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# Influences of Fermentation Time, Hydro-distillation Time and Fractions on Essential Oil Composition of Damask Rose (*Rosa damascena* Mill.)

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**Abstract:** Damask rose (*Rosa damascena* Mill.) is known for its high quality oil, used in the perfumery industry. The aim of this study was to determine the influences of fermentation time, hydro-distillation time and fractions with sequential intervals on essential oil composition, particularly on methyl eugenol content of Damask rose. Essential oil of the rose flowers was produced by hydro-distillation using a Clevenger-type apparatus. Six fermentation times (6, 12, 18, 24, 30 and 36 h at 25°C in sack), 6 distillation times (30, 60, 90, 120, 150 and 180 min.) and 7 fractions (0-15, 16-30, 31-60, 61-90, 91-120, 121-180, and 181-240 min.) during a hydro-distillation were used. The components in the essential oils were analyzed by GC-FID and GC-MS. Rose oil was characterized by high percentage of acyclic monoterpene alcohols, represented particularly by citronellol, geraniol and nerol, and long-chain hydrocarbons represented particularly such as nonadecane, nonadecene and heneicosane. The oil yield started to decrease through the fermentation (from 0.055 to 0.025 %). Fermentation increased the citronellol and methyl eugenol contents in opposition to the content of geraniol and nerol. Each one of hydrocarbons increased their percentages nearly two times and more during the fermentation. Extending of distillation time up to 150 min increased the essential oil yield. The longer distillation time gave a higher methyl eugenol concentration, whose content increased steadily up to last distillation time (from 0.69 to 1.65 %). Contents of monoterpene alcohols decreased, whereas the hydrocarbons steadily increased up to late fractions.

**Key Words**: Damask rose, essential oil, methyl eugenol, fermentation, hydro-distillation time and fractions.

**Introduction:** From an industrial perspective, only a few *Rosa* species are used for rose oil production [1]. Damask rose or oil-bearing rose (*Rosa damascena* Mill.) is the most

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important *Rosa* species for rose oil production in the world. It is not possible to cultivate roses everywhere, as these plants need optimal weather and soil conditions during the flowering season. Especially, air temperature and humidity, cloudiness and precipitation in the flowering season (May and June) contribute to obtain roses with high oil yield and quality [2].

The best quality rose oil in accordance with the world standards is produced from the roses grown in Isparta, Turkey and Kazanlik, Bulgaria. Essential oil content of rose flowers is very poor and far below 1 % (about 0.03-0.04 %). About 3.5 tons or 1.250.000 fresh rose flowers hand-picked in the early hours during the flowering season to produce only 1 kg rose oil after hydro-distillation in the factory type retorts or stills [3].

Hydro-distillation is the most widely used and an economical method to obtain rose oil. After 500 kg of rose flowers are loaded into the still along with 1500 liters of water, steam valve is turned on. Coils inside the bottom of the still carry the steam which heats the water. The water in the still boils and the steam is carried through the top of the distiller into the condenser. The distillate is collected in 200 liter florentine flasks of the copper stills. Essential oils which are lighter than water will float on the surface in the florentine flask. The oil that seperates out in the florentine flasks is called “decanted oil”, “first oil” or “direct oil”. The bottom water and decanted oil in the florentine flasks are then pumped into the stainless steel tanks to get the redistilled oil called “water oil”, “second oil” or “indirect oil”. Later, the first and second oils are blended to get the final product sold in the markets [4,5].

Rose oil is one of the most expensive essential oils, and called as “liquid gold” in the world markets. Most compounds of the rose oil can be isolated from other oils or synthesized, although the fine odour of the authentic rose oil has never been matched yet. Apart from rose oil, concrete and absolute are also main rose products [6]. Extraction of rose flowers by solvents, typically hexane or petrolether can be used to obtain rose concrete (a semisolid, reddish mass). The concrete yield (0.3-0.4 %) is nearly 10 times more than rose oil yield. 350-400 kg of flowers are needed to produce 1 kg of concrete, which explains why concrete is less expensive than the oil. Rose absolute is produced by extraction of concrete with ethyl alcohol, and 1 kg concrete gives over 0.6 kg of absolute [7].

Damask rose oil is characterized by high percentage of acyclic monoterpene alcohols representing in particular citronellol, geraniol and nerol as well as the aromatic alcohol phenylethyl alcohol, and long-chain hydrocarbons such as nonadecane, nonadecene, eicosane, heneicosane and tricosane. Sesquiterpene hydrocarbons like $\alpha$-guaiene, humulene, and $\delta$-guaiene, oxides and ethers like methyl eugenol, esters and aldehydes like geranyl acetate and geranial, phenols like eugenol are among of the other important components found in the rose oil [8-11].

Rose oil, which freezes at lower temperature is of superior quality, because the content of stearoptene is lower and the percentage of the fragrance-bearing alcohols, in return, is higher. Many other compounds in rose oil are present only in trace amounts but are also very important for the overall quality. For example, $\beta$-damascenone was found to be a useful marker for characterization the quality of rose oil [12].

Rose oil is one of the essential oils containing methyl eugenol at the high percentages (up to 5 %). Methyl eugenol is a high value aroma chemical used in cosmetic products and

flavoring agents. As a flavoring agent it has spicy, ginger-like undertones and its odor is musty tea-like warm and mildly spicy. However, methyl eugenol is not desired above a certain concentration in the essential oils due to negative side effects on human health [13, 14].

The aim of this study was to determine the influences of fermentation time, hydro-distillation time and fractions with sequential intervals on essential oil composition of Damask rose. Another aim of the study was to explore possible approaches to reduce the methyl eugenol content in rose oil.

**Experimental**

**Plant material:** Damask rose (*Rosa damascena* Mill.) was cultivated in a field located in Senir, a town 25 km. West of Isparta (latitude 37°45' N, longitude 30°33' E, elevation 950 m). The flowers were plucked early in the morning before sunrise during the days of May 2007.

**Experimental procedures:** While some of the freshly collected flowers was directly distilled, other part was distilled after 6, 12, 18, 24, 30 and 36 hours to detect the influence of fermentation time on essential oil yield and composition. The oil yields in the fresh (non-fermented) and fermented flowers were measured as percentage (v/w) by hydro-distillation in Clevenger apparatus.

The essential oil of the rose flowers was produced by hydro-distillation using a medium-scale Clevenger-type apparatus in the Rose and Rose Products Research and Implementation Center at Süleyman Demirel University (Isparta, Turkey). One kg rose petals were placed in a distillation apparatus with 3 L of water and hydro-distilled for 4 hours. Six different distillation times (30, 60, 90, 120, 150 and 180 min.) and 7 fractions (0-15, 16-30, 31-60, 61-90, 91-120, 121-180, and 181-240 min.) were used during hydro-distillation process. The oil yields of the individual fractions were measured as % (v/w).

**GC-FID and GC-MS analysis:** Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) analyses were done in Federal Centre for Breeding Research on Cultivated Plants, Institute of Plant Analysis in Ouedlinburg, Germany. The hydro-distilled rose oils were analyzed by gas chromatography-flame ionization detector (GC-FID) using a Hewlett-Packard gas chromatograph 5890, fitted with a HP Innowax column (60 m x 0.25 mm i.d.; film thickness 0.52 μm). Detector and injector temperatures were set at 265 and 250°C, respectively. The following oven temperature was used: beginning at 70°C and then 3°C/min up to 220°C (10 min hold). Carrier gas was nitrogen with a constant flow rate of 1 mL/min (spit 1:40). 2 μL rose oil was diluted in 1 mL of isooctane, and 1 μL of this sample was injected into GC. GC-MS analyses of the isolated oils were performed using an Hewlett-Packard MSD 5973/HP 6890 series plus 2, equipped with HP Innowax column. Detector and Injector temperatures were set at 265 and 250°C, respectively; column initial temperature was 70°C (3 min hold), rising at 3°C/min until 220°C (10 min hold). Ionization energy was set at 70 eV. Carrier gas was Helium with a constant flow rate of 1 mL/min. The percentage composition of the identified components was computed from the GC peak area without any correction factor. The components were

identified by comparing their retention times and mass spectra with those of pure reference compounds. Mass spectra were also compared with those in the National Institute of Standards and Technology (NIST) library database [15]. The component identification was finally confirmed by comparison of their retention indices with those of authentic compounds.

**Results and discussion:** The essential oil content (shown in Table 1 as oil yield) obtained from the non-fermented flowers, and found to be 0.055 %. It was found that oil yield started to decrease together with the incipient fermentation process. After a fermentation time of 6 hours, the essential oil content decreased from 0.055 to 0.050 %. Then a gradual decrease was observed up to the final fermentation time (0.025 % after 36 hours of fermentation). The losses of the essential oil observed during fermentation may be due to the increase in the internal temperatures of the petals resulting in a significant removal of volatile substances from special parenchyma cells in the petals. Therefore, in order to obtain a high oil yield from Damask roses, it is necessary to make the distillation as quickly as possible after hand-picking.

The duration of fermentation influenced the contents of essential oil components as shown in Table 1. The citronellol content increased dramatically with increased fermentation time, while geraniol and nerol contents decreased. In non-fermented flowers, citronellol, geraniol and nerol percentages were 15.75, 33.91, and 17.39, respectively. However, after fermenting 36 h citronellol, geraniol and nerol percentages were 37.62, 3.82, and 1.42. Among the monoterpene alcohols, the relations were also remarkable. There were negative changes between citronellol and geraniol, positive changes between geraniol and nerol. With some fluctuations, the contents of linalool, geranyl acetate and farnesol contents decreased, and phenylethyl acetate and phenylethyl alcohol increased through the fermentation (Table 1).

The experiments demonstrated that the methyl eugenol content is affected by the fermentation process. Rose oils distilled from long-term fermented flowers generally contained higher methyl eugenol values. Methyl eugenol contents of fermented flowers were 1.08, 2.43, 3.36, 3.91, 3.91 and 4.34 % in 6, 12, 18, 24, 30 and 36 hours of fermentation respectively, while the methyl eugenol content of non-fermented flowers was 0.96 % (Table 1). Phenylethyl alcohol is a major oil component, but because of its comparatively high solubility in water it is mainly present in the distillation water. Due to loss of phenylethyl alcohol, rose oil does not accurately represent the authentic odour of the rose flower. Contrary to that, extraction products like “rose concrete” and “rose absolute” contain comparatively high percentages of phenylethyl alcohol [16].

There were also remarkable changes in the contents of hydrocarbons like nonadecane, nonadecene, eicosane, heneicosane and tricosane, whose contents steadily increased during the fermentation (Table 1). The low contents of hydrocarbons causing solidification when the oil is chilled and high contents of monoterpene alcohols giving typical rosaceous and freshness character are desired to get high rose oil quality [4]. The total contents of the monoterpene alcohols decreased from 68.93 to 44.60 %, and the total contents of the hydrocarbons increased from 17.92 to 39.15 % at the end of the 36 h fermentation. Citronellol/geraniol (C/G) ratio was used for evaluating the odor quality of rose oil [9]. The best

odor of rose oil is produced when the ratio is between 1.25 and 1.30 [4]. The C/G ratios were higher in the oils distilled from long-term fermented flowers (e.g. 9.84 in 36 h fermentation) than non-fermented flowers (0.46). Based on these results, rose oils distilled from long-term fermented oils are poor in monoterpene alcohols with a high C/G ratio and rich in hydrocarbons causing the upset of the quality. Similar results from the previous studies reported by Baser [4] and Baydar and Göktürk Baydar [16].

The oil yield and chemical composition of the Damask rose distilled at times is shown in Table 2. Yields of essential oils obtained from rose flowers distilled for 30, 60, 90, 120, 150 and 180 min were 0.020, 0.030, 0.040, 0.040, 0.045, and 0.045 %, respectively. The increase in oil yield continued till 150 min, but no changes were detected after longer distillation times. In rose oil factories, hydro-distillation takes 120 min. If distillation time extends to 150 min, more oil could be produced. However, extending distillation time from 120 min to 150 min could not be economic due to the increases in energy and labor force expenses. In a former study, addition of 2.500 ppm of Tween 20 to the distillation water increased rose oil yield and decreased distillation time [17].

When a comparison was made between the six different rose oils obtained from six different distillation times from 30 min to 180 min, the same major compounds were found in different percentages. Percentages of citronellol, nerol and linalool increased, and the percentages of geraniol and phenylethyl alcohol decreased with increasing distillation time (Table 2). Decreases in the geraniol contents were more obvious and clear than the increases in the citronellol contents. Contents of geranyl acetate, phenylethyl acetate and farnesol increased steadily when distillation time was extended. Hydrocarbons like nonadecane and nonadecene did not exhibit a regular change with distillation time. However, contents of eicosane, heneicosane and tricosane increased with some fluctuations. Eugenol increased up to 90 min, after that time undetectable levels of this compound were found.

Content of methyl eugenol increased steadily from 0.69 to 1.65 % up to the final 180 min distillation (Table 2). Suggesting that distillation time could be limited to reduce methyl eugenol content in the rose oil at the expense of a little yield loss. In a study reported by Jui Chung [18], contents of linalool and camphor in rosewood oil decreased with increasing distillation time, but the safrole content increased with increasing distillation time.

The results of GC-FID analysis on separated fractions during sequential intervals are presented in Table 3. The oil yield increased rapidly at the beginning of the hydro-distillation, however it became slower thereafter. After 30 min distillation, more than 50 % essential oils were recovered from the flowers.

The essential oil composition was different in fractions. These differences might mainly be related to boiling point, degree of solubility and polarity of the compounds in rose petals. Boiling points of citronellol and geraniol are 176°C and 230°C, respectively. Both compounds were recovered in the order of their boiling points. However, citronellol, being more volatile, was collected at the same time as geraniol. A similar situation exists for some other compounds having different boiling points. According to Koedam [19], the compounds in the caraway oil vaporize according to their degree of solubility in the distillation water rather than following the order of their boiling points. In addition, Boutekedjiret *et al.* [20] suggested that the order of exit of compounds in the rosemary oil is determined by their polarity and not by their volatility.

The amount of monoterpene alcohols decreased, whereas the hydrocarbons steadily increased up to late fractions. Since the oxygenated constituents are polar, they are far better soluble in the boiling water than hydrocarbons, diffusion of the first was highly favored. During the first 15 min, the fractions consisted mostly from citronellol (22.39 %), nerol (19.74 %) and geraniol (35.03 %), and the fractions after 180 min contained only small amounts of citronellol (9.39 %), nerol (5.15 %) and geraniol (13.24 %). Concentrations of hydrocarbons like nonadecane (from 4.73 to 18.83 %), nonadecene (from 1.66 to 5.29 %), eicosane (from 1.05 to 2.61 %), heneicosane (from 1.78 to 13.03 %) and tricosane (from 0.31 to 3.87 %) were increased in the fractions from the first to the last fraction slide (Table 3). Although methyl eugenol content decreased slightly, the decreases were not clear. Its content increased over 0.90 % at first 60 min, and then decreased to 0.61 % at the last fraction of the hydro-distillation.

**Conclusion:** In practice, only fresh flowers should be used for commercial oil production. However, the rose flowers collected in sacks generally undergo varying degrees of fermentation prior to distillation in the rose oil factories. This study indicated that fermentation increased the citronellol and methyl eugenol contents in opposition to the content of geraniol and nerol. Methyl eugenol content higher than 4 % was detected, especially in those oils obtained from the long-term fermented flowers. For rose oil production with low methyl eugenol content, it is not recommended to use long-term fermented flowers for the distillation process. However, volatiles in rose flowers are bound in glycosidic form. Therefore, in order to release them during distillation a certain degree of fermentation is necessary, and this is done by leaving the flowers well aerated on the floor of a building for a short period. Hydro-distillation in the factory stills is generally carried out for 90 min. However, this study showed that distillation time affected essential oil yield and composition. Citronellol, nerol and linalool contents increased, and geraniol and phenylethyl alcohol contents decreased with increasing distillation time. A longer distillation time generally resulted in higher methyl eugenol content. In order to reduce methyl eugenol content in rose oil, distillation time should not be prolonged more then the optimum time giving economical oil yield.

During hydro-distillation of Damask rose flowers, essential oils are released from the storage cells in the petals by diffusion. Nevertheless, studies on the essential oil composition in separate fractions with sequential intervals during the hydro-distillation are insufficient. It was determined in the present study that each fraction of hydro-distillation had different essential oil composition. Contents of monoterpene alcohols decreased, whereas hydrocarbons steadily increased up to late fractions. Although no clear changes could be observed, higher contents of methyl eugenol were seen in the fractions between 16 and 60 min.

The results of the present study showed that it is principally possible to improve the essential oil composition of Damask rose by variation of fermentation time, distillation time as well as fractional distillation. The results could potentially be beneficial, since Turkey is one of the largest rose oil producers and this study might open new ways to the exploitation of Damask rose.

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**Table 1. Components (%) of essential oils detected at different fermentation times of *R. damascena* flowers**

| Rt[a] (min) | Components | RI[b] | Fermentation times (min) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Non-ferm. | 6 | 12 | 18 | 24 | 30 | 36 |
| 27.60 | Linalool | 1552 | 1.37 | 1.28 | 0.79 | 0.64 | 0.75 | 0.57 | 0.66 |
| 35.99 | Geranyl acetate | 1770 | 2.83 | 2.47 | 1.64 | 1.04 | 0.62 | 0.68 | 0.47 |
| 36.11 | Citronellol | 1772 | 15.75 | 16.94 | 30.41 | 38.92 | 42.56 | 38.54 | 37.62 |
| 37.48 | Nerol | 1810 | 17.39 | 17.04 | 8.30 | 3.31 | 2.45 | 1.95 | 1.42 |
| 38.53 | Phenylethyl acetate | 1841 | t | t | 0.26 | 0.39 | 0.33 | 0.37 | 0.45 |
| 39.07 | Geraniol | 1856 | 33.91 | 33.43 | 16.55 | 7.92 | 6.19 | 5.55 | 3.82 |
| 40.71 | Nonadecane | 1900 | 9.01 | 9.22 | 14.37 | 15.96 | 15.68 | 17.05 | 17.86 |
| 41.40 | Nonadecene | 1922 | 2.49 | 2.90 | 4.29 | 4.84 | 4.78 | 5.00 | 5.46 |
| 41.76 | Phenylethyl alcohol | 1933 | 0.51 | 0.72 | 0.41 | 0.67 | 0.71 | 0.66 | 1.08 |
| 44.11 | Eicosane | 2000 | 1.00 | 1.05 | 1.96 | 2.14 | 2.06 | 2.34 | 2.49 |
| 45.07 | Methyl eugenol | 2030 | 0.96 | 1.08 | 2.43 | 3.36 | 3.91 | 3.91 | 4.34 |
| 47.38 | Heneicosane | 2100 | 4.39 | 4.69 | 8.60 | 9.23 | 8.50 | 9.81 | 10.56 |
| 50.93 | Eugenol | 2188 | t | t | t | t | t | t | t |
| 53.53 | Tricosane | 2300 | 1.03 | 0.92 | 2.10 | 2.29 | 2.09 | 2.45 | 2.78 |
| 55.80 | Farnesol | 2359 | 2.08 | 1.95 | 1.49 | 0.54 | 0.29 | 0.20 | 0.00 |
| | Oil yield (%) | | 0.055 | 0.050 | 0.040 | 0.035 | 0.030 | 0.030 | 0.025 |

%, relative percentage obtained on HP Innowax column

[a] Retention times (Rt)

t (trace) = < 0.05%,

[b] Retention indices (RI)

**Table 2. Components (%) of essential oils detected at different distillation times of *R. damascena* Mill. flowers**

| Components | Distillation times (min) | | | | | |
|---|---|---|---|---|---|---|
| | 30 | 60 | 90 | 120 | 150 | 180 |
| Linalool | 0.66 | 0.85 | 1.13 | 1.17 | 1.08 | 1.24 |
| Geranyl acetate | 4.67 | 3.71 | 8.15 | 7.99 | 7.76 | 6.71 |
| Citronellol | 21.40 | 20.92 | 22.06 | 22.90 | 22.37 | 24.17 |
| Nerol | 12.21 | 13.12 | 14.78 | 13.97 | 13.14 | 13.00 |
| Phenylethyl acetate | 0.28 | 0.29 | 0.83 | 1.50 | 1.26 | 1.42 |
| Geraniol | 35.56 | 35.22 | 30.72 | 27.83 | 26.27 | 25.66 |
| Nonadecane | 6.46 | 7.47 | 5.48 | 5.53 | 6.78 | 6.98 |
| Nonadecene | 2.09 | 2.35 | 1.80 | 1.78 | 2.18 | 2.19 |
| Phenylethyl alcohol | 1.33 | 1.30 | 1.69 | 1.34 | 1.26 | 1.28 |

table 2. (continued).

| Components | Distillation times (min) | | | | | |
|---|---|---|---|---|---|---|
| | 30 | 60 | 90 | 120 | 150 | 180 |
| Eicosane | 0.84 | 0.86 | 0.65 | 0.76 | 0.92 | 0.95 |
| Methyl eugenol | 0.69 | 0.69 | 0.99 | 1.30 | 1.37 | 1.65 |
| Heneicosane | 3.62 | 3.80 | 3.02 | 3.66 | 4.58 | 4.70 |
| Eugenol | 0.22 | 0.53 | 0.60 | t | t | t |
| Tricosane | 0.76 | 0.76 | 0.68 | 0.92 | 1.24 | 1.21 |
| Farnesol | 0.99 | 1.03 | 0.91 | 1.14 | 1.29 | 1.35 |
| Oil yield (%) | 0.020 | 0.030 | 0.040 | 0.040 | 0.045 | 0.045 |

%, relative percentage obtained on HP Innowax column,
t = <0.05%

**Table 3. Components (%) of essential oils in the different distillation fractions of *R. damascena* Mill. flowers**

| Components | All oil | Distillation fractions (min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | (0-240) | 0-15 | 16-30 | 31-45 | 46-60 | 61-90 | 91-120 | 121-180 | 181-240 |
| Linalool | 1.28 | 0.40 | 0.62 | 0.71 | 1.03 | 0.79 | 1.06 | 1.12 | 1.03 |
| Geranyl acetate | 2.47 | 2.44 | 2.45 | 2.28 | 2.31 | 2.18 | 1.87 | 2.65 | 2.43 |
| Citronellol | 16.94 | 22.39 | 20.86 | 20.61 | 19.40 | 13.64 | 14.99 | 11.57 | 9.39 |
| Nerol | 17.04 | 19.74 | 18.85 | 16.82 | 15.84 | 9.13 | 9.63 | 6.58 | 5.15 |
| Phenylethyl acetate | t | 0.22 | t | t | t | t | t | t | t |
| Geraniol | 33.43 | 35.03 | 36.57 | 34.78 | 33.17 | 20.79 | 23.49 | 16.64 | 13.24 |
| Nonadecane | 9.22 | 4.73 | 5.46 | 6.00 | 6.94 | 15.93 | 13.01 | 16.51 | 18.83 |
| Nonadecene | 2.90 | 1.66 | 1.91 | 2.07 | 2.23 | 4.00 | 3.37 | 4.92 | 5.29 |
| Phenylethyl alcohol | 0.72 | 1.82 | 1.45 | 1.60 | 2.04 | 1.54 | 3.62 | 1.51 | 1.35 |
| Eicosane | 1.05 | 0.48 | 0.59 | 0.73 | 0.91 | 2.31 | 1.94 | 2.21 | 2.61 |
| Methyl eugenol | 1.08 | 0.79 | 0.92 | 0.92 | 0.91 | 0.67 | 0.69 | 0.69 | 0.61 |
| Heneicosane | 4.69 | 1.78 | 2.44 | 3.19 | 4.19 | 11.25 | 10.12 | 11.02 | 13.03 |
| Eugenol | 0.00 | t | t | 0.50 | 0.43 | t | 0.39 | 0.25 | 0.24 |
| Tricosane | 0.92 | 0.31 | 0.48 | 0.77 | 1.11 | 3.05 | 2.98 | 3.22 | 3.87 |
| Farnesol | 1.95 | 0.78 | 1.40 | 2.36 | 2.93 | 5.74 | 7.89 | 8.88 | 10.01 |

%, relative percentage obtained on HP Innowax column
t = <0.05%