Physiological and Psychological Effects of Rose ‘Wishing’ Flowers and Their Hydrosols on the Human Autonomic Nervous System and Mood State

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Physiological and psychological effects of rose aromas have been reported. Many of these reports focused on the effect of rose essential oils, but the effect of fresh rose flowers on humans has not been sufficiently reported. We were also interested in the possibility of using rose hydrosol, a byproduct of the rose essential oil manufacturing process, for aromatherapy. In this study, the physiological and psychological effects of rose ‘Wishing’ fresh flowers and their hydrosols on humans were evaluated. R–R power spectral analysis of heart beats revealed the sedative effect of the fresh flowers’ scent. On the other hand, hydrosols did not show such an effect. Gas chromatography (GC) and GC-mass spectrometry (GC-MS) analysis indicated the possibility that the sedative effect of the fresh flowers’ scent was derived from β-caryophyllene, phenylethyl acetate, and 3,5-dimethoxy toluene. It is possible that fresh rose flowers may be an alternative to rose essential oils in aromatherapy.

Key Words: aromatherapy, fresh flower, gas chromatography (GC), volatile component.

Introduction

Physiological/psychological effects of rose aromas have been reported; a sedative effect by decreasing sympathetic nerve activity (Haze et al., 2002), prevention of skin barrier disruption (Fukada et al., 2012), anxiolytic effects (Bradley et al., 2007), and an anti-conflict effect (Umezu et al., 2002). Many of these reports focused on the effect of rose essential oils, but the effect of fresh rose flowers on humans has not sufficiently reported.

Modern roses (often called hybrid tea roses) are the result of natural and artificial hybridization between European (Rosa damascena) and Chinese roses (R. chinensis) (Joichi et al., 2005). The hybridization has focused on vase life, flower form, flower shape, flower size, and productivity and it has been noted that many rose cultivars have inadvertently lost their aroma intensity (Borda et al., 2011). As a result, modern rose cultivars are generally not notable for their scent (Bergougnoux et al., 2007; Zuker et al., 1998). Breeding has, however, also resulted in 25000 cultivars (Baldermann et al., 2009) and more than 350 components with many distinctive notes as components of modern rose scents (Joichi et al., 2013). In other words, modern roses possess several types of notes, although their intensities are not high.

Recent studies have reported that volatile inhalation affects humans both physiologically and psychologically, even at quite low concentrations. For example, the sedative effects of a diluted jasmine tea odor too weak to elicit significant psychological effects due to a subjective preference for the odor have been confirmed (Kuroda et al., 2005). Our previous study using diluted lavender essential oil also showed sedative effects (Tomi et al., 2011). These facts indicate that a detectable odor intensity is not always necessary to obtain physiological/psychological effects. Therefore, we focused on the possibility of utilizing fresh flowers and hydrosols for aromatherapy.

Rose hydrosol, often refer to rose water or hydrolat, is a byproduct of hydrodistillation in essential oil extraction. Rose hydrosol is often sprinkled on meat dishes or used in the flavoring of desserts such as ice cream, jam, rice pudding, cake, and yoghurt (Pal, 2013). Hydrosol mainly consists of water and includes few aroma components, while essential oil includes highly concentrated aroma components. It has also been...
shown that hydrosol selectively contains polar volatile components (Dyer et al., 2008; Rao, 2012). Since oxygenated monoterpenes (citronellol, geraniol, linalool, nerol, and rose oxide) and benzoids (2-phenylethanol, methyl eugenol, and eugenol) are known as characteristic rose components (Baldermann et al., 2009), rose flower and hydrosols are thought to have relatively similar aroma component composition (Ulusoy et al., 2009). This fact implies that similar physiological/psychological effects can be expected from rose flowers and hydrosols.

In this study, we evaluated these physiological/psychological effects on humans. Aroma inhalation effects of fresh flowers and their extracts, hydrosols, were compared. In addition, it is considered that the visual characteristics of the fresh flowers, such as shape and color, may affect the physiological/psychological effects of rose aroma inhalation on humans, effects of aroma inhalation with/without visual effects were compared. From these results, we considered the novel use of fresh rose flowers and hydrosols in aromatherapy.

**Materials and Methods**

**Rose flowers and hydrodistillation**

Rose ‘Wishing’ fresh flowers were purchased from Imai Nursery (Hiroshima, Japan) and used in all the experiments. Petals (777 g) of ‘Wishing’ fresh flowers were gently collected and hydrodistilled with pure water (389 mL) using a distillation still, producing 270 mL of rose hydrosol.

**Volatile component composition of rose samples**

Volatile components in fresh flowers were analyzed by Head Space (HS)-Solid-Phase Micro Extraction (SPME)—gas chromatography (GC). Five fresh flowers (7.4 ± 0.1 g, mean ± SE) were used for GC analysis. Each flower was put into a 100 mL glass bottle. The bottle was sealed with plastic wrapping at 40°C. The headspace gas was equilibrated for 20 min prior to analysis. A SPME fiber (65 μL PDMS/DVB; Supelco, USA) was manually inserted into the headspace of the glass bottle for 40 min. After volatile component absorption, the needle of the SPME was inserted into GC.

Hydrosol samples were pretreated as mentioned below; hydrosol (10 mL) was put into 15 mL sample tubes and then hexane (200 μL) and NaCl (4 g) were put into the tubes to extract aroma components into the organic fraction. After vortexing, 1 μL of the organic fraction was injected into GC with a 5.0 μL micro syringe.

Analysis of volatile components in the essential oil was performed with GC and GC-mass spectrometry (MS). A GC2014 (Shimadzu, Japan) equipped with a flame ionization detector (FID) was used for quantitative determination. A GC (6890N; Agilent, USA)-MS (JMS-K9; JEOL, Japan) was equipped with a quadrupole detector and used for qualitative determination. GC systems were equipped with a DB-WAX (100% polyethylene glycol, 60 m × 0.25 mm i.d.; 0.25 μm film thickness) fused silica capillary column (J&W Scientific, USA), and the injector and detector were set at 250°C. The column oven temperature was programmed as follows: after holding at 80°C for 3 min, increased from 80 to 140°C at a rate of 1°C·min⁻¹, and from 140 to 180°C at a rate of 5°C·min⁻¹. Helium gas was used as carrier gas at 300 kPa, in the pressure control mode. A splitless mode was also applied.

The GC-MS system was operated in electron ionization mode at 70 eV. Analysis of the chromatograms was performed in scan mode, from 35 to 350 m/z. The identification of volatile compounds was made by comparison of their retention times and mass spectra with NIST08 library data. Each of the GC analyses was triplicated.

**Sample preparation**

Five samples were prepared as follows: fresh flowers, petals, blind (fresh flower samples with a blind cap made by filter paper), hydrosol (with a blind cap), and pure water (with a blind cap; control). Hydrosol and pure water (100 μL) were absorbed onto a piece of Kimwipes (20 × 10 mm) (Nippon Paper Crecia Co., Japan). Each of the samples was put on a plastic petri dish (60 × 15 mm) with a cotton stick (8 cm) on its side (Fig. 1). Subjects sniffed the samples 8 cm away from the petri dishes, by putting the edge of the cotton stick on their philtrum.

**Subjects of sensory test**

Fifteen healthy non-smoker students (6 males and 9 females) attended the sensory tests. They ranged in age from 20 to 22 years (the average age was 21.2 ± 0.7 years). Their mean body mass index was 20.2 ± 2.3 kg·m⁻². The experimental procedures were explained to the subjects, and informed consent was obtained from all the subjects according to the guidelines.

![Fig. 1. Sample preparation method. (a) Filter paper was put on a petri dish and then samples were put on the filter paper. This was used for evaluation of fresh flower and petal samples. (b) Sample was put on a petri dish and then covered by filter paper. This was used for blind, hydrosol and control (pure water) samples.](image-url)
established by the Declaration of Helsinki. Subjects were inhibited from drinking alcoholic beverages and stimulating food containing capsaicin or garlic and hard exercise on the day before the experiment. Furthermore, subjects were required to have breakfast 2 hours before the experiment and to abstain from food, drink, and exercise until the experiment was completed. All measurements were carried out between 9:00 am to 0:00 pm. The sensory test was conducted as a single-blind test and each of the subjects attended five times to evaluate the samples in randomized order.

Measurement of autonomic nervous system activity

The measurements were carried out in a quiet deodorized room (6 × 9 m²). The room temperature was set at 25°C. Power spectral analysis on R–R intervals was applied to monitor autonomic nervous system activity. This method is generally applied in the evaluation of aromatherapy effects on human (Inoue et al., 2003; Kuroda et al., 2005; Tomi et al., 2011). Active Tracer (GMS, Japan) was used for continuous electrocardiograph (ECG) monitoring. The time course for evaluation of physiological and psychological effects is expressed in Figure 2. The measurements were carried out by recording ECG for 30 min: 10 min for rest time and 20 min for sample inhalation. After 10 min from the beginning of measurements, a sample dish was given to the subjects and they inhaled the volatiles over the measurement time. During the measurement time, subjects answered a 100-cell calculation to avoid the influence of individual mood differences on the results.

R–R intervals were computed from ECG, and the maximum entropy method, a power spectral analysis, was carried out by means of MemCalc software (GMS). To evaluate the autonomic nervous system activity of each subject, a low frequency component (LFC, 0.04–0.15 Hz) and high frequency component (HFC, 0.15–0.40 Hz) were obtained. These two components were used to calculate autonomic nervous system parameters as follows:

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\text{Sympathetic nerve activity (SNA)} = \frac{\text{LFC}}{\text{HFC}}
\]

\[
\text{Parasympathetic nerve activity (PNA)} = \frac{\text{HFC}}{\text{total power spectrum}}
\]

Evaluation of mood states

To evaluate sample psychological effects on humans, the Japanese version of the Profile of Mood State (POMS) test with 65 questions was performed. Each of the questions is rated on a scale of 0–4, ranging from “not at all” to “extremely”. By analyzing the results, human mental conditions can be expressed by six emotional subscales: tension and anxiety (T–A), depression and dejection (D), anger and hostility (A–H), vigor (V), fatigue (F), and confusion (C). Total Mood Disturbance (TMD) scores of POMS were also utilized as an overall indicator of distress change in this measurement. The TMD score was calculated by summing up the five scores of T, D, A, F, and C subscales and subtracting the V score from these scores. The POMS test was performed both before and after the measurement of ECG to estimate the effect of inhalation of samples.

Salivary amylase activity

Salivary amylase activity is known to reflect psychological stress (Yamaguchi et al., 2004), so a salivary amylase monitor (Nipro, Japan) was applied. Salivary amylase activity was measured both before and after the measurement. To collect sublingual saliva, the subjects held a test stick in their mouth for 30 s. The saliva samples were soon analyzed by the salivary amylase monitor following the attached procedure.

Statistical analysis

The effects of aroma inhalation on autonomic nervous system and salivary amylase activity were evaluated by two-way repeated-measures ANOVA. Psychological evaluation by the POMS test was analyzed by the Kruskal-Wallis test. Statistical processing was performed with the SPSS base program (version 20.0; SPSS, USA).

Results

Volatile component composition of fresh rose flowers and hydrosols

Volatile component composition of the samples is summarized in Table 1. The value of each component in Table 1 shows the mean values of the relative peak area (%) against the total area of all component peaks. The major components of fresh flowers appeared to be 3,5-dimethoxy toluene (13.0%) and cis-3-hexenyl acetate (12.5%). Several types of sesquiterpene hydrocarbons (C_{15}H_{24}) were not identified because of the low similarity of their mass spectra between the sample peaks and MS library. The major volatile components of hydrosols were citronellol (34.7%), geraniol (24.1%), phenylethyl acetate (22.7%), and 3,5-dimethoxy toluene (10.5%). Our result showed that the ratio of citronellol was
higher in hydrosols than in fresh flowers. Since the hydrosol volatiles were extracted by an organic solvent in this study, actual volatilized components from the hydrosol may be different from the present results. It was also revealed that fresh flowers had a greater variety of volatile components than hydrosols.

**Physiological evaluation by power spectral analysis**

Fresh flower, petal, blind, hydrosol, and water (control) were applied to the physiological and psychological measurements. Odor intensity of each sample was almost equal among the samples. Autonomic nervous activity data obtained by power spectral analysis on R–R intervals are shown in Figures 3 and 4. Figure 3 shows the time-dependent changes in SNA. The SNA value of the control constantly increased during the test time, although it was expected to be constant. This increase in SNA can be attributed to the stresses caused by the enforced sitting quietly during the test time or the unaccustomed measurement situation. Similar increases in sympathetic nerve parameters have been observed previously (Inoue et al., 2003; Kuroda et al., 2005; Tomi et al., 2011). The effect of rose samples on human SNA varied between the samples (p ≈ 0.001, two-way ANOVA). Although time-dependent changes were seen, no significant interaction was observed among the samples. The SNA value of the control briefly increased between 10 and 25 min. Tukey’s honestly significant difference (HSD) test revealed that the blind sample significantly decreased SNA compared to the control (p ≈ 0.03). In contrast, the SNA mean value of hydrosol was higher than that of the control, but not significantly different. Tukey’s HSD test results showed significant differences between blind and hydrosol (p ≈ 0.001).

Figure 4 shows the PNA parameter. The effect of rose samples on human PNA were also different between the samples (p ≈ 0.001, two-way ANOVA). The PNA value of the control remained constant during the

**Table 1. Volatile component composition of ‘Wishing’ fresh flowers and hydrosols.**

| RT (min) | Fresh flower (%) | Hydrosol (%) | Component               |
|---------|------------------|--------------|-------------------------|
| 7.8     | 6.4              | 6.4          | hexyl acetate           |
| 9.2     | 12.5             | 12.5         | cis-3-hexenyl acetate   |
| 15.1    | 1.4              | 1.4          | C15H24                  |
| 17.0    | 2.7              | 2.7          | C15H24                  |
| 20.0    | 0.8              | 0.8          | C15H24                  |
| 23.9    | 2.5              | 2.5          | C15H24                  |
| 27.2    | 2.3              | 2.3          | C15H24                  |
| 30.7    | 1.5              | 1.5          | C15H24                  |
| 32.9    | 9.4              | 9.4          | C15H24                  |
| 36.5    | 0.3              | 0.3          | carvone                 |
| 37.3    | 9.1              | 9.1          | C15H24                  |
| 41.3    | 4.9              | 4.9          | citronellol             |
| 44.3    | 7.7              | 7.7          | phenylethyl acetate     |
| 46.9    | 13.0             | 13.0         | 3,5-dimethoxy toluene   |
| 49.1    | 4.4              | 4.4          | geraniol                |
| 54.0    | 4.5              | 4.5          | phenylethyl alcohol     |

Total 83.4 96.4

Data were obtained by GC-FID and GC-MS. Components are sorted by Retention Time (RT). Unidentified components were deleted from the list. C15H24 means unidentified but annotated as sesquiterpene hydrocarbons. Each of the values shows the mean value of the relative peak area (%) against the total area of all component peaks. Since the hydrosol volatiles were extracted by an organic solvent in this study, the actual volatilized components from the hydrosol may be different from the present data.

![Fig. 3.](image-url) Effects of rose sample inhalation on the human sympathetic nerve activity parameter. The mean value for 5–10 min was standardized as 100%. Relative values (%) were expressed as the mean line ± SE (n = 15). A significant difference was apparent between each group (p ≈ 0.03: blind sample vs. water (control) sample by two-way repeated-measures ANOVA and Tukey’s HSD test).

![Fig. 4.](image-url) Effects of rose sample inhalation on the parameter of human parasympathetic nerve activity. The mean value for 5–10 min was standardized as 100%. Relative values (%) were expressed as the mean line ± SE (n = 15). A significant difference was apparent between each group (p ≈ 0.02: blind sample vs. water (control) sample by two-way repeated-measures ANOVA and Tukey’s HSD test).
measurement time. Tukey’s HSD test revealed that the blind sample significantly increased PNA compared to the control (p ≈ 0.02) and also showed significant differences between blind and hydrosol (p ≈ 0.001). The mean values of hydrosol were smaller than those of water, but the differences were not significant. There was no significant interaction between the samples either.

Increases in salivary amylase activity (kIU·L\(^{-1}\)) conducted just before and after the measurements are presented in Figure 5. There were no significant differences between the rose samples (one-way ANOVA). The Student’s \(t\)-test was also applied to evaluate the salivary amylase activity difference between before and after the measurements of each sample.

Hydrosol and water samples both showed increased salivary amylase levels, but they were not significant. Only the hydrosol samples increased salivary amylase activity, as shown by the Student’s \(t\)-test (p ≈ 0.09).

**Psychological effects on humans**

Psychological effects of rose samples were examined by POMS test to examine the change in mood state before and after the measurements. Figure 6 shows the difference in POMS score between pre- and post-inhalation, calculated by the equation: (POMS score [post-inhalation] − POMS score [pre-inhalation]). Among the six emotional states, only V scores were significantly different among the samples (p ≈ 0.01, one-way ANOVA). Tukey’s HSD test revealed that the blind sample significantly decreased V compared to the control (p ≈ 0.01). Results of TMD are summarized in Figure 7. Although TMD scores were not significantly different among the samples (p ≈ 0.11, one-way ANOVA), some tendencies were observed such as that the fresh flower, petal, and blind samples decreased TMD and the hydrosol samples increased it.

**Discussion**

**Volatile component compositions of fresh rose flowers and hydrosols**

In this study, we performed volatile component analysis by means of GC and GC-MS. In fresh flower analysis, SPME was applied in order to trap a slight amount of aroma components volatized in the head space. Because preliminary experiments showed that the HS-SPME method did not sufficiently absorb the volatile components of hydrosol, hydrosol analysis was performed using organic solvent extraction combined with salting-out. The disturbance in volatile absorption into SPME may be caused by water vapor, because hydrosol
and without the shape of the rose flower), and blind on humans. These two suggest that fresh flowers and hydrosols have different monoterpene alcohols were known by their sweet, floral, and rose-like notes (Baser et al., 2003; Chen and Viljoen, 2010). These differences in major components of hydrosol appeared to be citronellol (34.7%) and geraniol (24.1%). This result follows a previous study which researched the volatile components in rose flowers, has a sedative effect on humans because a physiological relaxation effect of viewing rose flowers has been reported (Ikei et al., 2013). However, fresh flower and petal samples did not show any significant differences compared to controls in SNA and PNA analysis. These data imply that the sedative effect of the ‘Wishing’ aroma is influenced by visual effects of fresh flower and petal samples. It is reported that pink-colored Petunia hybrida gives an emotional lift (Kim and Fujii, 1995). As ‘Wishing’ flowers have a pale pink color, the sedative effect may be partially neutralized by the visual effect of the ‘Wishing’ flowers.

In hydrosol samples, increases in salivary amylase activity were observed. It has been demonstrated that secretion of salivary amylase is regulated by the sympathetic nervous-adrenomedullary system (Yamaguchi et al., 2004). As there were no significant differences between hydrosol and control samples, the mean value changes in hydrosol samples in SNA and PNA supports the salivary amylase increase.

Physiological and psychological effects of rose samples on humans

Fresh rose flowers have color and shape in addition to their scent. We hypothesized that visual characteristics could alter the effect of rose scent on humans physiologically or psychologically; thus, three rose samples were prepared: fresh flowers (visible), petals (visible, without the shape of the rose flower), and blind (blinded petals using filter paper, in order to remove visual effects). The other samples, hydrosol and pure water (control) were also blinded in order to equalize the condition of the samples.

Analysis of autonomic nervous system activity showed that the blind sample decreased SNA and increased PNA. This shows the sedative effect of rose ‘Wishing’ aroma on humans. In this case, sedative effect means the increase in parasympathetic nerve activity and subsequent alleviation from mental tension (Kaneko and Norimatsu, 2012). The blind sedative effect supports previous studies about rose aroma inhalation effects (Haze et al., 2002).

We had hypothesized that fresh flower and petals have a sedative effect on humans because a physiological relaxation effect of viewing rose flowers has been reported. However, fresh flower and petal samples did not show any significant differences compared to controls in SNA and PNA analysis. These data imply that the sedative effect of the ‘Wishing’ aroma is influenced by visual effects of fresh flower and petal samples. It is reported that pink-colored Petunia hybrida gives an emotional lift (Kim and Fujii, 1995). As ‘Wishing’ flowers have a pale pink color, the sedative effect may be partially neutralized by the visual effect of the ‘Wishing’ flowers.

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Psychological evaluation by POMS showed a decrease in the V value with the control (Fig. 6). This result means that inhalation of a water (control) sample decreased the mental vigor of subjects. In this case, vigor is recognized as a mood of ebullience and high energy (Dyer et al., 2001). It is possible that participation to the measurement itself could make subjects lose vigor. In blind, on the other hand, no decrease in V was observed. This implies that the decrease in mental vigor in the control was eliminated by the rose flower aroma. Similarly, a decrease in TMD was also observed in fresh flowers and petals. TMD is considered to be a single global estimate of affective state (Dyer et al., 2001). These data suggest that aroma inhalation of fresh flowers may alleviate a negative mood state.

Aroma components that affect the physiological and psychological effects on humans

From the evaluation of autonomic nervous system parameters, only the blind sample exhibited sedative effect. It is considered that the difference between these two samples comes from the difference in volatile component composition. For example, previous papers have suggested that β-caryophyllene, one of the characteristic components in rose flowers, has a sedative effect (Galdino et al., 2012). Different aroma components are likely to cause different effects on humans. Furthermore, phenylethyl acetate and 3,5-dimethoxy toluene have been reported to possess sedative effects on humans (Jäger et al., 1992; Tankam and Ito, 2013). These
components are thought to stimulate the autonomic nervous system. On the other hand, hydrosol did not show a sedative effect on humans, despite also containing phenylethyl acetate and 3,5-dimethoxy toluene, as in rose flower samples. The other constituents in hydrosol, citronellol or geraniol, have not reported in terms of their sedative or stimulant effects on humans. It is possible that these components affect the sedative effect. In order to clarify the key component in the rose sedative effect, evaluation of physiological and psychological effects of pure volatile components is necessary in further studies. Furthermore, in our present study, the amounts of volatile components were not quantitatively equalized. It is necessary to evaluate the relationships between the quantitative differences in the volatile components and physiological and psychological effects in further studies. Determining whether the effects of roses on humans are obtained only in ‘Wishing’ or also for other varieties is also needed.

In conclusion, our study indicated that the rose ‘Wishing’ flower scent appeared to possess a sedative effect on humans, as has been reported elsewhere, while hydrosols did not exhibit such effects. These results demonstrate that fresh rose flowers have a sedative effect on humans and can be used as alternative source of essential oil. A new use of fresh rose flowers is proposed for aromatherapy or phytotherapy.

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