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ANALYSIS OF VOLATILE ORGANIC COMPOUNDS IN ROSE PETALS USING HEADSPACE SOLID-PHASE MICROEXTRACTION COUPLED TO GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Headspace solid-phase microextraction coupled to gas chromatography and mass spectrometry (HS-SPME-GC/MS) has been optimised for the analysis of volatile compounds in six samples of rose petals (Rosa Mariyo, Rose Tara, Rosa Rhodos, Rosa Sudoku, Rosa Deep Purple, Rosa Tacazzi). Volatiles from roses were extracted using a SPME fibre by carrying out the subsequent separation and identification by comparison of their mass spectra with mass spectra libraries and retention indexes. Several factors influencing the SPME were taken into account; namely, extraction time, extraction temperature and the sample weight. The optimal parameters of the extraction of volatile compounds were obtained based on statistical evaluation; the most suitable conditions being the extraction time of 60 min., the extraction temperature of 90 °C and the sample weight of 0.7 g.

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Introduction

It is generally known that the rose flower slices contain mixtures of aromatic compounds forming numerous different species and varieties. For example, the *Rosa genus* includes from 100 to 200 species and more than 18 000 cultivars and hybrids [1]. More than 400 volatile compounds have been identified in rose scent of various rose cultivars.

The chemical composition of a rose scent is significantly complex due to the presence of several chemical groups: hydrocarbons (e.g. β-caryophyllene), alcohols (e.g. phenylethyl alcohol), terpenes (e.g. geraniol, nerol), esters (e.g. hexyl-acetate, geranyl acetate), aromatic ethers (e.g. 3,5-dimethoxytoluene), and some others [2,3]. Literary sources report on monoterpenes as the major components of rose scent (principally citronellol, geraniol, nerol, and linalool), 2-phenylethanol and sulphur-containing volatiles, such as dibenzothiophene [4-7]. One of the possibilities of how to extract volatile or semi-volatile compounds from the plant material is headspace solid-phase microextraction (HS-SPME) which offers several advantages. Some of these advantages of HS-SPME are easy and fast preparation of sample, as well as elimination of solvents or easily automated sampling [8-10]. SPME can be a fast, sensitive, and economical tool for sample preparation preceding the proper analysis employing gas chromatography [11]. Several papers have dealt with gas chromatography-mass spectrometry (GC/MS) analysis of volatile organic compounds in flowers [12-15].

Usage of SPME technique for analysis of volatiles in natural matrix enables application of different fibres with the subsequent comparison of efficiency. Bicchi *et al*. [15] has performed the juxtaposition of eight fibres in the study focused on gas chromatographic analysis of aromatic and medicinal plants.

The main goal of this study was the application of 50/30 μm DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) fibre for the extraction of volatile compounds from rose petals using headspace solid-phase microextraction (HS-SPME) combined with GC/MS. Process of extraction was optimized with emphasis on identification with the largest number of compounds.

Experimental

Chemicals

Two *n*-alkane standard mixtures (C_8-C_{20} in hexane; C_{21}-C_{40} in toluene; 40 mg l^{-1} each component) were purchased from Sigma-Aldrich (Prague, the Czech Republic).

Plant material

Six different types of rose petals (*Rosa genus*) were analysed: *Rosa Mariyo*, *Rosa Rhodos*, *Rosa Sudoku*, *Rosa Tara*, *Rosa Tacazzi*, and *Rosa Deep Purple*. All roses have been brought from the Netherlands within the period from October 2014 to March 2015.

Sample Preparation

Roses were air-dried on the filter paper at a room temperature of 20-25 °C. Residual moisture was determined by moisture analyser KERN MLB50-3 (Kern, Balingen; Germany) and dry weight ranged from 82.3 % to 86.1 %. Dried rose petals were milled into a powdered form using a 5100 Mixter Mill (SPEX SamplePrep, Metuchen; NJ, USA). Extraction by HS-SPME was carried out in 20 ml headspace vials closed by a cap with a Teflon septum (Supelco, Bellefonte, PA, USA).

Headspace Solid-Phase Microextraction

SPME experiments were carried out using 50/30 μm divinylbenzene/carboxen/ polydimethylsiloxane fiber (DVB/CAR/PDMS, Bellefonte, PA, USA). The fibre was conditioned before use, according to manufacturer's recommendation. The HS-SPME was carried out at optimised conditions, when 0.7 g of sample was taken into 20-ml glass vials and the extraction procedure run at a temperature of 90 °C for 60 min.

GC-MS Analysis

A gas chromatograph (model GC-2010 Plus, from Shimadzu; Kyoto, Japan) coupled with mass spectrometry detector TQ-8030 and auto-sampler AOC-5000 Plus (again from Shimadzu) was used for analysis. Injections were performed in splitless mode. The GC-MS system was equipped with a capillary column SLB™-5ms with length of 30 m, inner diameter of 0.25 mm, and the film thickness of 0.25 μm (Supelco, Bellefonte; PA, USA). Helium 5.0 (Linde Gas; Prague, the Czech Republic) was used as a carrier gas at a constant linear velocity of 30 cm s^{-1}. The injector and the interface temperature were maintained at 230 °C. The column temperature had been programmed as follows: the initial temperature was 40 °C (5 min), then increased in a gradient of 3 °C min^{-1} up to 280 °C (for 15 min). The mass spectrometer was operated in the full scan mode over a mass range of

m/*z* = 45-500 and in the electron-ionization mode (70 eV).

The mixtures of *n*-alkanes (C_8-C_{20}, C_{21}-C_{40}) were injected using the above temperature program in order to calculate the retention index (*RI*) for each peak. Identification of the components was done by comparison of mass spectral fragmentation patterns stored in MS data libraries NIST 11, Willey 209 and FFNSC 2 and verified by comparison of retention indexes of the already identified compounds with the published index data (NIST Chemical Webbook database) and RIs from MS data library FFNSC 2. The criterion of similarity for the mass spectra was at least 80 %.

GC-FID Analysis

GC-FID analysis was performed on a Shimadzu device (model GC2010, Shimadzu; Kyoto, Japan) connected to a flame ionization detector (FID) with a temperature adjusted at 300 °C. The other analytical conditions (the column type and temperature program, injector temperature, carrier gas, and its linear velocity) were the same as those for GC-MS analysis.

Optimization of the Extraction Conditions

The HS-SPME conditions were optimized using a central composite design (CCD). The statistical analysis and CCD were performed using Statistica CZ software (version 12; StatSoft CR; Prague, Czech Republic). The CCD was based on a 2^3 factorial design plus nine axial points plus three replicates in the centre of design. The independent variables in design were the extraction time, extraction temperature and sample weight. Twenty experiments (see Table I) were generated by CCD and executed in the respective order. All optimisation experiments were performed with milled rose petals of *Rosa Mariyo*.

Results and Discussion

Optimization of HS-SPME

A suitable combination of time and temperature of extraction, as well as the extraction parameters may improve the HS-SPME extraction efficiency via the vapour pressure and equilibrium of volatile compounds in the HS of the sample [16-18].

In the beginning of experiment, three parameters have been chosen for the optimization of HS-SPME technique — the time of extraction: 20-60 min), the

Table I Central composite design-coded independent variables (x_1, x_2, x_3), the corresponding experimental conditions (X_1, X_2, X_3) and the results represented by the total area in the respective chromatogram for 50/30 μm (DVB/CAR/PDMS) fiber. (C) – central point

Run No.	Extraction time min x_1 (X_1)	Extraction temperature °C x_2 (X_2)	Sample weight g x_3 (X_3)	Total area predicted	Total area observed
1	–1 (28)	–1 (58)	–1 (0.38)	3.02×10^8	2.43×10^8
2	–1 (28)	1 (82)	1 (0.62)	8.62×10^8	8.00×10^8
3	1 (52)	–1 (58)	1 (0.62)	4.40×10^8	3.61×10^8
4	1 (52)	1 (82)	–1 (0.38)	8.48×10^8	8.13×10^8
5 (C)	0 (40)	0 (70)	0 (0.50)	4.88×10^8	5.10×10^8
6 (C)	0 (40)	0 (70)	0 (0.50)	5.21×10^8	5.10×10^8
7	–1 (28)	–1 (58)	1 (0.62)	2.59×10^8	2.78×10^8
8	–1 (28)	1 (82)	–1 (0.38)	6.38×10^8	7.00×10^8
9	1 (52)	–1 (58)	–1 (0.38)	2.52×10^8	2.97×10^8
10	1 (52)	1 (82)	1 (0.62)	9.00×10^8	9.42×10^8
11 (C)	0 (40)	0 (70)	0 (0.50)	5.37×10^8	5.10×10^8
12 (C)	0 (40)	0 (70)	0 (0.50)	5.85×10^8	5.10×10^8
13	–α (20)	0 (70)	0 (0.50)	4.03×10^8	4.19×10^8
14	α (60)	0 (70)	0 (0.50)	5.74×10^8	5.82×10^8
15	0 (40)	–α (50)	0 (0.50)	1.82×10^8	2.18×10^8
16	0 (40)	α (90)	0 (0.50)	1.10×10^9	1.08×10^9
17	0 (40)	0 (70)	–α (0.30)	4.47×10^8	4.33×10^8
18	0 (40)	0 (70)	α (0.70)	5.31×10^8	5.70×10^8
19 (C)	0 (40)	0 (70)	0 (0.50)	4.55×10^8	5.10×10^8
20 (C)	0 (40)	0 (70)	0 (0.50)	4.79×10^8	5.10×10^8

temperature of extraction: 50-90 °C, and the weight of sample: 0.3-0.7 g. The distribution coefficient between the sample and the HS, as well as between the HS and the fibre is influenced by the extraction temperature [17]. The performance of SPME is dependent on the availability and selection of appropriate coating. Therefore, it is important to have fibre coatings that eis able of extracting this range of analytes [19]. When selecting an SPME sorbent, the polarity of the

sorbent coating should match the polarity of the analyte and the coating should be resistant to the high-temperature conditions [20].

The correct fibre was selected based on recommendations by the Sigma-Aldrich Guide [21]. The bipolar fibre (DVB/CAR/PDMS) has been examined for analysis of compounds in roses. Optimum extraction conditions were determined by the method of response surface modelling. The whole design consists of twenty experimental points as seen in Table I.

The response was based on the sum of the peak areas of all those could be detected, belonging to one of the most common parameters to optimise the SPME conditions [16,22].

These values were statistically processed in Statistica 12 program, which allows us to assembly the second-order models. Reliability of such a model, which includes linear terms, quadratic terms, and interaction between the linear terms could be expressed as $R^2 = 0.9644$. As seen in Eq. (1) (below), the second-order polynomial equation was constructed for the response of a variable (the total area of all detected peaks, coded "*TA*") related to the experimental conditions chosen

$$\begin{aligned} TA = {} & 1.079\times10^{9} - 3.737\times10^{6}X_1 - 2.330\times10^{4}X_1^2 - 3.742\times10^{7}X_2 + \\ & + 3.521\times10^{5}X_2^2 - 4.383\times10^{8}X_3 - 2.099\times10^{8}X_3^2 + 1.024\times10^{5}X_1X_2 + \\ & + 5.012\times10^{6}X_1X_3 + 1.131\times10^{7}X_2X_3 \end{aligned} \quad (1)$$

where: X_1 … extraction time (in min), X_2 … extraction temperature (in °C), X_3 … sample weight (in g), representing the above-mentioned experimental conditions of independent variables, as seen in Table I.

The significant factor (*p*-value less than 0.05 at a confidence interval of 95 %) of the equations was the extraction temperature. A correlation between the experimental data and the predicted values is shown in Table I. For this model, we have also found out the optimum conditions: the extraction time for 60 min., the temperature of extraction of 90 °C and the sample weight of 0.7 g.

Analysis of Real Samples

Table II shows the relative abundance expressed as a relative area in percent of compounds detected in the volatile fraction of all the samples. A sum of 211 volatile compounds were identified in the six samples of *Rosa genus* (*Rosa Mariyo*, *Rosa Rhodos*, *Rosa Sudoku*, *Rosa Tara*, *Rosa Tacazzi* and *Rosa Deep Purple*). Table II is arranged according to the chemical composition (namely: alcohols, hydrocarbons, carbonyls, terpenes, esters and others). However, a relatively large number of the peaks have not been not identified; often, due to the absence of appropriate mass spectrum in libraries or absence of the retention indexes calculated for the given column.

Table II Chemical composition of the volatile components of extracts of rose petals *Rosa genus*, when the contents of individual compounds are expressed as the average relative percent peak area of GC-FID in three replicates ($n = 3$), n.i. … not identified

Chemical species	Relative area, %					
	Mariyo	Rhodos	Sudoku	Tara	Tacazzi	D. Purple
Alcohols	8.29	5.25	4.43	40.89	2.84	2.74
Alicyclic hydrocarbons	n.i.	2	n.i.	n.i.	0.17	n.i.
Aliphatic hydrocarbons	13.58	14.13	27.81	17.19	14.96	12.47
Aromatic hydrocarbons	0.01	0.04	0.17	0.02	0.03	0.22
Esters	2.42	2.32	1.57	3.62	0.82	1.25
Aldehydes	1.62	16.2	7.15	2	10.22	12.72
Ketones	2.22	3.51	6.64	3.45	9.32	5.9
Acids	0.33	1.46	2.5	1.8	3.25	4.12
Monoterpenes	0.03	0.38	n.i.	0.05	0.06	0.22
Sesquiterpenes	0.02	0.68	n.i.	n.i.	n.i.	n.i.
Oxidated monoterpenes	0.14	0.17	0.38	0.1	n.i.	0.5
Oxidated diterpenes	n.i.	0.12	1.07	0.17	0.11	0.08
Oxidated sesquiterpenes	0.28	n.i.	n.i.	n.i.	0.1	n.i.
Phenolic derivates	0.64	n.i.	0.39	0.51	0.13	2.36
Apocarotenoids	0.8	1.39	0.93	1.01	0.47	3.42
Pyrazines	0.25	0.22	0.09	0.09	0.86	0.41
Furans	0.21	0.59	6.79	0.71	1.07	0.8
Lactones	0.11	0.08	0.14	0.39	0.43	0.26
Sulfides	0.02	n.i.	0.09	0.03	0.14	0.27
Others	n.i.	0.45	0.01	4.58	2.3	0.12

Leffingwell [23] reports on the list of compounds present in Rose oil. The main constituent of Rose oil is citronellol (38 %), C14-C16 paraffins (16 %), geraniol (14 %), nerol (7 %), phenethyl alcohol (2.8 %) , eugenol methyl ether (2.4 %), eugenol (1.2 %), farnesol (1.2 %), linalool (1.4 %), Rose oxide (0.46 %), carvone (0.41 %), Rose furan (0.16 %), β-damascenone (0.14 %), and β-ionone (0.03 %). The relative odour contribution (in % rel. of odour units) provides differences in the content for the minor constituents compared with the major

component – citronellol. The minor constituents of β-damascenone (70 %) and of β-ionone (19.2 %) are behind a significant majority of the odour contribution against citronellol (4.3 %).

Characteristic floral rose fragrance is chiefly influenced by a few compounds. The main compound is *cis*-rose oxide present in an isomeric form. Another compound that contributes to the scent of roses is β-damascenone known as rose ketone. Other compounds that make minor contributions to the overall aroma include *trans*-geraniol, nerol, citronellol, farnesol, and linalool [24]. Some substances were not detected in extracts; namely, *cis*-rose oxide, *trans*-geraniol, citronellol and farnesol.

Analysis of the volatile composition of rose petals has shown that alcohols, carbonyl-compounds, and hydrocarbons (mainly paraffins), were the most typical class of the compounds in all chromatographic profiles of six samples of rose petals, representing up to 60 % of the total volatile fraction, which is evident from Fig. 1.

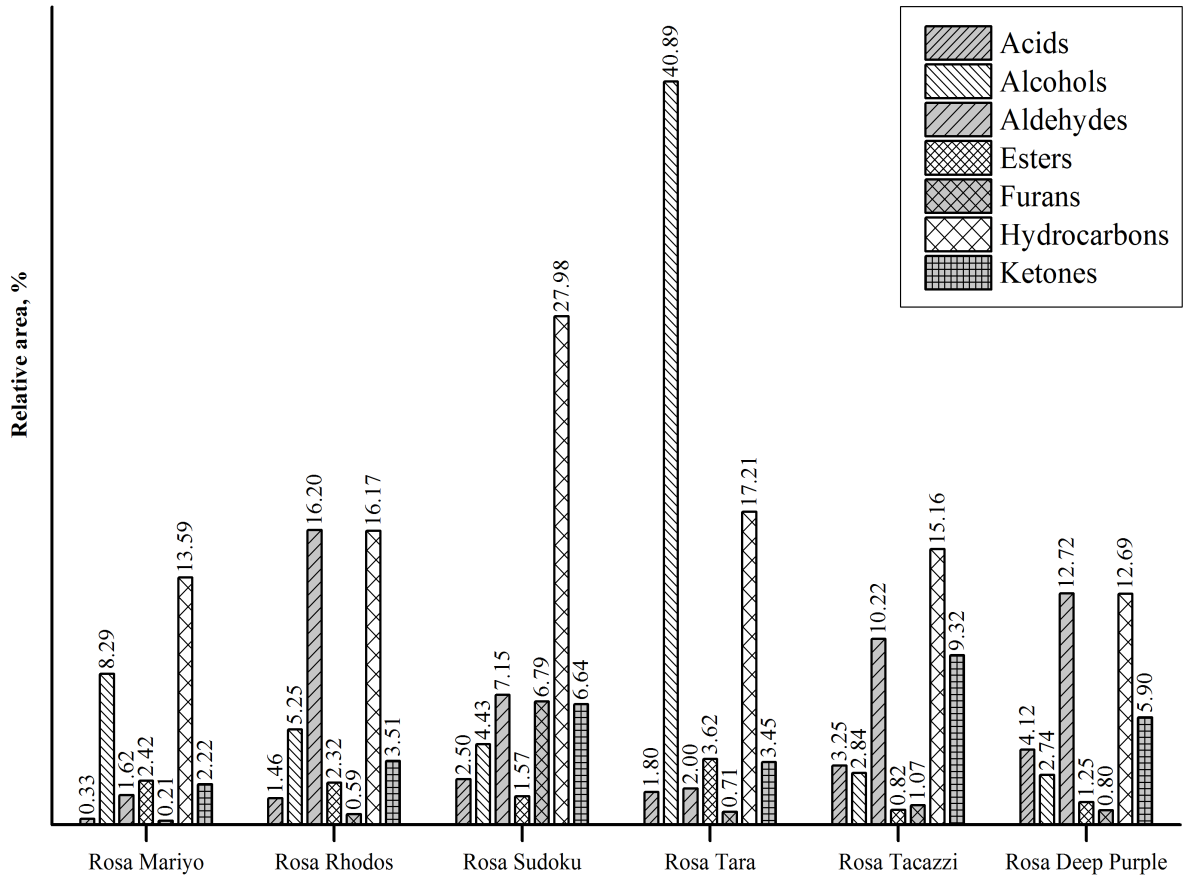

Fig. 1 Chemical comparison of rose petals (in %)

As seen in Fig. 2 showing aroma profiles of the individual rose petals samples using retention indexes and a relative peak area (GC-FID) of the identified compounds, a composition of all samples is very similar in some

respect; especially, in elution of hydrocarbons. The same figure shows the elution of higher aliphatic hydrocarbons (from $C_{21}H_{44}$ to $C_{29}H_{60}$) in all the samples except the sample *Rosa Mariyo*. Aliphatic hydrocarbons with an odd carbon number were more abundant in comparison with hydrocarbons of the even number of carbons. For example, in sample *Rosa Sudoku* it was 11.49 % heneicosane (C21), 0.64 % docasane (C22), 3.66 % tricosane (C23), 0.37 % tetracosane (C24), 1.57 % pentacosane (C25), 0.10 % hexacosane (C26), 1.16 % heptacosane (C27), 0.05 % octacosane (C28), and 0.14 % nonacosane (C29). Two higher hydrocarbons, more precisely heneicosane and tricosane, were identified more frequently than other higher hydrocarbons. Heneicosane was present in an interval from 3.62 to 11.49 % and tricosane from 1.31 % to 3.66 %.

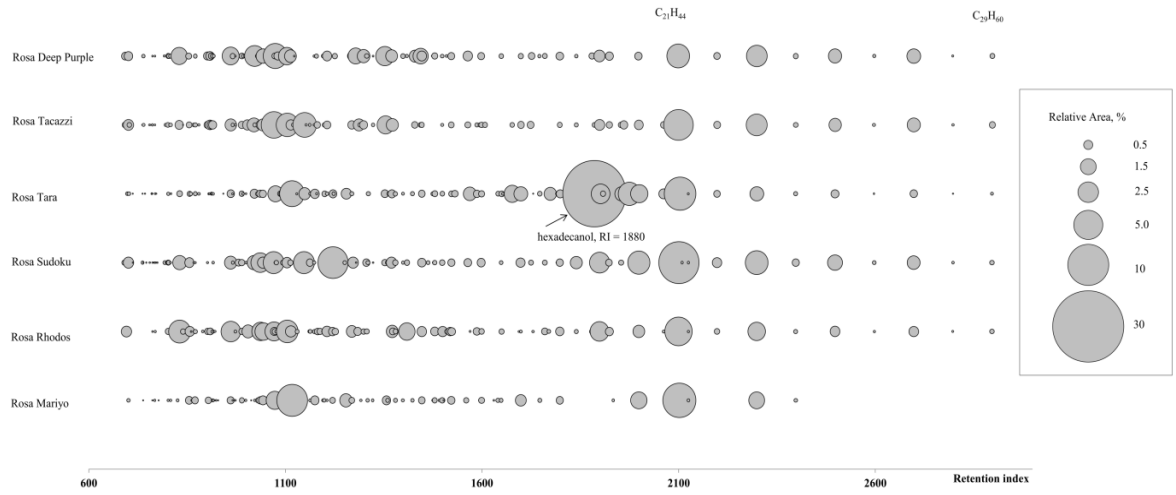

Fig. 2 Aroma-profiles of the individual rose petals. The area of "bubbles" represents a relative peak area of GC-FID

Heptadecane, eicosene, nonadecane, eicosane, and heneicosane have been reported in the essential oil of *R. brunonii* Lindl [25]. In our experiments, hexadecanol was observed with significantly higher response in chromatograms of *Rosa Tara* (28.27 %) in comparison to those of *Rosa Sudoku* (0.11 %), *Rosa Tacazzi* (0.15 %), and *Rosa Deep Purple* (0.26 %). Hexadecanol was not detected in extracts of petals *Rosa Mariyo* and *Rosa Rhodos*. Other significant alcohols, with the content higher than 1 %, were as follows: benzyl alcohol in *Rosa Rhodos* (2.18 %) and *Rosa Sudoku* (2.49 %); phenethyl alcohol in *Rosa Mariyo* (6.69 %); *Rosa Tara* (4.31 %) and *Rosa Deep Purple* (1.01 %); tetradecanol (1.88 %); pentadecanol (1.08 %) and heptadecanol (3.50 %) in *Rosa Tara*.

Rose petals significantly differed in an amount of carbonyl compounds,; namely: aldehydes (1.62-16.20 %) and ketones (2.22-9.32 %). Some compounds, such as 3-furaldehyde (3.40 %) in *Rosa Rhodos*, nonanal with a similar area in *Rosa Rhodos* (3.32 %), and *Rosa Tacazzi* (3.49 %) were also detected and pyrrole-α-methyl ketone had been dominant ketone in comparison with other ketones.

Except a very low content in *Rosa Rhodos* (0.44 %) extract, pyrrole-*α*-methyl ketone was present in all the remaining samples at a content 1.67-4.51 %. Esters are not the only group, being responsible for the odour of flowers, but they take a significant position. In the hybrid rose, esters, terpenes, and phenolic derivatives play a role in producing the fragrance. Geranyl acetate and 2-phenylethyl acetate are the two esters making major contributions to the fragrance of roses and other flowers [26].

Nevertheless, in the present work, geranyl acetate was not identified and 2-phenethyl acetate was found at a relatively low level of 0.73-1.11 %.

Yet another group of substances that contributes to the flavour and/or aroma of flowers are apocarotenoids, including β-cyclocitral, β-ionone, geranial, geranylacetone, theaspirone, α-damascenone and β-damascenone [27].

In this work, apocarotenoids were found in different contents of the individual samples of roses extracts: 0.47 % *Rosa Tacazzi*, 0.8 % *Rosa Mariyo*, 0.93 % *Rosa Sudoku*, 1.01 % *Rosa Tara*, 1.39 % *Rosa Rhodos*, 3.42 % *Rosa Deep Purple*. (*E*)-β-damascenone was detected in an amount corresponding to 0.12-0.21 % of the FID area. It is a compound belonging among the most aromatic ones [28], being typical in manufacturing of commercial perfumes [29].

Terpenes and their oxidised forms commonly generating a large part of the essential oils contained in aromatic flowers, were made up to 0.27-1.45 %. Monoterpenes (0.03-0.14 % limonene, 0.02 % γ-terpinene, 0.03-0.28 % *p*-cymenene) were identified at a very low content. In Chinese rose oil [30], limonene was found in similar representation area (0.02 %) as in our samples under investigation. A similar representation as in Chinese rose oil was observed also at sesquiterpenes (0.68 % α-muurolene and 0.02 % *(E,E)*-α-farnesene), oxidated monoterpenes (0.03-0.22 % linalool, 0.07-0.1 % α-terpineol, 0.18 % *cis*-geraniol, and 0.04-0.38 % carvacrol), oxidised diterpenes (0.08-1.03 % 6,10,14-trimethyl-2-pentadecanone, and 0.04 % phytol) and oxidised sesquiterpenes (0.2 % nerolidol and 0.08-0.1 % fokienol). Phenolic derivatives like, for example, eugenol were present in a range of 0.28-2.36 %. Eugenol was identified in extract of *Rosa Deep Purple* in relatively high amount; namely, as a 2.36 % portion. Maciąg *et al*. [31] reports on eugenol in hydrolate fractions of Rugosa rose petals (*Rosa rugosa* Thunb.) in a content of 0.1-3.0 %, in contrast to the essential oil where eugenol had not been present.

The large part of rose petals is characterized by carboxylic acids (0.33-4.12 %) and a smaller portion of substances, such as furans (0.21-6.79 %), pyrazines (0.09-0.86 %), lactones (0.08-0.43 %), sulfides (0.02-0.27 %), and others (0.01-4.58 %).

In the other sections, di-sec-butyl phthalate (4.2 %) was identified at a high amount being considered as a contaminant.

In general, most of volatile organic compounds (VOCs) were present at very low concentrations (representing less than 1 % of total sum of the relative area in

%). Also some compounds were identified, such as further probable contaminants, particularly toluene in all the samples (< 0.05 %), phthalates in three of all samples (< 5 %) and diisopropylnaphthalenes (< 0.2 %) (1,7- and 2,6-diisopropyl-naphthalene). The presence of diisopropylnapthalenes can be explained by its usage as a plant growth regulator in agriculture or as a solvent for the production of printed materials in which roses can be stored and/or transported [32].

Yet another potential contaminant, isomeric form α-Iso E Super, was identified in samples of *Rosa Mariyo* and *Rosa Rhodos*. This synthetic compounds is commercially available and used in various type fragrances, especially in perfumes. Procymidone (*RI* = 2080), belonging to the fungicide group [33], was detected in *Rosa Rhodos* at a very low content (0.07 %).

Conclusion

As shown in the previous text, application of HS-SPME-GC/MS is an attractive method for detection and identification of VOCs in rose petals. The optimal parameters of extraction (sample weight 0.7 g in 20 ml vial, extraction time 60 min and extraction temperature 90 °C) were evaluated using a central composite design for the DVB/CAR/PDMS fibre. More than two hundred compounds were identified in six samples of *Rosa genus*, i.e. *Rosa Mario*, *Rosa Rhodos*, *Rosa Sudoku*, *Rosa Tara*, *Rosa Tacazzi*, and *Rosa Deep Purple*. Aroma-profiles define obvious differences in each kind of roses. The most abundant groups were alcohols, carbonyl-compounds, and hydrocarbons. Terpenes and their oxidised forms were present at very low concentration compared to the most abundant substances.

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