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Variation in Scent Compounds of Oil-Bearing Rose (*Rosa damascena* Mill.) Produced by Headspace Solid Phase Microextraction, Hydrodistillation and Solvent Extraction

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Abstract: In this research, rose oil and rose water were hydro-distilled from the fresh oil-bearing rose flowers (*Rosa damascena* Mill.) using Clevenger-type apparatus. Rose concretes were extracted from the fresh rose flowers by using non-polar solvents, e.g. diethyl ether, petroleum ether, cyclo-hexane, chloroform and n-hexane, and subsequently by evaporation of the solvents under vacuum. Absolutes were produced from the concretes with ethyl alcohol extraction at -20°C, leaving behind the wax and other paraffinic substances. Scent compounds of all these products detected by gas chromatography (GC-FID/GC-MS) were compared with the natural scent compounds of fresh rose flower detected by using headspace solid phase microextraction (HS-SPME) with carboxen/polydimethylsiloxane (CAR/PDMS) fiber. A total of 46 compounds analysis were identified by HS-SPME-GC-MS in the fresh flower, and a total of 15 compounds were identified by GC-MS in the hydrodistilled rose oil. While main compounds in rose oil were geraniol (35.4%), citronellol (31.6%), and nerol (15.3%), major compound in fresh rose flower, rose water and residue water was phenylethyl alcohol (43.2, 35.6 and 98.2%, respectively). While the highest concrete yield (0.7%) was obtained from diethyl ether extraction, the highest absolute yield (70.9%) was obtained from the n-hexane concrete. The diethyl ether concrete gave the highest productivity of absolute, as 249.7 kg of fresh rose flowers was needed to produce 1 kg of absolute.

Keywords: Oil-bearing rose; *Rosa damascena*; Distillation; Extraction; Volatile oil compounds; Headspace solid phase microextraction.

1. Introduction

Isparta, a city in the Southwestern part of Turkey, is known as “Rose Valley of Turkey” because of the advanced industrial oil-bearing rose cultivation since 1888 when Ottoman Empire ruled in Turkey. The climatic conditions of the region are ideal and favorable for the cultivation of oil-bearing rose. The air humidity, cloudiness, insolation, and precipitation during the flowering season (May and June) contribute to obtaining the roses with high yield and quality [1].

In the rose valley of Turkey, about ten thousand families have been supporting their life by farming oil-bearing rose. Nearly ten thousand tons of fresh rose flowers from the area of 2.500 ha are hand-picked in the flowering season annually, and then daily hydrodistilled to produce rose oil and

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rose water, and/or extracted by a solvent to produce rose concrete and rose absolute by 16 rose oil companies having distillation/extraction facilities. Turkey with a total export of 15 million € in 2013 is the leading country that it meets over 50 % of the world rose oil, concrete and absolute production [2].

The oil-bearing rose (*Rosa damascena* Mill. f. *trigintipetala* Dieck) has a pink flower with 30 petals and heavy rosy scent. The main industrial products from oil-bearing rose are rose oil, rose water, rose concrete and rose absolute which are produced by hydrodistillation and solvent extraction processes [3-4]. Hydrodistillation with cohobation is a widely used method for producing volatile oils from oil-bearing rose, as flowers tend to aggregate and form lumps which cannot be distilled using water and steam distillation or direct steam distillation. In the industrial process of hydrodistillation, large stills with 3000 liter are filled with roses (500 kg) and water (1.5 tones), and then steam-heated for about 90 minutes. The vaporized water and rose oil exit the still and enter a condensing apparatus, and then accumulate in a florentine flask. The oil separated in the Florentine flask is known as "direct oil" which makes up about 20% of the total oil. The water which condenses along with the oil is drained off and redistilled, cohobation, in order to obtain the water-soluble fractions of the rose oil. After cohobation, the oil obtained is called "indirect oil" which makes up the large bulk, about 80%, of the total oil. The direct and indirect rose oils are combined and make the final rose oil of commerce (rose otto or Attar). The hydrosol portion of the distillate is known as rose water [5-6].

Concrete and absolute are the main solvent extraction products of oil-bearing rose. Concrete is not widely used in perfumery and cosmetics in their native form, but it is generally converted into an alcohol-soluble aromatic liquid known as absolute. To produce concrete, the flowers are agitated in an extractor with a non-polar solvent such as n-hexane, which draws out the aromatic compounds as well as other soluble substances like wax and pigments. The extract is subjected to vacuum processing which removes the solvent for re-use. The remaining waxy mass is known as concrete. The concrete is agitated with ethyl alcohol under at -15°C to -20°C for dissolving the aromatic constituents, leaving behind the wax and other paraffinic substances. The alcohol fraction is low-pressure evaporated, leaving behind the finished absolute [7-10].

Volatile oil content of rose flowers is very poor (0.03-0.04%). About 3.5 tons or 1.250.000 fresh rose flowers are hand-picked in the early hours during the flowering season to produce only 1 kg rose oil after hydrodistillation in the factory type retorts or stills [1]. Even with its high price in the world markets (over 9.000 €/kg with a value of 2015), rose oil is the most widely used essential oil in perfumery and cosmetics. 1 kg of concrete is extracted from 300-400 kg of fresh flower (0.3-0.4%) [9] and 1 kg of concrete produces about 0.6 kg of absolute [8], which explains why concrete and absolute prices are less than the oil. The rose residue (pomace), a waste material of hydrodistillation, is not economically used. However, it can be evaluated for the production of compost, methane biogas, phenylethyl alcohol, residue concrete, health sludge, cosmetics and also natural antioxidant [11].

Epidermal cells of the flower petals are the main source of fragrance compounds which are complex of organic volatile molecules, e.g. monoterpenes, sesquiterpenes, aromatic alcohols, oxides, ethers, esters and aldehydes [12]. The amounts and relative contents of the scent compounds are the most important parameters which determine the quality of the rose products [13]. Monoterpene alcohols such as linalool, citronellol, nerol and geraniol, hydrocarbons such as nonadecane, nonadecene, heneicosane, heptadecane, octadecane and tricosane, sesquiterpene hydrocarbons such as α-guaiene, humulene, λ-murolene and δ-guaiene, oxides and ethers such as methyl eugenol, esters and aldehydes such as geranyl acetate and geranial, phenols such as eugenol are among of the most important rosaceous compounds found in the Turkish rose oil [6, 14-16].

However, the processes of distillation and extraction significantly influence the yield and quality of the volatile oils and other aromatic extracts. From this point of view, the first objective of this study was to compare the volatile compounds of rose oil, rose water and residue water with the floral compounds of Rosa damascena by headspace solid phase microextraction (HS-SPME) and gas chromatography (GC-FID/GC-MS) analysis. These comparisons were also necessary to detect the changes in the floral scent compounds during the hydrodistillation process, and to test the hydrodistilled rose products. On the other hand, n-hexane, a solvent obtained from petrochemical sources, is the most widely used solvent to produce extract (concrete) from the oil-bearing rose. Is it possible to explore more productive and healthy alternatives to n-hexane among the other organic hydrocarbons for the solvent extractions? Based on this question, another objective of the study was to

determine the extract (concrete and absolute) yields and productivities of some non-polar solvents like cyclo-hexane, petroleum ether, diethyl ether and chloroform as an alternative to n-hexane, and finally to identify scent compounds of the extracts by using GC-FID and GC-MS analyses.

2. Materials and Methods

2.1. Materials

The fresh flowers of *R. damascena* Mill. were hand-picked in the early morning hours (from 8:00 to 10:00 a.m.) of the flowering season (May and June, 2011) from Rose and Rose Products Research and Implementation Center (GULAR) at Suleyman Demirel University in Isparta province of Turkey (latitude 37°45' N, longitude 30°33'E, altitude 997 m). The flowers were distilled using Clevenger-type apparatus, and extracted by using various organic solvents (Sigma-Aldrich Chemical Co., USA) in order to get rose concrete and rose absolute.

2.2. Methods

2.2.1. Hydrodistillation

Fresh rose flowers (1 kg) and tap water (3 L) were placed in a flask (6 L) connected to the condenser of a Clevenger apparatus according to standard procedure as described in the European Pharmacopoeia [17]. The volatile oil and the water mixture were finally separated by decantation. The rose oil content measured as a percentage (v/w) was 0.045 % on average in triplicate analyses. After hydrodistillation, rose water (hydrosol) collected under the rose oil and residue water in the distillation flask was also separated. The essential oils of the rose water and residue water were extracted by n-hexane to determine the fragrance compounds by GC-FID/GC-MS analysis. The essential oil was dried with anhydrous sodium sulphate, and stored at 4°C until used for analysis.

2.2.2. Solvent extraction

After the flowers of oil-bearing rose were collected in the morning, they were spread on wire shelves and kept for a while under shade at room temperature to remove extra moisture [18]. The flowers were extracted with each one of the non-polar solvents given in Table 1 with high purities by using separator funnels. The multistage extraction was performed (3 times) by the solvents with 30, 20 and 15 minutes, consecutively in the funnels in order to increase the yield of the extract. After the solvents were evaporated from the extracts by a rotary evaporator under vacuum at below 50°C, rose concrete, a pale reddish, waxy, semi-solid material, was obtained.

The concretes were dissolved in 96 % ethanol and stirred vigorously at temperatures of 35°C–40°C for 8 h. The alcoholic solution was chilled at -20°C for 8 h in a deep freezer; the crystallized stearoptens were filtered through filter paper under vacuum [9]. The separated stearoptens remained on the filter paper was redissolved in 50 mL ethanol (96% purity) to obtain the liquid filtrates. To yield the absolute, the filtrate left to stand overnight in a deep freezer and evaporated under vacuum using a rotary evaporator at 55°C to remove the ethanol and water. In the production of absolute, the concretes were treated with ethanol for approximately 10 times their own respective weights [8]. Apart from yield as % (v/w), the concrete and absolute efficiencies were also calculated. While concrete efficiency represents the amount of fresh flowers for extracting 1 kg of rose concrete, absolute efficiency represents the amount of the concrete for extracting 1 kg of rose absolute. Solvent extraction analyses were performed in triplicate and the data was analyzed with the analysis of

variance (ANOVA) using SAS [19]. Means were separated with by Duncan's Multiple Range Test ($P \leq 0.05$).

2.2.3. GC-FID and GC-MS analysis

Gas Chromatography/Mass Spectrometry (GC-MS) analysis of the rose samples was performed on Shimadzu 2010 Plus GC-MS equipped with a Quadrupole (QP-5050) detector. The analysis was performed under the following conditions: capillary column, CP-Wax 52 CB (50 m x 0.32 mm, film thickness 0.25 μm); injector and detector temperature, 240°C; stove heat program, from 60 °C (10 min. hold) to 90 °C rising at 4 °C/min., and increasing to 240 °C (11.5 min. hold) rising at 15 °C/min.; flow speed, 1 psi; detector: 70 eV; ionization type, EI; carrier gas, helium (20 mL/min.); sample injected 1 μL. Identification of constituents was carried out with the help of retention times of standard substances by composition of mass spectra with the data given in the Wiley, NIST Tutor library. The quantitative analysis was conducted using Gas Chromatography/Flame Ionization Detector (GC-FID), Shimadzu Model Thermo Ultra Trace, operating at the same conditions of GC-MS. 50 μL of the volatile oils and aromatic extracts was solubilized in 5 mL of n-hexane and injected in to the split mode 1/100. Based on liquid-liquid extraction, 50 mL of rose water and residue water was diluted with 2 mL of n-hexane overnight, and upper phase including rose oil was injected into the GC-FID and GC-MS systems.

2.2.4. HS-SPME-GC-MS analysis

The fresh flowers of oil-bearing rose were subjected to solid phase microextraction (SPME, Supelco, Germany) with a fibre precoated with a 75 μm-thick layer of Carboxen/Polydimethylsiloxane (*CAR*/*PDMS*). 2.5 g of fresh flowers newly hand-picked was put into a 10 mL vial, which was then immediately sealed with a silicone septum and a crimp cap. After incubation for 30 min at 60°C, SPME fibre was pushed through the headspace of a sample vial to adsorbed the volatiles, and then inserted directly into the injection port of the GC-MS (Shimadzu 2010 Plus GC-MS with the capillary column, Restek Rxi®-5Sil MS 30 m x 0.25 mm, 0.25 μm) at a temperature of 250°C for desorption (5 min) of the adsorbed volatile compounds for analysis. Identification of constituents was carried out with the help of retention times of standard substances by composition of mass spectra with the data given in the Wiley, NIST Tutor, FFNSC library. LRIs (Linear Retention Indices) were calculated by using a series of the standards of C_7-C_{30} saturated n-alkanes (Sigma-Aldrich Chemical Co., USA) for reference in the same column and conditions as described above for GC-MS analysis.

3. Results and Discussion

Headspace solid phase microextraction (HS-SPME) is a technique that uses a fused silica fiber, which is coated on the outside with an appropriated stationary phase for the adsorption of volatiles [20-21]. In this study, HS-SPME combined with GC-MS system using carboxen/polydimethylsiloxane (*CAR*/*PDMS)* fiber was applied for characterization of volatile compounds emitted from the pink oil-bearing rose flowers. HS-SPME technique was clearly faster sample preparation technique as compared to classical hydrodistillation (90 min/300 min), and performed with a much smaller amount of flower than hydrodistillation (2.5 g/1 kg). In addition, no organic solvent was needed.

Through the use of HS-SPME for the volatile extraction from the rose flower, it was possible to identify a total of 46 floral compounds, representing about 100% of the total composition by direct injection in GC-MS (Table 1). Phenylethyl alcohol, citronellol, geraniol were the major floral compounds (43.2%, 16.6% and 10.3%, respectively) of the fresh rose flowers. Dobreva [22] monitored the daily dynamics of compounds in *R. damascena* flowers using HS-SPME technique and

identified 37 compounds; geraniol (0.6-32.3%), citronellol (1.2-30.9%), nerol (0.4-8.6%) and phenylethyl alcohol (1.3-13.4%) whose presence was highly dependent on day moment and abiotic factors (air temperature, relative humidity, intensity of sunlight and wind) at the relevant time.

The rose oil yield after hydrodistillation was 0.045 % (v/w) on average (not tabulated). According to GC/GC-MS analysis of the hydrodistilled rose oil, a total of 15 volatile compounds were identified (calculated as %-peak area of GC–FID analysis) by high percentage of non-cyclic monoterpene alcohols, represented particularly by geraniol (35.4%), citronellol (31.6%), and nerol (15.3%), and long-chain hydrocarbons (alkanes) represented particularly such as nonadecane (7.2%), hexadecane (1.3%) and heneicosane (1.8%). Although phenylethyl alcohol, or 2-phenylethanol, was the major scent compound of the fresh flower, it was found only 1.3% in the hydrodistilled rose oil (Table 1).

The high quality rose oil is characterized by the ratio (citronellol+nerol)/geraniol, which should be between 1.2 and 1.3 [5]. In the studied sample, the ratio was 0.9. This ratio is mainly changed by fermentation of the fresh flowers. If the flowers are late harvested and late distilled during the day, they undergo fermentation. During fermentation, while citronellol ratio increases, geraniol and nerol ratios decrease [13, 16]. Because unfermented fresh flowers were used as material in this study, (citronellol+nerol)/geraniol ratio was lower than 1.2-1.3. In general, the percentage of total alcohols (55.3–83.4%) in rose oil increased with the increase in pressure and temperature of the distillation [23].

The percentages of hexadecane (1.3%), nonadecane (7.2%) and heneicosane (1.8%) were higher in the hydrodistilled rose oil than those of HS-SPME analysis in the fresh rose flowers (Table 1). Percentages of alkanes or steaoreptens in the rose oil increase, and the percentages of monoterpene alcohols decrease with extending hydrodistillation time and latter fraction slides [16]. While fresh rose flowers had only 0.2% of rose furan and *cis*-rose oxide, hydrodistilled products did not any contain them (Table 1). Many other compounds in rose oil such as β-damascenone and β-ionone are present only in trace amounts but are also very important for the overall quality [24].

GC scent compounds of the rose water and residue water were different from the compounds of the rose oil. The oil yields from rose water (hydrosol) and rose residue were about 0.1-0.2%.The main compound of the rose water (hydrosol) and residue water was phenylethyl alcohol, 35.6% and 98.2%, respectively (Table 2). Due to the solubility in water, rose water and residue water, by-products of the hydrodistillation, contained very high amounts of phenylethyl alcohol than rose oil. Therefore probably rose water better represent the natural fragrance of the oil-bearing rose due to its very high phenylethyl alcohol content. The other odorous constituents of the rose water oil were geraniol (27.9%), nerol (12.7%), citronellol (8.3%) and eugenol (6.2%). The volatile oils of the rose water and residue water did not contain long-chain hydrocarbons (stearoptens) except eicosane which was the major higher alkane remaining in the rose water and residue water, in both 1.8% (Table 1).

The amount of volatile compounds in hydrosols depends on the solubility and specific gravity of aromatic compounds. In conventional production, rose water contains very low amounts of (below 0.1%) essential oil and its main component is phenylethyl alcohol [25]. It is important to say that a main source of phenylethyl alcohol is 2-phenylethyl β-D-glucopyranoside which is accumulated in the oil at the harvest time and can be hydrolyzed easily during the hydrodistillation of petals inside the distillation still [26]. Although rose oil shows a strong antioxidant and antimicrobial effect [27-31], similar effects of rose water is rather low [30]. Since rose water provides an ideal growth environment for bacteria, yeasts and fungi, application of physical preservation methods such as pasteurization, and UV treatment or addition of approved chemicals after production of rose water are necessary to assure consumers health and quality of rose water [32].

Methyl eugenol is a high value aroma chemical used in perfume and cosmetic products. However, it is not desired above a certain concentration in the essential oils due to negative side and allergic effects on human health [33-34]. Rose oil is one of the essential oils containing methyl eugenol that its percentage can increase up to 5.0 %, especially in the rose oils distilled from excess or long-term fermented and hydrodistilled flowers [16]. The HS-SPME analysis of the fresh flower and GC-FID analysis of the hydrodistilled rose oil gave the similar percentages of methyl eugenol as 0.9% and 0.8%, respectively. While the essential oil of the rose water had a percentage of 1.23, residue

Table 1. The results of HS-SPME-GC-MS in fresh rose flower and GC-FID in distillation products.

LRI[a]	Compounds	HS-SPME-GC-MS (%) Fresh flower	GC-FID (%)		
			Rose oil	Rose water	Residue water
850	Hex-2(*E*)-enal	0.1	-	-	-
853	Hex-3(*Z*)-enol	0.2	-	-	-
866	Hex-2(*E*)-enol	0.2	-	-	-
867	n-Hexanol	0.9	-	-	-
933	α-Pinene	0.7	-	-	-
964	Benzaldehyde	0.5	-	-	-
972	Sabinene	0.2	-	-	-
978	β-Pinene	0.1	-	-	-
991	β-Myrcene	2.1	-	-	-
1018	α-Terpinene	0.1	-	-	-
1030	Limonene	0.3	-	-	-
1031	Benzyl alcohol	1.1	-	-	-
1035	(*Z*)-β-ocimene	0.2	-	-	-
1045	Phenylacetaldehyde	0.2	-	0.3	-
1046	(*E*)-β-Ocimene	0.3	-	-	-
1058	α-Terpinene	0.1	-	-	-
1086	Terpinolene	0.1	-	-	-
1090	Rose furan	0.1	-	-	-
1101	Linalool	0.2	0.3	-	-
1113	Phenethyl alcohol	43.2	1.3	35.6	98.2
1125	*cis*-Rose oxide	0.1	-	-	-
1165	β-Citronellal	0.3	0.6	-	-
1179	Verbenol	0.1	-	-	-
1212	Linalyl formate	4.5	-	-	-
1232	Citronellol	16.6	31.6	8.3	-
1238	Nerol	1.9	15.3	12.7	-
1250	Geraniol	10.3	35.4	27.9	-
1268	Geranyl acetate	2.4	2.3	-	-
1350	Citronellyl acetate	1.4	-	-	-
1357	Eugenol	0.1	0.5	6.2	-
1361	Neryl acetate	2.7	-	-	-
1397	Methyl eugenol	0.9	0.8	1.2	-
1418	β-Caryophyllene	0.2	0.7	-	-
1438	Aromadendrene	0.2	0.3	-	-
1452	*(E)*-Citral	-	-	-	-
1454	α-Humulene	0.1	-	-	-
1487	β-Selinene	0.1	-	-	-
1500	Pentadecane	0.8	-	-	-
1518	*d*-cadinene	0.1	-	-	-
1600	Hexadecane	0.1	1.3	-	-
1680	Tetradecanol	0.2	-	-	-
1700	Heptadecane	1.0	-	-	-
1800	Octadecane	0.1	-	-	-
1884	Hexadecanol	0.7	-	-	-
1900	Nonadecane	3.5	7.2	-	-
2000	Eicosane	0.2	0.5	1.8	1.8
2100	Heneicosane	0.4	1.8	-	-
Compound number		**46**	**15**	**8**	**2**

[a] Linear Retention Indices, as determined on a Restek Rxi®-5Sil MS column using a series of the standards of C_7-C_{30} saturated n-alkanes, -: not detected

water was totally free of methyl eugenol (Table 1). The yields and productivities of the concrete and absolute from different extraction solvents were presented in Table 2.

The yields of concretes from *R. damascena* flower by using n-hexane extraction were reported to be about 0.25% (1 kg from 400 kg of fresh flower) in Turkey [9], Bulgaria [35], and Pakistan [18]. The yields of rose absolutes from *R. damascena* concrete by using ethyl alcohol extraction were reported to range from 55% to 68% [8-10, 35]. In the present study, concrete yields were between 0.30% and 0.66%, and absolute yields were between 52.1% and 70.9%. While the highest concrete yield (0.66%) and productivity (1 kg from 150.1 kg of fresh flower) was obtained from diethyl ether extraction, the highest absolute yield (70.9%) and productivity (1 kg from 249.7 kg fresh flower) was obtained from ethyl alcohol extractions of n-hexane and diethyl ether concretes, respectively (Table 2).

Table 2. Yields and productivities of the concrete and absolute from different extraction solvents.

Solvents	Concrete yield[1] (%)	Concrete productivity[2] (kg fresh flowers/kg product)	Absolute yield[3] (%)	Absolute productivity[4] (kg fresh flowers/kg product)
Diethyl ether	0.66 a[*]	150.1 c	60.1 b	249.7 b
Petroleum ether	0.45 b	223.5 b	43.8 c	514.5 a
Cyclo-hexane	0.43 b	230.5 b	45.4 c	520.6 a
Chloroform	0.44 b	233.6 b	52.1 c	450.5 a
n-Hexane (control)	0.30 c	336.2 a	70.9 a	474.4 a
Mean	**0.456**	**234.8**	**54.5**	**441.0**
CV (%)[**]	**5.7**	**7.1**	**10.1**	**14.1**

[1] concrete yield (%) means amount of concrete recovered from 100 kg fresh flowers (v/w)
[2] concrete productivity means amount of fresh flower (kg) for production of 1 kg concrete
[3] absolute yield (%) means amount of absolute recovered from 100 kg of concrete (v/w)
[4] absolute yield means amount of fresh flower (%) for production of 1 kg absolute
[*] Values within each column followed by the same letter or letters are not significantly different at the 0.05 probability level according to Duncan's Multiple Range Test.
[**] CV (%): Coefficient of variation

The differences between concrete and absolute yields and productivities of petroleum ether, cyclo-hexane and chloroform were not statistically important ($P \leq 0.05$). These solvents produced more concrete yields, but gave less absolute yields in comparison with n-hexane extraction. Chloroform ranked second after diethyl ether in the absolute productivity with 450.5 kg fresh rose flowers needed to produce 1 kg of absolute (Table 2). GC-FID results of the rose concretes and absolutes were given in Table 4 and 5, respectively.

The volatile oil compounds of the concretes were rich in monoterpene and aromatic alcohols such as phenylethyl alcohol (16.6-23.3%), citronellol (3.8-5.4%), nerol (2.2-3.3%) and geraniol (5.0-7.4%), and long-chain hydrocarbons such as nonacosane (19.2-38.5%), hexadecane (6.1-17.3%), eicosane (0.0-13.1%) and nonadecane (0.0-11.8%). The number of the volatiles in the concrete samples changed from 11 (in the diethyl ether concrete) and chloroform concretes to 15 (in the n-hexane concrete). Phenylethyl alcohol and nonacosane was the major compound in all rose concretes tested. The highest percentage of phenylethyl alcohol (23.3%) and the lowest percentages of citronellol, nerol and geraniol (3.8, 2.2 and 5.0%, respectively) were found in the concrete from diethyl ether extraction. On the other hand, n-hexane concrete gave the highest percentages of geraniol, citronellol and nerol, (7.4, 5.4 and 3.3%, respectively) and the lowest percentage of nonacosane (19.2%). Solvents tested in the study were selective to some volatile compounds. For example, eicosane was extracted by only hexanes at similar percentages, whereas nonadecena was extracted by only ethers. Some of the hydrocarbons, e.g. nonacosane and hexadecane, were extracted by all solvents used in the study (Table 3). These results might mainly be related to the solubility, polarity and other chemical properties of the solvents.

The major compounds of the absolutes from different solvent concretes were phenylethyl alcohol (35.2-38.4%), hexadecane (28.3-36.3%), geraniol (6.9-10.8%), citronellol (5.6-8.4%) and

nerol (3.4-5.0%) (Table 5). The number of absolute compounds detected was between 10 and 11, less than the numbers of concrete compounds shown (Table 3 and 4).

Table 3. GC-FID results of the rose concretes from different solvent extractions.

Compounds	Concretes from different solvent extractions of fresh rose flower				
	Diethyl ether	**Petroleum ether**	**Cyclo-hexane**	**n-Hexane**	**Chloroform**
n-Heptadecane	2.4	1.6	1.4	1.5	2.0
Citral	-	0.5	-	0.6	-
β-Caryophyllene	-	-	0.3	-	-
Citronellol	3.8	5.0	5.1	5.4	5.1
Nerol	2.2	3.0	3.1	3.3	2.7
Geraniol	5.0	7.0	5.9	7.4	5.4
Benzyl alcohol	0.9	0.8	0.7	0.8	1.1
Eicosane	-	-	13.1	13.1	-
Phenylethyl alcohol	23.3	19.9	16.6	20.5	22.7
Nonadecane	11.8	11.0	-	-	-
Dodecane	-	-	-	2.1	-
Tetradecane	-	-	1.9	-	-
Heptadecane	-	-	-	-	1.7
Octadecane	1.5	-	-	-	-
Triacontane	-	3.3	-	-	-
Methyl eugenol	-	-	-	0.3	-
Eugenol	0.6	1.3	1.2	1.4	0.8
Nonacosane	31.7	38.5	34.2	19.2	34.2
Geranic acid	-	-	0.2	0.2	-
Farnesol	-	1.8	1.7	1.9	1.3
Hexadecane	14.2	6.1	10.5	15.2	17.3
Compound number	**11**	**13**	**14**	**15**	**11**

-: not detected

Table 4. GC-FID results of the rose absolutes from different solvent concretes.

Compounds	Absolutes from ethanol extractions of different solvent concretes				
	Diethyl ether	**Petroleum ether**	**Cyclo-Hexane**	**n-Hexane**	**Chloroform**
n-Heptadecane	1.7	-	-	-	-
Citral	-	-	-	1.1	-
β-Caryophyllene	-	-	0.7	-	-
Citronellol	5.6	8.4	8.2	8.4	8.2
Nerol	3.4	5.0	5.0	5.0	4.4
Geraniol	6.9	10.8	9.3	10.8	8.3
Benzyl alcohol	1.7	1.1	0.8	1.0	1.6
Phenylethyl alcohol	37.8	38.4	35.2	36.5	37.0
Tetradecane	1.7	2.0	1.7	1.8	1.8
Methyl eugenol	0.4	0.5	0.5	0.5	0.5
Eugenol	1.3	2.1	1.8	2.1	1.2
Heneicosane	1.4	2.5	2.1	2.3	1.6
Hexadecane	36.3	28.3	34.6	30.6	35.0
Compound number	**11**	**10**	**11**	**11**	**10**

-: not detected

Ayci et al. reported that rose absolute extracted from n-hexane concrete consisted of 14 compounds, mainly of phenylethyl alcohol, citronellol, geraniol, nerol, methyl eugenol, geranyl acetate, benzyl alcohol, nonadecane, nonadecene and farnesol [10].

The absolutes gave similar composition of volatile compounds in general. However, diethyl ether absolute gave less percentage of geraniol, citronellol and nerol (6.9, 5.6 and 3.4%, respectively) than the others and more percentage of phenylethyl alcohol (37.8%) after petroleum ether absolute. Methyl eugenol was detected in all absolutes to range from 0.4 to 0.5% with the exception of n-hexane concrete which had only 0.3% (Table 3). Another interesting finding, nonacosane the main compound

of rose concretes was not detected in the rose absolutes. This compound probably is not soluble in ethyl alcohol and keeps in solid residue by-product. This view is supported by a study carried out by Ayci et al. who emphasized that the hydrocarbon fractions of solid residue are composed of long-chain, saturated hydrocarbons of high molecular weight including nonacosane [10].

4. Conclusion

There are numerous methods for isolating floral compounds from the aromatic flowers. For industrial production of aromatic oils and other extracts from the roses, the common methods are hydrodistillation with cohobation and extraction with organic solvents [36]. The main target in the distillation and extraction processes is to produce the aromatic oils and extracts which have compounds close to genuine scent compounds secreted from the rose flowers. For this reason, it is important to identify the scent compounds not only in the products but also in the flowers. The use of headspace solid phase microextraction (HS-SPME) is shown to be a convenient and effective analytical tool for the sampling of floral compounds of oil-bearing rose by Dobreva [22] and Jirovetz et al. [37].

In our study, while a total of 46 floral compounds of the fresh rose flower were identified by SPME-GC-MS analysis, the aromatic products from hydrodistillation and solvent extractions contained between 10 and 15 compounds detected by GC-FID/GC-MS analysis. These results show that both hydrodistillation and solvent extraction processes significantly change the natural scent composition or chemical profile of the oil-bearing rose. For example, while phenylethyl alcohol was one of the main floral scent compounds in the fresh rose flower (>40%), hydrodistilled rose oil contained very small amount of it (<1.5%), which explain why the smell of rose oil does not resemble the genuine odour of rose flower.

Some of the compounds extracted from the rose flowers undergo denaturing or chemical breakdown mainly due to the high temperatures during hydrodistillation. Moreover some valuable floral compounds such as phenylethyl alcohol remain in the rose water and residual water by-products during the hydrodistillation process. While there is a commercial value of rose water, residue water does not. However, residue water can be economically utilized as a source of phenylethyl alcohol which is used in cosmetic industry as ingredient in perfume and other formulations because of its popular rose-like smell.

For perfume industry, production of concrete and absolute is accomplished by solvent extraction under low temperatures. As volatile oil compounds are generally non-polar molecules, they can be largely extracted through dissolving in a non-polar solvent. The most common non-polar solvent used in the extractions is n-hexane. As a result of this study, the most feasible alternative solvent to n-hexane seemed to be diethyl ether due to its higher concrete yield and absolute productivity, and also its higher phenylethyl alcohol content. However, diethyl ether is extremely flammable and may be explosive according to the standard operating procedures.

Due to the adverse effects on the human health and the environment, healthier and more productive alternatives to hydrocarbon solvents should be explored for the extractions. Nowadays, supercritical CO_2 is becoming an important commercial and industrial solvent due to its high purity, low toxicity and non-flammable property as compared to the traditional solvents [38]. As a conclusion, it is necessary to improve the most efficient distillation and extraction methods that capture the majority of the floral compounds without a significant change in their chemical and molecular structures. The findings obtained from this and similar studies may provide an important scientific contribution to these requirements.

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