

## Article

# Comparative Study of the Yield and Chemical Profile of Rose Oils and Hydrosols Obtained by Industrial Plantations of Oil-Bearing Roses in Bulgaria

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**Abstract:** Bulgaria is famous for its oil-bearing rose. *R. damascena* Mill. and *R. alba* L. are mainly cultivated in the country, but a recent survey of industrial plantations in 2020 revealed that *R. centifolia* L. and hybrids of *R. damascena* Mill. X *R. gallica* L. are also common in the rose valley. Although their essential oil cannot be compared in quality with the classic, these species are preferred by farmers with high yields of flowers and resistance to diseases and pests. All these roses are also used to produce rose water and extracts. The aim of this investigation was to compare the yield and chromatographic fingerprints of seven rose oils and hydrosols produced in Bulgaria. The quantitative composition of the main components of the oils was compared with the norms of the world standards. Our study showed that the yield of essential oil from these roses was in the range of 0.015–0.048%. The main group in the chemical composition is terpene alcohols, which vary in range: geraniol (15.85–34.02%), citronellol (6.70–28.72%), and nerol (5.80–11.90%) but with a different ratio. Hydrocarbons are represented by saturated aliphatic homologs with an odd number of carbon atoms, the main ones being nonadecane (8.10–22.67%), heneicosane (4.37–10.21%), heptadecane (1.07–2.98%), and triclosan (0.81–5.90%). In contrast, the chemical profile of the hydrosols was performed using phenylethyl alcohol (27.45–69.88%), geraniol (13.72–28.67%), and citronellol+nerol (4.56–17.37%). The results show that the presence of plantations with a genotype different from that of *R. damascena* implies differences in the quality of rose oils and hydrosols. This determines their properties of use.

**Keywords:** *R. damascena*; *R. alba*; *R. gallica*; rose valley; distribution; essential oil; hydrolats



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## 1. Introduction

The genus *Rosa* belongs to the family Rosaceae and includes about 200 species, of which the main ones are *Rosa damascena* Mill., *Rosa gallica* L., *Rosa centifolia* L., *Rosa alba* L., and *Rosa rugosa* L. They are used for essential oil production and are spread out across the Northern Hemisphere [1,2]. Rose production in Bulgaria has centuries-old traditions. Until the middle of the last century, various species and forms were present in rose oil plantations [3], but gradually they were replaced by high-yielding specimens of *R. damascena*. Its composition includes over 300 components and cannot be imitated by natural or synthetic substitutes. It is mainly used in the perfumery and cosmetics industry as a base element of many perfumes. It has rich pharmacological activity and can be used in pharmacy or as a food additive in various health preparations [4]. The traditional region of breeding is the Kazanlak valley in central Bulgaria, the so-called Rose Valley (Figure S1). It is situated between the Stara Planina (north) and Sredna Gora (south) mountain ranges and is around 90 km long and around 10 km wide. The average altitude is 350 m. By the end of the XX<sup>th</sup> century, the rose had an equal distribution throughout the valley [5]. A few years ago, the authors of [6] investigated the only *R. damascena* plantations along the Rose Valley

of Bulgaria and found the low variability of the produced essential oil. But pronounced uniformity leads to quality problems and, hence, new trends to diversify by introducing and cultivating hybrids or other roses [7]. The qualities of the white oil-bearing rose were rediscovered [8], oil-bearing roses of different geographical origins were introduced [9], and studies on hamurs (mixtures) with different types of rose oils were performed [10]. At the beginning of the XXI century, the distribution began to develop at a rapid speed, and the scale of rose gardens surpassed even those of the pre-crisis years of state agriculture. This, in turn, led to the need for a new Rose Law, according to which the primary task was to inventory the rose plantations and register all rose growers and processors in the country. The subsequent survey revealed that the plantations were not only pure species such as *R. damascena*, *R. alba*, *R. gallica*, and *R. centifolia* but also foreign hybrids (Raduga) or different genotypes mixtures, some of which were of an unknown origin. The farmers declared that they preferred them for rose water production or for extraction. Of the known oil-bearing roses, only *R. rugosa* has not been identified. This fact dictates the need for the present comparative study on the quantity and quality of the main rose products: essential oil and hydrosols from the industrial fields in the Kazanlak valley. Basically, the lipophilic phase (essential oil) and floral water of plant extracts are much less studied scientifically [11]. The aim of this investigation was to compare the yield and chromatographic fingerprints of oils and hydrosols from industrial roses in Bulgaria. The quantitative composition of the main components of the oils was compared with the norms of the world standard ISO 9842:2003 [12].

## 2. Materials and Methods

### 2.1. Materials Collection and Identification

The study was conducted during the flowering period of 2020, in parallel with the inventory and identification of the plant material of the rose fields. The roses were supplied by private producers in the Kazanlak valley. Six plantations were selected, where pure species or different ones were bearded. The locations are shown in Figure S1 and were designated as RD—*R. damascena* Mill.; RA—*R. alba* L.; RC—*R. centifolia* L.; RG—*R. gallica* L.; RD + Raduga—a mixture of *R. damascena*: Raduga (70:30); and unknown—unknown rose form.

Rosa “Raduga” is a complex hybrid with parental forms (*Rosa damascena* Mill. X *Rosa gallica* subsp. *Eryosyla* Kell var. *Austriaca* Br.) X *Rosa gallica* L.) [13]. It is high-yielding and unpretentious to growing conditions, but the essential oil has a different profile than the standard one. The flowers are multi-petalled and wavy-shaped with a red-violet color. For a better comparison with the hybrid form, a pure form of an *R. gallica* L. sample was added from the IREMK experimental field.

The rose of unknown origin is from a plantation near the village of Krun. The flowers are similar to those of Raduga in morphology, but the bush and leaves have the typical light green coloration of *R. damascena* Mill.

The authenticity of the rose species was confirmed by Dr. Nelly Grozeva from Trakia University, Stara Zagora (Bg), and the voucher specimens were deposited in the IBER-BAS herbarium with the following numbers: *R. damascene*—SOM 178 483; *R. alba*—SOM 178 484; *R. gallica*—SOM 178 485; *R. centifolia*—SOM 178 486; Rosa Raduga—SOM 178 487; and Rosa Krun—SOM 178 488.

### 2.2. Distillation of Rose Oil and Hydrolats—Technological Conditions

The rose blossoms were picked up early in the morning (6.00–8.00 a.m.) in the most suitable phase of bud opening [14]. The yield was established as essential oil content after water vapor distillation in a Clevenger-type apparatus. The samples per charge were as follows: 200 g; 800 mL of water; 2 h duration. The resulting essential oil was reported in the measuring part of the florentine in ml and was recalculated as a percentage. The values are presented on a moisture-free basis. The oil obtained was dried over anhydrous sulfate and stored in tightly closed dark vials at 4 °C until analysis.

The hydrolats were obtained using established technology in Bulgaria—double distillation of fresh rose flowers and water in a ratio of 1:5 for 3 h—until obtaining an amount equal to the inserted material.

### 2.3. Chromatographic Procedure

#### 2.3.1. GC-FID/MS Analysis of Essential Oils

The chemical composition of the rose oils was evaluated on an Agilent 7820A GC System coupled with a flame ionization detector and a 5977B MS detector. The protocol was decided according to ISO 9842 for the gas chromatographic analysis of rose oil. Two capillary columns: non-polar EconoCap<sup>TM</sup> EC<sup>TM</sup>-5 (30 m × 0.32 mm × 0.25 μm film of 5% phenyl, 95% methylpolysiloxane) and polar HP-20M (50 m × 0.32 mm × 0.30 μm) were used. Hydrogen (99.999%) was used as a carrier gas. The split ratio was 1:10, the inlet temperature was set to 250 °C, and the FID temperature was set to 300 °C. The non-polar column reveals a much richer spectrum of compounds and a better presentation of paraffins, but it is not suitable for dividing the main terpene alcohols citronellol and nerol. They have very similar retention times and could not be split and calculated. For this reason, the polar column was used for better separation. The component relative percentages were calculated based on GC peak areas without any correction factors.

The identification of constituents was established by comparing the retention indices (calculated using a standard calibration mixture of n-alkanes C<sub>8</sub>–C<sub>40</sub> in n-hexane) and MS spectra with those reported in the literature [15] and by computer matching with the NIST library, as well as, whenever possible, co-injections with authentic compounds.

#### 2.3.2. GC-FID/MS Analysis of Hydrosols

The essential oil content and chemical composition of the hydrosols were measured after exhaustive triple extraction with diethyl ether (with volumes of 100 mL and in a 1:1 ratio for 10 min for every procedure), separation, the collection of the eluents, and the subsequent evaporation of the solvent. The weight of the final product was measured and calculated as a percentage (*w/w*). The chemical composition of the essential oil in the hydrosol was performed on the same instrument under the same conditions described above. The only difference was that the non-polar capillary column was used because the results achieved with it were sufficiently representative for the study.

### 2.4. Chemicals and Solvents

We used sodium sulfate and diethyl ether (≥99.5%) from Honeywell/Riedel-de Haën. The water for distillation is from the top.

### 2.5. Statistical Analysis

The analyses were made in triplicate. The data were expressed as the mean ± SD. The statistical difference was evaluated by Student's *t*-test. The level of significance was set at *p* < 0.05. The statistical program Microsoft Excel 2010 was used.

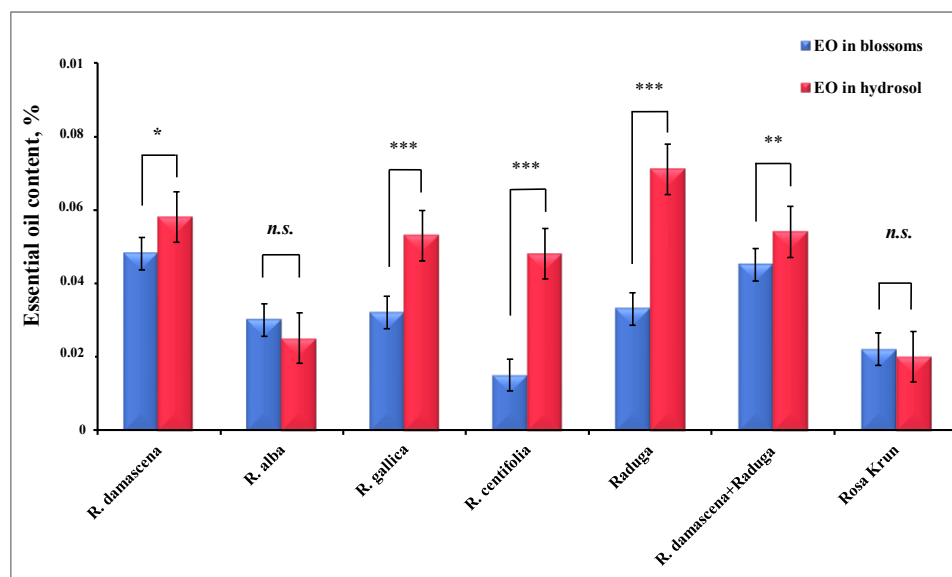
## 3. Results and Discussion

According to our preliminary studies on the problems related to the industrial processing of rose flowers from several geographical locations in Bulgaria, our study is the first large-scale and in-depth comparative analysis on the quantitative yield and qualitative composition of rose oils and hydrosols produced in this region. The new data obtained make it possible to eliminate to some extent the influence of geographical factors, climatic conditions, and variations in processing technology that inevitably occur in the industrial processing of the rose flower.

The locations of the studied plantations are shown in Figure S1. This is a close-up map of the Rose Valley in Bulgaria with the signs showing that different forms of oil-bearing roses are placed at the periphery of the valley. Along the central line of the valley

the plantations were presented with the main genotype, *R. damascena*, which is the same reported by Rusanov et al. [6].

The content of essential oil in the blossoms is a main feature for selection and breeding. This value meets the quality of the hydrosol produced later. The results for the rose oil content of the flowers and their aromatic waters are presented in Figure 1.



**Figure 1.** Comparative analysis of the essential oil content in the blossoms and hydrosols of the investigated roses. Quantitative features are the mean of three independent measurements. The values are presented in percentages. A significant statistical difference in the oil content of blossoms versus hydrolates is presented as follows: \*— $p < 0.05$ ; \*\*— $p < 0.01$ ; \*\*\*— $p < 0.001$ . n.s.—No significance.

Rose flowers are biological living raw materials. The essential oil in them is extremely dynamic and changes both as the bud dissolves and as the day progresses. In the bud phase or in an over-bloomed flower, the oil content is 30–90% less, and the terpene alcohols are reduced or in a different ratio. There is a similar progression as the hours of the day advance. The best quality and quantity moment of the oil is in the morning hours [2,13,14]. For comparative studies, this is very difficult. This fact makes the harvesting of the roses the most stressful moment in rose production and shows the high value of the results of our research. The yield of rose oil, depending on the species, varies between 0.015% and 0.048%. The maximum was reported for *R. damascena*. This result is reasonable, but it seems that the values are in the upper limit or are higher than those reported in the literature: 0.03 ÷ 0.04% [3,7,16]. In the case of the white roses, the yield was also within the upper limits of the results reported to date [7,8,17]. This, in turn, means that the conditions of cultivation and processing were optimal. For Raduga, the result of 0.033% was lower than the published information about the variety [11,18]. The reasons may be complex, given the strong influence of abiotic and endogenous factors on the biosynthesis of rose oil [19–21]. Compared to that of *R. gallica*, the yield is practically identical and confirms the close genetic relationship of the two roses. Other authors cite values of 0.016% [17] or 0.032% [22], but there is a question of another origin and geographical location. *R. centifolia* is found in countries such as Pakistan or Morocco [23], but the essential oil yield data in these countries are usually related to extractive products and may not be relevant. The scarce information about the distillation product in the conditions of cultivation and processing in Bulgaria showed that the oil content was in the range of 0.01 ÷ 0.04% [7,18]. Our result fits within these limits, confirming the putative biosynthesis limits of the genotype.

The unknown rose (that from the village of Krun) had a low oil content (0.022%), which is comparable to that of centifolia and white roses. According to this indicator, it cannot be classified as any of the putative species.

Figure 1 showed that the content of the rose oil in the hydrosols was higher than that in the blossoms. The only exception was the products of *R. alba*. As we noted above, *R. centifolia* and Raduga are favored for rosewater production. They have a higher oil content in the hydrosol, despite the low oil content in the blossoms—obviously, they are suitable for the product. The same content levels were reported for *R. damascena* and *R. centifolia* [24]. All the values corresponded with the requirements of the Bulgarian national standard for rose water (an essential oil content  $\geq 0.025$ ). The biggest producer of rose water is Iran. According to Yazdanfar et al. [25], the Iranian standard requires a minimum of 0.012% essence levels. Our rose hydrolates were high above this level.

The chemical composition of the essential oils is presented in Table S1. Twenty-two components with concentrations higher than 0.1% were identified. They represent from 82.99% to 96.14% of the total detected components. The number of the total compounds (as peaks in the chromatogram) was from 184 to 228. Even if some remain unidentified, this number reveals the abundance or the complexity of the fragrance [26] and immediately allows us to infer the low potential of the *Rosa* Krun. The cultivar Raduga also had a relatively small number of components, and its presence was also reported in the mixed plantation.

The corresponding data of ISO 9842:2003 were applied. The standard refers to the rose oil of *R. damascena* Mill. Its oil is characterized by a high content of terpene alcohols, the main of which is citronellol, followed by geraniol, nerol, and linalool. The ratio between them is also an important feature. Oils with a citronellol/geraniol ratio of 2.50–4.30 [27] or 1.25–1.30 [28] are considered to be of high quality. The main hydrocarbons have a C<sub>17</sub>, C<sub>19</sub>, and C<sub>21</sub> skeleton [29]. A small number of sulfur-containing components have been identified [30]. Our result for the whole composition of *R. damascena* oil fits the standard perfectly. The rest of the minor components, which are not standardized, have the typical chemical characteristics of the oil [6,31,32]. In this sense, the composition of oil from the damask rose plantation fully confirmed the genotype data and the results from previous publications on non-typical compositions as large numbers of sesquiterpenes (44.05%) and a lack of major terpene alcohols (citronellol, geraniol, and nerol) might be related to nonauthentic origins, flower harvesting time, or a compromised analysis method [4,30].

In the case of white roses, there is a dynamic in constituents, from typical citronellol [33] to a decided geraniol pattern [7]. This is probably due to the variability within the population itself [6]. Geraniol is known to carry the pharmacological effects of rose oil [34,35]. In our case, geraniol is the main component overall, its amount being twice that of citronellol. The compound methyl eugenol, which was monitored due to its potential genotoxic and carcinogenic properties [36], is noted to make up less than 1% of the composition. The low content of methyl eugenol and, at the same time, the high content of geraniol is a guarantee of the safe use of *R. alba* oil with a proven biological effect. The number of individual hydrocarbons (as well as the total number of them) approaches that of pink roses, with distinct heneicosane. The latter confirms the different structure of the paraffins in the white rose, namely, a shift of the characteristic homologs to higher members. Overall, our sample has a balanced composition (a high content of terpene alcohols, of which geraniol is the main one), which is typical of the best samples of the genotype.

The *R. gallica* essential oil has a genotype-typical pattern with high geraniol presence [7]. This rose is widely grown in the countries of the former Soviet Union, and its oil often has a high content of phenylethyl alcohol (over 50%), but this is due to the common use of simultaneous distillation/extraction processing technology in Russia and Ukraine [37]. Another comparative study of *R. damascena* and *R. gallica* essential oils from Iran reported negligible geraniol content and an absence of nerol in both samples [22]. In this case, the reason is the analytical technique (using the non-polar GC column, where citronellol and nerol are coupled and, usually, citronellol, the major compound, is detected, but nerol is missing). Our oils from the pure form and from the hybrid (variety Raduga) have a typical geraniol pattern, but the second one contains significantly higher levels of nerol and citronellol. This, in turn, affects the sum of terpene alcohols (35.17% for *R. gallica* and 54.3% for the Raduga variety). Referring to the hydrocarbons, the level of heptadecane

and heneicosane is more than twice as low in the hybrid form. As a result, the ratio of alcohols and paraffins in *R. gallica* is 1.03, while that in the Raduga variety is 3.37. Our results confirm the data in the literature regarding the oils of both species [7,17,18] and explain to some extent the great interest of farmers in the Raduga variety.

In the rose oil of *R. centifolia*, the total content of terpene alcohols is low [7]. The chromatographic profile typically describes twofold lower levels of the major terpene alcohols compared to *R. damascena* and high levels of hydrocarbon components [18]. The high paraffin values make it undesirable for perfumery purposes, but, in practice, it is used to make rose water and extracts. Some references report a composition with a high content of phenylethyl alcohol, but this is due to the different technology, as mentioned above [38,39]. The chemical composition of our sample reflected the data reported in the literature.

The chemical composition of the hydrosols from the same rose genotypes is presented in Table 1. The content of the terpene alcohols citronellol and nerol are given together because their amount is now negligible and their separation with another GC run was not economically justified, nor did it contribute to the value of the study. Figure S2 presents parallel chromatograms of the oil and hydrolates. It clearly shows the differences in the profile of the volatile ingredients.

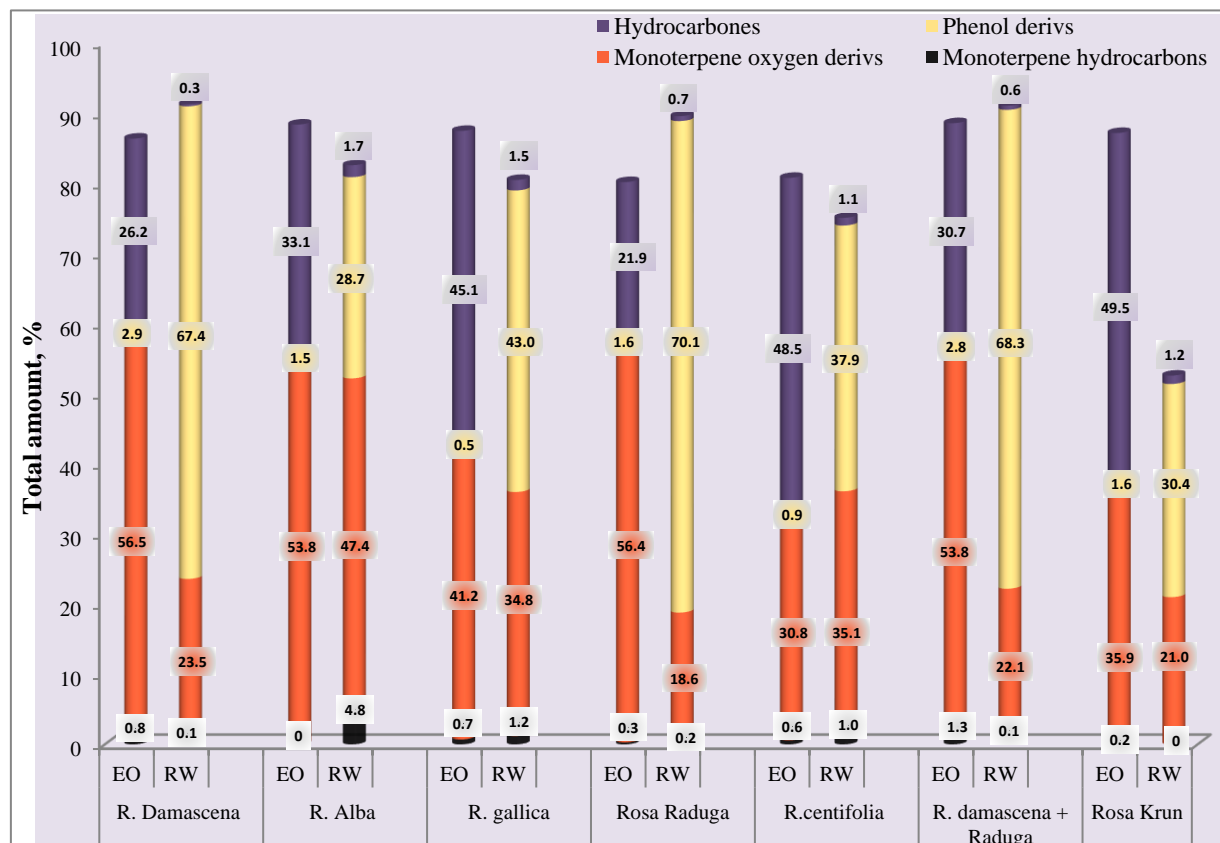
It is obvious that the chemical structure of the scent waters is different from that of the essential oil. The hydrolats mainly consist of phenylethyl alcohol—from 27.45% in *R. alba* to 69.88% in Raduga. The compound with the second highest content was the terpene alcohol geraniol with levels of 13.72–28.67%, and citronellol + nerol had the third highest content, which was in the range of 4.56–17.37%. The content of the plant paraffins was also minimized. While in the essential oil, the main hydrocarbons reached the values of 3.28% ( $C_{17}$  in *R. gallica*), 21.62% ( $C_{19}$  in *R. centifolia*), 14.03% ( $C_{21}$  in *Rosa* Krun), and 5.01% ( $C_{23}$  in *R. gallica*), in the waters, the same rates were much lower than 1%. Generally, the water-soluble constituents, such as alcohols, were better presented in the hydrolates (Figure S2). The data reported in the literature on hydrosols refer mainly to *R. damascena* and very rarely to other types of roses [11,24,40,41]. Unlikely components for rose oil, such as p-cresol, phthalic acid, or 6,6-dimethyl-2-Vinylidenebicycloheptane, have been encountered [42], but these reports could be explained with the treatment of the feedstock or with the analytical procedure. Our study, for the first time, presented a comparison of the volatile composition of hydrolats from different industrial oil-bearing roses, including the hybrid Raduga.

We grouped the chemical components and, after comparing the essential oil and hydrosols, we obtained the volatile profile of the different roses (Figure 2).

**Table 1.** Chemical composition of the hydrosols from oil-bearing roses in the Kazanlak valley.

№	Compound	RI Calc.	RI Lit.	Main and Character Constituents, Rel. %						
				<i>R. damascena</i>	<i>R. alba</i>	<i>R. gallica</i>	<i>R. centifolia</i>	<i>Rosa Raduga</i>	<i>R. damascena + Rosa Raduga</i>	<i>Rosa Krun</i>
1	Ethanol	489	489	tr	tr	tr	tr	tr	tr	tr
2	$\alpha$ -Pinene	939	939	tr	0.01 $\pm$ 0.00	tr	0.21 $\pm$ 0.01	tr	0.01 $\pm$ 0.00	tr
3	Limonene	1031	1033	tr	4.81 $\pm$ 0.03	1.19 $\pm$ 0.01	0.78 $\pm$ 0.03	0.19 $\pm$ 0.03	0.10 $\pm$ 0.01	tr
4	Linalool	1097	1098	0.06 $\pm$ 0.01	1.34 $\pm$ 0.05	1.54 $\pm$ 0.01	1.26 $\pm$ 0.03	0.36 $\pm$ 0.05	0.15 $\pm$ 0.02	0.01 $\pm$ 0.00
5	Phenylethanol	1110	1110	65.52 $\pm$ 2.50	27.45 $\pm$ 0.92	42.47 $\pm$ 1.54	36.61 $\pm$ 0.92	69.88 $\pm$ 0.72	67.21 $\pm$ 1.12	29.54 $\pm$ 0.92
6	Cis-rose oxide	1106	1106	0.02 $\pm$ 0.00	n.d.	n.d.	n.d.	0.01 $\pm$ 0.00	tr	n.d.
7	Trans-rose oxide	1126	1126	0.01 $\pm$ 0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
8	Citronellol + Nerol	1229 *	1228 *	8.94 $\pm$ 0.92	17.37 $\pm$ 0.78	8.80 $\pm$ 1.10	16.25 $\pm$ 0.92	4.56 $\pm$ 0.82	8.01 $\pm$ 0.12	6.11 $\pm$ 0.12
9	Geraniol	1248	1246	14.48 $\pm$ 1.12	28.67 $\pm$ 0.92	24.42 $\pm$ 1.10	17.55 $\pm$ 0.10	13.72 $\pm$ 1.12	13.96 $\pm$ 0.92	14.87 $\pm$ 0.52
10	Eugenol	1348	1351	1.58 $\pm$ 0.92	1.18 $\pm$ 0.03	0.67 $\pm$ 0.03	1.22 $\pm$ 0.05	0.13 $\pm$ 0.03	0.90 $\pm$ 0.05	0.78 $\pm$ 0.03
11	Geranylacetate	1352	1352	0.33 $\pm$ 0.03	0.39 $\pm$ 0.05	0.62 $\pm$ 0.001	0.38 $\pm$ 0.03	0.39 $\pm$ 0.01	0.39 $\pm$ 0.03	0.42 $\pm$ 0.03
12	Methyl eugenol	1405	1405	0.22 $\pm$ 0.03	0.08 $\pm$ 0.01	0.04 $\pm$ 0.00	0.03 $\pm$ 0.00	0.04 $\pm$ 0.00	0.18 $\pm$ 0.00	0.04 $\pm$ 0.00
13	Heptadecane (C <sub>17</sub> )	1700	1700	0.01 $\pm$ 0.00	0.06 $\pm$ 0.01	0.09 $\pm$ 0.01	0.14 $\pm$ 0.03	0.02 $\pm$ 0.00	0.01 $\pm$ 0.00	0.02 $\pm$ 0.00
14	Farnesol	1712	1713	0.15 $\pm$ 0.01	0.85 $\pm$ 0.03	0.13 $\pm$ 0.03	0.52 $\pm$ 0.05	0.12 $\pm$ 0.01	0.15 $\pm$ 0.03	0.12 $\pm$ 0.01
15	Nonadecene	1880	1880	0.02 $\pm$ 0.00	0.33 $\pm$ 0.03	0.13 $\pm$ 0.03	0.12 $\pm$ 0.01	tr	0.01 $\pm$ 0.00	0.02 $\pm$ 0.00
16	Nonadecane (C <sub>19</sub> )	1901	1901	0.10 $\pm$ 0.00	0.53 $\pm$ 0.01	0.66 $\pm$ 0.05	0.29 $\pm$ 0.03	0.17 $\pm$ 0.03	0.21 $\pm$ 0.01	0.57 $\pm$ 0.03
17	Eicosane	2000	2000	0.01 $\pm$ 0.00	0.05 $\pm$ 0.01	0.06 $\pm$ 0.01	0.03 $\pm$ 0.00	0.03 $\pm$ 0.00	0.01 $\pm$ 0.00	0.02 $\pm$ 0.00
18	Heneicosane (C <sub>21</sub> )	2100	2100	0.04 $\pm$ 0.00	0.25 $\pm$ 0.03	0.17 $\pm$ 0.03	0.14 $\pm$ 0.01	0.06 $\pm$ 0.01	0.06 $\pm$ 0.01	0.14 $\pm$ 0.00
19	Tricosane (C <sub>23</sub> )	2300	2300	0.01 $\pm$ 0.00	0.05 $\pm$ 0.00	0.07 $\pm$ 0.01	0.07 $\pm$ 0.01	0.12 $\pm$ 0.03	0.01 $\pm$ 0.00	0.05 $\pm$ 0.00
20	Pentacosane	2500	2500	0.09 $\pm$ 0.01	0.37 $\pm$ 0.03	0.30 $\pm$ 0.03	0.26 $\pm$ 0.07	0.20 $\pm$ 0.03	0.24 $\pm$ 0.01	0.31 $\pm$ 0.03
21	Heptacosane	2700	2700	0.02 $\pm$ 0.00	0.05 $\pm$ 0.00	0.05 $\pm$ 0.01	0.03 $\pm$ 0.00	0.07 $\pm$ 0.01	0.02 $\pm$ 0.00	0.04 $\pm$ 0.00

Legend: The RI indices apply to an EconoCap™ ECTM-5 column. \*—Citronellol; tr—traces < 0.01%; n.d.—not identified. Data expressed as mean  $\pm$  SD.



**Figure 2.** Distribution of the main chemical groups in the rose oils and rose waters. Data are calculated based on mean values.

The figure shows that the tree of the roses had the products with the same pattern—a low content of phenyl derivatives in the essential oil and a high content of them in the hydrosol. These are the results recorded for *R. damascena*, Raduga, and a mixture of the species. The others had another shape: although they had the same levels of the phenyl derivatives in the oil, their flower waters contained much less of the same substances. This fact could be explained by the water solubility of the phenylethyl alcohol—the main aroma carrier in the flowers that was passed through the water during the distillation process and that was finally present in minimal amounts in the oil. Its levels in the hydrosol could be an indirect indication of its amount in the natural essential oil of the fresh rose flowers. The same relation was reported by Tomi et al. [43]. Another author divided hydrosols according to their chemotype: (1) high phenylethyl alcohol (69.7–90.2%), (2) moderate phenylethyl alcohol (12.0–47.8%), (3) citronellol + geraniol (17.5–47.4% and 12.3–36.4%, respectively), and (4) eugenol + geraniol (52.0% and 13.3%, respectively) [41]. Our results (Figure 2) listed the products from *R. damascena*, Raduga, and a mixture of them in the first group; from *R. centifolia*, *R. gallica*, and *Rosa Krun* in the second group, and from *R. alba* in the third group. Eugenol type chemotype was not noted.

#### 4. Conclusions

A quantitative and qualitative analysis of rose oils and hydrosols from industrial plantations in the Kazanlak valley was performed. The results showed that the rose fields consist of the pure forms, or a mixture of the main genotypes, of oil-bearing roses. The main products possess the typical characteristics of the investigated roses. There were also unidentified roses in the fields. It is interesting that a mixed plantation with more than 70% *R. damascena* preserves the quality of the oil. Our studies shed light on the quantitative yield and qualitative composition of both rose oils and hydrosols produced under industrial conditions in Bulgaria. The results obtained allow us to extract to some extent the influence



of geographical factors, climatic conditions, and variations in processing technology that inevitably occur in the industrial processing of roses. Of the seven types of oil studied, *R. damascena* Mill. and *R. damascena* + Raduga fully cover the parameters of ISO 9842:2003. The other oils of *R. alba* L., *R. gallica* L., *R. Raduga*, *R. centifolia* L., and *Rosa Krun* were poorer in citronellol and nerol but richer in heneicosane (C<sub>21</sub>). This implies differences both in their biological activities and in their organoleptic qualities, which opens up new possibilities for their inclusion as components in various perfume compositions.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/resources12070083/s1>, Figure S1: Location of the rose plantations in Kazanlak valley; Table S1: Chemical composition of the rose oils from plantations in Kazanlak valley; Figure S2: Model of the essential oil and hydrosol GC chromatograms in parallel.

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## References

- Pal, P.K. Evaluation, genetic diversity, recent development of distillation method, challenges, and opportunities of *Rosa damascena*: A review. *J. Essent. Oil Bear. Plants* **2013**, *16*, 1–10. [CrossRef]
- Rusanov, K.; Kovacheva, N.; Dobрева, A.; Atanassov, I. *Rosa x damascena* Mill. (Rose). In *Medicinal, Aromatic and Stimulant Plants*; Springer: Cham, Switzerland, 2020; pp. 467–500.
- Astazhkov, N. Studies on the Diversity of the Population of the Kazanlak Rose and Some Issues of Reproduction and Breeding to Improve Rose Production in Bulgaria. Ph.D. Thesis, Plovdiv, Bulgaria, 1988; p. 317.
- Naquvi, J.K.; Ansari, H.S.; Ali, M.; Najmi, A.K. Volatile oil composition of *Rosa damascena* Mill. (Rosaceae). *Int. J. Pharmacogn Phytochem.* **2014**, *2*, 177–181.
- Kovacheva, N.; Rusanov, K.; Atanassov, I. Industrial Cultivation of Oil-Bearing Rose and Rose Oil Production in Bulgaria During 21ST Century, Directions and Challenges. *Biotechnol. Biotechnol. Equip.* **2010**, *24*, 1793–1798. [CrossRef]
- Rusanov, R.; Kovacheva, N.; Rusanova, M.; Atanassov, I. Low variability of flower volatiles of *Rosa damascena* Mill. plants from rose plantations along the Rose Valley, Bulgaria. *Ind. Crops Prod.* **2012**, *37*, 6–10. [CrossRef]
- Kovatcheva, N.; Zheljazkov, V.; Astatkie, T. Productivity, oil content, composition, and bioactivity of oil-bearing rose accessions. *HortScience* **2011**, *46*, 710–714. [CrossRef]
- Dobрева, A.; Tintchev, F.; Heinz, V.; Schulz, H.; Toepfl, S. Effect of pulsed electric fields (PEF) on oil yield and quality during distillation of white oil-bearing rose (*Rosa alba* L.). *J. Herbs Spices Med. Plants* **2010**, *15*, 127–132.
- Kovacheva, N.; Rusanov, R. Introduced Species and Varieties of Oil-Bearing Roses in Kazanlak Field Conditions. *Plant Sci.* **2013**, *50*, 88–91.
- Kovatcheva, N.; Dobрева, A.; Petkov, M. Assessment of Essential Oils from Oil-Bearing roses. 20<sup>th</sup> International Scientific Conference—St. Zagora. *Agric. Sci. J. Plant Stud.* **2010**, *1*, 187–192.
- Acimovic, M.; Tešević, V.; Smiljanic, K.; Cvetkovic, M.; Stankovic, J.; Kiprovski, B.; Sikora, V. Hydrolates: By-Products of Essential Oil Distillation: Chemical Composition, Biological Activity and Potential Uses. *Adv. Technol.* **2020**, *9*, 54–70. [CrossRef]
- ISO 9842:2003; Oil of Rose (*Rosa x damascena* Miller). International Organization for Standardization: Geneva, Switzerland, 2003. Available online: <https://www.iso.org/standard/28611.html> (accessed on 30 March 2023).
- Nazarenko, L.G. *Raduga—New Sort Essential Oil Rose*; Scientific Works of VNIEMK: Simpheropol, Ukraine, 1983; Volume 15, pp. 49–54.
- Staikov, V.; Balinova-Tsvetkova, A.; Decheva, R.; Kalaidjiev, I. Rose flower storage conditions and their influence on the quantity and quality of Rose Oil. *Riv. Ital.* **1975**, *57*, 176–180.
- NIST Chemistry WebBook. Available online: <https://doi.org/10.18434/T4D303> (accessed on 10 January 2023).
- Baydar, H. Oil-bearing rose (*Rosa damascena* Mill.) cultivation and rose oil industry in Turkey. *Eur. Cosmet. Mark.* **2006**, *14*, 13–17.
- Kovacheva, N. Investigation on introduction species of oil—bearing roses. In Proceedings of the International Science Conference, Stara Zagora, Bulgaria, 4–5 June 2008; pp. 1–6.
- Nedkov, N.; Kanev, K.; Kovacheva, N.; Stanev, S.; Djurmanski, A.; Seikova, K.; Lambev, H.; Dobрева, A. *Handbook of major essential oil crops*; Helicon: Kazanlak, Bulgaria, 2005.

19. Baydar, H.; Baydar, G. The effects of harvest date, fermentation duration and Tween 20 treatment on essential oil content and composition of industrial oil rose (*Rosa damascena* Mill.). *Ind. Crops Prod.* **2005**, *21*, 251–255. [CrossRef]
20. Erbas, S.; Baydar, H. Variation in scent compounds of oil-bearing Rose (*Rosa damascena* Mill.) produced by Headspace Solid Phase Microextraction, hydrodistillation and solvent extraction, Academy of Chemistry of Globe publications. *Rec. Nat. Prod.* **2016**, *10*, 555–565.
21. Karami, A.; Zandi, A.; Khosh-Khui, M.; Salehi, H.; Saharkhiz, J.M. Analysis of essential oil from nine distinct genotypes of Iranian Damask rose (*Rosa damascena* Mill.). *J. Med. Plants Res.* **2012**, *6*, 5495–5498.
22. Shamspur, T.; Mostafavi, A. Chemical Composition of the Volatile Oil of Rosa Kazanlik and *Rosa gallica* from Kerman Province in Iran. *J. Essent. Oil-Bear. Plants* **2010**, *13*, 78–84. [CrossRef]
23. Khan, M.A.; Rehman, S.U. Extraction and analysis of essential oil of Rosa species. *Int. J. Agric. Biol.* **2005**, *7*, 973–974.
24. Labadie, C.; Ginies, C.; Guinebretiere, M.H.; Renard, C.; Cerutti, C.; Carlin, F. Hydrosols of orange blossom (*Citrus aurantium*) and rose flower (*Rosa damascena* and *Rosa centifolia*) support the growth of a heterogeneous spoilage microbiota. *Int. Food Res. J.* **2015**, *76*, 576–586. [CrossRef]
25. Yazdanfar, N.; Mohamadi, S.; Zienali, T.; Sadighara, P. Quantification of Methanol, Ethanol, and Essential Oil Contents of Commonly Used Brands of Rosewater (*Rosa damascena*) in Iran. *J. Nutr. Food Secur.* **2022**, *7*, 374–378. [CrossRef]
26. Ohloff, G.; Demole, E. Importance of the odoriferous principle of Bulgarian rose oil in flavor and fragrance chemistry. *J. Chromatogr. A* **1987**, *406*, 181–183. [CrossRef]
27. Nicolov, N.; Tsoutsulova, A.; Nenov, N. Essence de Roses et aures huiles essentielles Bulgares. *Riv. Ital.* **1976**, *58*, 349–365.
28. Baser, K.H. Turkish rose oil. *Perfum. Flavor.* **1992**, *17*, 45–52.
29. Kovats, E. Composition of essential oils. 7. Bulgarian oil of rose (*Rosa damascena* Mill.). *J. Chromatogr.* **1987**, *406*, 185–222.
30. Omata, A.; Yomogida, K.; Nakamura, S.; Ota, T.; Toyoda, T.; Amano, A.; Mura, S. New sulphur components of roses oil. *Flavour Fragr. J.* **1991**, *6*, 149–152. [CrossRef]
31. Tambe, E.; Gotmare, S. Study of Variation and Identification of Chemical Composition in Rosa Species Oil Collected from Different Countries. *J. Appl. Chem.* **2016**, *9*, 11–18.
32. Toluei, Z.; Hosseini; Tafreshi, S.; Arefi; Torkabadi, M. Comparative Chemical Composition Analysis of Essential Oils in Different Populations of Damask Rose from Iran. *J. Agric. Sci. Technol.* **2019**, *21*, 423–437.
33. Dobрева, A.; Velcheva, A.; Bardarov, V.; Bardarov, K. Chemical composition of different genotypes oil-bearing roses. *Bulg. J. Agric. Sci.* **2013**, *19*, 1213–1218.
34. Neshev, G. Bulgarian Rose Oil—Pharmacological and Clinical Studies. Ph.D. Thesis, Medical Academy, Sofia, Bulgaria, 1990.
35. Wittig, C.; Scheuer, C.; Parakenings, J.; Menger, M.D.; Laschke, M.W. Geraniol Suppresses Angiogenesis by Downregulating VEGF. *PLoS ONE* **2015**, *10*, e0131946. [CrossRef]
36. Report of Carcinogens, 15th ed.; Methyleugenol. Available online: <http://ntp.niehs.nih.gov/go/roc> (accessed on 7 June 2023).
37. Chipiga, A.; Volchenkov, V. On the intensification of the main technological processes of the essential oil-bearing raw material processing. In Proceedings of the 7th International Congress of Essential Oils, Kyoto, Japan, 7–11 October 1977; pp. 93–94.
38. Ahsan, M.; Younis, A.; Nafees, M.; Tufail, A.; Shakeel, Q.; Raheel, M.; Nawaz, F.; Jaskani, M.; Amin, M.; Sajid, M.; et al. Marginal quality water arbitrated essential oil contents in metal hoarded flower petals of scented roses. *Ecotoxicol. Environ. Saf.* **2021**, *226*, 112853. [CrossRef]
39. Younis, A.; Riaz, A.; Khan, M.A.; Khan, A. Effect of time of growing season and time of day for flower harvest on flower yield and essential oil quality and quantity of four *rosa* cultivars. *Flori. Orn. Biotech.* **2009**, *3*, 98–103.
40. Roques, D. My Favourite Rose. IFEAT World. 2018. Available online: [www.ifeat.org](http://www.ifeat.org) (accessed on 1 July 2023).
41. Tanjga, B.B.; Lončar, B.; Aćimović, M.; Kiprovska, B.; Šovljanski, O.; Tomić, A.; Travičić, V.; Cvetković, M.; Raičević, V.; Zeremski, T. Volatile Profile of Garden Rose (*Rosa hybrida*) Hydrosol and Evaluation of Its Biological Activity In Vitro. *Sci. Hortic.* **2022**, *8*, 895. [CrossRef]
42. Katekar, V.; Rao, A.; Sardeshpande, V. A cleaner and ecological rosewater production technology based on solar energy for rural livelihood. *Clean. Circ. Bioeconomy* **2022**, *2*, 100022. [CrossRef]
43. Tomi, K.; Sakaguchi, E.; Ueda, S.; Matsumura, Y.; Hayashi, T. Physiological and Psychological Effects of Rose ‘Wishing’ Flowers and Their Hydrosols on the Human Autonomic Nervous System and Mood State. *J. Hortic.* **2017**, *86*, 105–112. [CrossRef]

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