VOLATILE COMPOUNDS OF FRESH FLOWERS

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ABSTRACT

HS-SPME/GC-MS was used for qualitative analysis of volatile compounds emitted from four types of fresh flowers: Dok Kaew, Dok Mok, Dok Phakakrong and Dok Tanyong. Volatile compounds in headspace were adsorbed on PDMS/DVB fiber at room temperature for 15 minutes, the adsorbed compounds were thermally desorbed at 200 °C in an injection port of GC and analysis by GC-MS. The results showed that the emitted volatile compounds in headspace have the differences in the profiles of compounds depend on type and the time after the flowers were picked. Total 75 compounds were detected and the important groups of compound were alkene, ester, aldehyde, ketone and alcohol with various quantity. The 53, 20, 35 and 2 compounds were found in headspace of Dok Kaew, Dok Mok, Dok Phakakrong and Dok Tanyong, respectively. The main compounds of Dok Kaew were benzaldehyde (53.6 %), benzene acetaldehyde (13.0 %) and β-cubebene (10.3 %); in Dok Mok were benzaldehyde (28.6 %) and benzene acetaldehyde (28.2%) in Dok Phakakrong were trans-β-ocimene (35.0 %) and trans-caryophyllene (10.7 %). High level of p-methylanisole (98.90%) was found in Dok Tanyong.

KEYWORDS: Headspace Solid Phase Microextraction, Gas Chromatography-Mass Spectrometry flowers, Dok Kaew, Dok Mok, Dok Phakakrong and Dok Tanyong

1. INTRODUCTION

Floral fragrances are the primary means by which plants attract potential pollinators. The characteristic aroma of the flowers support the interest in 1950s of the perfume industry in France and Spain. These organic compounds can be identified. Previous methods for the extraction and analysis of these compounds used techniques such as: solvent extraction, and steam distillation [1] followed by capillary chromatography. These methods either require large sample sizes, the use of solvents, or considerable time and effort to achieve the analysis. Santford V. Overton and John J. Manura used purged and trap Technique subsequently analyzed by thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) for the identification and comparison of the volatile organic, aroma, and flavor components.[2] Deng C., Song G. and Hu Y. used headspace SPME (a65 microm carbowax/ divinylbenzene, extraction temperature of 22 °C and extraction time of 10 min) combined with gas chromatography-mass spectrometry (GC-MS) for characterization of volatile compounds emitted from two varieties Osmanthus flowers.[3] Liu B. Z. and Gao Y. studied headspace constituents of fresh Gardenia flower by GC/MS. The headspace volatiles were sampled by solid-phase microextraction (SPME) and dynamic headspace sampling (DHS). They reported that SPME afforded a simpler and more sensitive sampling method, and much more accurate information about headspace volatiles of Gardenia flower.[4]

For this study, headspace volatiles compounds emitted from four kinds of flowers: Dok Kaew, Mok, Phakakrong and DokTanyong were compared.The emitted compounds were adsorped on SPME fiber followed by desorbing in a heated injection port of GC and subsequently analyzed by gas chromatograph-mass spectrometer (GC-MS).

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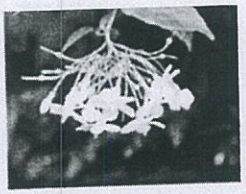
2. MATERIALS AND METHOD

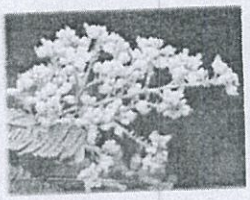
2.1. Four kinds of fresh flower samples:

Dok Kaew
Common name: Orange Jessamine, Satin-wood
Botanical name: Murraya Paniculata
Family: RUTACEAE



Dok Mok
Common name: Moke
Botanical name: Wrightia Religiosa
Family: APOCYNACEAE

Dok Phakakrong
Common name: Lantana; Wild sage; Cloth of gold
Botanical name: Lantana L
Family: VERBENACEAE



Dok Tanyong
Common name: Divi-divi
Botanical name: Caesalpinia Coriaria
Family: CAESALPINIOIDEAE

2.2 SPME

The SPME used in this experiment was the manual type, consisting of a SPME fiber holder (manual) and a PDMS/DVB coated fiber (Supelco, Bellefonte, PA). The fiber was immersed into a injection port of GC (200 °C) for 1 hour before initial using. The fresh flower was placed in 20 mL headspace vial and capped. The SPME needle was pierced through the septum, and the plunger was depressed to expose the fiber to the headspace region of the sample vial. The volatile compounds in headspace were adsorbed at room temperature (26 °C) for 15 minutes. The adsorbed volatile compounds were desorbed at 200 °C in the GC injection port and flushed into the GC column.

2.3 GC-MS analysis

Analyses were performed on a gas chromatograph–mass spectrometer (Agilent 6890 and HP 5973 mass-selective detector, Agilent Technologies) equipped with a fused silica capillary column, HP-5MS (30 m × 0.25 mm i.d. × 0.25 μm, Agilent Technologies). The injection port temperature was set at 200 °C. The column temperature program started at 60 °C held for 2 minutes and then was increased at a rate of 10 °C/min to 250 °C and held for 4 minutes. Helium gas, at a flow rate of 2 ml/min was used as a carrier gas. The GC–MS transfer line was set to 180 °C. The mass spectrometer was operated in the electron impact (EI) mode with an electron energy of 70 eV, ion source temperature, 230 °C. Compounds were identified by comparison with reference spectra from Wiley 275 database.

3. RESULTS

An analysis was performed for headspace of each flower. The chromatograms obtained from the total ion current (TIC) in Figure 1 and Figure 2 were integrated without any correction for coelutions. The peaks area of identified compounds were shown in Table 1.

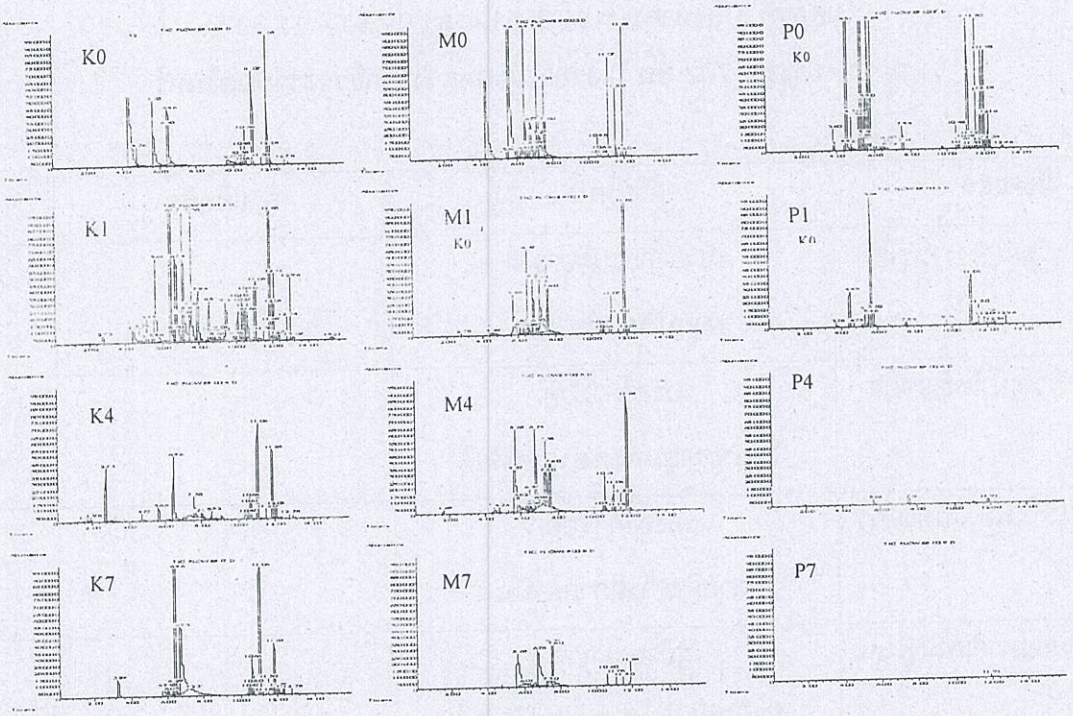


Figure 1 GC chromatograms showed different profiles of volatile compounds depend on type and time after the flowers were picked. (K=Dok Kaew, M=Dok Mok and P=Dok Phakakrong 0=freshly flower, 1= 1 day after picked, 4= 4 days after picked and 7= 7 days after picked.)

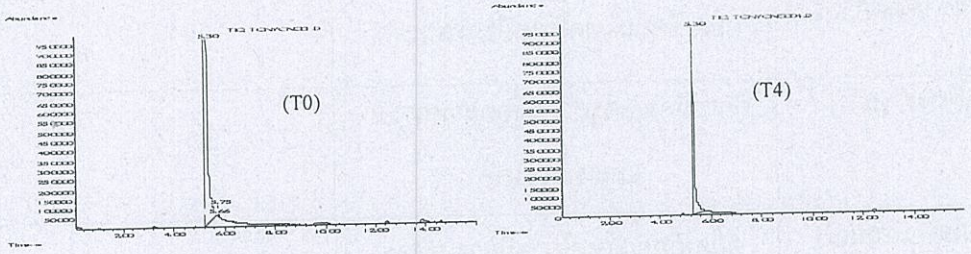


Figure 2 Chromatograms of Dok Tanyong, (T0) freshly flower and (T4) 4 days after picked.

Table 1 Volatile compounds identified by using SPME/GC-MS technique.

No. Compound: Peak area (Code of flower)

1. Ethyl propinoate: 1532350(K4)
2. Isoamyl alcohol: 942178(K4), 635791(M1),1244595(M4)
3. Ethyl isobutyrate: 1119584(K4), 802658(M1),
4. Butaric acid 2-methyl ester: 608410(K0), 505991(K1), 1120654(K4)
5. Butaric acid 2-ethyl ester: 1751646(K1), 10966278(K4)
6. Isoamyl acetate: 391526(K4)
7. Ethyl senecioate: 649819(K4)
8. alpha-Thujene: 2999895(P0), 701640(P1)
9. 2-Pinene: 811847(P0), 246706(P1)
10. Benzaldehyde: 119823533(K0), 10514992(K1), 148971586(M0), 2773758(M1), 2921546(M4), 2453827(P0)
11. Sabinene: 33096513(P0), 4642015(P1)
12. Myrcene: 2640203(K0),2960949(K1), 966691(K4), 886527(M0), 787029(M4), 450489(M7), 5709498(P0), 549142(P1)
13. Ethyl n-caproate: 736106(K1)
14. 2,3-Dimethyl-1,3-butadiene: 452526(K1)
15. l-Phellandrene: 653353(P0)
16. Hexyl acetate: 646224(K1)
17. 2-Hexen-1-ol acetate: 4967543(K1)
18. alpha-terpipene: 4148929(P0), 409373(P1)
19. Limonene: 317889(K4), 447630(P1)
20. o-Cymene: 29996763(P0), 2345486(P1)
21. 3-Methyl-4-brendene: 2136643(P0)
22. 1,8-Cineole: 2235910(P0)
23. cis-Ocimene: 699175(K1), 370017(K4), 786876(M0), 262351(M4), 6536878(P0), 1757591(P1)
24. Benzene acetaldehyde: 31149659(K0), 147923501(M0)
25. trans-beta-Ocimene: 30510844(K4), 2048771(K7), 18131901(M1), 30626274(M4), 132424977(P1)
26. Benzenemethanol: 2329624(K4), 5929942(K7), 179794(M1), 8968828(M4), 27503477(M7)
27. gamma-Terpinene: 36708629(P0), 2509980(P1)
28. cis-Linalool oxide: 1677265(K7), 2870936(M0), 2701424(M1), 2387941(M4), 771843(M7), 1382182(P0)
29. Methyl benzoate: 4747900(K0), 20123457(K1)
30. Linalool L: 13881744(K0), 67708568(K1), 12195812(K4), 32731476(K7), 65385279(M0), 11454172(M1), 4877666(M4)
31. 3-Carene: 1566345(P0), 430101(P4)
32. 2H-Pyran-3(4H)-one, 6-ethenyldihydro-2,2,6-trimethyl-: 1306132(P0), 390556(P1)
33. trans-2-hexenyl propionate: 2221267(K1)

Table 1 *Continued...*

34. 2,6-Dimethylene-7-octen-3-one: 620867(P0)
35. Benzene ethanol: 36417049(K1), 48841241(K4), 54647270(M0), 52165953(M1), 45952386(M4), 36968396(M7),
36. p- Cymene: 2822206(M0), 4075948(M1), 5444516(M4)
37. Benzene acetonitrile: 55273655(K1), 2039361(K7), 11153594(M0), 29710150(M1), 6839802(M4)
38. Benzyl acetate: 7527354(K1)
39. Epoxylinalol: 2879069(P0), 661899(P1), 707306(P4)
40. Ethyl benzoate: 39180755(K1), 8653125(K4)
41. Llinalool Z-pyranic oxide: 884589(P0), 614728(P1)
42. Methyl salicylate: 15703052(K0), 4401788(K1)
43. trans-2-hexenyl isovalerate: 5745605(K1), 1248089(K4)
44. Ethyl phenylacetate: 3349799(K1)
45. Phenylethyl phenylacetate: 1975002(K1)
46. Ethyl salicylate: 3623594(K1), 1642258(K4)
47. 1H-indole: 3482462(K0), 30487074(K1)
48. 2,3,4,5-Tetramethylcyclopent-2-en- 1-ol: 331571(P0), 695582(P1)
49. Ethyl 3- phenyl propionate: 12324232(K1)
50. Cyclosativen: 1133046(K0), 4685692(K1), 548969(K4)
51. alpha-Copaene: 2194139(K0), 3551174(K1), 780082(K4), 780082(K7), 2798196(P0)
52. beta-Bourbonene: 429221(K0), 6399020(K1), 1859593(K4), 1352004(K7), 467755(M0),
53. beta- cubebene: 8740585(K0), 4396575(K1), 2635186(K4), 4702107(K7) , 805621(P0), 231557(P4)
54. alpha-Gurjunene: 217298(K1)
55. beta-Caryophyllene: 10663865(K0), 11303972(M0), 40265213(P0), 5548574(P1), 332932(P4)
56. alpha.-Cedrene: 1658002(P0), 238921(P1)
57. alpha.-Bergamotene: 2026825(P0), 335583(P1)
58. beta.-sesquiphellandrene: 472436(P0), 241049(P1)
59. Germacrene-D: 388401(K1), 327418(K4), 530652(K7), 8815406(P0), 2623593(P1), 650904(P4)
60. Amylbenzoate: 444943(K1)
61. alpha-humulene: 1025599(K0), 1260461(K1), 7833866(M0), 16143590(P0), 2491072(P1)
62. alpha.-Longipinene: 373820(P0)
63. Aromadendrene: 422864(K0), 538440(M0)
64. gamma.-curcumene: 11471046(P0), 1195081(P1)
65. beta.-Selinene: 1435519(K0), 678039(K1), 2088627(K4), 11777843(M0),
66. Aristolene: 304776(K1), 1417730(K4), 2032769(K7)
67. Beta Phenyl ethyl isovalerate: 3637528(K1), 8035300(K4), 6464599(K7)
68. Bicyclogermacrene: 2213092(K0), 5107292(K1), 95321(K4), 1006833(M0), 1435519(P0), 678039(P1), 2088627(P4)

Table 1 *Continued...*

69. Farnesene: 169989(K0), 9740550(K1), 1517220(K4), 1333416(K7)
70. Germacrene-B: 752752(K0), 2981585(K1), 280965(K4), 802260(K7)
71. gamma.-curcumene: 4760141(P0), 819200(P1)
72. beta.-cadinene: 240959(K0), 1164564(M0)
73. Farnesyl acetate: 535971(K0), 8555635(K1), 752752(K4), 885000(K7), 694847(P0), 1370911(P1)
74. Benzyl benzoate: 2343925(K1), 396005(K7)
75. Phenethyl benzoate: 2834668(K1), 1742688(P0)

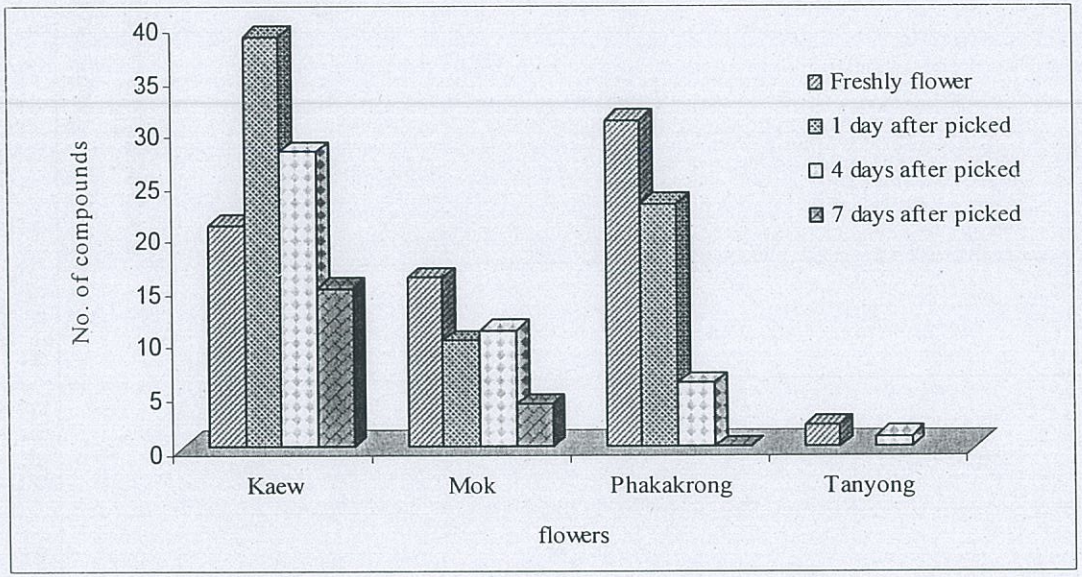


Figure 3 Number of compounds which were found at different times after the flowers were picked.

4. DISCUSSION AND CONCLUSIONS

The 75 compounds identified comprise of 34 alkenes, 24 esters, 6 alcohols , 4 ketones, 2 aldehydes and 5 others. The compounds 53, 20, 35 and 2, were found in headspace of Dok Kaew, Dok Mok, Dok Phakakrong and Dok Tanyong, respectively. The 26 esters were found only in Dok Kaew. The 19 of 20 compounds in headspace of Dok Mok were the same compounds as in headspace of Dok Kaew. The results showed that the emitted volatile compounds in headspace have the differences in the profiles of compounds which depend on the time after the flowers were picked as shown in Figure 3.

Dok Kaew has the widest variations in composition. Number of compounds of freshly flower was 21 compounds and changed to 39, 28 and 15 compounds on 1 day, 4 days and 7 days after flowers were picked, respectively. There are many esters occurred on 1 day after picked. The main compounds of freshly Dok Kaew were benzaldehyde (53.61%), benzene acetaldehyde (13.02 %) and β-cubebene (10.29 %); in Dok Mok were benzaldehyde (28.57 %) and benzene acetaldehyde (28.22%); in Dok Phakakrong were trans-β-cymene (35.03%) and trans-caryophyllene (10.66%). Dok Tanyong was differed from those 3 studied flowers, which found very high level of p-methyl anisole (98.90%).

Headspace-Solid Phase Microextraction (HS-SPME) proved to be a fast, useful, convenient and environmental friendly technique (solvent free method). It can be used with GC/MS technique for comparing of volatile organic compounds emitted from these four kinds of flowers. It can be stated that this technique is a real tool for the qualitative analysis of volatile compound. This method would be used to follow volatile composition changes in various samples. The procedure is flexible, and it may be transported in the field.

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