

## **CHEMICAL INVESTIGATION ON *Rose damascena* Mill. VOLATILES; EFFECTS OF STORAGE AND DRYING CONDITIONS**

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**Abstract.** The oil bearing rose (*Rosa damascena*) is the most important rose species in terms of fragrances and flavourings. Due to the very short blooming period and excessive amount of flowers, considerable amount of the rose flowers wait for a long time until distillation. There are losses of essential oil yield and quality use of before waited petals. The cold storage and drying applications may be alternative method for evaluate of excessive amount of flowers. Therefore in this study it was aimed to determine the effects of storage on cold (4°C) and room condition (25°C) and convective drying with different temperatures (40, 50 and 60°C) in terms of changes in volatile compositions of oil rose flowers based on direct hexane extraction. Totally 20 volatile compounds were identified in fresh, stored and dried rose petals. The detected compounds varied according to the various storage and drying conditions. It was determined that phenylethyl alcohol, citronellol, geranyl acetate, nonadecane were predominant compounds on all treatments. In the study, storing treatments led to increase on the percentage of oxygenated monoterpenes (OM) while drying treatments led to decrease on OM. It was determined that storing and drying treatments led to increase on the percentage of benzenoid compounds (BC) and aliphatic hydrocarbons (AH).

**Key words:** oil bearing rose, volatiles, storage, solid phase micro-extraction, GC/MS

### **INTRODUCTION**

Roses known as garden plants, cut flowers, potted plants cultivated in nearly all of the countries of the world since many centuries [Weiss 1997]. The economic importance of roses also lies in the use of their petals as a source of natural and enthusiastic fragrances and flavourings [Kovacheva et al. 2010]. The fragrance of rose is appreciated

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by most people due to the having desirable compounds. Generally, red rose smells as purely sweet, sometimes pungent-sweet; purple rose smells vigorously sweet; pink rose smells clear sweet; yellow rose smells woody sweet; white rose smells waxy sweet [Zhiping et al. 2006]. Among the roses, oil bearing rose (*Rosa damascena*) is the most common in Rosaceae family in terms of its fragrances and flavourings. Therefore, the products of oil bearing rose are used many different industries such as perfume, cosmetic, pharmaceutical and food [Lavid et al. 2002]. *Rosa damascena* is cultivated and used in Turkey, Bulgaria, Iran, India, Morocco, France, China, Italy, Libya, South Russia and the Ukraine in the world [Weiss 1997]. In addition, it has traditionally been grown and used in Turkey (Isparta) and Bulgaria (Kazanlik) [Göktürk Baydar et al. 2004, Kazaz et al. 2009].

Due to the difficulties of production of oil rose, the low oil content and the lack of natural and synthetic substitutes, rose oil is one of the most valuable essential oil. Quantification of rose oil is very low due to the production procedure. Essential oil composition is varied over the flower stages, flower parts, and the harvesting period [Verma et al. 2011]. Approximately, One kg of rose oil can be obtained from approximately 3.000 kg of petals [Baser 1992]. Oil rose is flowered only once annually and flowering lasts for almost 4–6 weeks [Kazaz et al. 2009]. The rose bushes produce daily a large number of blooming flower buds, which are picked by hand and subjected to distillation within the same day. Because of the short blooming period and excessive amount of flowers, considerable amount of the rose flowers wait for a long time until distillation. There are not only losses of essential oil yield but also losses of quality depending on waiting of petals. Therefore, only fresh rose petals are preferred for oil production [Baydar and Göktürk-Baydar 2005].

More than 400 volatile compounds have been identified in the floral scent of various rose cultivars until now. These compounds can be classified into five major groups based on their functions: hydrocarbons, alcohols, esters, aromatic ethers, and others [Lavid et al. 2002]. So far, volatile compounds of *Rosa damascena* Mill. of rose oil has been studied by some research groups [Baser 1992, Babu et al. 2002, Rezaei et al. 2003, Loghmani-Khouzani et al. 2007, Mostafavi and Afzali 2009, Dobрева and Kovacheva 2010]. Effects of storage and drying of oil rose flowers on content and volatile compounds of oil have been reported [Baydar and Göktürk-Baydar 2005, Baydar et al. 2008a, Kazaz et al. 2009, Kazaz et al. 2010, Verma et al. 2011]. However, there is little report on the volatile compounds in *R. damascena* flowers, based on direct extraction [Picone et al. 2004, Héthelyi et al. 2010, Rusanov et al. 2011a, b]. It is well known that storage is a technique for maintaining desirable qualities in material, when low storage temperatures are used [Tano et al. 2007]. Therefore, the cold storage of rose petals until distillation gains great importance in order to decrease losses of oil yield and quality. In addition to drying of flowers may be alternative method for prevent to fermentation and decay while petals are waiting for a distillation. The removal of moisture prevents the growth and reproduction of microorganisms causing decay and minimizes many of the moisture-mediated deterioration reactions [Alibas 2009]. Therefore, this study aimed to determine the effects of storage on cold (4°C) and room condition (25°C) and convective drying with different temperatures (40, 50 and 60°C) in terms of changes in volatile composition of oil rose flowers based on direct hexane extraction.

## MATERIALS AND METHODS

This study was conducted in Cukurova University, Agriculture Faculty, and Horticulture Department in Adana city of Turkey. The experimental materials of the roses oil bearing rose (*Rosa damascena* Mill.) were grown Egirdir Fruit Research Station in Isparta provinces of Turkey. The rose petals were separated immediately after the harvesting and the samples were prepared for flavour analysis. The material was not spread. The material was layered 2 cm thickness in the cloth bags.

Their flavour analyses were done in 6 various treatments to compare based on their volatile variation. Firstly, fresh rose petals were immediately analysed after the harvesting. Secondly the rose petals were stored under +4°C conditions containing a relative humidity of 85% ±5 for 4 days. Thirdly, the rose petals were dried under room temperatures (25°C). Finally, the rose petals were dried under various thermal degrees such as 40, 50 and 60°C using thermal incubator (Memmet IF30 plus) for 4 days.

**SPME analysis of volatile compounds.** A supelco fiber holder (Bellefonte, PA-USA) and a 100 µm polydimethylsiloxane (PDMS) coated fused-silica fiber were used due to the most suitable fiber for adsorbing volatiles compound from the rose petals [Arthur and Pawliszyn 1990]. Prior to first extraction, the fiber was conditioned in the GC injector port at 250°C for 1h according to manufacturer's recommendation. HS-SPME technique was used in the extraction of the volatile compounds. Rose petals were homogenized with saturated sodium chloride (5 g) for HS-SPME and 50 g of sample for each extraction was placed into a 100 ml glass vial. In HS-SPME analysis, the PDMS fibre was inserted into the headspace of the glass vial and PDMS fibre was immersed into the sample during 30 min at 30°C. During this time rose petal samples were stirred with a magnetic stirrer. After equilibration the fibre was removed from the sample and the analytes were thermally desorbed in the injector port of the GC/MS instrument for analysis. Thermal desorption in the injector glass liner at 250°C, for 10 min. The analyses were carried out in triplicate.

**GC/MS analysis.** Aroma compounds of the rose petals were analysed by GC-MS. A Perkin Elmer Clarus apparatus equipped with CPSil5CB (25 m × 0.25 mm *i.d.*, 0.4 µm film thickness) fused-silica capillary column was used. The flow rate of helium as carrier gas was 1 ml·min<sup>-1</sup>. The injector temperature was 250°C, set for splitless injection. The column temperature was 60°C/5°C/min/260°C for 20 min. Mass spectra were taken at 70 eV. Mass range was between *m/z* 30–425. A library search was carried out using the Wiley GC-MS Library and Flavor Library of Essential Oil Constituents. The mass spectra were also compared with those of reference compounds and confirmed with the aid of retention indices from published sources. Relative percentage amounts of the separated compounds were calculated from total ion chromatograms by the computerized integrator.

## RESULTS AND DISCUSSIONS

The weight loss of rose petals stored at both 4 and 25°C during storage approximately at rate of 6 and 80%, respectively (data not shown). On the other hand, the

weight loss of rose petals dried by 40, 50 and 60°C approximately at rate of 83, 85 and 86 %, respectively.

The results of changes on volatile composition of *Rosa damascena* Mill petals which were stored at cold (4°C) and room (25°C) and dried at various conditions (40, 50 and 60°C) for four days were given in Table 1. It was determined that some differences were detected between storage and drying conditions in terms of the volatile composition. Totally, 20 compounds were identified based on the treatments.

It was detected phenylethyl alcohol (~25%), 4-Amino-furazan-3-carboxylic acid (3-morpholin-4-yl-propyl)-amide (~23%) and citronellol (~21%) as major components in fresh petals. On the other hand, it was found that 2-trifluoromethylbenzoic acid 2-octyl ester, undecanoic acid isopropyl ester, 2-amino-propionic acid,  $\alpha$ -pinene, nonadecane, N-butyl-2-decanamine were also present in considerable concentrations. In addition  $\alpha$ -limonene  $\beta$ -myrcene, geraniol, geranyl acetate, eugenol, methyleugenol, citronellyl acetate, dimethyl-lauramine, N,n-dimethyl-1-hexadecanamine and trans rose oxide were also determined in fresh petals.

Héthelyi et al. [2010] was pointed out that phenyl ethyl alcohol was the principal component of fragrant rose flowers among the 13 rose varieties. Addition to phenyl ethyl alcohol, orcinol dimethyl ether was the main constituents of the fragrant pink and white rose varieties. Rusanov et al. [2011b] demonstrated that the main compound on flower extract of five oil bearing rose genotypes was phenylethyl alcohol (7.99–8.44%) and followed by nonadecane, heneicosane, 9-nonadecene, heptecosane, tricosane, nonacosane, beta-citronellol, nerol, trans geraniol, n-heptedecane, pentacosane. Similarly, in this paper we detected phenyl ethyl alcohol was the major component of *Rosa damascena* Mill both fresh and treated petals. As shown in Table 1 phenyl ethyl alcohol percentage increased very sharply in various storage and drying conditions and ranged from 63.76 to 81.09%. Piccone et al. [2004] showed that 2-phenylethanol was the major volatile emitted of *Rosa damascena semperflorens*. Rusanov et al. [2011a] demonstrated that major volatile compound of *R. damascene* flower were designated by  $\beta$ -citronellol, trans-geraniol, phenylethyl alcohol, heneicosane, nonadecane, heptedecane. Similar results with previous papers were obtained in this study. However, some of the various compounds were detected in this paper. These differences can be due to using various extraction techniques.

It was determined that changes of volatile compounds of petals which are stored and dried compare to fresh ones in this study. It was revealed that some of the compounds were lost with the different treatments. In the study, 6 compounds were identified by cold storage treatment, whereas 5 compounds were identified by storage on room condition. It was found out that the rates of phenylethyl alcohol, geraniol, geranyl acetate, methyl eugenol and nonadecane increased with cold storage condition (4°C) compared to the fresh petals whereas, citronellol were remained same level. On the other hand, phenethyl alcohol, geraniol, geranyl acetate and nonadecane increased on room condition (25°C) compared to the fresh petals. However, the rates of citronellol decreased in same condition. In this study, it was found out that the rates of phenylethyl alcohol, geranyl acetate and nonadecane were increased but the rates of citronellol and methyl eugenol were decreased with convective drying treatments compared to the fresh petals. In addition, methyleugenol which were undesirable component, lost at 60°C. On the

Table 1. The percentage of volatile composition of *R. damascena* Mill. flowers stored at cold and room conditions and dried for four days

Compounds	RT	Fresh petal	Storage condition				Convective drying			
			4°C		25°C		50°C			60°C
			4°C	25°C	40°C	50°C	60°C			
2-Amino-propionic acid	2.29	2.55 ±0.37	nd	nd	nd	nd	nd	nd	nd	
α-Pinene	6.27	2.05 ±0.30	nd	nd	nd	nd	nd	nd	nd	
α-Limonene	6.59	0.30 ±0.04	nd	nd	nd	nd	nd	nd	nd	
β-myrcene	7.67	0.75 ±0.11	nd	nd	nd	nd	nd	nd	nd	
Trans rose oxide	11.07	0.94 ±0.14	nd	nd	nd	nd	nd	nd	nd	
Phenylethyl alcohol	11.43	25.06 ±3.61	68.54 ±10.85	63.76 ±7.63	81.09 ±17.75	79.32 ±11.37	77.29 ±21.16			
Citronellol	14.62	21.09 ±3.04	21.00 ±3.33	14.55 ±1.74	8.94 ±1.96	8.40 ±1.20	10.69 ±2.93			
Geraniol	15.38	0.21 ±0.03	1.79 ±0.28	10.38 ±1.24	nd	nd	nd	nd	nd	
Citronellyl acetate	18.03	0.90 ±0.13	nd	nd	nd	nd	nd	nd	nd	
Geranyl acetate	18.88	0.69 ±0.10	1.24 ±0.20	3.79 ±0.45	2.26 ±0.49	2.33 ±0.33	2.18 ±0.60			
Eugenol	18.40	0.82 ±0.12	nd	nd	nd	nd	nd	nd	nd	
Methyl eugenol	19.58	1.98 ±0.28	2.24 ±0.35	nd	1.28 ±0.28	0.98 ±0.14	2.87 ±0.79			
α-Phenyl cinnamic acid	21.76	nd	nd	nd	nd	1.65 ±0.24	nd	nd	nd	
Dimethyl-lauramine	22.04	0.43 ±0.06	nd	nd	nd	nd	nd	nd	nd	
2-Trifluoromethyl	25.65	2.66 ±0.38	nd	nd	nd	nd	nd	nd	nd	
N-butyl-2-decanamine	26.37	5.80 ±0.83	nd	nd	nd	nd	nd	nd	nd	
4-Amino-furazan	31.28	23.45 ±3.37	nd	nd	nd	nd	nd	nd	nd	
Nonadecane	31.37	3.58 ±0.52	5.19 ±0.82	7.52 ±0.90	6.43 ±1.41	7.32 ±1.05	6.97 ±1.91			
N,n-dimethyl	31.58	1.81 ±0.26	nd	nd	nd	nd	nd	nd	nd	
Undecanoic acid	37.04	4.93 ±0.71	nd	nd	nd	nd	nd	nd	nd	

2-Trifluoromethyl – 2-Trifluoromethylbenzoic acid, 2-octyl ester

4-Amino-furazan – 4-Amino-furazan-3-carboxylic acid (3-morpholin-4-yl-propyl)-amide

N,n-dimethyl – N,n-dimethyl-1-hexadecanamine

Undecanoic acid – undecanoic acid isopropyl ester

RT – retention time

nd – correct isomer not determined

The data are corresponding standard deviation (±) of the three reading

other hand,  $\alpha$ -phenyl cinnamic acid, which was not seen in other treatments, was detected at 50 and 60°C.

There is limited previously report on volatile compounds of oil rose petals which are stored and dried, based on direct extraction up to now. On the other hand, it was demonstrated to the some effects of storage and drying of oil rose flowers in terms of the oil content and volatile composition of oil [Kazaz et al. 2009, 2010]. It was also reported in previous studies that oil content decreased significantly as the waiting duration for distillation increased. Kazaz et al. [2010] reported that the concentration of citronellol, a main component of rose oil, increased during a storage duration of 10 days. They also emphasized that nerol and geraniol in stored petals were lower than those of unstored petals. On the other hand, the percentage of nonadecane, heneicosane and eicosane were greater than the control group during storage. Verma et al. [2011] reported that the volatiles compositions of oil and water of *R. damascena* obtained from the shade-dried petals were quite different from ones obtained from fresh flower. They also implied that heneicosane, nonadecane, tricosane, pentacosane were predominant in oil and water obtained from shade-dried rose flowers.

Similar to our results, it was reported in previously studies that the main components of rose flower and products from *R. damascena* were oxygenated monoterpenes such as citronellol, geraniol and nerol; benzenoid compounds such as phenylethyl alcohol, 2-phenyl ethyl alcohol and benzyl alcohol; aliphatic hydrocarbons such as heneicosane, nonadecane, tricosane, pentacosane and eicosane [Piccone et al. 2004, Rusanov et al. 2011a, b].

The chemical class of volatile compound rose petals which were detected in this study based on the treatments were shown in Table 2. Oxygenated monoterpenes (OM) were increased at both storage conditions. It was found that OM of petals were 1.0 and 1,2 times higher with storage cold and room conditions compared to fresh ones. On the other hand, petals which were dried (at 40, 50 and 60°C) had approximately 2.2 times lower OM than fresh ones. As an oxygenated monoterpenes, citronellol and geranyl acetate were detected on petals which are fresh or treated. On the other hand, geraniol was detected just in petals which are fresh and stored (at both storage conditions). Trans rose oxide and citronellyl acetate were detected just on fresh petals as an oxygenated monoterpenes.

As shown in Table 2, It was found that benzenoid compounds (BC) of petals which are stored at cold and room conditions were 2.5 and 2.3 times higher than fresh ones. Furthermore, total BC content of petals which were dried at 40, 50 and 60°C were 3.0, 2.9 and 2.8 times higher than fresh petals. Phenylethyl alcohol which was major benzenoid compound was increased with storage at 4 and 25°C at the rate of 2.7 and 2.5 times respectively compare to fresh petal. In addition, drying of petals at 40, 50 and 60°C increased to percentage of phenylethyl alcohol at the rate of 3.2, 3.2 and 3.1 times, respectively. It was determined that Eugenol including the benzenoid compound was not present in petals stored and dried in contrast to fresh ones. Lastly, the changing of methyl eugenol which was the other benzoic compound was not consistent in terms of the storage and drying treatments. Methyl eugenol which was undesirable component was not detected on petals stored at 25 and dried at 60°C.

Only nonadecane was found as an aliphatic hydrocarbon (AH). It was determined that nonadecane of petals which was stored at cold (4°C) and room (25°C) conditions at the rate of 1,4 and 2,1 times higher than fresh ones. However the percentage of nonadecane of petals which were dried at 40, 50 and 60°C were at rate of 1.8, 2.0 and 1.9 times higher than fresh petals (tab. 2).

Table 2. Consists of class compositions (%) of volatile compounds of *R. damascena* Mill. petals stored at cold and room conditions and dried for four days

Class composition	Fresh petal	Storage condition			Convective drying	
		4°C	25°C	40°C	50°C	60°C
Monoterpene hydrocarbons (MH)	3.1	nd	nd	nd	nd	nd
Oxygenated monoterpenes (OM)	23.83	24.03	28.72	11.12	10.73	12.87
Benzenoid compounds (BC)	27.86	70.78	63.76	82.37	80.3	77.29
Aliphatic hydrocarbons (AH)	3.58	5.19	7.52	6.43	7.32	6.97
Others	41.63	nd	nd	nd	1.65	2.87

nd – correct isomer not determined

Baydar et al. [2008a] reported that the concentrations of monoterpene alcohol were increased in oil of rose flowers stored for one month under storage conditions at +4°C. Kazaz et al. [2009] pointed out that citronellol among the monoterpene alcohol in oil obtained from of *R. damascene* flowers were increased by at various storage duration at 0 and +3°C. In another study, Kazaz et al. [2010] reported that the concentration of monoterpene alcohols (citronellol, geraniol, nerol) which are a main component of rose oil, increased during storage (0°C) duration of 10 days. Similarly, in our study we detected that geraniol was increased as a monoterpene whereas citronellol was at same level in storage at +4°C for four days.

Baydar et al. [2008b] also demonstrated that citronellol among the monoterpene in rose oil were increased with flower storage under room conditions for 36 hours. Similarly content of Oxygenated monoterpenes (OM) were increased with storage at both +4 and +25°C in our study. However, in this study opposite to the work of Baydar et al. [2008b] the percentage of citronellol was decreased whereas geraniol was increased with flower storage at room conditions for four days.

Kazaz et al. [2009, 2010] found that hydrocarbons (nonadecane, heneicosane and eicosane) which are second highest component after monoterpene alcohols of rose oil were increased with petal storage. Baydar et al. [2008a] also reported that the concentrations of hydrocarbon in oil from flower stored at +4°C displayed higher scores than in oil of petals distilled immediately. Baydar et al. [2008b] reported that the concentrations of hydrocarbon in oil rose flowers stored for various durations under room conditions displayed higher scores than the petals distilled immediately. Similarly in our study aliphatic hydrocarbon (nonadecane) of petals were increased with both storage condition compare to fresh petal.

On the other hand, Verma et al. [2011] reported that the rose oil and water were dominated by aliphatic hydrocarbons (56 and 46% respectively) obtained from shade-dried rose flowers. They also implied that shade-dried petals were quite suitable as a good source of aliphatic hydrocarbons. Similarly in our study aliphatic hydrocarbons of petals were increased with drying conditions compare to fresh petal.

Compare to previous reports with this study, some of variation were detected due to using not only various extraction techniques but also the various storage and drying conditions. Addition to this study we analysed fresh rose petals whereas in most previous studies they analysed rose oil or rose water.

## CONCLUSIONS

The results revealed that changes were on volatile composition of *Rosa damascena* Mill petals which were fresh, stored at cold (4°C) and room (25°C) and dried at various conditions (40, 50 and 60°C) for four days. Totally, 20 compounds were identified based on fresh petals. It was revealed that some of the compounds lost based on the treatments. Totally, five or six compounds were identified by storage and drying treatments.

It was detected phenylethyl alcohol (~25%), 4-Amino-furazan-3-carboxylic acid (3-morpholin-4-yl-propyl)-amide (~23%) and citronellol (~21%) as major components in fresh petals. But it was found phenylethyl alcohol were considerably increased petals stored and dried. Moreover considerably amount of geranyl acetate and nonadecane were also detected in petals which are both stored and dried. On the other hand, considerably amount of geraniol was identified in both storage conditions especially in 25°C (approximately 10%) as a major component whereas it was not detected on drying treatments.

In this study, the chemical class of volatile compound rose petals were changed by storage and drying treatments. In the study, storing treatments led to increase on the percentage of oxygenated monoterpenes (OM) while drying treatments led to decrease on OM. It was determined that storing and drying treatments led to increase on the percentage of benzenoid compounds (BC) and aliphatic hydrocarbons (AH). In the results the percentage of phenylethyl alcohol as a BC and nonadecane as an AH were considerably increased.

It was reported in previous studies that oil content decreased significantly as the waiting duration for distillation increased. On the other hand, it is important that some volatile components are protected with storage and drying for four days in this study. The results of this study showed that storage and dried of oil rose petals may be an alternative method for density material evaluation.

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## **BADANIE CHEMICZNE OLEJKÓW LOTNYCH W *Rosa damascena* Mill.; WPLYW WARUNKÓW PRZECHOWYWANIA I SUSZENIA**

**Streszczenie.** Róża damasceńska (*Rosa damascena*) jest najważniejszym gatunkiem róży w kategoriach aromatów i zapachów. Ze względu na krótki okres kwitnienia i dużą liczbę kwiatów, znaczna liczba kwiatów róży czeka przez długi czas na destylację. Oznacza to straty olejków lotnych oraz pogorszenie jakości. Składowanie w chłodni i suszenie może być alternatywną metodą. Niniejsze badanie miało na celu określenie wpływu przechowywania w chłodni (4°C) i w warunkach pokojowych (25°C) oraz suszenia konwekcyjnego w różnych temperaturach (40, 50 i 60°C) na zmiany składu olejków lotnych kwiatów róży damasceńskiej w oparciu o ekstrakcję heksanu. W świeżych, magazynowanych i suszonych płatkach róży zidentyfikowano w sumie 20 składników lotnych. Różniły się one w zależności od warunków przechowywania i suszenia. Stwierdzono, że alkohol fenylloetylowy, cytronelol, octan geranylu oraz nonadekan były głównymi składnikami w przypadku wszystkich zabiegów. W niniejszym badaniu przechowywanie spowodowało wzrost utlenionych monoterpenów (OM), natomiast suszenie prowadziło do spadku OM. Stwierdzono, że zabiegi przechowywania i suszenia prowadzą do wzrostu zawartości składników benzenoidowych (BC) oraz węglowodorów alifatycznych (AH).

**Słowa kluczowe:** róża damasceńska, olejki lotne, przechowywanie, mikroekstrakcja do fazy stacjonarnej, GC/MS

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