NEW PRODUCT FROM BULGARIAN ROSE

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Abstract: *The chemical composition of extract from rosa (Rosa damascena Mill.) by extraction with tetrafluoroethane was analyzed using GC and GC/MS. The main compounds (concentration higher than 3%) of extract were: phenylethyl alcohol (59.08%) and citronellol (12.31%).*
Keywords*: Rosa damascena, extraction, tetrafluoroethane*

Introduction. Bulgaria is known throughout the world as the land of the oleaginous rose (*Rosa damascena* Mill.). Oleaginous roses belong to the family Rosaceae, genus *Rosa*, subgenus *Eurosa*, section *Gallicanae* D.C. In Bulgaria, a number of difference roses are cultivated – Damask rose (*R. damascena* Mill.), White rose (*R. alba* L.) and a few others. Of commercial interest to the essential oil industry is only *R. damascena* Mill. The oleaginous rose was first introduced in Bulgaria during the 14th century and found most favorable growing conditions in the Eastern sub-Balkan valleys. The industrial processing of rose sets its beginning in the middle of 17th century. The former is considered as being the major type, while the latter occupies areas along the Sredna Gora ridge being more resistant to the less favorable soil and climatic conditions (Georgiev and Stoyanova, 2006).

The essential oil is deposited mostly in the petal leaves (about 93% of the total content of the flower) – in the cells of the epidermis and the parenchyma. The average oil content in the flowers is 0.035%, but varies among varieties from 0.009% to 0.062%. The composition of the essential oil is also influenced by the age of the plant or of the respective organ that is processed (Staikov et al. 1975b, 1975c). The climatic conditions both prior to rose-picking and during the picking period, for example, are crucial for the yield and the quality of the essential oil. This is the reason why during the rose-picking period from one and the same growing area different outcomes with respect to oil yield and oil quality are registered for each of the picking days or for shorter several day-periods The unification of the essential oils obtained during the whole rose-picking campaign, aimed at quality uniformity, is the step that secures the final type of rose oil, characteristic in its quality for the respective crop year and growing region (Georgiev and Stoyanova, 2006; Staikov et al., 1975a).

Traditional rose products are rose oil, rose water, concrete and absolute.

The essential oil is obtained from rose blossoms by water distillation. The oil which floats upon the water is separated by decantation and the oil is known as the "primary oil". The yield of this oil is derived (10 – 15%). The distillation waters are re-processed by distillation (cohobation) and the secondary rose oil is obtained (85 – 90% of total oil yield). The two fractions are then combined in their natural proportion and the Bulgarian type of rose oil is finally obtained.

The distillation waters after the cohobation are known as rose water.

Rose oil is an oily, clear liquid or a heterogeneous mass (at temperatures below 23°C), with yellow to yellow-green color and specific odor. Approximately 400 oil components have been identified. They are divided in two categories of substances: carriers and fixators of fragrance. The fragrance carriers comprise the liquid part of the oil, the so-called "eleoptene". The fragrance fixators are scentless and solid at room temperature. They constitute the solid fraction of the oil, the so-called "stearoptene". Eleoptene composes 75–92% of the oil, and stearoptene – 8–25%. About 62–77% terpene compounds are found in oil's composition: hydrocarbons (monoterpenes up to 2% and sesquiterpenes 3–5%), oxygen-containing derivatives (monoterpenes 64–71% and sesquiterpenes 0.5–2%), fatty hydrocarbons and their oxygen derivates (18–25%), phenylpropanoids (3–5%), and others (0.5–2%) (Georgiev and Stoyanova, 2006).

In the end of the picking campaign rose water containing about 0.2–1.2% essential oil is also obtained, which composition incorporates ethyl alcohol and phenylethyl alcohol (33% each), citronellol and geraniol (11% each), nerol (7%) (Georgiev and Stoyanova, 2006).

By extraction of rose blossoms with petroleum ether rose concrete is obtained in Bulgaria, the yield being 0.20–0.25%. Conrete's composition incorporates over 200 components, of which hydrocarbons, aldehydes, ketones, alcohols, esters, fatty acids, *etc*. (Georgiev and Stoyanova, 2006).

The absolute of rose is prepared from the concrete by extraction with alcohol. This extract is cooled to precipitate the waxes. Absolute's composition includes the components of the essential oil, with phenylethyl alcohol being the major ingredient (70%), followed in order by: citronellol (11 – 12%), geraniol and nerol (2% each). Rose absolute contains fatty acids from the C_{10-28} homologous order (mainly C_{22} and C_{24}), homologs belonging to the groups of substances found in rose wax, but dominated by lower molecular weight members. Polycyclic compounds are also identified – ursolic and other acids, the pentacyclic triterpenoid amyrin, β-sitosterol, sterol, *etc*. (Georgiev and Stoyanova, 2006).

The aromatic products obtained from rose blossoms – essential oil, concrete, absolute and rose water, are extensively applied in perfumery, cosmetics and medicine. They all have well-defined antimicrobial activity (Georgiev and Stoyanova, 2006).

Instead of above listed classical methods for production of aroma preparations from rose blossoms, in 2012, Bulgarian scientists applied new and nontraditional methods for obtaining the aromatic products based on the manipulation of pulsed electric fields (Dobreva et al., 2013; Tintchev et al., 2012), surfactant and maceration (Dobreva and Lambrev, 2011; Dobreva et al., 2011). Authors reported that these methods are unsuitable in industrial conditions, independently of higher yield of oil.

New perspective method for production of rose aromatic product is extraction with liquid gases. The installations for extraction with liquefied gases characterized with high working pressure (0.6 MPa when butane is used, 1.5 MPa when propane is used and 4-7 MPa when CO_2 is used) and increased capital investments are needed. There are one laboratory installations with liquefied $C_2H_2F_4$, situated in University of Food Technologies in Plovdiv, and two industrial working with liquefied CO_2

and one with liquefied $C_2H_2F_4$ These installations are used for processing of different essential oil bearing plants in Bulgaria, for example lavender, coriander, fennel and *etc.*

In this paper we showed also the possibility for obtaining the rose aromatic products by extraction with liquid $C_2H_2F_4$.

The aim of present study is producing of new plant extracts from rose growing in Bulgaria, by using liquefied gas (1,1,1,2-tetrafluorethane) in laboratory installation and determination of their chemical composition and characteristics for possible application in cosmetics products.

Materials and methods. The rose flowers were harvested in 2014 in the vicinity of the town of Karlovo, Bulgaria.

The conditions of the extraction are following: The extract obtained by a 1 dm^3 volume $C_2H_2F_4$ (1,1,1,2-tetrafluorethane) laboratory-extractor under temperature 20 - 25°C, pressure 5,7 - 6,5 bar and time 90 min.

The physical-chemical properties of extract were measured according to ISO 9842:2006. GC analysis was performed using gas chromatograph Agilent 7890A; column HP-5 ms (30m x 250µm x 0.25µm); temperature: 35°C/3 min, 5°C/min to 250°C for 3min, total 49min; carrier gas helium 1ml/min constant speed; split ratio 30:1. GC/MS analysis was carried out on a mass spectrometer Agilent 5975C, carrier gas helium, column and temperature as the same as the GC analysis. The identification of chemical compounds is made by comparison to their relative retention time and library data. The identified components are arranged in order to the retention time and quantity in percentage.

Antimicrobial activity of extract was determined against pathogenic and spoilage bacteria and yeasts from clinical and food isolates and also against reference strains, which are deposited in the microbial culture collection of Department of Biochemistry and microbiology, "Paisii Hilendarski" University of Plovdiv, Bulgaria. Minimal Inhibitory Concentration (MIC, µg/ml) and Minimal Bactericidal Concentration (MBC, µg/ml) of extract were determined by reference methods for broth dilution antimicrobial susceptibility tests for bacteria that grow aerobically and reference method for broth dilution antifungal susceptibility testing of yeasts (National Committee Clinical Laboratory Standards, 1990).

Results and Discussions. The produced extract is liquids with characteristic odor. The yield and some of physical and chemical characteristics are following: yield - 1.2 – 1.5% (w/w); appearance - light yellow solid, liquid bellow 20 – 22 °C; dry substance – 20.5% (105°C); refractive index (n_D^{20}) – 1.4800; acid value (mg KOH/g extract) – 1.2. As seen the results for the yields of extract is comparable with literature data and values of the physical and chemical characteristics of the extract are almost equal with these for the absolute.

Chemical composition of the extract is listed in Table 1. As seen 25 components representing 93.90% of the total content were identified. Eight of them were in concentrations over 1 % and the rest 17 constituents were in concentrations under 1%. The main compounds (concentration higher than 3%) of extract were: phenylethyl alcohol (59.08%) and citronellol (12.31%). According to qualitative and quantitative content of the major constituents the produced extract is equal to the rose absolute, published by above listed authors.

Distribution of major groups of aroma substances in the extract is shown in Figure 1. Phenylpropanoids are the dominant group in the extract, followed by oxygen monoterpenes and aliphatic hydrocarbons.

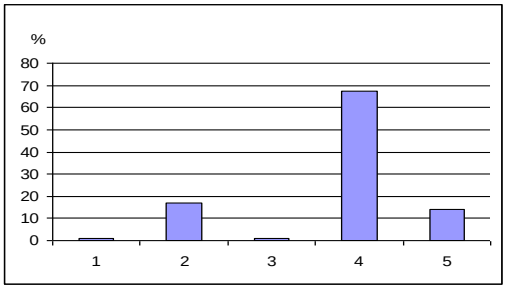

Fig. 1. Group of components in the extract, %.
1- monoterpene hydrocarbons (1.09%), 2 – oxygen monoterpenes (16.55%), 3 – sesquiterpene hydrocarbons (0.95%), 4 – phenyl propanoids (67.45%), 7 – aliphatic hydrocarbons (13.96%).

Table 1. Chemical composition of rose extract.

№	Compounds	RI	%
1.	α-Pinene	939	0.35
2.	β-Pinene	979	0.41
3.	Limonene	1029	0.26
4.	Eucalyptol	1031	0.33
5.	Phenylethyl alcohol	1107	59.08
6.	(R)-(+)-Citronellal	1145	0.49
7.	beta-Citronellol	1226	12.31
8.	beta-Citral	1238	0.46
9.	trans-Geraniol	1253	0.43
10.	Phenethyl acetate	1259	2.59
11.	alfa-Citral	1267	0.43
12.	Citronellyl acetate	1353	0.21
13.	Eugenol	1359	1.32
14.	Geranyl acetate	1381	0.88
15.	Methyleugenol	1404	0.35
16.	beta-Caryophyllene	1425	0.89
17.	n-Pentadecane	1500	0.47
18.	n-Heptadecane	1700	0.34
19.	(9E)-9-Nonadecene	1878	2.05
20.	n-Nonadecane	1900	3.52
21.	n-Eicosane	2000	0.64
22.	(9E)-9-Icosene	2017	0.80
23.	n-Heneicosane	2100	2.18
24.	n-Tricosane	2300	0.44
25.	n-Tetracosanol-1	2690	2.67

According to qualitative and quantitative content of the major constituents the produced extract is equal to the rose absolute (Table 2).

The results of antimicrobial testing are presented in Table 3. The studied rose extract demonstrated antibacterial activity against used test bacterial cultures, with the exception of both species belonging to the genus *Pseudomonas* tested by Agar Disc Diffusion Test. Probably it is due either to the diffusion limitations into the agar medium or to the increased resistance of these bacterial species. It is well known that Serial Broth Dillution Method is more reliable and reproducible and overcome the disadvantages of the Agar Diffusion Method. For these reasons it was applied parallel to the diffusion test. The results obtained by both methods shown that Gram-positive bacteria belonging to the species Bacillus and Staphylococcus are more sensitive in comparison with Gram-negative species Citrobacter, Escherichia and Pseudomonas.

Table 2. Comparative chemical composition of rose aromatic products

Compounds, %	extract with $C_2H_2F_4$	essential oil ISO 9842:2006	absolute 17381-96 (H-65)
Phenylethyl alcohol	59.08	max 3.5	45.0 -71.0
Citronellol	12.31	20.0 – 34.0	6.0 – 12.0
Nerol	0.43	5.0 – 12.0	1.5 – 6.5
Geraniol	-	15.0 – 22.0	2.5 – 7.5
Eugenol	1.32	-	0.9 – 2.2
Methyleugenol	0.35	-	0.3 – 0.8
n-Heptadecane	0.34	1.0 – 2.5	-
n-Nonadecane	3.52	8.0 – 15.0	-
n-Heneicosane	2.18	3.0 -5.5	-

Probably the so called "*external membrane*" which is typical for the Gram-negative bacteria deteriorated the diffusion of the extract from the nutritive medium trough the cell wall and membrane into the cytoplasma.

Table 3: Antimicrobial activity of rose extract

Test microorganisms	Origin	Rose extract		
		IZ, mm	MIC, µg/ml	MBC, µg/ml
B. cereus	ATCC 11778	15	512	512
C. diverus	Clinical isolate	12	1024	1024
E. coli	ATCC 8739	12	1024	1024
E. coli	Food isolate	12	1024	1024
P. aeruginosa	ATCC 9627	0	1024	2048
P. aeruginosa	Clinical isolate	0	1024	2048
P. fluorescens	Food isolate	0	1024	2048
S. abony	Clinical isolate	10	1024	1024
S. abony	ATCC 6017	10	1024	1024
S. aureus	ATCC 6538	12	512	512
S. aureus	Food isolate	14	512	512
S. epidermidis	Clinical isolate	14	512	512
C. albicans	ATCC 10231	12	512	512
C. albicans	Clinical isolate	10	512	512
C. glabrata	ATCC 90030	9	1024	1024
C. glabrata	Clinical isolate	9	1024	1024

The rose extract also demonstrated antimicrobial activity against three species of medically important yeasts belonging to genus *Candida*. Non albicans candida (NAC) species *C. glabrata* are more resistible in comparison with *C. albicans*. The antimicrobial activity of rose extract is equal to the traditional aromatic products from rose, published by above listed authors.

Conclusion. For the first time in Bulgaria new extract from rose (*Rosa damascena* Mill.) was produced by extraction with tetrafluoroethane. This method is suitable for industrial production of absolute, because it is energy saving and also the obtaining of absolute and application of dangerous and combustible solvents is suspended.

The produced extract was almost quantitative and quality identical with absolute from the rose flowers, according to its chemical composition and antimicrobial activity. The extract is prospective for possible application in cosmetics products.

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