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Diurnal fluctuation of volatile compounds emitted from *Four Seasons* Rose (*Rosa damascena* Mill.) cultivated in Beijing

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Summary

Four Seasons Rose is an old variety of *Rosa damascena* Mill. Using the technique of dynamic headspace collection, the volatiles emitted from the flowers of *Four Seasons* Rose were collected at five time points of a day. Then, by the means of thermal-desorption cold trap/gas chromatography/mass spectrometer technique (TCT-GC/MS), constituent compounds of the volatiles were identified. After that, the daily dynamic variation of the floral scents from *Four Seasons* Rose was analyzed. The results demonstrated that the daily fluctuation of volatile components changed in different weather conditions. A total of 93 volatile compounds were identified. There are the most diversity of compounds at 14:00 in a fine day, and the compounds are as many as 78, whereas only 50 of volatile compounds are detected in a rainy day.

Introduction

Rosa damascena Mill., commonly known as Damask rose, is indigenous to Europe and Middle East countries, Iran and Turkey (ALMASIRAD et al., 2007). Some varieties are of great importance for rose oil production. Rose oil is mainly used in the perfumery and cosmetics industry (ARIDOGAN et al., 2002). Besides, it also finds application in the food industry as a flavor additive (MOLLOV et al., 2007; SHIKOV et al., 2012). Because of the low oil content and the lack of natural and synthetic substitutes, rose oil is one of the most expensive essential oils in the global markets (BAYDAR and BAYDAR, 2005). This rose is also used worldwide for manufacture of products with diverse applications such as aroma therapeutic, antibacterial (SEYHAN et al., 2009), antimicrobial (BASIM and BASIM, 2003; OZKAN et al., 2004), antioxidizing (ACHUTHAN et al., 2003), and anti-HIV effects (MAHMOOD et al., 1996). Anyway, Fragrance is one of the most desirable traits of damask rose.

The amount and composition of the rose oil distilled from the rose petals is strongly affected by the genotype, the climatic conditions, the time of rose petals harvesting, and the technology used for processing and distillation (RUSANOV et al., 2009). As far as genotype concerned, several damask rose varieties dominated among oil bearing roses over centuries, due to higher rose oil content and superior essential oil quality. Moreover, being a hybrid, the progeny members of damask rose incline to emitting lower quality and quantity of volatile compounds (MAGALI et al., 2007; KOVACHEVA et al., 2010). So it seems that plantation location and harvesting time probably affect rose oil production. Therefore, being raw material, the flower of oil bearing rose is necessary to be investigated completely so as to clearly understand the changing composition of floral scents emitted from damask rose flowers. In fact, damask rose flower volatiles vary at different stages of flower development, and there is fluctuation of flower volatiles were profiled at different time points during the day (RUSANOVIR et al., 2011). So identifying the constituents of floral volatile and detecting their variations is conducive to tuning of the rose oil composition by precise flower harvesting practices is proposed. However, previous studies on constituents of volatile emissions of damask rose flower focused on the rose oil or flower extraction. Due to the subjects measured indirectly acquired from flower, the measurement results were inevitably affected by processing technology or extraction method, even the storage time.

Here, directly collecting volatile emissions via technique of dynamic headspace collection, we detect the variation of floral volatiles emitted from an old variety of damask, named as *Four Seasons* Rose, investigating variation of floral volatiles in line with flower development stages and daytime periods. Given that samples are intact volatiles emitted directly from rose flowers, we hope the results could provide support for the possible applications of more precise flower harvesting in China, a new industrial plantation base of oil bearing rose.

Material and methods

Plant material

Four Seasons Rose used in this study is an old variety of damask rose, blooming in late spring to early summer, with medium pink and very fragrant flower. The plants were cultivated in botanical trial base affiliated to Chinese Forestry Academy. The experimental site locates in western suburb of Beijing, at an elevation of 150 m, and has a temperate climate.

Volatiles collection

In order to determine the temporal variation of the floral scent over a day, volatiles were sampled from 3 full-blooming flowers (stages 6, as determined by RUSANOV et al. (2012)) of 4 selected plants for 30 min. once every three hours, starting at 5:00 AM and ending at 6:00 PM. There were 5 time points throughout daytime, summing up to 15 samples per plant individual, 60 samples in total. Trials were conducted under different weather including rainy day, cloudy day and sunny day, which were the 11th, 12th and 13th of June 2011.

Volatiles were collected using an improved dynamic headspace air-circulation method (GAO et al., 2005). At first, three well growing flowers borne on the same node of each plant were enclosed in a loosely fitted polyester oven bag (Reynolds Oven Bags, 482 mm× 596 mm, Richmond, VA, USA) , and air was extracted from the bags by vacuum pump (QC-1S Air Sampler, Beijing Municipal Labor Protection Institute, China) at a rate of approximately 100 ml/min. Secondly, Air was pumped (1.1 l/min) through a double filter consisting of charcoal and porous polymer GDX-101 and introduced purified air into the bags, balanced for 30 min. Thirdly, linking a 6mm Dynatherm Centerless Ground SS Sample Tube filled with 200 mg Tenax-TA (mesh 60-80) to a QC-1S Air Sampler, a closed air circulation system was set up. Finally, volatiles were trapped for 30 min via the closed air circulation system at a flow rate of 200 ml/min.

Thermal desorption

Tubes were desorbed using a Perkin Elmer Turbo Matrix 650 automatic thermal desorber (ATD). The ATD was adjusted to the

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mode of 2-Stage desorption. At the first stage, it was programmed to desorb the tubes at 260 °C with a 25 mL/min desorption flow, lasting 10 minutes. The desorbed compounds from the tubes were trapped in the cooled trap of Tenax (set at -25 °C), then quickly heated at 40 °C/s to 300 °C, with a setting time of 5 minutes.

GC-MS analysis

Analyses were carried out using a Perkin Elmer Clarus 600gas chromatograph (GC) coupled to a Perkin Elmer Clarus 600T mass spectrometer. An Elite-5ms Capillary column 30m×0.32mm ×0.25μm was used to analyze the samples. The oven temperature was initially set at 40 °C for 2 min; increased at 6 °C/min to 180 °C, and held for 2min; and increased at 15 °C/min to 270 °C, maintaining at this temperature for 3 min. The carrier gas was nitrogen with a flow rate of 2.0 mL/min. The EI mass spectra of the eluted volatile compounds were obtained with an electron energy of 70 eV over a scan range of 29-500 amu.

Statistics and calculation

Identification of the volatile components was carried out by deconvolution using the AMDIS ver. 2.69 software (National Institute of Standards and Technology (NIST), USA) and the NIST 2008 mass spectral library searched by NIST MS Search v2.0 software. The retention index for individual compound was determined by C10-C40 n-alkanes mixture (Sigma-Aldrich). Using the software TurboMass Ver 5.4.2, the relative contents of each compound were calculated based on total ion current without standardization, by the means of the chromatographic peak area normalization method.

Data analysis was performed using Microsoft Excel (Microsoft Corporation). The relative content of each identified volatile compound was indicated by mean of 4 replicates plus standard error in the Figs. ranging from 4 to 7.

Result and analysis

Total ion current of volatile compounds

The preservation of the traditional odor and composition of the floral volatile is an ultimate prerequisite for production of high quality of rose oil. In order to preserve the authentic scent of the oil rose, monitoring the floral scent from raw material flower is of critical importance. The characteristic fragrance of damask rose results from its specific composition of volatiles and certain proportion between individual constituent compounds, and the volatile emissions change rhythmically under stable environmental conditions. For most roses, their volatile emissions reach summit during their full-blooming stage as the flower development process concerned (Joanne et al., 2004).

In Fig. 1, 2 and 3, GC/MS total ion current of volatile compounds from *four seasons* rose show the relative abundance of emission constituents in different weather conditions. Among them, the 5 graphs ranging from A to E represent profiles of emission constituents at different time points including 5:00 am, 8:00 am, 11:00 am, 2:00 pm and 5:00 pm, in a rainy day; from F to J, the graphs stand for the dynamics emission constituents at the same time points as above in a cloudy day; and the 5 graphs marked K, L, M, N and O, indicate the dynamics emission constituents at the same five time points in a sunny day.

The analysis of chromatograms of volatile components and corresponding relative abundance at the five time points is based on net values after rejecting control air background. A total of 93 volatiles compounds were identified in daily fluctuation, in which alkanes, terpenes and aldehydes compounds are the major components. Moreover, under different weather conditions, volatile composition are significant different. On the fine day, as many as 78 compounds are detected at 11:00 am, while only 50 compounds detected at the same time point on a cloudy day.

Diurnal dynamics of alcohols among volatile emissions

Alcohols among volatile emissions from *four seasons* rose mainly include 2-ethylhexyl alcohol, citronellol and phenylethyl alcohol, in addition, composition and amount of these group of compounds exhibit diurnal fluctuation (Fig. 4).

As showed in Fig. 4, on a rainy day, total alcohols sharply increase in the morning, then remaining stable at noon, after that, there is a rapid rise in the afternoon; there is a small amount of citronellol at noon, and its relative content is just 0.227%. On a cloudy day, total alcohols grow swiftly in early morning, but subsequently fall down until noon, and seeing a steady soaring after noon; as for citronellol, there is a continued rise from early morning, and reaching summit at 3:00 pm, with maximal relative content of 0.741%, there followed a decline. On a sunny day, total alcohols increase rapidly from early morning, and peaking at 2:00 pm, then plunging dramatically; while citronellol rise in the morning, and reaching the peak at 9:00 am, with relative content of 0.89%. Citronellol is an important contributor of pleasant floral odor of valuable, high-priced rose oils (Jirovetz et al., 2005), and is highlighted in the International Rose Oil Standard (ISO 9842, 2003). On a sunny morning, harvesting of *four seasons* rose flower for production of rose oil probably obtains a high yielding of citronellol. So the result supports the traditional harvesting practices of rose flowers prevailing among the dominant rose oil producers in Bulgaria (Rusanovir et al., 2011).

As regard to Benzenethanol, also known as phenylethyl alcohol, its aroma is described as floral, rose-like, honey notes. On a rainy day, it increases moderately in the morning and then undergoing a fast rise after 11:00 am, however, dropping substantially after 2:00 pm; on a cloudy day, Benzenethanol increases slightly, and seeing a quick rise after 3:00 pm; on a sunny day, there is a rapid increase topping at 4.572% before noon, and followed by a fast decrease afterwards.

Diurnal dynamics of terpenes among volatile emissions

Based on analysis of chromatograms in Fig. 1, 2 and 3, it is found that the main terpene compounds are α-pinene, β-myrcene and D-limonene from fresh flowers of *four seasons rose,* and the terpenes fluctuate in daily rhythm. Fig. 5 shows terpenes always grow first and then decreasing no matter what weather condition. But there is slightly difference in changing rhythms of terpenes under different weather conditions. The high peak appears at 9:00 am in rainy day, while at noon in cloudy day or sunny day. In the rainy day, cloudy day and sunny day, relative content of total terpenes reaches 40.511%, 45.491% and 16.389%, respectively. The obvious different from the other compounds is that the maximal emission occurred when sunlight is not very intensive.

Among terpenes of the volatiles, α-Pinene, β-Myrcene and D-Limonene are the major component compounds. Their daily changing trends are different from one another. In Fig. 5, the maximal level of α-Pinene was detected at the beginning of the diurnal period, and its relative abundance is as high as 10% in a rainy day, while the relative abundance remains on a low stable level on fine days. In the contrast, the fluctuation of β-Myrcene relative content is consistent in three different weather conditions. β-Myrcene firstly rises and then drops, peaking at noon. The maximal abundance reaches 12.201%, 17.978% and 4.887% in rainy day, cloudy day and sunny day, respectively.

Several minor terpenes were detected, and their amounts were very small. In Fig. 6, the relative contents of three terpenes are less than 5%. But they probably play an important role in characteristic aroma.

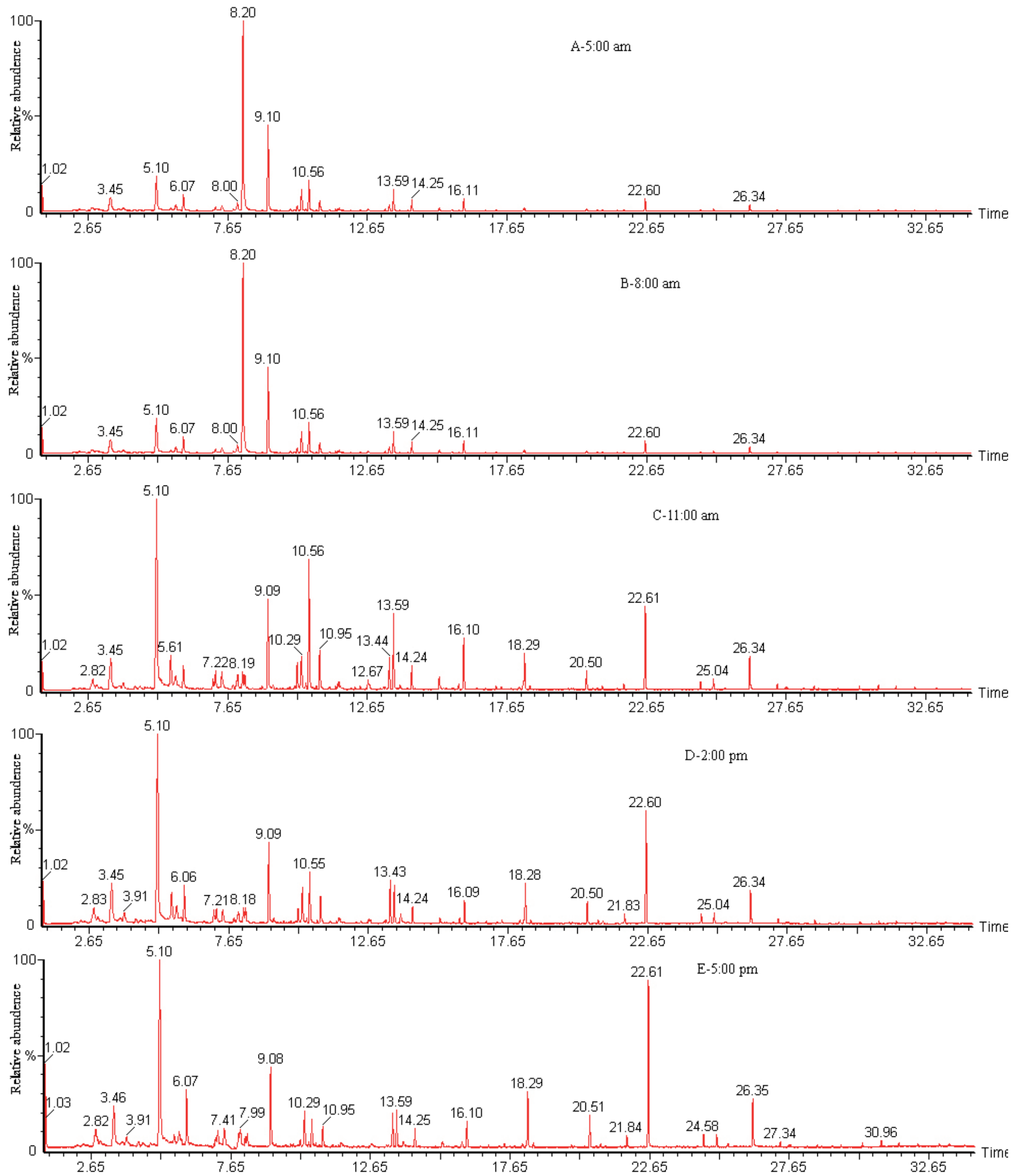

Fig. 1: GC/MS total ion current chromatograms of volatile compounds from four seasons rose in rainy

Sabinene is a natural bicyclic monoterpene with weak turpentine-like, spicy, warm-woody aroma. It usually increases in the morning, reaching summit at noon, and declines in the afternoon.

Beyond expectation, the familiar major monoterpenols such as nerol and geraniol (RUSANOVIR et al., 2011; JIROVETZ et al., 2005) were not detected in the flower volatiles of four seasons rose. But the oxidised monoterpenols including nerol acetate and geraniol acetate were detected on the sunny day (Fig. 3). In addition, relative abundance of 0.588%, the maximum of nerol acetate was emitted at 8:00 am only on the sunny day, whereas geraniol acetate peaking at 2:00 pm, with maximal relative abundance of 0.290%.

Diurnal dynamics of alkanes among volatile emissions

The three major hydrocarbons also show diverse accumulation patterns. While, the maximal level of pentadecane accumulation was detected at the end of the diurnal period in different weather conditions, the trace amount of heptadecane is detected only in the early morning of a rainy day. heneicosane increases moderately after noon, but it firstly declines in the morning of cloudy or sunny days. In terms of relative content in total of volatile compounds, the alkanes of flower volatile accumulation levels for the time points 2:00 p.m. and 6:00 p.m., which are out of the traditional daytime flower harvesting period are higher than those of the 5:00 a.m. to 12:00 p.m. period. Given that good quality rose oil should possess a higher amount of monoterpene alcohols and a lower amount of alkanes (BASER, 1992). So the results in this research support the rationality of the traditional rose harvesting, which is carried out in the morning.

Besides, this assay is also identified the ketones, esters, the phenyl/Phenylpropanes and acids, etc. The benzene compounds including ethylbenzene and o-xylene present higher level in a cloudy day, and the maximum is 8.298% and 11.781% at noon, respectively, while the lowest level occurred on a fine day. The amounts and components of esters were little in different weather conditions. The highest content was found at 9:00am (1.099%) on fine days as compared to

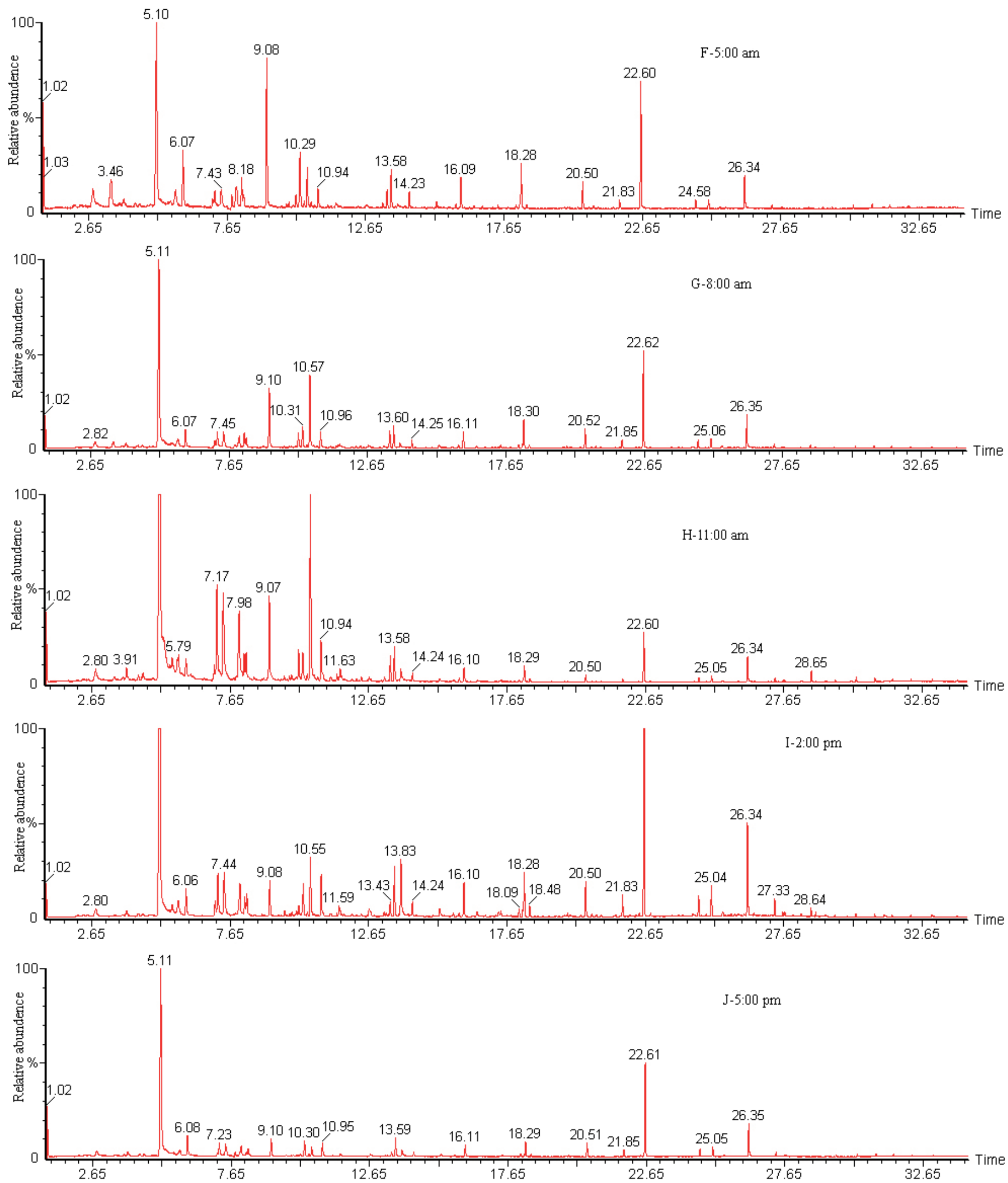

Fig. 2: GC/MS total ion current chromatograms of volatile compounds from four seasons rose in cloudy day

other time. There are five compounds of ketone could be detected, only 6-methyl-5-heptene-2-ketone can be measured at every time point. The other components appeared in some specific individual time points, for instance, 3,5,5-trimethyl-2-cyclohexene-1-ketone and cyclopentanone only can be found at 8:00-9:00 am and 9:00-12:00, respectively.

Discussion

The study of volatile compounds of *Rosa damascene* showed that volatiles were emitted rhythmically, with maximum peaks coinciding with the time period after 8-10h of illumination (JOANNE et al., 2004), or even to the time period after 6-9h of illumination (HELSPER et al., 1998).The fluctuation exists not only in relative contents but in component compounds. Duo to the surroundings damask rose located in changeable, the volatile components incline to varying accordingly. The aromatic components released from *four seasons* are most abundant at 11:00-12:00 am on sunny day. The reason probably is intensive light and high temperature, which make the volatile easier to emit. The volatile emission of many plants has a relation to light and temperature. In cold experiment, for example, orchid aroma is very little even no fragrance can be detected, while the same orchid would release a big amount of floral scent in sunny days (WILLIAMS and WHITTEN, 1983). The emission of aroma from *Trifolium repens* L. in 20 °C is 54% higher than in 10°C (JAKOBSEN and OLSEN, 1994). Anyway, the diurnal regulation of scent emission is complex (HENDEL et al., 2007). Although the daily emission of most scent compounds is synchronized, various independently evolved mechanisms control the production, accumulation and

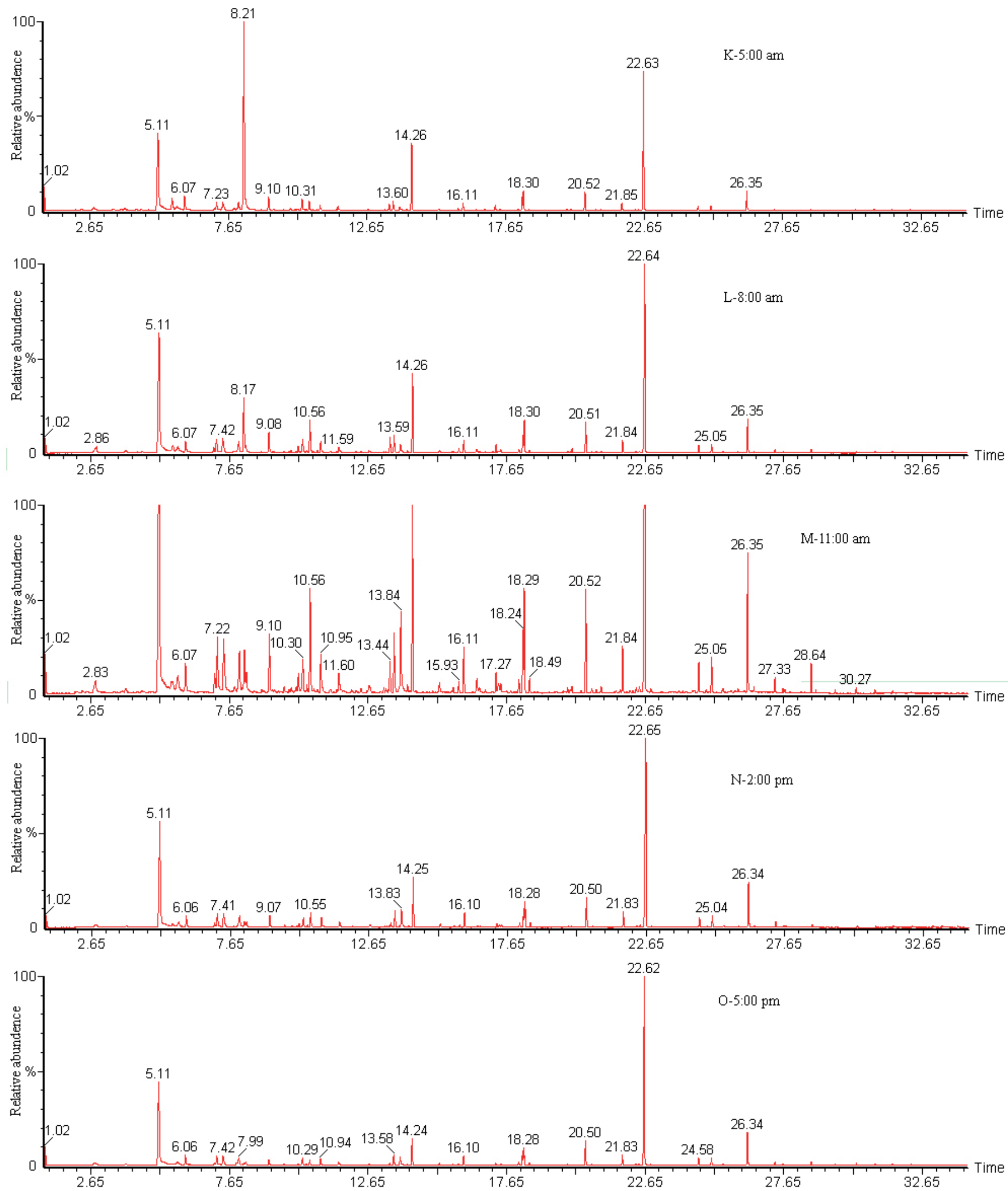

Fig. 3: GC/MS total ion current chromatograms of volatile compounds from four seasons rose in sunny day

release of divergent volatiles. It is noticed that there are not geraniol and nerol detected in this research, which is abnormal to some certain compared to previous studies (Rusanovir et al., 2011; Jirovetz et al., 2005). The reason may lie in the improper flower developmental stage for sampling of volatiles from the damask rose flower or inappropriate environmental conditions.

Conclusion

Fluctuation of flower volatiles at different time points during a day is investigated in this research. The aromatic components released from four seasons are most abundant at 11:00-12:00 am on sunny days. The daily fluctuation rhythm of volatile component compounds is different in varying weather, for instance, the relative content of terpene compounds is higher in rainy day than in a fine day. Above all, it is concluded that every individual compound possibly has its own daily fluctuation rhythm in accordance with weather conditions. The results in this research suggest that there are obvious variations among different time points of flower sampling, which could provide more delicate guide for the harvesting oil bearing rose.

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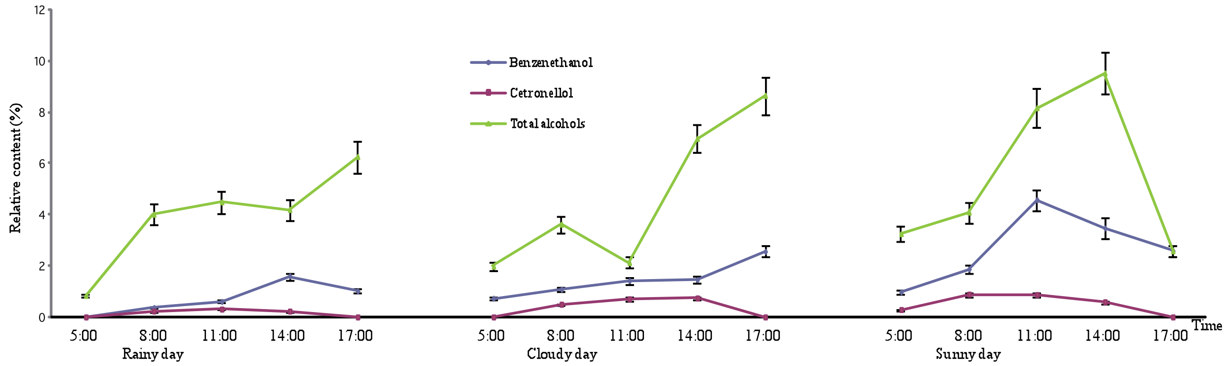

Fig. 4: Diurnal dynamics of alcohols in volatile emissions from flowers of four seasons rose

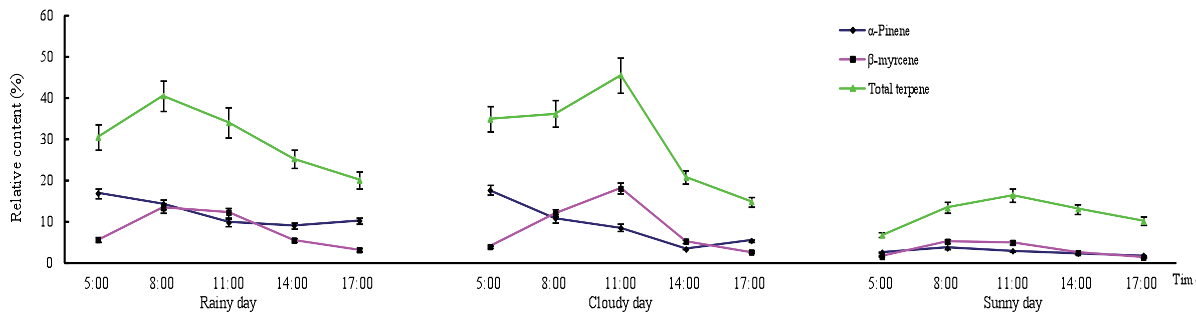

Fig. 5: Diurnal dynamics of terpenes in volatile emissions from flowers of four seasons rose

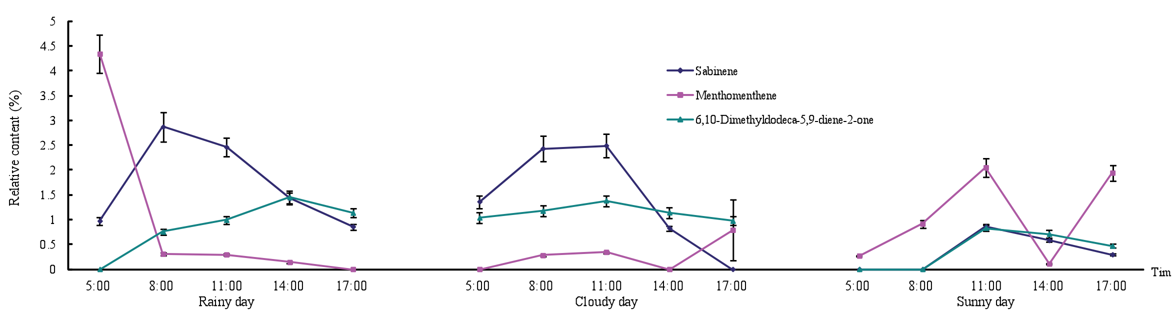

Fig. 6: Diurnal dynamics of three minor terpenes in volatile emissions from flowers of four seasons rose

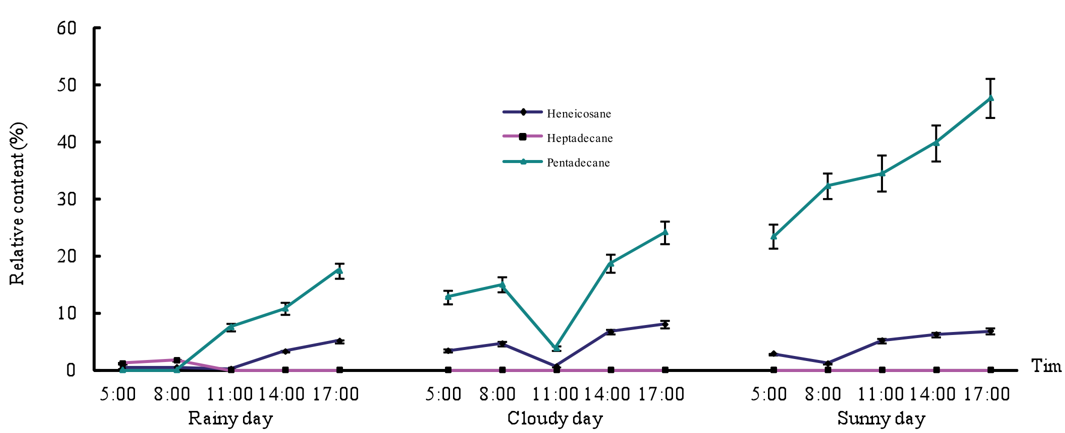

Fig. 7: Diurnal dynamics of alkanes in volatile emissions from flowers of four seasons rose

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