

Effect of Different Extraction Methods on Yield and Quality of Essential Oil from Four *Rosa* Species

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ABSTRACT

In the present study rose oil was extracted from the petals of four *Rosa* species i.e. *R. damascena*, *R. centifolia*, *R. borboniana* and *Rosa* '*Gruss an Teplitz*' through solvent extraction through hexane, solvent extraction through ether and steam distillation. *R. damascena* yielded (0.145%) of absolute oil, *R. centifolia* yielded 0.11% whereas *R. 'Gruss an Teplitz*' yielded the least (0.035%) absolute oil. Solvent extraction through hexane yielded more absolute oil (0.11%) than steam distillation (0.075%) and solvent extraction (0.07%) through ether on petal weight basis. Gas-chromatography of the rose oil was carried out for the qualitative and quantitative analysis of the oil constituents. Major compounds identified were citronellol, methyl eugenol, geraniol, geranyl acetate, phenyl ethyl alcohol, linalool, benzaldehyde, benzyl alcohol, rhodinyl acetate, citronellyl acetate, benzyl acetate and phenyl ethyl formate. Both techniques (solvent extraction and steam distillation) yielded oil with differences in the percentage composition of each component, but solvent extraction through hexane proved better (i.e. higher yield and more components) than steam distillation for extraction of essential oil from roses.

Keywords: citronellol, essential oil composition, Rosa centifolia, solvent extraction, steam distillation

INTRODUCTION

Roses belong to one of the most popular groups of ornamental plants and have a long quintessential romantic history. During 4700 years, the rose has been a companion to humankind, mainly for its fragrance, but also for rose oil and rose water, and for cosmetic and medical purposes (Gustavsson 1998). The genus *Rosa* includes 200 species and 18,000 cultivars (Weiss 1997; Gudin 2000) however, only a few of them exhibit the marked fragrance that is sought by perfumeries around the world.

Rosa damascena (Damask rose), R. gallica and R. centifolia are the most important species, producing high-value aromatic oil, which is used in the pharmaceutical, flavour and fragrance industries. In modern times, their popularity is reflected by the fact that 98% of women's fragrances and 46% of men's fragrances contain some form of rose. Rose oil is used primarily as a fragrance component in pharmaceutical preparations (e.g. ointments and lotions), and is extensively used as a fragrance ingredient in perfumes, creams, and soaps. The fragrance of the rose flower is captured by using a variety of methods. Previously, solvent extraction, water distillation and steam distillation methods were used, nowadays CO₂ extraction is used. In order to get the best quality and quantity of essential oils, the extraction procedure seems to be the key step. It is the type of plant material that determines which method will be used to obtain the essential oil. Rose oil, for example, which is highly volatile, can not tolerate high temperature during steam distillation. Although the extraction of essential oils may sound only to be of technical interest, it is one of the key points which determines the quality of the oil that is used, since a wrong, or wrongly executed extraction, can damage the oil, and alter the chemical signature of the essential oil. Previously the most popular method for extracting the best quality essential oils was steam distillation, ideally in a copper or stainless still that separates the plant material and steam. The separate chamber ensures hot water will not break down or dilute the

essential oil, which is slowly liberated from the plant material (Durst and Gokel 1987; Wilson 1995). Many old-time distillers favor this method for most oils, and say that none of the newer methods produces better quality oils, but as technological advances are made more efficient and economical methods have being developed. Zoebelein (1997), on the other hand reported that rose otto (rose oil), is not a distilled oil as this would seriously damage the petals and the essence obtained from them, but it is a blend of decanted and recovered oil. Rose essential oil can be extracted by using solvent extraction method (hexane), stream distillation and high-pressure CO₂ techniques (Zoebelein 1997; Amanullah 2002). Solvent distillation is more frequently used to extract the aroma of rose blossoms. The product of solvent extraction is a waxy, light brown, semi-solid material, known as a concrete. Rose absolute is prepared from the concrete by extraction with alcohol. The absolute is a reddish liquid with a typical rose odour. Solvent extraction yields about 10 times that obtained by steam distillation, in the order of 0.1-0.2% (1-2 ml concrete/kg flowers) (Lawrence 1991). In the present study, we extracted the essential oil from four Rosa species, R. damas-cena, R. centifolia, R. borboniana and R. 'Gruss an Teplitz' using different extraction methods with the aim of examining the differences in the oil content of these Rosa species.

MATERIALS AND METHODS

Collection and preparation of flowers

Flowers of four *Rosa* species, *R. damascena*, *R. centifolia*, *R. borboniana* and *R. 'Gruss an Teplitz*' were collected from the Rose Project Area, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, during 2003 and 2004. Flowers were harvested at random at a half-open stage, 35 petals/flower. They were kept for 24 hours in shade only to remove extra moisture, but not in order to dry. Petals were separated, weighed and spread in a tray under shade at room temperature in order to remove moisture and then used for extraction.

Methods of essential oil extraction

Steam distillation

Steam distillation was the first method used for obtaining rose oil. With this method, rose water was obtained from 20 kg rose petals by using a steam distillation unit. Rose water obtained from a steam distillation unit has a thin layer of oil on the surface of the water, which was collected by a further process. The thin layer of oil was recovered from hydrosols by using an organic solvent (*n*-hexane; 99% GC grade; hexane was selected over pentane because it has higher boiling point). Oil was separated from *n*-hexane by distillation.

In detail, a thin layer of oil was recovered from hydrosols by using organic solvent, *n*-hexane, which was added to hydrosols. All the oil was extracted from hydrosols and two layers formed (organic and aqueous layer). These two surfaces were separated by using a separating funnel. The upper organic layer was preserved for further processing.

Oil was separated from organic solvent (*n*-hexane) by using a process of distillation using a recovery evaporator at 45° C and the remaining concrete oil was collected into another flask. To remove moisture from concrete oil sodium sulphate (2 g) was added to this concrete oil and was filtered through a filter paper. In this way, concrete oil was obtained.

To get absolute oil from concrete oil a minimum volume of absolute alcohol was added in the concrete oil. All the natural waxes were removed by absolute alcohol. These natural waxes were filtered through filter paper. Now-absolute alcohol was removed by distillation by using a rotary evaporator, as explained above. Last traces of *n*-hexane were removed by bubbling nitrogen gas through this oil.

In this way rose oil was obtained by using steam distillation.

Solvent extraction

For solvent extraction, a Soxhlet apparatus was used. n-Hexane and ether were used as solvents to extract the essential oil from the four Rosa species. Twenty kilograms of petals from each Rosa spp. were used for this method. Solvent extracts of all the volatile compounds from the petals were collected in a flask (2L, Kinax, USA). This was the concrete oil, which was further processed to remove any remaining solvent from rose oil. A distillation process was performed to recover the solvent from the concrete oil (organic solvent and rose oil) using a rotary evaporator. In this way all the organic solvent was recovered. Distillation by using rotary evaporator is useful because the active ingredients of rose oil are not lost. Absolute oil from concrete oil was recovered by adding 2 ml of absolute alcohol in 20 ml of concrete oil. The alcohol removes all the natural waxes present in the essential oil. The oil was filtered and the absolute alcohol was removed by performing distillation with a rotary evaporator. Final traces of alcohol were removed by bubbling nitrogen gas through this oil. In this way we obtained rose absolute oil by using solvent extraction.

Identification and gas chromatography (GC)

Gas chromatography (GC) for separation and qualitative and quantitative analysis of constituents of rose oil was also carried

Table 1 Effect of extraction method on properties of Rosa centifolia oil

out. A Shimadzu 17-A gas chromatograph was used (Sp 2330 capillary columns, BPX70 - 70% phenyl column; column length 30 m; column thickness 25 μ m). Other analytical conditions were: sample size (1 μ l); carrier gas, N_{2(g)} flow velocity = 5 ml/min; initial column temperature, 50°C; final column temperature, 120°C. Initial hold up time* was three min, final hold up time was nine min and ramp rate was 5°C/min. Identification of constituents of essential oil of all four *Rosa* species was done by comparison with gas chromatograms of a mixture of standards (Fluka, 99% GC grade, Switzerland) while quantitative analysis were carried out by calculating the area under the peak using software CSW-32 (http://www.dataapex.com/products/csw32.php).

Statistical analysis

The data were analyzed statistically for significance within species and means were compared by Duncan's multiple range test at the 5% probability (Steel *et al.* 1996).

RESULTS

The objective of this study was to find out difference in the properties of essential oils from four different Rose spp. extracted by two different methods. The properties of the essential oil from Rosa centifolia are defined in Table 1. The results showed that there was little variation (except total alcoholic contents) in the properties of the essential oil when extracted with different methods. To explore the most suited method for extraction of the oil, a comparative study was designed with different methods of extraction for four different species of Rosa. Solvent extraction using n-hexane as the organic solvent proved to be the best extraction method for all Rosa species. Extraction by steam distillation was the second best method for oil extraction in all four Rosa species. The quantity of oil recovered by solvent extraction (*n*-hexane) was highly significantly different from other two methods (**Table 2**). Among the *Rosa* species, R. damascena was best (quanti- and qualitatively) for the recovery of absolute oil. R. centifolia was closest to R. damascena in terms of recovery of absolute oil while the least amount of absolute oil was recovered from R. 'Gruss an Teplitz'. In conclusion, highly significant differences were observed depending on the extraction method and Rosa species.

Different constituents of the essential oil of the four *Rosa* species were determined by using GC, listed in **Tables 3** and 4. In the rose essential oil extracted through solvent (*n*-hexane) 13 components were characterized. The major components were citronellol, phenyl ethyl alcohol, methyl eugenol, geranyl acetate, rhodinyl acetate, benzyl alcohol and linalool. The percentage composition of the components of essential oil were compared in the four *Rosa* species. Citronellol (62.1%), the principal constituent for fragrance, was highest in *R. damascena*, followed by 54.7% in *R. centifolia*, 27.2% in *Rosa* '*Gruss an Teplitz*' and 12.9% in *R. borboniana*. The second major constituent of rose essential oil was phenyl ethyl alcohol. The highest percentage (47.2%) of this component was identified in *Rosa* '*Gruss an Teplitz*' followed by *R. borboniana* (40.2%), and the least

Extraction method	Specific gravity at 15°C	Optical rotation	Refractive Index at 25°C	№ esters	Total alcoholic content (%)
Distillation	0.8650	-32.7+46.4	1.4630	15.5	75.6
Solvent (ether)	0.873	-33.2+45.3	1.4721	14.3	80.0
Solvent (n-hexane)	0.877	-32.5+45.3	1.4256	14.10	84.2

Extraction method	R. centifolia	R. 'Gruss an Teplitz'	R. damascena	R. borboniana
Solvent extraction (<i>n</i> -hexane)	$21.3 \pm 0.67 \text{ c}$	7.7 ± 0.33 g	28.0 ± 0.58 a	$9.7\pm0.33~f$
Solvent extraction (ether)	13.7 ± 0.33 e	3.5 ± 0.29 i	$19.7 \pm 0.33 \text{ d}$	$8.4 \pm 0.31 \text{ g}$
Distillation	$14.0 \pm 0.58 \text{ e}$	6.3 ± 0.33 h	26.3 ± 0.33 b	7.7 ± 0.33 g

Different letters indicate significance at P < 0.05 according to DMR1.

* EXPLANATION: The volume of the mobile phase (or the corresponding time) required to elute a component the concentration of which in the stationnary phase is negligible compared to that in the mobile phase. In other words, this component is not retained at all by the stationary phase. Thus, the holdup volume (time) is equal to the *retention volume (time) of an unretained compound*. The hold-up volume (time) corresponds to the distance and it includes any volumes contributed by the sample injector, the detector, and connectors.

Table 3 Relative percentage composition of the components identified through gas chromatography in essential oil of Rosa extracted through solvent	
extraction (<i>n</i> -hexane).	

Component	R. damascena	R. centifolia	R. borboniana	R. 'Gruss an Teplitz'
Citronellol	62.134	54.745	27.231	12.854
Methyl eugenol	4.214	3.901	2.534	3.564
Geraniol	1.254	2.684	1.364	2.974
Geranyl acetate	3.524	2.524	4.235	14.587
Phenyl ethyl alcohol	19.254	30.691	40.234	47.235
Linalool	1.247	1.687	1.243	1.875
Nerol	0.234	-	0.456	1.351
Benzaldehyde	0.234	1.156	1.020	2.045
Benzyl alcohol	1.257	0.085	5.254	3.214
Rhodinyl acetate	2.345	1.951	5.364	2.985
Citronellyl acetate	1.810	0.789	2.458	0.786
Benzyl acetate	0.941	0.245	1.257	0.568
Phenyl ethyl formate	0.781	0.879	2.354	3.014

Table 4 Relative percentage composition of the components identified through gas chromatography in essential oil of *Rosa* extracted through solvent extraction (ether).

Component	R. damascena	R. centifolia	R. borboniana	R. 'Gruss an Teplitz'
Citronellol	57.247	52.737	21.789	13.483
Methyl eugenol	3.781	3.324	2.131	3.217
Geraniol	1.132	1.982	1.432	2.792
Geranyl acetate	3.532	2.501	4.234	15.245
Phenyl ethyl alcohol	22.721	33.789	43.145	46.432
Linalool	1.107	1.723	0.979	0.899
Nerol	-	-	-	-
Benzaldehyde	0.432	0.973	1.972	1.321
Benzyl alcohol	1.543	0.321	4.351	4.512
Rhodinyl acetate	-	1.512	2.498	3.710
Citronellyl acetate	1.243	1.832	1.234	2.481
Benzyl acetate	-	-	-	-
Phenyl ethyl formate	-	-	-	-

was recorded in *R. damascena* (19.3%). It was found that nerol, benzyl acetate and phenyl ethyl formate could not be identified in rose essential oil when extracted with ether in solvent extraction, indicating that ether has a lower ability to extract all the components from rose petals.

The oils of all four *Rosa* species contained more or less the same compounds differing only in their relative percentages. A comparison of the composition of the oils showed that the oil of *R. damascena* contained higher amounts of citronellol and methyl eugenol whereas the oil of *Rosa 'Gruss an Teplitz'* had higher concentration of phenyl ethyl alcohol, geranyl acetate and geraniol. The detailed percenttage composition of components of the four *Rosa* species is presented in **Table 4**.

DISCUSSION

The results of this research showed that different Rosa species had variation in their oil content, ranging from 0.03%to 0.1%, in agreement with the findings of Kokkini and Vokon (1989) who reported 0.1% oil content in Damask rose. The oil percentage in R. centifolia ranged from 0.075% to 0.11%. A range of 0.1-1% absolute oil was reported in *R. centifolia* by Karousou *et al.* (1998). *R. damascena* growing wild in Greece ranged from 0.1% to 0.9% (Kokkini and Papageorgion 1998). The difference in oil content of the tested species in turn indicates the positive response for selection for oil contents, i.e. an understanding of the variation in quality and quantity of oil will be helpful for the selection of species for oil extraction to promote farmers to adopt it as a commercial crop It is well known that the chemical composition of volatile oils isolated from aromatic plants depends strongly on the extraction method, among other variables (Muzika et al. 1990; Stashenko et al. 1996). Rose possesses very delicate aromatics and cannot survive the process of distillation to capture their "magical" aromas, and a process of solvent extraction is thus used. The method of extraction with volatile solvents was first applied to flowers in 1835 by Robiquest. From the results in this study we found that



Fig. 1 Comparison of oil from Rosa species through different extraction methods.

more concrete oil and absolute oil was obtained through solvent extraction compared to distillation (**Fig. 1**). *n*-Hexane can dissolve more volatile compounds than ether and yielded essential oil which contained relatively little wax, albumin and coloring matter, but correspondingly more of the odoriferous compounds (Wang 2000). We observed that the solvent *n*-hexane had an ability to dissolve all the odoriferous principles of the flower and possessed a low boiling point (69°C), allowing it to be easily removed (distilled off), without resorting to higher temperature. The odorous principles present in rose flowers can be isolated more efficiently by extraction with volatile solvents than by steam distillation. The concrete and absolute oil of rose can be extracted more efficiently by solvent extraction.

Although hexane was the best solvent found so far for

essential oil extraction from roses it poses some inherent disadvantages. There are relatively high solvent losses in the course of the extraction process; these losses result primarily from evaporation due to a low boiling point. In steam distillation, R. damascena gave a higher yield of essential oil than the other three Rosa species. But the overall yield of essential oil through steam distillation is not satisfactory (i.e. lower) compared to solvent extraction. Many authors have reported that steam distillation produces lower extraction yields (Stashenko et al. 1997; Tuan and Iiangantileke 1997; Simandi et al. 1999). The reason could be that rose possessed highly volatile aromatics that cannot survive the process of steam distillation. Oleum (1993) stated that rose oil is not distilled oil as this would seriously damage the petals and essence obtained from them, but it is a blend of decanted and recovered oil. Essential oil isolated by the steam distillation are different in composition to those naturally occurring in the oil bearing petals of roses, since the steam distillation conditions cause chemical reactions to occur which result in the formation of certain artificial chemicals, called artifacts. Therefore essential oil is scathed by the thermal conditions of steam distillation (Clifford 1999). Semen and Hiziroglu (2005) examined that the yield of oil from Juniperus virginiana was higher when extracted through solvent extraction method than those of others obtained by steam distillation (3.5%). The influence of the method of extraction on oil composition and the labiality of the constituents of essential oil explain why the composition of the product obtained by steam distillation is most often different from that which is initially present in the secretary organs of the vegetable (Bruneton 1995).

Therefore, it can be concluded that solvent extraction exhibited higher extraction efficiency than the steam distillation method. Comparison of all experiments under the chosen conditions showed solvent extraction is the most effective extraction method for rose essential oil.

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