Headspace Solid-Phase Microextraction Analysis of Volatile Components in *Narcissus tazetta* var. *chinensis* Roem

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Received: 9 October 2013; in revised form: 28 October 2013 / Accepted: 1 November 2013 / Published: 6 November 2013

**Abstract:** The volatile components in single-flowered and double-flowered Chinese narcissus were identified by headspace-solid phase microextraction (HS-SPME) coupled with GC and GC/MS. Changes in aroma during the vase-life (days 0, 1, 2, 3, 4, 5 and 6) of two samples were also studied. A total of 35 compounds were identified, of which all were present in single-flowered and 26 in double-flowered samples. The main aroma components were (E)-β-ocimene, and benzyl acetate. Single-flowered narcissus have a higher percentage of benzyl acetate, while double-flowered narcissus have a higher percentage of 1,8-cineole. In vase-life, the total volatile component content peaked on day 2 for single-flowered and day 3 for the double-flowered narcissus. For both single-flowered and double-flowered narcissus flowers, the total content of volatile components had decreased significantly by day 4.

**Keywords:** Chinese narcissus; *Narcissus tazetta* var. *chinensis* Roem; HS-SPME; volatile compound
1. Introduction

Chinese narcissus (*Narcissus tazetta* var. *chinensis* Roem) is a member of the Amaryllidaceae family and the Narcissus genus. It is a monocot plant whose flowers develop at high temperatures and bloom at lower temperatures. Featured during the Chinese Spring Festival, Chinese narcissus is a traditional and well-known Chinese flower with high economic and ornamental value [1]. It is a popular ornamental flower worldwide, especially in China and Taiwan, and narcissuses are widely cultivated in southern China [2]. Narcissus has a strong fragrance [3], that is highly valued in the fragrance industry [4].

Table 1. Comparisons of major volatile compounds reported in *Narcissus* flowers in published papers.

| Plant species/variety          | Major compound                                                                 | Methods of testing/extraction                  | Ref.   |
|--------------------------------|--------------------------------------------------------------------------------|------------------------------------------------|--------|
| *Narcissus poeticus* L.        | α-terpineol, methyl-(E)-isoeugenol, methyl-(E)-isoeugenol, and benzyl benzoate | headspace                                      | [3]    |
| *N. trevithian* and *N. geranium* | Authors did not present any quantitative data on the individual compounds. However, they reported *N. trevithian* contains more phenolic compounds and fewer esters compared to *N. geranium* | high vacuum distillation                       | [4]    |
| *N. tazetta* var. *chinensis*  | benzyl alcohol, α-terpineol, γ-phenylpropyl alcohol, 1,8-cineole, benzyl acetate, and linalool | hydrodistillation                              | [5]    |
| *N. tazetta* florepleno        | benzyl acetate, methyl anthranilate, benzyl alcohol, and linalool              | hydrodistillation                              | [6]    |
| Narcissus, two cultivars       | benzyl acetate, benzyl alcohol, linalool, and indole                           | hydrodistillation                              | [7]    |
| Fresh narcissus flowers        | benzyl alcohol , and α-terpineol                                               | headspace                                      | [8]    |
| Narcissus flowers              | (E)-β-ocimene and α-terpineol                                                   | Likens-Nickerson extraction                   | [9]    |
| Narcissus flowers              | benzyl acetate, (E)-β-ocimene, 3,4-dimethoxytoluene, and 3,5-dimethoxytoluene   | headspace                                      | [10]   |
| Zhangzhou narcissus flowers    | benzyl acetate, benzyl alcohol, indole, 3,7-dimethyl-1,6-octadien-3-ol, and p-mentha-1,8-dien-4-yl acetate | hydrodistillation                              | [11]   |
| *N. tazetta* var. *chinensis*  | benzyl acetate, linalool, and 1,8-cineole                                       | hydrodistillation                              | [12]   |
| *N. tazetta* and               | γ-terpinene for *N. tazetta*                                                    | lab-prepared absolutes                        | [13]   |
| *N. tazetta* subsp. tazetta    | γ-terpinene, linalool, and benzyl acetate for *N. tazetta* subsp. tazetta      |                                                |        |
| *N. tazetta* var. *chinensis*  | benzyl acetate and (E)-β-ocimene                                               | headspace and simultaneous distillation        | [14]   |
| *N. tazetta* and *N. serotinus*| trans-ocimene for *N. tazetta*                                                 | hydrodistillation                              | [15]   |
| *N. poeticus* L.               | cinnamy alcohol, methyl isoeugenol, isoeugenol, methyl eugenol, α-terpinol, and phenyl propyl alcohol | hexane and supercritical CO₂ extraction       | [16]   |
| *N. pseudonarcissus* L.        | (E)-β-ocimene                                                                  | headspace                                      | [17]   |
Several studies on the volatile compounds of *Narcissus spp.* have been performed. Table 1 summarizes plant species/variety, method of testing/extraction, major volatile compounds and reference number in the published papers.

It was also recently confirmed that the use of headspace analysis provides a more natural profile that studies using hydrodistillation of plant volatiles [18]. SPME allows the sampling of the volatiles emitted by living plants in a fast and easy way [19], allowing the volatile compounds present in the headspace of odoriferous flowers in different flowering to be studied, including compounds that may not be detectable by conventional methods, such as solvent extractions, and hydrodistillation, but can be detected using HS-SPME [20].

Many previous studies have investigated the aroma of Chinese narcissus flowers, but few of them have discussed changes in narcissus flower aroma during the vase period. The aim of this work was to utilize HS-SPME method to investigate the volatile components and to understand the changes in these aromas during various stages of the vase period of fresh Chinese narcissus flowers.

2. Results and Discussion

2.1. Analysis of the Volatiles in Fresh Single- and Double-Flowered Chinese Narcissus Flowers

The volatile compounds in the narcissus flowers were analyzed by headspace solid-phase microextraction coupled with GC and with GC-MS. Tables 2, 3, and 4 show the 35 components that were identified in single-flowered samples, including 13 monoterpenes, three terpene alcohols, one terpene aldehyde, four terpene esters, one terpene oxide, one aromatic aldehyde, one aromatic alcohol, four aromatic esters, one sesquiterpene, three aliphatic esters, two hydrocarbons, and one other compound. A total of 26 components were identified in double-flowered samples, including 13 monoterpenes, three terpene alcohols, one terpene oxide, one aromatic alcohol, three aromatic esters, two aliphatic esters, two hydrocarbons, and one other compound. The main constituents of the Chinese narcissus flowers were \((E)-\beta\text{-ocimene (62.73}–66.06\text{%})\), benzyl acetate (11.65–25.02%), \((Z)-\beta\text{-ocimene, 1,8-cineole, and linalool.}

In a study by Sakai *et al.* [5], \((Z)-\beta\text{-ocimene was not detected. However, in a later study by Sakai [7], this component was detected. In a study by Surburg *et al.* [17], different headspace methods were used to analyze the aroma of *Narcissus pseudonarcissus* L. The main component was \((E)-\beta\text{-ocimene; a contribution of 75% was detected by the dynamic headspace method, while 30.1% was detected by the vacuum headspace method. In a study by Arai [14], the headspace method was used to analyze *Narcissus tazetta* var. *chinensis*, and a 51.19% contribution of \((E)-\beta\text{-ocimene was detected. In this study, we used HS-SPME for analysis and found 62.73 and 66.06% contributions of \((E)-\beta\text{-ocimene for the two samples used in this study. However, as shown in Table 1, no form of \(\beta\text{-ocimene was reported as one of the major volatile components in distilled samples, therefore we postulate that heating during distillation decreased the content of this compound that could be detected. In addition, we found that single-flowered narcissus are rich in esters, including benzyl acetate, phenethyl acetate, isoamyl acetate, prenyl acetate, and 3-hexyl acetate. Among these ester components, the benzyl acetate content was the highest. Benzyl acetate was also a major component of narcissus flower aroma [3–7,10–14]. As shown in Table 1, benzyl acetate was reported as the first major volatile component in several
In studies, probably because of the low content of ocimene. In this study, the content of benzyl acetate was 25.02%, next to (E)-β-ocimene (67.02%). Volatile compound isolation methods by headspace or by distillation gave different data from this comparison. It was confirmed again that the use of headspace analysis provides a more natural profile than studies using hydrodistillation of plant volatiles [17].

In a study by Van Dort et al. [4] prenyl acetate was detected as a minor component. Regarding the terpene alcohols, linalool, and α-terpineol were detected. Among these compounds, linalool is the major contributor to the aroma [5–7,10,12–14]. α-Terpineol was reported as a major aromatic component of narcissus flowers in studies by Joulain [8], Loo and Richard [9], and Ehret et al. [3]. Indole is known as being very important in floral odors [4]. Although the indole content is low, its aroma threshold is low; thus, a pleasing and strong fragrance is emitted, even at low concentration.

Fresh Chinese Narcissus flowers were picked and immediately analyzed and could therefore be considered as fresh at the time of SPME. As for aliphatic hydrocarbons, pentadecane was not detected by Sakai et al. [5] or Sakai [7], but this component was detected by Loo et al. [9]. The presence of n-alkanes, as a biomarker of fresh flowers, is ascribed to the Narcissus tazetta var. chinensis Roem flowers, and not contamination. Li et al. [20] and Shang et al. [21] also cited n-alkanes as a biomarker of living flowers for Michelia alba and Syring oblate flowers. In addition, sesquiterpene-type components are almost undetectable, which may be due to the poor absorption of sesquiterpene components by SPME [22,23]. To summarize, the main components of the floral scent of narcissus flowers were (E)-β-ocimene, benzyl acetate, linalool, and indole. Among these components, benzyl acetate and (E)-β-ocimene have floral aromas [24].

### 2.2. Volatile Compounds over the Vase-Life of Narcissus tazetta var. chinensis Roem

Table 3 shows the aroma constituents of Narcissus tazetta var. chinensis Roem at different flowering stages (flower buds, day 0; early flower blooming, day 1; flower blooming, day 2–3, late flower blooming, day 4; senescence, day 5–6 as analyzed by GC and GC/MS. All samples were placed in a plant tissue culture laboratory with a controllable environment, and the room temperature was set at 25 °C. The vase life was 6 days. Every morning, the SPME method was used to extract aroma for analysis. The GC method was used for analysis and to compare the peak area content. As shown in Table 2, a total of 35 volatile compounds were identified for the single-flowered samples, for which the volatile components content peaked on day 2, decreasing significantly by day 3, and being lowest at day 6. Among these main components, (E)-β-cimene, prenyl acetate, and benzyl acetate have the highest aroma content on day 2, with contents that decreased significantly thereafter. Among the monoterpane components, α-pinene, sabinene, β-myrcene and γ-terpinene have the highest content on day 0, decreasing with time thereafter. 6-Methyl-5-hepten-2-one was undetected on days 0-5, but it was detected in trace amounts on day 5 and peaked on day 6. The odor of 6-methyl-5-hepten-2-one is metallic and wet-rubber-like as described by Chen et al. [24]. The compound was a speculated off-odor of the Narcissus tazetta var. chinensis Roem. A total of 25 volatile compounds were identified for the double-flowered samples, and the content of their volatile components peaked on day 3 of the vase period. Among these major components, the aroma contents of (Z)-β-ocimene, (E)-β-ocimene, benzyl acetate, and linalool peaked on day 3, decreasing significantly by day 4. Oyama-Okubo et al. [25]
analyzed of the major scent compounds in cut flowers of ‘Casa Blanca’ lilies reported that total emissions of scent compounds peaked on the third day and then decreased.

Table 2. Volatile compounds of fresh flowers of *Narcissus tazetta* var. *chinensis* Roem.

| Compound                      | RI x | RI y | Content (%) | Single-flowered | Double-flowered |
|-------------------------------|------|------|-------------|-----------------|-----------------|
| Monoterpenes                  |      |      |             |                 |                 |
| α-Pinene                      | 936  | 941  | 0.10 ± 0.01 | 0.31 ± 0.05     |                 |
| Sabinenne                     | 973  | 967  | 0.01 ± 0.00 | 0.15 ± 0.05     |                 |
| β-Pinene                      | 978  | 971  | 0.02 ± 0.01 | 0.12 ± 0.06     |                 |
| Myrcene                       | 987  | 983  | 0.61 ± 0.05 | 1.22 ± 0.09     |                 |
| α-Phellandrene                | 1002 | 1002 | <0.01       | <0.01           |                 |
| δ-3-Carene                    | 1010 | 1004 | 0.05 ± 0.02 | 0.40 ± 0.15     |                 |
| α-Terpineole                  | 1013 | 1011 | 0.05 ± 0.01 | 0.08 ± 0.01     |                 |
| Limonene                      | 1025 | 1030 | <0.01       | <0.01           |                 |
| (Z)-β-Ocimene                 | 1029 | 1037 | 1.80 ± 0.49 | 4.64 ± 0.78     |                 |
| (E)-β-Ocimene                 | 1041 | 1040 | 62.73 ± 6.04 | 66.06 ± 11.01  |                 |
| γ-Terpineole                  | 1051 | 1051 | 0.42 ± 0.11 | 0.27 ± 0.08     |                 |
| α-Terpinolene                 | 1082 | 1085 | 0.04 ± 0.01 | 0.12 ± 0.03     |                 |
| *allo*-Ocimene                | 1113 | 1116 | 1.21 ± 0.07 | 1.22 ± 0.48     |                 |
| Monoterpene alcohols          |      |      |             |                 |                 |
| Linalool                      | 1086 | 1087 | 1.14 ± 0.59 | 1.71 ± 0.35     |                 |
| α-Terpineol                   | 1176 | 1174 | 0.28 ± 0.02 | 0.06 ± 0.02     |                 |
| Myrtenol                      | 1178 | 1176 | 0.04 ± 0.00 | 0.04 ± 0.01     |                 |
| Monoterpene aldehyde          |      |      |             |                 |                 |
| Citronellal                   | 1129 | 1129 | <0.01       |                 |                 |
| Monoterpene esters            |      |      |             |                 |                 |
| Neryl acetate                 | 1342 | 1345 | <0.01       |                 |                 |
| Geranyl acetate               | 1362 | 1362 | <0.01       |                 |                 |
| Methyl cinnamate              | 1354 | 1373 | 0.10 ± 0.01 |                 |                 |
| Cinnamyl acetate              | 1420 | 1422 | 0.02 ± 0.01 |                 |                 |
| Monoterpene oxide             |      |      |             |                 |                 |
| 1,8-Cineole                   | 1025 | 1025 | 1.49 ± 0.50 | 4.05 ± 0.35     |                 |
| Aromatic aldehyde             |      |      |             |                 |                 |
| Benzaldehyde                  | 964  | 964  | <0.01       |                 |                 |
| Aromatic alcohol              |      |      |             |                 |                 |
| Benzyl alcohol                | 1006 | 1032 | 0.06 ± 0.01 | 0.01 ± 0.00     |                 |
| Aromatic esters               |      |      |             |                 |                 |
| Benzyl acetate                | 1134 | 1165 | 25.02 ± 5.66 | 11.65 ± 4.22   |                 |
| Phenethyl acetate             | 1230 | 1269 | 1.12 ± 0.16 | 0.38 ± 0.08     |                 |
| 3-Phenylpropyl acetate        | 1335 | 1357 | 0.07 ± 0.03 | 0.51 ± 0.04     |                 |
| Benzyl benzoate               | 1730 | 1769 | 0.17 ± 0.02 |                 |                 |
| Sesquiterpene                 |      |      |             |                 |                 |
| β-Caryophyllene               | 1431 | 1430 | 0.04 ± 0.02 |                 |                 |
| Aliphatic esters              |      |      |             |                 |                 |
| Isoamyl acetate               | 893  | 886  | 0.06 ± 0.01 | 0.03 ± 0.01     |                 |
| Prenyl acetate                | 979  | 906  | 1.26 ± 0.29 | 2.18 ± 0.38     |                 |
| 3-Hexenyl acetate             | 985  | 985  | 0.13 ± 0.03 |                 |                 |
| Hydrocarbons                  |      |      |             |                 |                 |
| Pentadecane                   | 1500 | 1500 | 0.23 ± 0.09 | 0.02 ± 0.01     |                 |
| Eicosane                      | 2000 | 1983 | 0.08 ± 0.03 | 0.06 ± 0.03     |                 |
| Other                         |      |      |             |                 |                 |
| Indole                        | 1257 | 1295 | 0.33 ± 0.11 | 0.26 ± 0.04     |                 |

* Literature Retention indices obtained from [26–28] and reference were checked for all compounds on DB-1 column; * Retention indices obtained using series of *n*-alkanes (C₅-C₂₅) on DB-1 column; * Values are means ± SD of six replicates.
| Compound                  | RI ¹ | Single-flowered (peak areas) ² | Double-flowered (peak areas) |
|--------------------------|------|-------------------------------|-----------------------------|
|                          |      | 0  | 1  | 2  | 3  | 4  | 5  | 6  | 0  | 1  | 2  | 3  | 4  | 5  | 6  |
| Monoterpenes             |      |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| α-Pinene                 | 941  | 8.99bc | 4.82d | 4.28de | 2.01e | 11.28bc | 13.23b | 17.30a | 18.19 a | 9.65bc | 9.50bc |
| Sabinene                 | 967  | 1.30d  | 0.69e  | 0.10f  | 5.17a  | 1.82c  | 2.61b  | 1.70c  |
| β-Pinene                 | 976  | 1.21c  | 0.84e  | 0.73c  | 3.54b  | 2.03c  | 9.48a  | 1.87c  |
| Myrcene                  | 983  | 30.88d | 22.61e | 25.15d | 8.82fg | 0.51g  | 29.16d | 46.25c | 68.39b | 107.22a | 25.84d | 16.46ef | 13.34f |
| α-Phellandrene           | 1002 | <0.10  |        |        |        |        |        |        |        |
| δ-3-Carene               | 1004 | 2.43f  | 1.91f  | 1.90f  | 1.32f  | 1.11f  | 10.19e | 20.35b | 36.72a | 21.80b | 18.30c | 17.10c | 13.30d |
| α-Terpinepine            | 1011 | 1.00cde | 1.99b  | 2.74a  |        |        | 1.23cd | 0.91e  | 0.89e  | 1.26c  | 1.27c  | 0.98de | 0.42f  |
| Limonene                 | 1030 | <0.10  |        |        |        |        |        |        |        |
| (Z)-β-ocimene            | 1037 | 43.81de | 139.76c | 144.4c | 64.98d | 9.12fg | 2.81g  | 1.83g  | 28.70efg | 137.02c | 221.85b | 698.81a | 237.87b | 72.20d | 35.13ef |
| (E)-β-ocimene            | 1040 | 792.65ef | 2271.78d | 2299.77d | 645.21f | 27.79h | 4.64h  | 2.24h  | 311.77g | 2694.95c | 3147.59b | 9914.71a | 3371.10b | 1024.45e | 659.79f |
| γ-Terpinene              | 1051 | 18.09c | 9.50d  | 7.47d  | 4.20e  | 3.89e  | 1.48e  | 1.39e  | 8.72d  | 16.68c  | 18.88bc | 21.77ab | 22.62a  | 21.67ab | 18.75c |
| α-Terpinolene            | 1085 | 2.41e  | 2.01e  | 1.76f  |        |        | 2.24e  | 3.23d  | 4.33c  | 8.31a  | 5.83b  |
| Allo-ocimene             | 1116 | 37.71f | 44.83e | 49.57d | 15.48h | 1.28j  | 7.87i  | 48.03de | 58.36c | 184.16a | 70.96b | 26.29g | 16.65h |
| Monoterpene alcohols     |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Linalool                 | 1087 | 58.85g | 112.42f | 109.84f | 51.48g | 20.99i | 17.78i | 1.21j  | 21.43i | 147.11d | 188.91c | 280.72a | 243.05b | 159.81d | 32.88h |
| α-Terpineol              | 1174 | 12.11b | 15.21a | 3.61c  | 3.06c  |
| Myrtenol                 | 1176 | 1.34g  | 1.80d  | 2.03b  | 1.45f  | 1.62e  | 1.95bc | 3.48a  | 1.90c  |
| Monoterpene ketone       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 6-Methyl-5-hepten-2-one  | 965  | 0.10b  | 11.10a  |      |      |      |      |      |      |      |      |      |      |      |      |
| Monoterpene esters       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Geranyl acetate          | 1362 | 4.49a  | 3.01b  | 2.10c  |
| Methyl cinnamate         | 1373 | 3.70e  | 3.76de | 4.71cd | 4.43cd | 5.23c  | 4.22de | 3.72de | 11.06a | 9.56b  |
| Cinnamyl acetate         | 1422 | 3.20c  | 6.08a  | 4.86b  |
| Monoterpene oxide        |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 1,8-Cineole              | 1025 | 143.23d | 158.73d | 144.65d | 64.14g | 11.56h | 2.76h  | 1.45h  | 85.13f | 222.04e | 268.56b | 450.91a | 271.20b | 122.62e | 65.85fg |
| Compound                  | RI  | Single-flowered (peak areas) | Double-flowered (peak areas) |
|--------------------------|-----|------------------------------|------------------------------|
|                          |     | 0 | 1  | 2  | 3  | 4  | 5  | 6  | 0  | 1  | 2  | 3  | 4  | 5  | 6  |
| Aromatic alcohol         |     |   |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Benzyl alcohol           | 1032| 1.40b | 1.82a | 1.51b |     |    |    |    | 0.72d |    |    |
| Aromatic esters          |     |   |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Benzyl acetate           | 1165| 675.59e | 1324.56b | 1390.43b | 119.50hi | 20.52j | 7.10j | 4.47j | 186.44gh | 927.25d | 1023.59c | 1598.03a | 568.64f | 256.70g | 99.44i |
| Phenethyl acetate        | 1169| 15.59g | 24.91e | 18.12fg | 6.90h | 2.78i | 1.78i |     | 46.14b | 32.15d | 20.86f | 53.12a | 38.77c | 14.91g | 7.81h |
| 3-Phenylpropyl acetate   | 1357|     |     |     |     |     |     |     |     | 6.43d | 12.02c | 63.45a | 34.34b | 6.37d |     |
| Benzyl benzoate          | 1769|     |     |     |     |     |     |     |     | 5.31a | 3.79b | 1.79c |     |     |     |
| Sesquiterpene            |     |   |    |    |    |    |    |    |    |    |    |    |    |    |    |
| β-caryophyllene          | 1430|     | 8.15a | 2.56c | 2.50c |     | 4.32b | 4.22b |     |     |     |     |     |     |     |
| Aliphatic esters         |     |   |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Isoamyl acetate          | 886 | 2.41g | 2.94g | 3.10g | 11.15bc | 8.13d | 11.98b | 6.10e | 3.69g | 4.15fg | 5.80ef | 5.85ef | 9.64cd | 17.54a | 17.59a |
| Prenyl acetate           | 906 | 50.71e | 62.61d | 65.91d | 40.82f | 3.27h | 2.05h |     | 24.15g | 108.26c | 147.73a | 125.11b | 123.04b | 44.73f | 21.61g |
| 3-Hexenyl acetate        | 985 |     | 1.03b | 2.79a |     |     |     |     |     |     |     |     |     |     |     |
| Hydrocarbons             |     |   |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Pentadecane              | 1500| 6.80e | 11.61de | 15.22d | 18.73d | 15.70d | 15.00d | 5.51e | 50.10a | 43.63abc | 37.21c | 47.12ab | 42.67bc |     |     |
| Eicosane                 | 1983| 7.87b | 4.31d | 3.49e | 2.84f |     |     |     |     | 5.03c | 4.02d | 11.61a | 5.11c | 2.08g |     |
| Other                    |     |   |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Indole                   | 1295| 12.50ef | 36.07b | 34.48b | 18.03a | 1.81h | 0.61h |     | 30.26c | 41.90a | 19.40d | 14.22e | 10.52fg | 9.35g |     |

* Retention indices, using paraffin (C5-C25) as references; † Values are means of six replicates. Values having different superscripts are significantly (p < 0.05) different.
Table 4. Peak areas of chemical groups of volatile compounds on vase life of *Narcissus tazetta* var. *chinensis* Roem.

| Compounds            | Single-flowered (peak areas) | Double-flowered (peak areas) |
|----------------------|-----------------------------|-------------------------------|
|                      | 0   | 1   | 2   | 3   | 4   | 5   | 6   | 0   | 1   | 2   | 3   | 4   | 5   | 6   |
| Monoterpenes         |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| alcohol              | 940.48 | 2500.74 | 2537.91 | 742.07 | 45.00 | 8.93 | 5.46 | 419.87 | 2984.5 | 3586.4 | 10979.8 | 3763.44 | 1186.83 | 757.38 |
| Monoterpenes alcohol | 72.30 | 129.43 | 115.48 | 55.99 | 20.99 | 17.78 | 1.21 | 21.43 | 148.73 | 190.86 | 284.2 | 244.95 | 159.81 | 32.88 |
| Monoterpenes ketone  | 0.10 | 11.10 |     |     |     |     |     |     |     |     |     |     |     |     |
| Monoterpenes esters  | 8.19 | 6.77 | 6.81 | 4.43 | 8.43 |     |     | 6.08 | 9.08 | 3.72 | 11.06 | 9.56 |     |     |
| Monoterpenes oxide   | 143.23 | 158.73 | 144.65 | 64.14 | 11.56 | 2.76 | 1.45 | 85.13 | 222.04 | 268.56 | 450.91 | 271.2 | 122.62 | 65.85 |
| Aromatic alcohols    | 1.40 | 1.82 | 1.51 |     |     |     |     |     | 0.72 | 1.12 |     |     |     |     |
| Aromatic esters      | 691.18 | 1349.47 | 1408.55 | 131.71 | 27.09 | 10.67 | 4.47 | 232.58 | 965.83 | 1056.47 | 1714.60 | 641.75 | 277.98 | 107.25 |
| Sesquiterpene        | 8.15 | 2.56 | 2.50 | 4.32 | 4.22 |     |     |     |     |     |     |     |     |     |
| Aliphatic esters     | 53.12 | 65.55 | 69.01 | 51.97 | 12.43 | 16.82 | 6.10 | 27.84 | 112.41 | 153.53 | 130.96 | 132.68 | 62.27 | 39.20 |
| Hydrocarbons         | 14.67 | 15.92 | 18.71 | 21.57 | 15.70 | 15.00 | 5.51 | 50.10 | 48.66 | 41.23 | 58.73 | 47.78 | 2.08 |     |
| Other                | 12.50 | 36.07 | 34.48 | 18.03 | 1.81 | 0.61 |     | 30.26 | 41.90 | 19.40 | 14.22 | 10.52 | 9.35 |     |
| Totals (peak areas)  | 1928.88 | 4274.07 | 4339.63 | 1094.79 | 143.33 | 85.32 | 35.30 | 843.03 | 4521.51 | 5343.39 | 13650.78 | 5125.58 | 1822.11 | 1011.91 |

Notes: All the definitions of the symbols used in Table 3 mean values were also used in Table 4.
For both single-flowered and double-flowered narcissus flowers, the total content of volatile components had decreased significantly by day 4, and the total volatile component was lowest on day 6.

2.3. Comparison of Volatile Compounds from Single-Flowered and Double-Flowered of Narcissus tazetta var. chinensis Roem

Due to their different relative contents, the two narcissus flower types might have different scents. More ester compounds were identified in single-flowered samples than in double-flowered samples, including benzyl benzoate, and 3-hexenyl acetate, which may contribute to the aroma profile of the flowers. As shown in Table 4, Single-flowered samples have lower total peak areas and higher percentages of sesquiterpenes than the double-flowered samples.

3. Experimental

3.1. Plant Materials

Single-flowered and double-flowered narcissus bulbs were purchased from Zhangzhou, Fujian Province, China. These bulbs were placed in pots containing water and then left under outdoor light. The bulbs were incubated for 35 days until they blossomed.

3.2. Methods

3.2.1. Volatile Components of Narcissus Flowers

(1) Aroma components of the single- and double-flowered Chinese narcissus flowers: Fresh single- and double-flowered narcissus flowers in full bloom (ten each) were picked and immediately placed into sealed bottle. The SPME method was used to extract the aroma components. This experiment and all other experiments in this study were performed with six replicates.

(2) Volatile compounds during the vase-life of Narcissus tazetta var. chinensis Roem: Samples of narcissus bulbs about to flower were placed in a tissue culture laboratory controlled at 25 °C. The samples were exposed to 12 h of light and 12 h of darkness every day. Fresh budding single-flowered and double-flowered narcissus flowers (one flower each) were selected on a day then defined as day 0. These two samples were cut and inserted into two water-containing test bottle (precleaned # 27343 22-mL clear screw cap vials; Supelco, Bellefonte, PA, USA). From day 0 to day 6 at 10:00–12:00 in the morning, the SPME method was used to extract aroma to monitor the changes thereof.

3.2.2. Analysis of Volatile Compounds

(1) HS-SPME analysis. A 50/30-μm divinylbenzene/carboxen/polydimethylsiloxane fiber (Supelco, Inc.) was used for aroma extraction. The SPME fiber was exposed to each sample for 30 min at 25 °C, after which each sample was injected into a gas chromatograph injection unit. Peak area data reported from the integrator was used for quantification.

(2) Analysis of volatile components of samples by GC: Qualitative and quantitative analyses of the volatile compounds were conducted using an Agilent 6890 GC equipped with a 60 m × 0.25 mm i.d. DB-1 fused-silica capillary column with a film thickness of 0.25 μm and a flame ionization detector.
The injector and detector temperatures were maintained at 250 °C and 300 °C, respectively. The oven temperature was held at 40 °C for 1 min and then raised to 200 °C at 2 °C/min and held for 9 min. The carrier gas (nitrogen) flow rate was 1 mL/min. Kovats indices were calculated for the separated components relative to a C5-C25 n-alkanes mixture [29].

(3) Analysis of volatile components of samples by GC-MS: The volatile compounds were identified using an Agilent 6890 GC equipped with a 60 m × 0.25 mm i.d. DB-1 fused-silica capillary column with a film thickness of 0.25 μm coupled to an Agilent model 5973 N MSD mass spectrometer (MS). The injector temperature was maintained at 250 °C. The GC conditions in the GC-MS analysis were the same as in the GC analysis. The carrier gas (helium) flow rate was 1 mL/min. The electron energy was 70 eV at 230 °C. The constituents were identified by matching their spectra with those recorded in a MS library (Wiley 7n). In addition, the constituents were confirmed by using the Kovats indices or GC retention time data with those of authentic standards or by publication literature.

(4) Statistical Analysis: The data were subjected to a mono-factorial variance analysis, with Duncan’s multiple range method used by a significance of differences of \( p < 0.05 \) (SPSS Base 12.0).

4. Conclusions

Thirty-five volatile components were identified in Narcissus flowers. The main aroma components for narcissus flowers were (Z)-ocimene, benzyl acetate, linalool, and indole. More ester compounds were identified in single-flowered samples than in double-flowered samples, which may contribute to the aroma profile of the flowers. The double-flowered samples had higher contents of 1,8-cineole than the single-flowered samples. During the vase life, it was found that the total volatile content peak area was greatest for single-flowered samples on day 2, while that for double-flowered samples occurred on day 3. The volatile constituents throughout the vase life of Narcissus tazetta var. chinensis Roem were reported for the first time in this study.

Acknowledgments

This work was supported by a research grant from China Medical University (CMU100-T-15 and CMU101-S-31), Taiwan.

Conflicts of Interest

The authors declare no conflict of interest.

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Sample Availability: Not available.

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