02	0.01	0.02	0.02	0.01	0.01	ND	ND	0.01	42.05
trans-Geraniol	7	1.52	1.51	1.87	2.25	1.64	2.08	2.49	1.86	0.12	0.06	0.11	0.10	0.08	0.05	0.08	0.06	0.08	29.94
Tricosane	6	2.57	2.40	2.55	2.73	2.33	2.36	4.04	2.75	0.82	0.28	0.36	0.37	0.27	0.19	0.29	0.31	0.36	53.35
Z-12-Pentacosene	7	0.41	0.43	0.46	0.33	0.46	0.55	0.24	0.21	2.28	0.77	1.06	0.69	0.68	0.49	0.09	1.97	1.00	74.61
Z-5-Nonadecene	13	0.03	0.02	0.03	0.03	0.03	0.04	ND	ND	0.21	0.08	0.10	0.09	0.09	0.12	ND	ND	0.12	42.06
1-Docosene #2	4	0.012	0.010	0.013	0.012	0.011	0.043	0.016	0.009	0.39	0.12	0.17	0.16	0.11	0.19	0.29	0.27	0.21	44.86
1-Nonadecene	21	0.008	0.008	0.007	0.009	0.010	0.006	ND	0.050	0.47	0.08	0.12	0.15	0.14	0.05	ND	2.92	0.56	186.37
5-Eicosene, (E)-	15	0.007	0.007	0.008	0.007	0.007	0.022	0.003	0.003	0.25	0.07	0.10	0.08	0.08	0.16	0.22	0.15	0.14	49.2
6,9-Heptadecadiene	11	0.013	0.014	0.017	0.011	0.014	0.123	0.179	0.157	0.14	0.07	0.08	0.05	0.08	1.17	0.25	0.25	0.26	143
alfa-Citral	1	0.007	0.008	0.016	0.013	0.010	0.009	0.050	0.011	0.01	0.01	0.02	0.01	0.01	0.00	0.01	0.00	0.01	74.69
alfa-Terpineol	13	0.003	0.002	0.002	0.001	0.002	ND	0.002	0.006	0.02	0.01	0.01	0.00	0.01	ND	0.00	0.01	0.01	60.45
benzoic acid (-) -menthyl ester	13	0.003	0.003	0.003	0.001	0.004	ND	ND	ND	0.14	0.05	0.05	0.02	0.05	ND	ND	ND	0.06	72.86
beta-cis-Ocimene	14	0.009	0.004	0.002	0.001	0.005	ND	ND	ND	0.12	0.04	0.02	0.02	0.09	ND	ND	ND	0.06	82.38
beta-Elemen	10	0.023	0.013	0.009	0.004	0.015	0.003	0.002	0.008	0.13	0.03	0.03	0.03	0.04	0.03	0.02	0.04	0.04	83.31
beta-Linalool	7	0.025	0.032	0.033	0.022	0.027	0.042	0.006	0.055	0.04	0.02	0.03	0.02	0.03	0.01	0.00	0.04	0.02	57.25
beta-Terpinyl acetate	5	0.022	0.012	0.011	0.005	0.011	0.001	0.004	0.016	0.34	0.12	0.12	0.09	0.13	0.01	0.03	0.10	0.12	84.47
Butanoic acid, 3,7-dimethyl-6-octenyl ester	8	0.076	0.102	0.082	0.071	0.091	ND	0.028	0.114	1.05	0.54	0.66	0.51	0.30	ND	1.51	0.73	0.76	53.42
Caryophilen oxide	24	0.002	0.003	ND	ND	0.004	0.026	ND	0.002	0.03	0.13	ND	ND	0.28	0.00	ND	0.01	0.09	129.45
Elemol	8	0.006	0.006	0.008	0.006	0.006	0.047	0.469	ND	0.03	0.03	0.05	ND	0.02	0.08	0.11	ND	0.05	67.37
gama-Gurjunene	8	0.019	0.011	0.009	0.003	0.011	ND	ND	0.006	0.15	0.03	0.05	0.03	0.05	ND	ND	0.04	0.06	76.56
Nonanal	6	0.002	0.004	0.003	0.003	0.002	0.010	0.002	0.003	0.05	0.05	0.04	0.03	0.02	0.04	0.03	0.04	0.04	25.29
Pentadecanal- #1	10	0.008	0.031	0.013	0.034	0.011	0.033	0.012	0.020	1.00	0.46	0.30	0.72	0.14	0.30	0.54	2.19	0.71	93.18
Pentadecanal- #2	4	0.004	0.012	0.006	0.02	0.006	0.014	0.013	0.006	0.37	0.16	0.10	0.23	0.08	0.13	0.20	ND	0.18	53.98

Symbols # indicate compounds which showed similar match factor results when compared to the NIST MS library in the process of building the custom AMDIS library but were detected with different retention times; The symbol ND stands for not determined; Names shown in *italic* indicate compounds which were detected after raising the sensitivity option of the AMDIS deconvolution module from very low to medium; RSD- Relative Standard Deviation; AFE- Average Amount in Flower Extract in % calculated on the basis of three parallel extractions (area of the deconvoluted component (Area) relative to the total ion count for the entire chromatogram); AFE/ARO ratio between AFE and ARO normalized to the AFE/ARO ratio of the internal standard 2-nonadecanone

the GC/MS analysis of flower extracts will be applied to pre-select quality oil rose breeding lines for next analysis of distilled rose oil and evaluation of agronomic characteristics. That's why the possibility to extrapolate the metabolite data derived from GC/MS analysis of rose flower extracts to the composition of rose oil distilled from the same flower sample is crucial for the successful application of the procedure. One of the major drawbacks for extrapolation of the flower to distilled oil volatile data could originate from the rose oil distillation procedure *per se*. The composition and relative abundances of the flower volatiles depends on the rose genotype, flower stage, flower collection manner, etc. Due to the specificity of hydrodistillation procedure used for production of rose oil, one could expect that recovery of particular compound in the distilled oil could depend on its relative abundance and overall volatile composition of the processed flower sample, i.e. to depend on the rose genotype.

In order to evaluate the influence of the flower volatile composition and abundances on the rate of recovery of the volatiles in distilled rose oil, a comparative analysis of GC/MS detected volatiles in flower extracts and distilled rose oils of eight oil bearing rose genotypes was carried out. The analyzed genotypes belong to three groups: five 'traditional' *R. damascena* genotypes, two interspecific hybrids involving

R. damascena and one accession of *R. alba*. GC/MS analysis showed that they differ both in the composition and relative abundances of the detected volatiles in the flower extracts and distilled rose oil (**Table 1**). To carry out the comparative analysis a data set consisting of ratios of relative abundance for each volatile in the flower extract to relative abundance in the distilled rose oil (AFE/ARO) was generated. A "core" subset of AFE/ARO ratios of 27 volatiles, detected in the flower extract and rose oil spectra of all analyzed rose genotypes, was subjected to single factor ANOVA test at significance level of 0.01 in order to evaluate the genotype influence. The results revealed that the studied genotypes do not significantly influence the determined AFE/ARO ratio, (p-value of 0.98 and F=0.21 with Fcrit=2.72). The AFE/ARO dataset was further used for calculation of the average and relative standard deviation /RSD/ values of the AFE/ARO ratio for each detected compound. Prior calculation the AFE/ARO ratios were normalized with the AFE/ARO ratio of the internal standard calculated for each genotype. The obtained data (**Table 1**) indicate that the relative abundance of volatiles in the flower extract could be reliably extrapolated for estimation of the relative abundance of the same volatile in the distilled rose oil with RSD ranging from 16.85% to 94.26% for the 50 most abundant compounds (**Table 1**). Taken together the results from the comparative study suggest that the described procedure could be successfully applied for overall assessment of the composition of the flower distilled rose oil and prediction of the relative abundance of the majority of compounds. The higher throughput makes the procedure very useful for screening and comparison of large sets of wide range of oil bearing rose genotypes and rose segregating populations on the base of direct GC/MS analysis of flower volatiles.

Conclusions

The described procedure for solvent extraction and GC/MS analysis of rose flowers volatiles allows reliable detection of the majority of volatile compounds that are present in distilled rose oil. The protocol is based on micro volume extraction, consumes small solvent volume and could be applied at high throughput manner for large scale flower volatiles analysis. The procedure shows very good reproducibility. The relative abundances of the detected flower volatiles correlated well to their relative abundances in the distilled rose oil, without significant influence from the rose genotype and flower volatile composition. The calculated ratio of flower extract to rose oil relative abundances AFE/ARO for each detected compound could be used reliably for prediction of rose oil composition on the base of GC/MS analysis of rose flower extract. The described procedure could be applied as routine for early testing and assessment of the volatile composition of the distilled rose oil, without waiting rose plants to be fully developed and produce sufficient volume of flowers for Clevenger distillation of rose oil. This makes possible to substantially accelerate oil bearing rose breeding and to reduce the employed essential oil distillation resources.

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