Bulgarian Journal of Agricultural Science, 27 (No 4) 2021, 785–791

On the subcritical extraction of *Rosa damascena* Mill.

Ana Dobreva[1*], Ivayla Dincheva[2] and Nenko Nenov[3]

[1]*Agricultural Academy, Institute for Roses and Aromatic Plants, 6100 Kazanlak, Bulgaria*
[2]*Agricultural Academy, AgroBioInstitute, 1164 Sofia, Bulgaria*
[3]*University of Food Technologies, 4000 Plovdiv, Bulgaria*
[*]*Corresponding author:* anadobreva@abv.bg

Abstract

Dobreva, A., Dincheva, I. & Nenov, N. (2021). On the subcritical extraction of *Rosa damascena* Mill. *Bulg. J. Agric. Sci., 27 (4)*, 785–791

Subcritical extraction of *Rosa damascena* Mill. was performed with Freon 143a. The pressure and duration of the process were studied to obtain the yield. It was found that a short-time triple extraction at low pressures of 5-6 bar resulted in the highest yield – 0.151%. The chemical composition of the product revealed a high scent content: β-phenylethyl alcohol (25.6 – 54.1%), citronellol (1.5 – 2.3%), geraniol (1.0 – 3.9%) and nerol (1.9 – 2.9%). The distribution of paraffins' content was: nonadecane (2.7 – 4.9%), nonadecene (3.1 – 5.6%), eicosane (4.4 – 11.6%) and heneicosane (4.4 – 9.8%). The deviations in the constituents of the day product and the year batch production were discussed. The yield and composition were compared with other rose aromatic products. The content of methyleugenol, potential genotoxic and carcinogenic agent was regular low (0.4 – 0.8%), comparing the essential oil (up to 4%).

Keywords: Rosa damascena Mill.; subcritical extraction; scent compounds; methyleugenol
Abbreviations: TFE – 1,1,1,2-tetrafluoroethane

Introduction

Rosa damascena Mill. originates from Middle Asia but has developed its potential in Southeastern Europe and the Eastern Mediterranean coast, on the territory of present-day Bulgaria and Turkey. The plant is of strategic importance for the economy. The rose products have a high value and cannot be replaced by synthetic (or other) substances. Worldwide, about 3000 kg of rose oil, 5000 kg of absolute and tons of rose water are consumed annually, with major manufacturers being Bulgaria, Turkey and Iran (Kovacheva et al., 2010; Rusanov et al., 2009). The aroma is unique and the healing properties of the petals and the various extracts have been applied for centuries in medicine. The essential oil is famous for its rich composition (more than 300 components) and there is no alternative in perfumery (Ohloff & Demole, 1987). The modern pharmacology uses it to alleviate a wide range of physical and mental disorders (Boskabady et al., 2011; Farnia et al., 2015; Neshev, 1990; Nikolova et al., 2016).

In addition, the petals contain flavonoids, glycosides and anthocyanins that have beneficial effects on the human body or can be used in the food industry. There are no risk components (eg. alkaloids) and their use is safe (Boskabady et al., 2011). The rose industry wastes can be used for composting, animal forage or for bio-sorbtion of hazardous substances from aqueous solutions. After processing, additional amounts of aromatic or biologically active products can be obtained. Polyphenols, glycosides and polysaccharides are the mosty recovered by-product. There are several strategies for utilization – acid hydrolysis, microbiological cultivation with beta-glucosidase producing strains with consequent schemes of distillation and extraction increased the overall yield to 0.50 – 0.55% (Slavov, et. al., 2017). Even waste wa-

ters can be a source of phenolic compounds (Rusanov, et. al., 2014). Adding the massive use of rose water and dried flowers in the lifestyle and folk medicine, the oil-bearing rose is perhaps the most universal plant.

The products from roses are extracted by distillation and extraction. These methods have preserved their traditional principles for centuries, but modern technologies modernize them by applying the latest developments in science and technology, with the ultimate goal of increasing yields while preserving or improving the quality of the final result. Roses are biologically viable raw material and the amount of essential oil is deposited in the upper layers of the petals (Bergougnoux et al., 2007; Mihailova et al., 1977a) in very small amounts: 0.030 – 0.055%. This makes the technological intervention extremely difficult. Efforts are focused on storage processes (Kazaz et al., 2010), pre-treatment of the petals (Dobreva et al. 2011; Tintchev et al., 2013), changes in pressure and temperature (Babu et al., 2002), fractionation (Baydar et al., 2008), ultrasound or thermal methods (Manouchehria et al., 2018; Mohamadi et al., 2013; Ozel et al., 2006), solvents with different polarities (Erbaş & Baydar, 2016). All of them are associated with an increase in temperature, which inevitably leads to loss or destruction of the terpenes.

The use of liquefied gases is an alternative to traditional industrial methods – in this case, the solvent has the characteristics of gas and liquid. There is a very high permeability in the raw material, hence very high diffusion coefficient at low density, low surface tension and, as a result, optimized mass transfer of target aromatic and biologically active substances (Wang & Weller, 2006). The treatment of the feedstock and the separation of the solvent do not require an increase in temperature. Selectivity is controlled in a narrow range with changes in temperature and pressure.

Sequential extraction of *Rosa damascena* with supercritical CO_2 can be used for total extraction of valuable substances. Initially, the dried rose petals was treated with pure CO_2 (15 MPa and 40°C) and the solvent was removed in sequential separators (online) at 7 MPa/25°C and 5 MPa/15°C. The resulting aromatic product is low (0.021%) and very different in composition from the traditional essential oil or absolute – phenylethyl alcohol is 3.94%. The subsequent extraction with CO_2:H_2O and CO_2:EtOH:H_2O produces polyphenols with 80% more than MeOH (Da Porto et al., 2015). Upon processing of a rose concrete with CO_2 (8 MPa/40°C) and subsequent separation of the solvent into two types of products – primary, rich in paraffins and secondary – analogue of absolute, which is high in phenylethyl alcohol (50%) and low in paraffins (15.1%) (Reverchon et al., 1997).

The extraction of *Rosa damascena* with CO_2 and co-solvent ethanol can be used to produce quercetin (Ghoreishi et al., 2016).

Suitable for the extraction of terpenes is the subcritical 1,1,1,2-tetrafluoroethane (TFE), chemically inert and well compatible with copper and carbon steel, harmless to the human body and fire and explosion-proof and is authorized for use in the food industry (Corr, 2002). It is also known as R134a. Can be operate at relatively low pressures and its separation from the product and the raw material is accomplished by an equilibrium evaporation-condensation cycle. It also has a high volatility and a boiling point (26.2°C) at atmospheric pressure, which means that it is a negligible solvent residue in the products. Due to the flexible selectivity of the solvent, the resulting products can be high quality analogs of essential oils (Gochev et al., 2012; Nenov et al., 2011), food or bio-pharmaceuticals substances (Babu et al., 2014; Han et al., 2012; Lapkin e. al., 2014; Setapar et al., 2014).

Wilde & McClory (1994) and Wilde (1996) mention the application of this method to *Rosa damascena,* but there is a lack of details of the extract. Baser et al. (2003) also report for the mixture of 90% tetrafluoroethane: 10% diethyl ether, but as factor only the treatment time was noticed. Another publication focuses on the antimicrobial activity of the product (Nenov et al., 2016). All of this has led the team to explore the extraction of the oil rose with R134a and to answer questions about yield and the quality of the result.

Material and Methods

Raw material and extraction parameters

As a raw material were used fresh flowers of *Rosa damascena* Mill., from the plantations of Comerg Ltd., in the territory of Pavel Banya, nearby Kazanlak, Bulgaria. The blossoms were picked from 8 to 10 am in the phase of semi-opened to fully-opened petals (Staikov et al., 1975), and extracted immediately.

The technological study was carried out in the period May-June 2016 of the installation, company development, with a volume of the extractor 1 liter. Sample weights for one charge was 300 g. Periodic stationary extractions were carried out – the raw material was sputtered with solvent and after the contact time, the supernatant was drained into a separator, where the solvent is evaporated at a lower pressure, and in the last few minutes, low heat was used to completely vaporize the vapor.

The annual batches of 2015 and 2016 were obtained at an installation, company development, with two 50 liter extractors, during the period May-June. It is a sum of the daily extractions of the raw material, mixed in their natural ratio.

Table 1. Variants of *Rosa damascena* Mill. extraction

Variants	Pressure, Bar	Temperature, °C	Repeat number	Duration, min
Variant 1	5 – 6	20 – 25	1	60
Variant 2	8 – 10	35 – 40	1	60
Variant 3	5 – 6	20 – 25	2	5-30
Variant 4	5 – 6	20 – 25	3	5-30-15

Process parameters: quantity per charge 6 – 7 kg; pressure 5 – 6 bar; duration 60 min. The process is a periodic.

All the products were dehydrated with Na_2SO_4 and stored until analysis in a tightly closed alumina containers under cold conditions.

The solvent is food grade 1,1,1,2-tetrafluoroethane (CAS number 811-97-2), purchased from the Frigo Chem Ltd. (Bulgaria).

Based on the experience of the team in the extraction technology in roses, the study was conducted on the variants, shown in Table 1.

Each technological variant was performed triplicate. The extract yields were measured as % (v/w).

Analysis and identification of the compounds

GC-MS analysis was carried out on a 7890A gas chromatograph (Agilent Technologies) interfaced with a 5975C mass selective detector (Agilent Technologies). Separations were performed using a 30 m × 0.25 mm (i.d.) DB-5ms silica-fused capillary column coated with 0.25 µm film of poly (dimethylsiloxane) as the stationary phase. The flow rate of carrier gas (helium) was maintained at 1.0 ml/min. The injector and the transfer line temperature were kept at 250°C. The oven temperature program used was 60°C for 5 min then 5°C/ min to 300°C for 10 min. The injection volume was 1 µl in a split mode 10:1. The mass spectrometer was scanned from 50 to 550 m/z. All mass spectra were acquired in electron impact (EI) mode with 70 eV.

A mixture of aliphatic hydrocarbons (C_8-C_{40}) (Sigma) was injected into the system under the above temperature programme in order to calculate the retention index RI (as Kovàts index) of each compound. Identification of compounds was obtained by comparing the RI and the spectral data from the NIST'08 (National Institute of Standards and Technology, USA).

Results and Discussion

After extraction the exhausted flowers seemed like the fresh one for a while. This was due to the gentle technology and the specifics of the solvent. The resulting extracts were an orange mass with a brown tint. The efficacy of the extraction was a function of the solvent properties and process conditions. A good solvent for a given analyze should provide favorable interaction with it in order to promote solubility and selectivity, but minimizing losses with solvent vapors. The task is easier when it comes to a particular substance. In our case there was a complex of compounds, each with a different polarity. The yield results are represented in Figure 1.

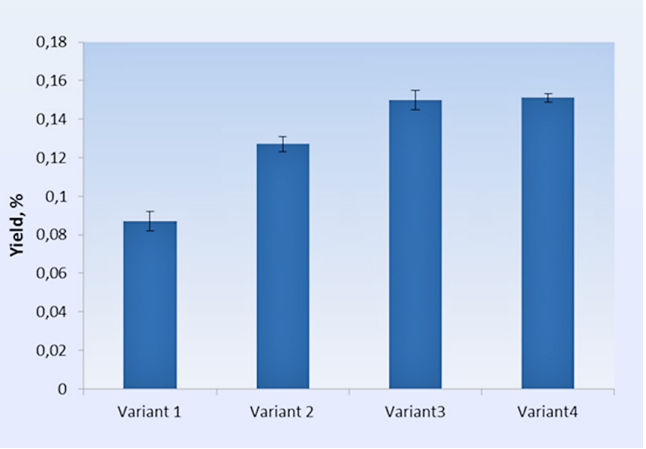

Fig. 1. The influence of extraction parameters on the yield of *Rosa damascena* Mill. TFE extracts

In the variants 1 and 2, the importance of pressure as a factor for extraction efficiency was clearly highlighted. At the same time, an increase of only 3-4 Bar gave 46% higher yields. This means that the permeability of the solvent in the plant matrix was enhanced and, in parallel, a greater number of substances become soluble. The result is related to the thermodynamic characteristics of R134a (dipole moment 2.06 D, polarization 13.8 cm^3/ mol) and density decrease occurring with pressure increase, retaining selectivity within certain limits. TFE is a higher polar solvent than CO_2 at lower pressures and the degradation of materials is much less, yielding much improved extraction results for sensitive natural products (Roth, 1996).

The following two variants give an idea of the effect of multiplicity (number of extractions) with pure freone. It is known that once the extractant enters the cell of the feedstock, the dissolution step stops at the equilibrium concentration of the solute in both the solvent phase and the cellulose

juice. Driving force is the concentration gradient. Due to the high water content in the fresh rose flowers and the polarity of the solvent, the saturation coefficient is low. After separation of the specimen, there are quantities left in the matrix that requires further treatments. For roses, the conventional hexane/petroleum ether extraction requires multiple processing with a solvent or an unsaturated miscelle. The first extraction should be brief to remove the paraffins from the surface of the petals (Shlyapnikoff, 1975).

In the present work, at the same pressure, two- or threefold extraction led to an increase in the same extent of 72% or 74% (Variants 3 and 4) versus Variant 1, which proved that the dynamics of extraction with a subcritical solvent was the same as in the conventional at normal pressure and temperature. As for the yield values, the results correlate with Nenov et al. (2016) – 0.14%, higher than Wilde (1996) – 0.1% and significantly higher than those obtained with supercritical CO_2 of 0.02% (Da Porto et al., 2015).

Table 2. Chemical compositin of the *Rosa damascena* Mill. TFE extracts

Compound	KI	Variant 1	Variant 2	Variant 3	Variant 4	Batch 2015	Batch 2016
		Content (% total peak area)					
α-Pinene	939	0.3	0.3	0.3	0.3	0.1	0.1
Camphene	954	0.1	0.1	0.1	0.1	0.1	0.1
Sabinene	971	0.1	0.1	0.1	0.1	0.1	0.1
β-Pinene	979	0.5	0.6	0.5	0.6	0.4	0.4
β-Myrcene	991	0.1	0.2	0.1	0.2	0.1	0.1
Phenylmethyl alcohol	1001	0.5	0.6	0.5	0.6	0.2	0.2
β-Linalool	1097	1.0	1.2	1.0	1.0	0.5	0.5
cis-Rose oxide	1106	0.2	0.3	0.2	0.3	0.1	0.1
Phenylethyl alcohol	1110	36.8	25.6	36.0	37.2	54.1	51.5
trans-Rose oxide	1124	0.1	0.1	0.1	0.1	0.1	0.1
Limonene	1129	0.2	0.2	0.2	0.2	0.1	0.1
Eucalyptol	1131	0.1	0.1	0.1	0.1	0.1	0.1
Terpinene-4-ol	1179	0.2	0.3	0.2	0.3	0.2	0.1
α-Terpineol	1187	1.0	1.2	1.0	1.2	0.8	0.8
Nerol	1225	2.2	1.9	2.4	2.9	2.3	2.3
β-Citronellol	1229	1.9	1.5	1.8	2.1	2.3	2.2
Geraniol	1248	1.2	1.0	2.5	3.0	3.9	1.1
Eugenol	1336	1.6	1.8	3.3	4.0	2.2	2.2
Geranyl acetate	1352	0.7	1.0	0.8	0.9	0.6	0.6
Methyleugenol	1405	0.6	0.5	0.7	0.8	0.4	0.4
β-Caryophyllene	1419	0.3	0.3	0.3	0.3	0.3	0.2
α-Caryophyllene	1454	0.1	0.1	0.1	0.1	0.1	0.1
n-Pentadecane	1525	1.1	1.4	1.1	1.1	0.8	0.9
n-Hexadecane	1600	1.2	1.5	1.2	1.2	0.8	0.9
n-Heptadecane	1700	1.8	2.3	1.9	1.8	1.3	1.4
n-Octadecane	1800	1.1	1.4	1.2	1.1	0.8	0.9
Nonadecene	1880	4.4	5.6	4.6	4.0	3.1	3.4
n-Nonadecane	1901	3.9	4.9	4.0	3.8	2.7	3.0
n-Eicosane	2000	9.1	11.6	9.4	8.9	4.4	6.0
n-Heneicosane	2100	7.7	9.8	7.9	7.3	4.4	5.0
n-Docosane	2200	0.3	0.4	0.3	0.3	0.2	0.2
(Z)-9-Tricosene	2294	1.2	1.5	1.2	1.2	0.8	0.9
n-Tricosane	2300	3.4	4.3	3.5	3.3	2.4	2.6
n-Tetracosane	2400	2.4	3.1	2.5	2.4	1.7	1.9
n-Tetracosanol-1	2495	3.7	4.7	3.8	3.6	2.6	2.8
n-Pentacosane	2500	1.2	1.6	1.3	1.2	0.9	1.0
n-Hexacosane	2600	0.9	1.1	0.9	0.9	0.6	0.7

The commercial product absolute is obtained after two separate extraction procedures – initially a non-polar solvent is obtained, and after that it is extracted with cold-absolute ethanol. Each stage is a separate technology, with different equipment associated with service costs, while subcritical extraction is a single process, uses one installation, and solvent removal costs are minimal (only a pressure difference is used). Taken in this way, the yield TFE extract of 0.150 – 0.151% was in favor of the new technology compared to the yields of absolute 0.03% (Khan and Rehman 2005); 0.14% (Younis et al., 2008); 0.16 – 0.17% (Georgiev & Stoyanova, 2006).

The chemical composition of the products obtained is presented in Table 2.

The main component was phenylethyl alcohol. This propanoid is highly soluble in water and during distillation process remains in the distillation water. Its presence in essential oil is up to 3.5% (Georgiev & Stoyanova, 2006). At the same time, it is responsible for the aroma of the rose flowers and the high quantities of the resulting absolute (over 50%), whereas is a guarantee of good quality. In our technological samples, phenyethyl alcohol was presented in the range of 25.6 – 37.2%, the minimum being taken into account for single, continuous high pressure extraction (Variant 2). The maximum was for three-stage short-term low pressure extraction (Variant 4). The Variant 4 was the technological model with the best balance between the ingredients of odor and paraffins. The major alcohols – citronellol, geraniol and nerol were presented in maximum values – totally 8.04%, as well as the ratio of the main terpenes to the main paraffins was the highest one. The lowest value of this relationship had Variant 2 – the single prolonged extraction at high pressure. In the case of supercritical extraction with CO_2 the pressure rise also leads to the increase of hydrocarbons (Baser et al., 2003; Da Porto et al., 2015). It is noteworthy that in the batches of both years phenylethyl alcohol was in larger quantities. It can be explained with the storage processes and the enhanced activity of its enzymes at higher temperatures (Guseva et al., 1969). Methylengenol is a carrier of certain odor qualities, but it has gathered considerable attention due to it is could be genotoxic and carcinogenic agent (European Commission, 2001). Its presence in aromatic products is restricted in safety limits (IFRA standard, 2009). The aim is to reduce it as much as possible – both in the raw material itself and through the processing technology (Baydar et al., 2008; Rusanov et al., 2012). Our results revealed that the content was regular low (0.4 – 0.8 %), comparing the essential oil, which is up to 4% (Georgiev & Stoyanova, 2006). Component levels are comparable to liquefied gas extraction products (Boelens & Boelens, 1997; Kurkcuoglu & Baser, 2003), despite the indication that rising in pressure would increase its content (Babu et al., 2002).

Considering that extraction technology with liquid gases has not yet been validated, it is interesting to compare the quality with the absolute commercial product. Since the origin of the raw material is important and the Bulgarians products are a quality standard, we have compared them. The data were shown in Table 3.

The composition of the extract was an intermediate level between the essential oil and the absolute. It is well known that the balance between liquid phase (odor) and solid phase (hydrocarbons) determines the quality (Nikolov et al., 1977). Essential oil is rich with terpene alcohols and paraffins, but the content of phenylethyl alcohol is low. The rose absolute is structured with high content phenylethyl alcohol, but the fixative constituents are in minimums. The TFE extract contain both the odor and fixative constituents in enough amount. Practically contains aromatic constituents of rose blossom in their natural ratio (Mihailova et al., 1977b).

Conclusion

We found that it is necessary, subcritical extraction of *Rosa damascena* Mill. should take place in several stages. The resulting extract was an original aromatic product similar to rose essential oil and absolute but not identical with them. The main odor was due to phenylethyl alcohol, nerol, citronelol, geraniol and eugenol. The specifics of the solvent preserve the availability of scent fixation (some waxes), thus ensuring durability. The components of the solid stearoptene were eicosane, heneicosane, nonadenecene and nonadecane.

Table 3. The groups of basic odor compounds in *Rosa damasena* Mill. industrial products

Compounds	Essential Oil (Georgiev & Stoyanova, 2006; ISO 9842: 2003; Nikolov et al., 1977)	TFE extract	Absolute (Georgiev & Stoyanova, 2006; Nedeltcheva et al., 2017; Nikolov et al., 1972)
Phenylethyl alcohol, %	max 3.5	51.5 – 54.1	45 – 70
Terpene alcohols, % (Citronellol+Nerol+Geraniol)	40 – 68	5.1 – 8.5	7 – 25
Hydrocarbones, % (C_{17}+C_{19}+C_{20}+C_{21}+C_{23})	18 – 25	15.2 – 18.0	1.4 -5.0

Acknowledgments

The investigation was supported by Comerg Ltd. (www.comerg.com), Bulgaria.

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Received: August 13, 2020; *Accepted:* September 18, 2020; *Published:* August, 2021