Chemical Composition of Flower Volatiles and Seeds Fatty Acids of *Rosa iliensis* Chrshan, an Endemic Species from Kazakhstan

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**Abstract:** In the present work, the flower volatiles and seed fatty acids compositions of three populations (P-I, P-II, P-III) of *Rosa iliensis* were investigated for the first time. *R. iliensis* is a rare, endangered, narrow-endemic species growing in floodplains of the Ili and Sharyn rivers of Almaty region. The flower volatiles have been investigated with the tandem of MSD-SPME and GC-MS/FID techniques. The seed lipids were extracted from the ripe fruits with microextraction technique. The flower volatiles of *R. iliensis* three populations were characterized by the abundance of oxygenated monoterpenes with benzaldehyde (13.3-38.7 %) and citronellol (2.6-8.8 %) as the major constituents. There were detected significant differences in floral scents between the populations. The flowers of P-I (from Sharyn River) contain sesquiterpene γ-elemene (8.8%), the flowers of P-II (upper reaches of the Ili River) were rich in α-gurjunene (12.8%), while flowers of P-III (lower reaches of the Ili River) contained any sesquiterpenes. Seven fatty acids were determined in the seeds and unsaturated acids were found to be dominant for studied populations. Linoleic (43.0-51.0%), α-linolenic (26.5-28.1%), and oleic (12.0-16.1%) acids were detected as the major constituents. The present study shows that *R. iliensis* species is rich source of valuable volatiles and fatty acids.

**Keywords:** *Rosa iliensis*; population; volatiles; fatty acids; MSD-SPME; microextraction; GC-MS/FID.

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1. Introduction

The genus *Rosa* L. (Rosaceae family) grows naturally throughout the temperate and subtropical regions of the northern hemisphere and consists of approximately 120 species that are
widely distributed in Europe, Asia, the Middle East and North America. The genus is divided taxonomically into four subgenera: Hulthemia (Dumort) Focke, Platyrrhodon (Hurst) Rehder, Hesperhodos Cockerell and Rosa. The subgenus Rosa comprises nine sections: Cinnamomeae (DC. Ser.) (incl. Caroliniae Crép.), Caninae (DC.Ser.), Synstylae DC., Pimpinellifoliae (DC. Ser.), Banksianae Lindl., Bracteatae Thory, Indicae Thory, Laevigatae Thory, and Rosa [1]. In the CIS (Commonwealth of Independent States) there are 63 species of rose hips and 10 types of cultivated roses, including 21 species of rose hip and 3 types of cultivated roses in Kazakhstan [2].

*Rosa L.* species are an object of great scientific interest as a source of biologically active substances and are widely used as medicinal, vitamin, and food raw materials. For food purposes, rose hip is used in tea [3], syrups [4], jam, marmalade [5], wine [6], soups, and noodles [7, 8].

The main commercial value of *Rosa* species is known to be as essential oil (rose oil) [9, 10] and fruits (rose hip) that are good source of valuable bioactive metabolites such as vitamin C [11], carotenoids, polyphenols (anthocyanins, etc.) [12], organic acids (citric, malic etc.), fatty acids, and carbohydrates (glucose etc.). In the scent of roses, more than 400 volatiles have been reported [13].

The fruits of the rose hip have been used in folk medicine for a long time. Rose hip positive effects have been demonstrated in reducing the risk of heart disease [14], various forms of cancer [15-17], diarrhea [18], inflammation, and arthritis [19], pre obese and diabetes [20]. Rose hips have prophylactic and therapeutic actions against the common cold, infectious diseases, gastrointestinal disorders, urinary tract diseases, and inflammatory diseases [21, 22]. Rose hip as the richest natural source of vitamin C [23] was used for the treatment of osteoarthritis in several clinical studies [24].

The aim of this study was to characterize the floral scent and seed’s fatty acids profile of *R. iliensis* three populations for subsequent characterization of the distribution and the raw material reserves that formed potential commercial element for national pharmaceutical industry. To the best of our knowledge, there is no previous report about flower volatiles and seed fatty acids of *R. iliensis*.

### 2. Materials and Methods

#### 2.1. Chemicals

Lipid Extraction Kit, boron trifluoride reagent (BF₃), and n-hexane (Sigma-Aldrich, Germany) were purchased from Sigma-Aldrich (St. Louis, USA). A C₅–C₄₀ n-alkane standard solution was purchased from Fluka (Buchs, Switzerland). A manual SPME holder (57330-U, SUPELCO, Bellefonte, PA) and the PDMS-DVB 65 mm fiber (blue type) were used for SPME procedure of volatiles.

#### 2.2. Plant Material

The samples of *Rosa iliensis* (Figure S1) were collected in 2018 (September - October) during the flowering and fruiting stages along floodplain of the Ili and Sharyn rivers in Almaty Region of the Republic of Kazakhstan (Figure S2). The coordinates of the populations are given in Table 1. Botanical identification was performed by Dr. Ametov A.A. (Al-Farabi Kazakh National University). The plant material was presented as the flowers and the fruits with seeds. Voucher specimens are deposited at the Herbarium of the Institute of Botany and Phytointroduction of the Forestry and Wildlife Committee of the Ministry of Ecology, Geology and Natural Resources of the Republic of Kazakhstan (index AA, voucher specimen number U. Fisyun 3389).

| Table 1. Collection data of *Rosa iliensis* populations |
|---------------------------------|-------------------------------|-----------------------------|----------------|
| Population | Plant material | Location | GPS coordinate | Altitude |
| P-I | Flowers, seeds | Sharyn River | 79°15′44.1″ north latitude, 43°31′26.4″ east longitude | 629 m |
| P-II | Flowers, seeds | upper reaches of the Ili River | 79°34′46.4″ north latitude, 43°58′19.8″ east longitude | 494 m |
| P-III | Flowers, seeds | lower reaches of the Ili River | 76°57′37″ north latitude, 44°09′50.6″ east longitude | 417 m |
2.3 Extraction of Volatiles with MSD-SPME Technique

Microsteam distillation - solid phase microextraction (MSD-SPME) of the volatiles was carried out using an assembly reported previously [25]. MSD-SPME technique involved concurrent solid-phase microextraction combined with continuous hydrodistillation of the volatiles. This method significantly reduces the time required for the isolation of volatiles. It should be noted that this technique allows the isolation of volatiles from a very small amount of plant material [26, 27]. Tandem of MSD-SPME with GC-MS/FID techniques is simple, sensitive, rapid, solvent-less, and non-toxic green technique for analysis of the volatile compounds at microscale level. In experiment, the ground flowers (0.3 g) were placed in 25 mL round bottom flask along with 3 mL of water. The flask was fitted with a Claisen distillation head with plug and a condenser set up for refluxing rather than distillation. The threaded plug was used for SPME fiber assembly. A SPME holder equipped with PDMS-DVB “blue type” fiber was used for the extraction of volatiles. Previously, the fiber was conditioned at 250 °C for 10 min before the experiment. After the SPME needle pierced the plug, the fiber was expressed through the needle and exposed to the headspace above the plant sample. The extraction time for the flower volatiles was 3 min. After trapping of the volatiles, the loaded SPME fiber was withdrawn into the needle, and then the needle was removed from the plug and subsequently used for GC-MS/FID analyses. Thermal desorption of analytes from the fiber coating was performed by injection of the fiber in the injection port (at 250°C) for 5 min.

2.4. Lipid Extraction and Fatty Acid Derivatization

The protocol for fatty acid analysis comprises the following steps: sample preparation, total lipid extraction, methylation of fatty acids, and analysis of the fatty acid methyl esters using GC-MS/FID techniques. The Lipid Extraction Kit was used for extraction of the total lipids from the seeds [28]. The lipids were typically extracted using a dual solvent partition system containing a lipophilic solvent and an aqueous solvent. The lipids were retained in the lower chloroform layer; whereas, aqueous-soluble compounds were retained in the upper methanol-water layer. According to the kit protocol, 0.15 g mill-ground plant material was homogenized with 3 mL extraction solvent consisting of chloroform/methanol (2:1, v/v). After homogenizing and vortexing, 0.5 mL of an aqueous buffer of the kit (composition is not disclosed by the company) was added and the sample was vortexed again. Subsequently, the extraction solution was poured into a syringe system containing a filter (trapping the water). The eluted solvent contained the chloroform phase with total lipids that comprised all extracted lipids from the seeds. The aliquot 200 μL of the total lipids was dried under a stream of nitrogen for subsequent transesterification. After drying, 1 mL of Boron trifluoride-methanol solution and 0.3 mL of n-hexane were added. The mixture was heated at 95°C for 1 hour under reflux. Then, 1 mL of n-hexane and 1 mL of distilled water were added to the reaction vessel, vortexed and centrifuged at 500 × g for 5 min. The top n-hexane layer was transferred into vial and then injected into GC-MS/FID system without solvent evaporation prior to injection.

2.5. Gas Chromatography-Mass Spectrometry (GC/MS) Analysis

GC-MS analysis was performed with an Agilent 5975 GC-MSD system (Agilent Technologies, Santa Clara, CA, USA), as reported previously [25]. An Agilent Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness) was used with He as the carrier gas (0.8 mL/min). The GC oven temperature was kept at 60°C for 10 min, increased to 220°C at a rate of 4°C/min, kept constant at 220°C for 10 min, and then increased to 240°C at a rate of 1°C/min. The split ratio was adjusted to 40:1, and the injector temperature was 250°C. MS spectra were monitored at 70 eV with a mass range of 35 to 450 m/z.

2.6. Gas Chromatography (GC-FID) Analysis
GC analysis was carried out using an Agilent 6890N GC system. To obtain the same elution order as with GC-MS, the line was split for FID and MS detectors, and a single injection was performed using the same column and appropriate operational conditions. Flame ionization detector (FID) temperature was 300°C.

2.7. Identification of the Volatile Constituents

The essential oil constituents and fatty acid methyl esters were identified by co-injection with standards (whenever possible), which were purchased from commercial sources or isolated from natural sources. In addition, compound identities were confirmed by comparison of their mass spectra with those in the Wiley -NIST GC/MS Library (Wiley, NY, USA) [29], MassFinder software 4.0 (Dr. Hochmuth Scientific Consulting, Hamburg, Germany) [30], and Adams Library [31]. Confirmation was also achieved using the in-house “Başer Library of Essential Oil Constituents” database, obtained from chromatographic runs of pure compounds performed with the same equipment and conditions. A C₈–C₄₀ n-alkane standard solution (Fluka, Buchs, Switzerland) was used to spike the samples for the determination of relative retention indices (RRI). Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

3. Results and Discussion

Volatile substances are important attributes used for the assessment of the quality of Rosa species fragrance. In the present work, the flower volatiles of Rosa iliensis three populations, endemic species of the Flora of Kazakhstan, have been investigated with tandem of MSD-SPME and GC-MS/FID techniques. In addition, the lipids were obtained from the seeds of R. iliensis ripe fruits with microextraction technique and methyl esters of the fatty acids were prepared using Boron trifluoride reagent for subsequent gas chromatographic analysis.

The chemical composition of the flower volatiles was investigated using GC-FID and GC/MS simultaneously, and Tables 2 and 3 summarize the identified constituents, their percentage composition, and their relative retention indices (RRI) (compounds are listed in order of their elution on Innowax column). Qualitative and quantitative distinctions in the flower’s volatile profiles of the three R. iliensis populations were demonstrated. A total of 51 compounds were identified in the flowers of R. iliensis three populations. The flowers of P-I and P-II contained 25 and 23 constituents, representing 97.3% and 97.7% of the total volatile’s composition. The volatile profile of the flowers of P-III was found to be the most complex with 37 constituents, representing 97.7% of the total volatile’s composition.

Table 2. Chemical composition of the flower volatiles of Rosa iliensis collected from three populations

| No | RRIa | RRIb | Compound | %c | P-I | P-II | P-III |
|----|------|------|----------|----|-----|------|-------|
| 1  | 1032 | 1008–1039 [32] | α-Pinene | -  | 2.5 | -    | -     |
| 2  | 1093 | 1056–1106 [32] | Hexanal | 8.0 | 3.8 | 11.4 |       |
| 3  | 1194 | 1163–1208 [32] | Heptanal | -  | -   | 4.6  |       |
| 4  | 1225 | 1118–1160 [32] | (Z)-3-Hexenal | 17.3 | 9.8 | 8.0  |       |
| 5  | 1348 | 1317–1357 [32] | 6-Methyl-5-hepten-2-one | - | 1.3 | -    |       |
| 6  | 1244 | 1232 [32] | Amyl furan (=2-Pentyl furan) | 3.8 | -   | 1.4  |       |
| 7  | 1300 | 1300 [33] | Tridecane | -  | -   | 0.7  |       |
| 8  | 1335 | 1289–1339 [32] | (E)-2-Heptenal | -  | -   | 1.3  |       |
| 9  | 1360 | 1316–1377 [32] | 1-Hexanol | 3.1 | 1.7 | 0.8  |       |
| 10 | 1362 | 1331–1369 [32] | cis-Rose oxide | -  | -   | 1.2  |       |
| 11 | 1376 | 1341–1386 [32] | trans-Rose oxide | -  | -   | 0.4  |       |
| 12 | 1400 | 1400 [34] | Tetradecane | -  | -   | 1.3  |       |
The data are presented as relative % by flame ionization detector data. Trace amounts (t) were present at <0.1%. RRI\textsuperscript{a}, relative retention index experimentally calculated based on retention of \textit{n}-alkanes, RRI\textsuperscript{b}, relative retention index reported in literature for constituents analyzed on polar column; \textsuperscript{c} The data are presented as relative % by weight for each component detected in \textit{R. iliensis} flowers.; \%, calculated from flame ionization detector data. Trace amounts (t) were present at <0.1%. P-I, P-II, P-III: populations I, II and III.
Rosa iliensis volatiles and seeds fatty acids

Distribution of the main compound groups presented in the flower volatiles of *R. iliensis* from three populations is presented on Table 3. In general, the flower volatiles of *R. iliensis* were characterized with abundance of oxygenated monoterpenes constituting 38.2%, 45.7%, and 33.8% in populations P-I, P-II, and P-III, respectively. The main components of the volatiles were benzaldehyde (32.9%, 38.7% and 13.3%) and citronellol (2.6%, 3.7%, and 8.8%). The populations P-I (near to Sharyn River) and P-II (upper reaches of Ili River) were distinguished by noteworthy content of sesquiterpene hydrocarbons (17.3% and 18.6%, respectively). γ-Elemene (8.8%) was detected in P-I, while α-gurjunene (12.8%) and β-caryophyllene (5.4%) were found in P-II. Overall, there were significant differences in the flower volatiles compositions between *R. iliensis* populations from lower and upper reaches of the Ili River. However, the flowers of *R. iliensis* of P-III (lower reaches of the Ili River) contained any sesquiterpenes.

Aliphatic aldehydes were found to be the next noteworthy compound group constituting the flower volatiles of *R. iliensis*. The highest content (33.4%) of the aliphatic aldehydes was detected in P-III, then in P-II (25.3%) and P-I (14.4%). Hexanal (8.0%, 3.8%, and 11.4%) and (Z)-3-hexenal (17.3%, 9.8% and 8.0%) were found to be the major aldehydes in P-I, P-II, and P-III, respectively. Ten other compounds were present in P-I and P-II at concentrations ≤1.0%. In P-III twelve compounds were present at concentrations ≤1.0%.

| Compound group                  | P-I     | P-II    | P-III   |
|---------------------------------|---------|---------|---------|
| Monoterpene hydrocarbons        | 3.8     | 2.5     | 1.4     |
| Oxygenated monoterpenes         | 38.2    | 45.7    | 33.8    |
| Sesquiterpene hydrocarbons      | 17.3    | 18.6    | 0       |
| Oxygenated sesquiterpenes       | 0.6     | 0       | 0       |
| Aliphatic alcohols              | 6.2     | 4.4     | 2       |
| Aliphatic aldehydes             | 25.3    | 14.4    | 33.4    |

Comparison of the chromatographic profiles of *R. iliensis* floral volatiles presented on Figure S3, showed the obvious differences and similarities between three populations.

The chemical compositions of the floral scent from two distinct genotypes of Iranian *R. damascena* flowers have earlier been reported by Karami et al. (2013) [44]. The main floral headspace components were phenyl ethyl alcohol (2.2-40.6%), β-citronellol (0.1-45.1%), α-pinene (67.2-1.2%), benzyl alcohol (2.6-64%) and geranyl acetate (0.7-22.7%). The percentage of the constituents varied according to development of the flowers. Phenyl ethyl alcohol was one of the dominant scent compounds emitted from European roses such as *R. damascena* and *R. hybrida* ‘Hoh-Jun’ which were used for the commercial production of rose oils [13]. In our work, the flowers of *R. iliensis* from Kazakhstan contained this constituent in scarce amount (between 0.5 and 1.5%).

One of important healthy functions of *Rosa* species is their essential fatty acids that humans cannot synthesize and have to get through diet [45]. Among these acids the polyunsaturated ones have important role in preventing of neurodegenerative and different metabolic disorders [46, 47]. Earlier, linoleic and α-linolenic acids were reported as major fatty acids in lipid profile of *R. canina* seeds [48].

In the present work, the fatty acids of *R. iliensis* were microextracted from the seeds gathered from ripe fruits. Subsequent transesterification and GC-MS/FID analysis resulted with seven fatty acids, three of them were designated as major ones (Table 4). There was no noticed a great variation of fatty acids between the three populations of *R. iliensis*. The major unsaturated fatty acid in the seed lipids was linoleic acid (18:2ω6, 45.1%, 51.0%, and 44.0%) in populations P-I, P-II and P-III, respectively. It was followed by α-linolenic (18:3ω3, 31.1%, 29.0%, and 28.5%) and oleic (18:1ω9,
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14.0%, 13.0%, and 17.1%) acids. Among saturated fatty acids, nonadecanoic (9:0 dioic), palmitic (16:0), stearic (18:0), and palmitoleic (18:1ω7) acids have been detected.

It is well known that α-linolenic and linoleic acids are two essential fatty acids that humans require [49, 50]. Results of the seed oil investigation allowed us to think that the rose hip seed and seed oil may be proposed as ingredients in functional food formulations and dietary supplements.

Table 4. Fatty acid composition of the seed lipids of Rosa iliensis collected from three populations

| No | RRIa | RRIb | Compound                   | % c   |
|----|------|------|----------------------------|-------|
| 1  | 2105 | 2101 | 9:0 dioic (Dimethyl azelate)| P-I   |
| 2  | 2223 | 2223 | 16:0 (Methyl palmitate)    | t     |
| 3  | 2436 | 2445 | 18:0 (Methyl stearate)     | t     |
| 4  | 2468 | 2472 | 18:1ω9 (Methyl oleate)     | 14.0  |
| 5  | 2509 | 2502 | 18:2ω6 (Methyl linoleate)  | 45.1  |
| 6  | 2512 | 2513 | 19:0 (Methyl nonadecanoate)| t     |
| 7  | 2572 | 2590 | 18:3ω3 (Methyl α-linolenate)| 31.1  |

RRIa, relative retention index experimentally calculated based on retention of n-alkanes, RRIb, relative retention index reported in literature for constituents analyzed on polar column; %, calculated from flame ionization detector data. Trace amounts (t) were present at <0.1%.

The residue products from rose hips have usually been used as animal fodder, but nowadays these residue products are gaining in importance as they can be used in cosmetics, pharmacology and in food applications due to containing the oil with a high degree of unsaturated fatty acids. The quantity of oil contained in the seeds depends on the species and ranges from 5 to 18% [55]. The fatty acid profile of seed lipids and the ratio of saturated / unsaturated fatty acids have contribution into medicinal importance of Rosa fruits due to their beneficial in cardiovascular issues (thrombosis, arteriosclerosis), lowering blood pressure, antimicrobial effect, etc. The efficacy of rose hip seed oil together with an oral fat-soluble vitamins on different inflammatory dermatitis such as eczema, neurodermatitis, and cheilitis, with promising findings of the topical use of rose hip seed oil on these inflammatory dermatoses has been reported by Lin et al. [56]. The rose hip oil has shown to reduce skin pigmentation, discoloration, acne lesions, scars and stretch marks, as well as retaining the moisture of the skin and delaying the appearance of wrinkles [57].

In literature there are a number of reports about fatty acids of Rosa species. Review on Rosa species has recently been reported by Ahmad et al. [58]. Szentmihályi et al. (2002) applied different techniques for extraction of oil from R. canina seeds [48]. Ilyasoglu (2014) has recently characterized the seeds and seed oil of R. canina [59]. The rose hip seed oil was rich in polyunsaturated fatty acids, linoleic acid (54.05%), linolenic acid (19.37%), and phytosterols, mainly β-sitosterol (82.1%). α-Linolenic acid was found to be dominant for the fruits of R. canina L., R. dumalis subsp. boissieri O. Nilsson, R. dumalis subsp. antalyensis (Manden.) Ö. Nilsson, R. villosa L., R. pulverulenta M. Bieb and R. pisiformis (H. Christ) Sosn. [60, 61]. Oleic, linoleic, and α-linolenic have been reported as major fatty acids in the fruits of R. rubiginosa L., R. subcanina (H. Christ) Vuk., R. dumalis (besseriana), R. inodora Fr., R. villosa, R. rugosa Thunb., while palmitic, linoleic, and α-linolenic were determined in noteworthy amounts in R. dumalis Bechst., R. pisiformis, R. villosa L. and R. pulverulenta [62-64].

4. Conclusion

We report here about qualitative and quantitative distinctions in the flower’s volatile profiles and seed fatty acids of the three R. iliensis populations. It was found that flower volatiles of R. iliensis contain a high amount of the oxygenated monoterpenes with benzaldehyde and citronellol as major
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constituents. Moreover, we determined the distinguish between populations distributed on upper and lower reaches of Ili River. The findings of the study indicated that R. iliensis volatiles rich in benzaldehyde and citronellol may be interesting for the further studies as components of different cosmetic products. It has earlier been well illustrated in the case of otto of roses that aromatic constituents like the alcohols (citronellol, phenyl ethyl alcohol etc.) as well as the aldehydes (benzaldehyde), although present in very small quantities, undoubtedly play an important role in the aromatic profile and commercial value of Rosa species [44, 65, 66]. In this respect, the commercial value of these volatiles should be evaluated as a production of concrete (extraction of the petals with hydrocarbon solvents) instead of pure essential oil.

On the other hand, it worth to notice that unsaturated part of the fixed oil of R. iliensis fruit seeds has quite high amount (90%) together ω3, ω6 and ω9 fatty acids which play important role and required for human’s health. So, the results of the seed oil investigation allowed us to think that the rose hip seeds and seed oil may be proposed as ingredients in functional food formulations and dietary supplements. The nutritional composition and the presence of bioactive compounds make the rosehip seed a valuable source of phytonutrients.

Thus, our data provide a previous basis to explain at least part of the beneficial properties of the flower volatiles and seed lipids from R. iliensis.

Conflicts of Interest: The authors declare no competing financial interest.

Author Contributions:
G.Ö. and A.Ch. conceived and designed the project.
A.Ch. and A.A. collected and identified plant material.
G.Ö., A.Ch., and T.Ö. performed the experiments.
G.Ö. and A.Ch. analyzed and interpreted the data, drafted and revised the manuscript.
All authors have read and agreed to the published version of the manuscript.

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Supporting Information

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