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Chemical profiling of Bulgarian rose absolute (*Rosa damascena* Mill.) using gas chromatography–mass spectrometry and trimethylsilyl derivatives

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ABSTRACT

Rosa damascena Mill. is one of the most important plants from Rosaceae family, with a long historical use in the traditional medicine and as a valuable oil bearing plant. The rose scent derived as oil, concrete or absolute, is one of the world most sought-after products and the development of reliable analytical methods for the origin assessment and quality assurance is of substantial fundamental and practical interest.

A comprehensive chemical profiling of Bulgarian rose absolute (*Rosa damascena* Mill.) was performed by means of GC/MS and GC–FID. A protocol for simultaneous analysis of compounds with high structural diversity and volatility, involving a multistep temperature gradient and trimethylsilyl derivatives was applied for this purpose. As a result 132 compounds, mainly mono- and sesquiterpenoids were identified. The main constituents, representing 80.0–95.5% of the total content of the detected compounds, were quantified by means of GC-FID. The developed approach was applied to rose absolute samples from Egypt and Morocco, as well. The specific chromatographic fingerprint, characteristic for the country of production (climatic-geographical origin) could be used for a fast classification of the rose absolute and for authenticity control.

1. Introduction

The genus Rosa consists of more than 200 species with 18,000 cultivars, but only few of them have found industrial scale application for their fragrance and flavouring properties – *Rosa centifolia* L., *Rosa gallica* L. and *Rosa damascena* Mill. (Mahboubi, 2016). Among others, *Rosa damascena* Mill. is considered as superior in terms of the essential oil quality and has been traditionally cultivated in Bulgaria and Turkey, the two main world producers of rose oil. Rose oil is also produced in Iran, India and Morocco. Recently, countries such as China, France and Romania have been trying to develop their own rose oil production without significant success due to the specific climatic conditions required by *Rosa damascena* to produce high quality oil.

Traditionally grown and used in Bulgaria for more than 400 years, *Rosa damascena* Mill., world-wide known as “oil rose”, is an emblematic plant species for the country. The industrial rose cultivation in Bulgaria involves exclusively *Rosa damascena* Mill. f. Trignipetalia Dieck (Kovacheva et al., 2010). Both the climate (mild winter and humid spring) and the soil structure in the area in central Bulgaria, known as the Valley of Roses, proved to be the most favourable for its cultivation. The relatively cooler spring temperatures prevent the formation of a thick protective wax layer on its petals. Instead, the dew and the more abundant rains cause them to become more strongly saturated with essential oil.

The chemical composition of Bulgarian rose oil, recognized as one of the world most sought-after products for its quality and fine aroma, has been extensively studied during the last century (Babu et al., 2002; Buccellato, 1980; Dobreva and Kovacheva, 2010; Garnero et al., 1976; Koksall et al., 2015; Kováts, 1987; Nedkov et al., 2009; Ohloff and Demole, 1987; Pellati et al., 2013). The first comprehensive study on commercial essential oil of *Rosa damascena* Mill., produced by steam distillation in Bulgaria, was carried out by Kováts from 1962 to 1967, involving two-dimensional preparative gas chromatography and counter-current separation. As a result, 127 compounds, from which only 40 of them had been previously reported, were isolated and identified by various spectroscopic methods, representing 98.6% of the volatile part (Kováts, 1987). It is interesting to note the dramatic fate of this masterpiece of the essential oil analysis – the paper, prepared in 1972, was not made free for publication until 1987 due to the enormous economic importance and industrial application of the results. Ohloff and Demole discussed the odoriferous principle of Bulgarian rose oil and the impact of the various rose oil constituents in flavour and fragrance chemistry (Ohloff and Demole, 1987). A comprehensive review on the composition of rose oil has been published (Lawrence, 1991). Up to now, 400 different volatile compounds have been identified in various rose species (Koksall et al., 2015) and 300 different components

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have been identified in the rose oil from *R. damascena* flowers with main constituents – β-citronellol, geraniol, nerol, 2-phenylethyl alcohol, eugenol, methyleugenol, *trans*-β-farnesol, linalool and aliphatic hydrocarbons. The rose oil, defined as "essential oil obtained by steam distillation of the flowers of *Rosa* × *damascena* Miller, of the Rosaceae family, cultivated in Turkey, Morocco and Bulgaria", and its main aroma components are under international regulation through ISO standard ("ISO 9842:2003 Oil of rose (Rosa x damascena Miller)," 2003).

Many other components that are presented in trace amounts are very important for the overall quality of the rose oil. β-Damascenone, β-ionone and rose oxides are key flavour components that contribute to the distinctive scent of the rose. Despite their low concentration, β-damascone and β-damascenone are considered as important markers for the rose oil quality. In particular, β-damascenone has a very low odour threshold of 0.002 ppb, compared with 300 ppb for nerol, 40 ppb for citronellol, 40–75 ppb for geraniol and 1.5–100 ppb for α-damascone (depending on the isomer), and a flavour threshold of only 0.009 ppb (Leffingwell and Leffingwell, 1991).

The botanical and geographical origin, environmental conditions, production method and storage are some of the factors affecting the chemical composition and quality of the essential oil and the sensorial and organoleptic characteristics of the product (Babu et al., 2002; Dobreva and Kovacheva, 2010; Koksall et al., 2015; Krupčík et al., 2015; Rusanov et al., 2011a, 2011b).

At the same time, the composition of the volatile constituents in rose petals usually differs from the composition of the corresponding rose oil. As a typical example, 2-phenylethanol, being highly polar and water soluble is found in the rose water in much higher concentration than in the rose oil (Agarwal et al., 2005). In addition, the traditional rose oil production, based on the steam distillation from fresh rose flowers, requires about 100 °C in the vapour phase, thus causing thermal decomposition of the labile compounds. It has been pointed out (Wabner and Beier, 2012) that constituents such as rose oxides and β-damascenone, presented in the rose oil, are not naturally found in the rose flower and are a by-product of the high temperature distillation process. Alternatively, the rose scent could be extracted as a wax-like substance called rose concrete by solvent extraction, which followed by ethyl alcohol re-extraction produces rose absolute (Aydinli and Tutaş, 2003; Babu et al., 2002). Following the production process, it is considered, that the rose absolute (RA) much better reflects the chemical composition of the rose flower.

In the literature, there are very few studies on the rose absolute chemical composition, focused on few main constituents and predominantly on the base of the absolutes produced in Turkey (Aycı et al., 2005; Aydinli and Tutaş, 2003; Garnero et al., 1976; Krupčík et al., 2015; Kurkcuoglu and Baser, 2003; Özkan et al., 2004; Ulusoy et al., 2009). The chemical composition of the rose absolute, produced from rose concrete at two different temperatures using 96% and 80% ethanol as a solvent, was determined by GC and GC/MS (Aydinli and Tutaş, 2003). It was found that the absolute mainly consists of phenylethyl alcohol, citronellol, geraniol, nerol, nonadecene, methyl eugenol, eugenol, nonadecane and benzyl alcohol. The amounts of these compounds were found to depend largely upon the conditions of production of absolute and the concentration of the ethanol used. Variation in scent compounds of R.damascena Mill. produced by hydrodistillation, solvent extraction and head space – solid phase microextraction (HS-SPME), were recently studied by using GC/MS and GC-FID (Erbaş and Baydar, 2016). In total fifteen compounds in rose concrete and absolute were identified. In addition, a metabolomic approach for the authentication of the rose absolute on the base of non-volatile compounds was proposed by means of UHPLC-ToFMS (Saint-Lary et al., 2016).

There are very few studies on the chemical constituents of Bulgarian rose absolute, with extremely low number of components being discussed (Krupčík et al., 2015; Saint-Lary et al., 2016). Therefore, the goal of this paper is a comprehensive chemical profiling of the Bulgarian rose absolute by means of GC/MS and GC-FID. To our best knowledge no such detailed investigation has been performed up to now. The aroma profile as well as the health influence are direct result of the chemical composition, this study could therefore provide a base for more adequate quality assessment, authenticity and allergen traceability in respect of the use of rose absolute in fine perfumery, natural cosmetics and clinical aromatherapy. The accumulated information could have strong practical effect on the development of fast and high throughput methods for analysis.

2. Experimental

2.1. Materials

2.1.1. Rose absolute samples

Rose absolute (*Rosa damascena* Mill.) samples from five consecutive years (2011–2015 harvest, denoted as BG1-BG5 respectively) were kindly provided by Galen-N Ltd (Sofia, Bulgaria). In addition two commercial rose absolute samples with different geographical origin (as declared by the trader – from Egypt and Morocco) were purchased from Eden botanicals (California, USA). The samples have the following characteristics: dark orange to orange-red viscous liquid with a strong, lasting scent of roses.

2.2. Methods

2.2.1. Derivatization

Trimethylsilyl derivatives were prepared by using a mixture of BSTFA (N,O-bis(trimethylsilyl) trifluoroacetamide and TMCS (trimethylchlorosilane), 99:1 (Supelco, Bellefonte, PA) as derivatization reagent. Fifty microliter dry pyridine and 75 µ l BSTFA/TMCS were added to 5 mg of each sample, dried under a stream of nitrogen and stored over the night in desiccator; the mixture was then heated for 30 min at 80 °C, cooled to the ambient temperature and then analyzed by GC/MS.

2.2.2. Analysis

Gas Chromatography-Mass Spectrometry (GC/MS): The GC/MS analysis was performed on a gas chromatograph HP 6890 GC System Plus coupled to a mass selective detector HP 5973 MSD (Hewlett Packard, Palo Alto, CA). Two fused silica capillary columns (J & W Scientific, Folsom, CA) with 60 m column length, 0.25 mm i.d., 0.25 µ m film thickness – an ultra-inert non-polar DB–5 ms UI (polydimethylsiloxane with 5% phenyl siloxane) and a mid-polar DB-17HT (polydimethylsiloxane with 50% phenyl siloxane), were used. The oven temperature was programmed from 60 °C (2.5 min held) to 120 °C at a rate of 10 °C/min, from 120 to 175 °C at a rate of 2.5 °C/min, and from 175 to 325 °C at a rate of 5 °C/min (30 min held at the final temperature). Helium (99.999%) was used as a carrier gas at a constant flow rate of 0.8 ml/min. The split ratio was 1:75, the inlet temperature was set to 300 °C and the transfer line temperature was 320 °C. The mass selective detector was operated in electron impact ionization (EI) mode at 70 eV electron energy, the ion source temperature was set to 230 °C, and the quadrupole temperature was 150 °C. The mass scan range was 30–750 amu. Instrument control and data collection were carried out using MSD Productivity ChemStation (E.02.02 SP2, Agilent Technologies).

Gas Chromatography with Flame-Ionization Detector (GC-FID): The GC-FID analysis was performed on a Thermo GC Ultra gas chromatograph equipped with flame ionization detector and a Thermo TriPlus autosampler (Thermo Scientific, Bremen, Germany) under the same temperature gradient as described above. Instrument control and data collection were carried out using Thermo Xcalibur software (Rev.2.0, SR1, Thermo Scientific).

Identification, quantitative analysis and chemometrics: The identification of the compounds was performed using commercial mass spectral