Article

Comparative Study of the Yield and Chromatographic 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, they 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 standard.

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 the range: geraniol (17.60 - 34.02 %), citronellol (8.38 - 28.7 %), and nerol (4.8 - 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 by phenyl ethyl alcohol (27.45 - 69.88 %), geraniol (13.72 - 28.67 %), and citronelol+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 predetermines their properties of use.

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

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 in 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 oil rose 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 Kazanlak valley in central Bulgaria, so-called Rose valley (Figure 1). It is situated between the Stara Planina Mountain (North) and the Sredna Gora Mountain (South), around 90 km long and around 10 km wide. The average altitude is 350 m. By the end of the XXth century, the areas with the population were completely equalized [5]. A few years ago [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, hence new trends to diversify with the introduction and cultivation of 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], study on hamurs (mixtures) with different types rose oils was done [10]. With the beginning of the XXIst century, the branch 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 is 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 *R. damascena*, *R. alba*, *R. gallica* or *R. centifolia*, but also foreign hybrids (Raduga) or different genotypes mixture, some of them of 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 Kazanlak valley. Basically, the comparison of lipophilic phase (essential oil) and floral water of plant extracts is 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 from the private producers in the Kazanlak valley. Six plantations were selected, where pure species or different one was bereded. 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 - 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, wavy shaped, with red-violet color. For a better comparison with the hybrid form, a pure form of R. gallica L. sample was added, from the IREMK experimental field.

The rose of unknown origin is from a plantation near the village 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.

2.2. Distillation of rose oil and hydrolats - technological conditions.

The rose blossoms were picked up early in the morning (6.00 – 8.00b am) in the most suitable phase of bud opening [14]. The yield was established as essential oil content after water-vapor distillation in Clevenger type apparatus. The sample per charge: 200 g; water 800 ml; duration 2 hours. The resulting essential oil was reported in the measuring part of the florentine in ml, it was recalculated as percent. The oil obtained was dried over anhydrous sulfate and stored in tightly closed dark vials at 4 °C until analysis.

The hydrolats were proceeded at established technology in Bulgaria – double distillation of fresh rose flowers and water in ratio 1:5 for 3 hours and the second one - until obtaining an amount equal to the inserted material.

2.3. Chromatographic procedure

2.3.1. GC/ MS profile of essential oils

The chemical composition of the rose oils was performed on an Agilent 7820A GC System coupled with a flame ionization detector and 5977B MS detector. The protocol was made according to ISO 9842 for gas chromatographic analysis of rose oil. Two capillary columns: non-polar EconoCap™ EC™ (30 m x 0.32 mm x 0.25 μm) and polar HP-20M (50 m × 0.32 mm x 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 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 identification of constituents was established by comparison of the retention indices and MS spectra with those reported in literature [15] and by computer matching with the NIST library, as well as, whenever possible, co-injections with authentic compounds.

2.3.2. GC/ MS profile of hydrosols

The essential oil content and chemical composition of the hydrosols were measurement after exhaustive triple extraction with diethyl ether, collection, and subsequent evaporation of the solvent. The weight of the final product was measured and calculated as percent (w/w). The chemical composition of the essential oil in the hydrosol was performed on the same instrument and the same conditions, described above.

2.4. Chemicals and solvents

We used sodium sulfate and diethyl ether (≥ 99,5 %) of a 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 $p< 0.05$. Statistical program Microsoft Excel 2010 was used.

3. Results and discussion

According to our preliminary studies on the problem related to the industrial processing of rose flowers from several geographical locations in Bulgaria, our study is the first of its kind 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.

As can see in Figure S1, the locations of the unclear or different forms of the oil-bearing roses were placed at the periferia of the Rose valley. Along the central line the plantations were presented with the main genotype - *R. damascena*, the same reported [6].

The content of essential oil in the blossoms is a main feature for selection and breeding work. 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 the Figure 1.

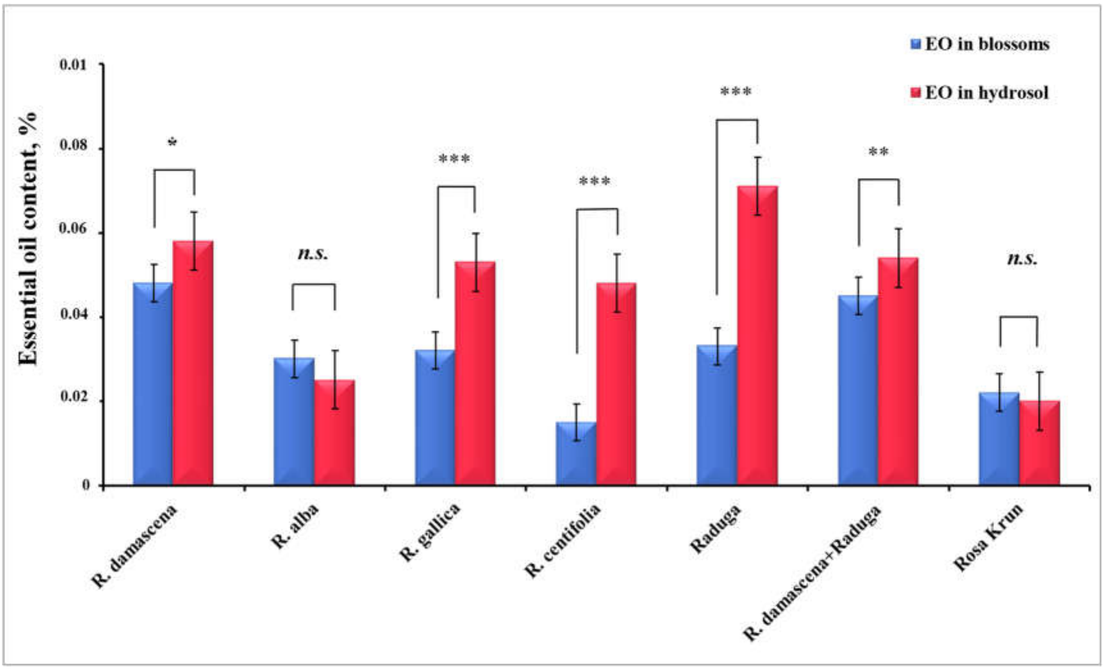

Figure 1. Comparative analysis of the essential oil content in the blossoms and hydrosols of the investigated roses. Quantitative features are the mean of three independent measurements. The values are presented in percentages. A significant statistical difference of the oil content of blossoms versus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

The rose flowers are biological living raw material. The essential oil in it is extremely dynamic and changes both as the bud dissolves and as the day progresses. In the bud phase or 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 dependence on the advancing hours of the day. The best quality and quantity moment of the oil is in the morning hours [2, 13, 37]. 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*. The result is reasonable, but it seems that the values are in the upper limit or higher than those in the literature 0.03÷0.04 % [3,7,16]. In the case of the white rose, the yield was also within the upper limits of the data known to date [8,7,17]. This, in turn, means that the conditions of cultivation and processing were optimal. At Raduga, the result – 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 quote values of 0.016% [17] or 0.032% [22], but there it is a question of another origin and geographical location. *R. centifolia* is traditional to 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 (Village Krun) had a low oil content (0.022 %), which is rather comparable to that of centifolia and white one. According to this indicator, it cannot be classified to any of the putative species.

Figure 1 showed that the content of the rose oil in the hydrosols was higher than the 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 (essential oil content ≥ 0.025 %). If refer the Iranian standard (ISO 1487), their essence levels were high above the minimum acceptable amount 120 ppm [25].

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. The number of the total detected compounds were from 184 to 228. Even unidentified, this number reveals the abundance or the complexity of the fragrance [26] and immediately infers the low potential of the Rosa Krun. The cultivar Raduga had also a relatively small number of components, its presence was also reported in the mixed plantation.

The comparable 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. The oils with a citronellol/geraniol ratio 2.5 - 4.3 [27] or 1.25 - 1.30 [28] are considered to be of high quality. The main hydrocarbons have a C_{17}, C_{19} and C_{21} 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, not standardized, have the typical chemical character of the oil [6,31]. In this sense, the composition of oil from the damask rose plantation fully confirmed the genotype, and publications with atypical composition and lack of major terpene alcohols might be related to the different origin, flower harvesting time or compromised analysis methodology [4, 30].

In the case of white rose, there is a dynamic in constituents, from a typical citronellol [33] to a decidedly 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 element methyleugenol is monitored due to its potential genotoxic and carcinogenic properties [36], here is less than 1%. The low content of methyleugenol and, at the same time, the high content of geraniol is a guarantee of safe use with a proven biological effect of *R. alba* oil. The number of individual hydrocarbons (as well as their sum) approaches that of pink rose, with distinct heneicosane. The latter confirms the different structure of the paraffins in the white rose, namely a shift of the characteristic homologues to the higher members. Overall, our sample has a balanced composition 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 traditions in the processing technology, used in Russia and Ukraine [37]. Another comparative study

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of R. damascena and *R. gallica* essential oils from Iran reported negligible geraniol content and absence of nerol in both samples [22]. In this case, the reason is the analytical technique. 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 Raduga variety). Refering 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, that in the Raduga variety: 3.37. Our results confirm the data in the literature about the oils of both species [7,17,18] and explain to some extent the great interest of farmers for Raduga variety.

In 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* Mill. and high in 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, also as mentioned above [38,39]. The chemical composition of our sample reflected data in the literature.

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

№	Compound	Main and character consituents, Rel.%						
		R. Damascena	*R .alba*	*R gallica*	*R.centifolia*	Raduga	*R.damascena* + Raduga	*Rosa* Krun
1	Ethanol	tr	tr	tr	tr	tr	tr	tr
2	α - Pinene	tr	0.01±0.00	tr	0.21±0.01	tr	0.01±0.00	tr
3	Limonene	tr	4.81±0.03	1.19±0.01	0.78±0.03	0.19±0.03	0.10±0.01	tr
4	Linalool	0.06±0.01	1.34±0.05	1.54±0.01	1.26±0.03	0.36±0.05	0.15±0.02	0.01±0.00
5	Phenylethanol	65.52±2.50	27.45±0.92	42.47±1.54	36.61±0.92	69.88±0.72	67.21±1.12	29.54±0.92
6	Cis-Rose oxide	0.02±0.00	n.d.	n.d.	n.d.	0.01±0.00	tr	n.d.
7	Trans-Rose oxide	0.01±0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
8	Citronellol+Nerol	8.94±0.92	17.37±0.78	8.80±1.10	16.25±0.92	4.56±0.82	8.01±0.12	6.11±0.12
9	Geraniol	14.48±1.12	28.67±0.92	24.42±1.10	17.55±0.10	13.72±1.12	13.96±0.92	14.87±0.52
10	Eugenol	1.58±0.92	1.18±0.03	0.67±0.03	1.22±0.05	0.13±0.03	0.90±0.05	0.78±0.03
11	Geranylacetate	0.33±0.03	0.39±0.05	0.62±0.00.1	0.38±0.03	0.39±0.01	0.39±0.03	0.42±0.03
12	Methyl eugenol	0.22±0.03	0.08±0.01	0.04±0.00	0.03±0.00	0.04±0.00	0.18±0.00	0.04±0.00
13	Heptadecane (C_{17})	0.01±0.00	0.06±0.01	0.09±0.01	0.14±0.03	0.02±0.00	0.01±0.00	0.02±0.00
14	Farnesol	0.15±0.01	0.85±0.03	0.13±0.03	0.52±0.05	0.12±0.01	0.15±0.03	0.12±0.01
15	Nonadecene	0.02±0.00	0.33±0.03	0.13±0.03	0.12±0.01	tr	0.01±0.00	0.02±0.00
16	Nonadecane (C_{19})	0.10±0.00	0.53±0.01	0.66±0.05	0.29±0.03	0.17±0.03	0.21±0.01	0.57±0.03
17	Eicosane	0.01±0.00	0.05±0.01	0.06±0.01	0.03±0.00	0.03±0.00	0.01±0.00	0.02±0.00
18	Heneicosane (C_{21})	0.04±0.00	0.25±0.03	0.17±0.03	0.14±0.01	0.06±0.01	0.06±0.01	0.14±0.00
19	Tricosane (C_{23})	0.01±0.00	0.05±0.00	0.07±0.01	0.07±0.01	0.12±0.03	0.01±0.00	0.05±0.00
20	Pentacosane	0.09±0.01	0.37±0.03	0.30±0.03	0.26±0.07	0.20±0.03	0.24±0.01	0.31±0.03
21	Heptacosane	0.02±0.00	0.05±0.00	0.05±0.01	0.03±0.00	0.07±0.01	0.02±0.00	0.04±0.00

Legend: tr – traces <0.01 %; n.d. – not identified. Data expressed as mean±SD

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It is obvious that the chemical structure of the scent waters is different than the essential oil. The hydrolats mainly consist of phenylethyl alcohol – from 27.45 % in *R. alba*, to 69.88 % in Raduga. The second one was the terpene alcohol geraniol with levels of 13.72 - 28.67 % and the third one – citronellol/nerol in limits 4.56 - 17.37 %. The content of the plant paraffins was also minimized. While in the essential oil the main hydrocarbons C_{17}, C_{19}, C_{21} and C_{23} reached the values of 3.28 % (in *R. gallica);* 21.62 % (in *R. centifolia*); 147.03 % (in *Rosa* Krun) and 5.01% (in *R. gallica*) respectively, 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.

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

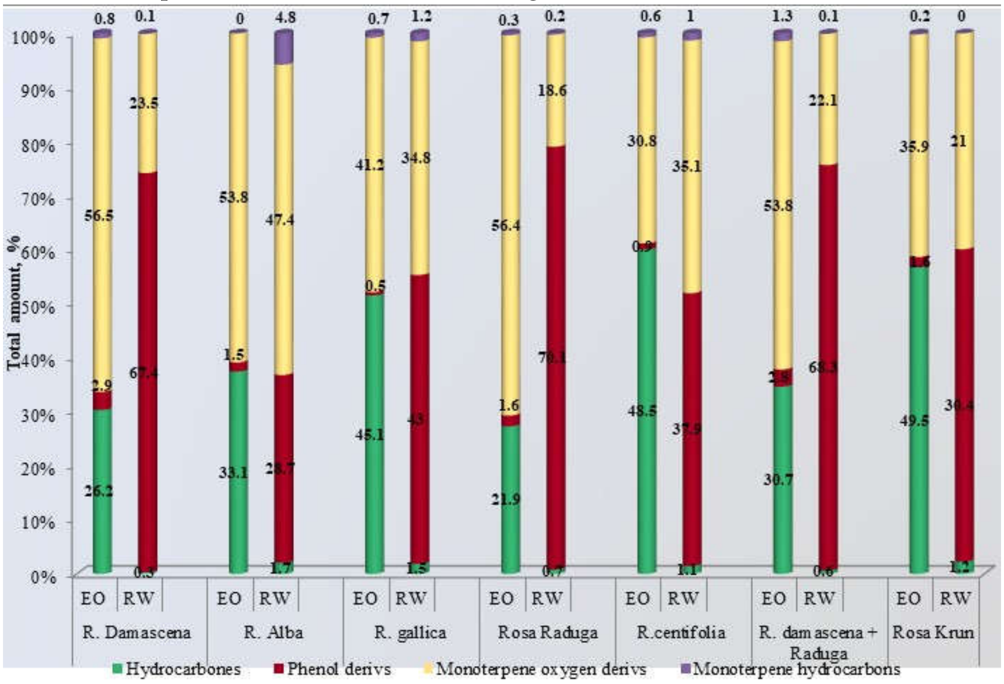

Figure 2. Distribution of the main chemical groups in the rose oils and rose waters. Data are calculated based on mean values.

The figure showed that tree of the roses had the products with the same pattern – low content of phenyl derivates in the essential oil and high in the hydrosol. These are R. *damascena,* Raduga, and mixture of the species. The others had another shape - although the same levels of the phenyl derivates in the oil, their flower waters contained much less the same substance. This fact could be explained with the water solubility of the phenylethyl alcohol – the main aroma carrier in the flowers passed in the water during the distillation process and finally being 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 flower. The same relation was reported by Tomi et al. [43]. Another author divided hydrosols according 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) [44]. Our results listed the products from *R. damascena,* Raduga and mixture of them in first group; from *R. centifolia*, *R. gallica* and *Rosa* Krun in the second one, and *R. alba* meet the third one. Eugenol type was not noted.

5. Conclusions

A quantitative and qualitative analysis of rose oils and hydrosols from industrial plantations in the Kazanlak valley was made. The results showed that the rose fields consist of pure forms or a mixture of the main genotypes of oil-bearing roses. The main products possess the typical characteristics of the investigated roses. The unidentified rose in the village of Krun cannot be

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identified or joined by similarity to them. It is interesting that in 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 abstract to some extent from the influence of geographical factors, climatic conditions, and variations in processing technology that inevitably occur in the industrial processing of the rose flower. 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_{21}). This implies differences both in their biological activities and also in their organoleptic qualities, which opens up new possibilities for their inclusion as components in various perfume compositions.

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References

1. 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.
2. Rusanov, K.; Kovacheva, N.; Dobreva, A.; Atanassov, I. *Rosa x damascena Mill*. (Rose). J Appl Res Med Aromat Plants; Springer: Cham, Switzerland. 2020, 467–500.
3. Astadzhov, 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. Thesis, Plovdiv. 1988, 317.
4. 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(5): 177 -181.
5. 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, 24:2, 1793-1798, DOI: 10.2478/V10133-010-0032-4.
6. 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(1), 6-10. https://doi.org/10.1016/j.indcrop.2011.11.010.
7. Kovatcheva, N.; Zheljazkov, V.; Astatkie, T. Productivity, oil content, composition, and bioactivity of oil-bearing rose accessions. ISSN International Centre. 2011, 46, 710-714. https://doi.org/10.21273/HORTSCI.46.5.710.
8. Dobreva, 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(3), 127-132.
9. Kovacheva, N; Rusanov R. Introduced Species and Varieties of Oil-Bearing Roses in Kazanlak Field Conditions. Plant Sci. 2013, 50(1), 88 – 91.
10. Kovatcheva, N.; Dobreva A.; Petkov, M. Evaluation of essential oils from oil- bearing roses 20th International Scientific Conference - St. Zagora. Agricultural science J. Plant Stud. 2010, I, 187 – 192.
11. Acimovic, M.; Teševic, 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.
12. ISO 9842:2003. International Organization for Standardization: ISO 9842:2003, Oil of Rose (Rosa x damascena Miller). Available online: https://www.iso.org/standard/28611.html (accessed on 30 March 2023)
13. Nazarenko, L.G. Raduga – new sort essential oil rose. Scientific Works of VNIEMK, Simpheropol, Ukraine. 1983, 15: 49-54.
14. Staikov, V.; A. Balinova-Tsvetkova, R; Decheva, I; Kalaidjiev. Rose flower storage conditions and their influence on the quantity and quality of Rose Oil, Riv. Ital. 1975, 57(4), 176 – 180.
15. NIST Chemistry WebBook. DOI: https://doi.org/10.18434/T4D303. Available online: https://webbook.nist.gov/chemistry/ (accessed on 10 Jan. 2023)

16. Baydar, H. Oil-bearing rose (*Rosa damascena Mill.*) cultivation and rose oil industry in Turkey. Eur.Cosmet.Mark. 2006, 14(6),13–17.
17. Kovacheva, N. Investigation on introduction species of oil - bearing roses. Proceedings of Int. Sci. Conference, Stara Zagora, Bulgaria, 2008, 1 – 6.
18. Nedkov, N.; Kanev, K.; Kovacheva, N.; Stanev, S., Djurmanski, A.; Seikova, K.; Lambev, H; Dobreva, A. JEOBP. Kazanlak, Bulgaria: Helicon. 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(2) 251-255, https://doi.org/10.1016/j.indcrop.2004.04.004.
20. Erbas, S.; Baydar, H. Variation in scent compounds of oil-bearing Rose (*Rose damascena Mill.*) produced by Headspace Solid Phase Microextraction, hydrodistillation and solvent extraction, Academy of Chemistry of Globe publications Rec. Nat. Prod. 2016, 10(5), 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 (42), 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(1), 78-84, DOI: 10.1080/0972060X.2010.10643794
23. Khan, MA; Rehman SU. Extraction and analysis of essential oil of Rosa species. Int. J. Agric. Biol. 2005, 7(6), 973-974.
24. Labadie, C.; Ginies, C.; Guinebretiere, MH.; 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(3), 576-586. https://doi.org/10.1016/j.foodres.2015.07.014.
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. (JNFS). 2022, 7(3), 374-378 http://dx.doi.org/10.18502/jnfs.v7i3.10203
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.
27. Nicolov N.; A. Tsoutsulova; N. Nenov. Essence de Roses et aures huiles essentielles Bulgares. Riv. Ital. 1976, LVIII, (10), 349 – 365
28. Baser, KH. 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, Sh.; Ota, T.; Toyoda, T.; Amano, A.; Mura, Sh. New sulphur components of roses oil. Flavour and Fragr. J. 1991, 6(2), 149–152
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), 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(2): 423-437
33. Dobreva A.; Velcheva A.; Bardarov V.; Bardarov K.; Chemical composition of different genotypes oil-bearing roses, Bulg. J. Agric. Sci. 2013, 19 (6), 1213-1218.
34. Neshev, G. Bulgarian rose oil – pharmacological and clinical studies. PhD 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. 2015, 10(7): e0131946. https://doi.org/10.1371/journal.pone.0131946
36. Report of Carcinogens. Methyleugenol. US NTP, Fourteenth Edition. 2016, http://ntp.niehs.nih.gov/go/roc
37. Chipiga, A.; Volchenkov, V. On the intensification of the main technological processes of the essential oil-bearing raw material processing. In: 7th International Congress of Essential Oils, Kyoto, Japan. 1977, 93 – 94.
38. Ahsan, M.; Younis, A.; Nafees, M.; Tufail, A.; Shakeel, Q.; Raheel, M.; Nawaz, F.; Jaskani, M.; Amin, M.; Sajid, M.; Akhtar, G.; Bukhsh, A.; Siddiqua, U.; Raza, M.; Schwinghamer, T.; Zulfiqar, H. Marginal quality water arbitrated essential oil contents in metal hoarded flower petals of scented roses. Ecotoxicol. Environ. Saf. 2021, 226, https://doi.org/10.1016/j.ecoenv.2021.112853.
39. Younis, A; Riaz, A; Khan, MA; 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. Ornam. Biotech. 2009, 3:98-103.
40. Katekar, V.; Rao, A.; Sardeshpande, V. A cleaner and ecological rosewater production technology based on solar energy for rural livelihood. CLCB, 2022, 2 https://doi.org/10.1016/j.clcb.2022.100022.

41. Roques, D. My favourite Rose. IFEAT World, 2018. www.ifeat.org
42. 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. (JNFS). 2022, 7 (3), 374-378 http://dx.doi.org/10.18502/jnfs.v7i3.10203
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(1), 105-112. doi: 10.2503/hortj.MI-098
44. Tanjga, B.B.; Lončar, B.; Aćimović, M.; Kiprovski, 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 https://doi.org/10.3390/horticulturae8100895.