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Analysis of Major Fragrant Compounds from *Citrus grandis* Flowers Extracts

(Analisis Sebatian Wangian Utama daripada Ekstrak Bunga *Citrus grandis*)

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ABSTRACT

Various sampling techniques such as hydrodistillation, Soxhlet extraction and solid phase micro extraction (SPME) were used to extract compounds, i.e. the active components attributed to fragrance of C. grandis *flowers. Gas chromatograph mass spectrometry was then used to identify and quantify the active components obtained from the techniques. The results thus far showed that, buds and blossoms of this flower which possesses a strong floral-, jasmine- and orange-like aroma contain β-myrcene, limonene, ocimene, linalool and caryophyllene as the major compounds. In hydrodistillation technique the levels of ocimene and linalool detected in blossom were higher than in the bud at 7.37 and 15.93%, respectively, while in the bud, limonene was the highest, i.e. 4.57%. In Soxhlet extraction, however the bud was found to consist of limonene (27.25%), ocimene (5.55%) and linalool (2.97%). The performance of three available SPME fibers was compared to evaluate the recoveries of volatile compounds in C.grandis flowers and 100 µm polydimethylsiloxane was found to be the most effective.*

Keywords: Citrus grandis*; fragrance; limonene; solid phase micro extraction*

ABSTRAK

Pelbagai teknik persampelan iaitu penyulingan hidro, pengekstrakan Soxhlet dan pengekstrakan mikro fasa pepejal (SPME) telah digunakan bagi mengekstrak sebatian-sebatian komponen aktif yang terlibat kepada kewangian bunga C. grandis. *Kromatografi gas spektrometri jisim kemudiannya digunakan bagi mengenalpasti dan mengukur komponen aktif yang diperolehi daripada teknik tersebut. Keputusan telah menunjukkan bahawa putik dan bunga ini, mempunyai aroma kuat menyamai keharuman bungaan, melor dan oren mengandungi β-mirsena, limonena, osimena, linalool and kariofilena sebagai sebatian utama. Dalam teknik penyulingan hidro ke atas bunga, kandungan osimena dan linalool telah dikesan lebih tinggi berbanding putik dengan peratusan masing-masing adalah 7.37 dan 15.93%, sementara di dalam putik, kandungan limonena adalah yang tertinggi iaitu 4.57%. Dalam pengekstrakan Soxhlet, keadaan adalah sebaliknya dengan kandungan limonena (27.25%), ocimena (5.55%) dan linalool (2.97%) di dalam putik adalah lebih tinggi. Prestasi ketiga-tiga fiber SPME juga telah dibandingkan daripada segi keupayaan mengukur sebatian meruap dari bunga C. grandis dan didapati 100 µm polidimetilsiloxane adalah paling berkesan.*

Kata kunci: Citrus grandis*; limonene; pengekstrakan mikro fasa pepejal; wangian*

INTRODUCTION

Citrus grandis also known as pomelo is a member of Rutaceae family. This largest citrus fruit is a native to southeastern Asia and Malaysia (Morton 1987). The flowers are fragrant, and described as having a floral-, jasmine- and orange-like aroma that showed great potential for the fragrance industry (Svoboda & Greenway 2003). Morton (1987) found that these flowers are highly aromatic and were gathered in northern Vietnam for making perfume.

Natural floral essential oils are used as ingredients for perfumes and fragrances. Therefore, the knowledge about specific components in floral fragrances can be used for production of natural and synthetic perfumes (Li et al. 2006). Researchers have an interest in *Citrus* species because of its valuable compounds especially for flavonoid and monoterpenoid production. The monoterpenoid compounds are the major components of many essential oils and as such, have economic importance as flavours and perfumes (Svoboda & Greenaway 2003).

Analytical methods have been developed to determine the volatile compounds present in the flowers. These involve the use of hydrodistillation, Soxhlet or solvent extraction. However, methods based on the manipulation of the headspace in contact with odorous materials are getting popular and very suitable for compounds analysis of fragrance. The headspace method like solid phase micro extraction (SPME), which is a rapid, simple and involved convenient sample preparation methods has gained increasing popularity for fragrance analysis (Augusto et al. 2003; Bicchi et al. 2000; Flamini et al. 2003; Kim & Lee 2002; Li et al. 2006;). Thus, this study was aimed at comparing the three different extraction techniques such as hydrodistillation, Soxhlet extraction and SPME in

extracting fragrances from bud and blossom of *C. grandis* flowers.

EXPERIMENTAL DETAILS

PLANT MATERIALS AND REAGENTS

Fresh flowers of *C. grandis* were collected from established plantation in Kodiang, Kedah. Two categories of flowers were used in this study i.e. blossom and bud. Opened and closed petals were categorized as blossom and bud, respectively. In extraction techniques, dichloromethane purchased from Fisher Scientific was used as an extracting solvent. The SPME fiber holder and fibers were purchased from Supelco (Bellefonte, PA\USA). Three different type of fibers were used i.e., 65 µm Polydimethylsiloxane/Divinylbenzyl (PDMS/DVB), 75 µm Carboxen/ Polydimethylsiloxane (CAR/ PDMS) and 100 µm PDMS.

HYDRODISTILLATION EXTRACTION

About 50 g of fresh flowers (buds or blossoms) were placed in 250 mL round bottom flask which contained 100 mL distilled water. The mixture was heated up to 100ºC and the distillate was then extracted three times using 15 mL portions of dichloromethane. The organic layer was collected and was drawn into a beaker which contained anhydrous magnesium sulphate for drying. The concentrated extracts were later analyzed using GC-MS system for compounds identification.

SOXHLET EXTRACTION

About 10-15 g of small pieces of cut fresh flowers (buds or blossoms) were placed in an extraction thimble which were lightly plugged with cotton wool. The samples were extracted with 200 mL of dichloromethane for about 3 hours until the solvent become colorless. The solvent were then removed from the extracts by using rotary evaporator at 40°C before drying overnight in an oven at 50°C. The dried extracts were stored in an amber glass vial and dissolved with 2 mL dichloromethane prior to GC-MS analysis.

SOLID PHASE MICRO EXTRACTION

All fibres were conditioned by heating them in a hot GC injection port at 250-300°C for 30-60 min prior to sample extraction in order to remove contaminants. About 30 g of fresh buds or blossoms were placed into a 15 mL septum cap vial and allowed to equilibrate for 20 minutes at 25ºC[6]. The SPME needle was then injected into the vial and the fiber was exposed in the headspace for 15 minutes at 25ºC. An analysis was performed by injecting the needle and exposed the fiber into the injection port of GC-MS system.

GAS CHROMATOGRAPHY - MASS SPECTROMETRY

GC-MS analysis was performed using Agilent 6890 Chromatograph linked to a Agilent 5973 Mass Spectrometer system. The column used was an HP 5MS (Crosslinked 5 % PH ME Siloxane) capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Helium was used as a gas carrier with the flow rate of 1 mL/min. The injector temperature was maintained at 220°C. Samples of 1 µL were injected manually in a splittless mode. Electron impact mass spectra were recorded in the 400-500 mass range. An electron ionization system was used with ionization energy of 70 eV. The oven temperature was held at 40°C for 3 min, raised to 80°C at 4°C/min and then to 250°C at 10°C/min (5 min hold). Identification of the volatile compounds was based on comparison of the mass spectra obtained with those of the reference compounds in the data systems of the NIST Mass Spectral Search Program (NIST 02 version mass spectral database, National Institute of Standards and Technology, Washington, DC, USA). Compounds were identified with a resemblance percentage above 90% (Alissandrakis et al. 2003).

RESULTS AND DISCUSSION

COMPARISON OF EXTRACTION TECHNIQUES FROM *C. GRANDIS* FLOWERS BY HYDRODISTILLATION AND SOXHLET EXTRACTION

Table 1 lists of the fragrance compounds and their peak identification detected by GC-MS by hydrodistillation and Soxhlet extraction from *C. grandis* flowers. Most of the compounds were found in both the bud and blossom. Peak identification for the chromatogram from hydrodistillation were linalool (15.93%), benzoic acid (10.02%) and ocimene (7.37%) which were higher in blossom compared to the bud. In the bud, the major compounds were geraniol (13.35%), farnesol isomer a (9.14%) and indole (8.20%). In Soxhlet extraction, limonene showed the highest percent in both bud (9.41%) and blossom (27.27%) followed by heptacosane (8.07-16.62%) and 1-hexacosene (2.06-2.79%). Ocimene, linalool, indole, benzoic acid and 1,6,10-dodecatrien-3-ol also were among the high volatile compounds detected in both extraction techniques.

There were slightly fewer compounds present in the chromatograms of *C. grandis* flowers extracted by Soxhlet extraction compared with those obtained by hydrodistillation. Water is a polar solvent, which accelerates many reactions, especially reactions via carbocation as intermediates. In addition, the avoidance of water in Soxhlet extraction avoids the degradation of compounds by hydrolysis, transesterification, or oxidation, and hence there are fewer degradation products noted in the analysis (Lucchesi et al. 2004).

COMPARATIVE ANALYSIS OF *C. GRANDIS* FLOWERS BY DIFFERENT SPME FIBRES COATINGS

In this study, the objective of using different types of fiber is to determine which fibres is most suitable for the analysis of *C. grandis* flowers. This was made based on which fiber can adsorp the highest concentrations of

TABLE 1. Compounds of the bud and blossom of *C. grandis* flowers from hydrodistillation and Soxhlet extraction

Peak	Compounds	RT (min)[1]	Peak Area (%)			
			Hydrodistillation		Soxhlet	
			Bud	Blossom	Bud	Blossom
6	α-Pinene	12.69	-	-	0.38	-
9	β-Pinene	14.55	0.05	0.08	-	-
11	Heptane	15.51	-	-	2.26	0.58
17	3-Carene	16.43	-	-	0.65	-
19	Limonene	16.67	4.57	3.11	27.25	9.41
20	Carveol	16.72	0.36	-	-	-
21	Ocimene	16.87	5.29	7.37	5.55	1.62
24	Benzyl alcohol	18.64	-	0.10	-	-
25	*cis*-Linalool oxide	19.60	-	6.43	-	-
26	Linalool	19.95	6.42	15.93	2.97	1.93
27	Linalool oxide	20.10	-	-	1.17	0.30
28	2-Furanmethanol	20.18	7.60	4.24	-	0.69
29	2,6-Dimethyl-2,4,6-octatriene	20.33	-	-	0.18	-
31	*trans*-p-Mentha-2,8-dienol	21.48	1.06	-	-	-
32	2,6-Dimethyl-1,3,5,7-octatetraen	21.76	0.10	-	-	-
34	Limonene oxide	21.81	0.24	-	-	-
36	3,7-Dimethyl-, -6 (R)-octenal	22.42	0.07	-	-	-
37	2-Cyclohexen-1-ol	24.02	1.06	-	0.56	-
38	3-Cyclohexene-1-methanol	24.17	4.88	-	-	-
39	p-Mentha-1-en-8-ol	24.63	-	-	0.29	-
40	2-Cyclohexen-1-one	26.48	-	-	0.35	-
41	Citral	26.63	-	1.79	0.38	-
42	Geraniol	26.79	13.35	6.43	0.68	-
43	Indole	28.36	8.20	4.03	5.93	1.58
48	Methyl-2-amino-Benzoic acid	29.48	1.61	10.02	0.21	2.92
50	2,7-Octadiene-1,6-diol	30.57	-	-	0.79	-
51	Caryophyllene	30.62	0.17	0.12	0.94	0.35
58	Benzaldehyde	31.87	-	-	0.68	-
59	1,4,7-Cycloundecatriene	31.95	-	-	1.00	-
62	1,6,10-Dodecatriene	32.64	0.58	1.00	0.68	0.17
66	Naphthalene	33.37	0.38	0.19	2.85	0.69
67	Di-epi-a-cedrene	33.92	-	0.15	-	-
68	α-Farnesene	34.34	0.19	0.27	-	-
71	1,6,10-Dodecatrien-3-ol	36.38	3.18	6.87	1.91	1.51
72	Hexadecane	36.47	-	-	-	0.17
73	Tetracosane	36.75	0.07	-	-	-
74	Dodecanoic acid	36.81	0.79	0.52	-	-
76	Farnesol	38.09	0.02	-	-	1.99
79	α-Cadinol	38.92	1.56	0.67	-	-
82	Farnesol isomer α	40.55	9.14	1.40	-	-
84	Octadecane	41.55	0.70	-	-	-
85	Nonadecane	41.63	5.41	0.61	-	-
86	2,6,10,14-Hexadecatetraen-1-ol	41.98	-	0.25	-	-
87	Curan-17-oic acid	42.29	-	0.60	-	-
88	Tricosane	42.73	2.38	0.50	-	1.58
89	Caffeine	42.97	-	-	-	4.67
90	1,2-Benzenedicarboxylic acid	43.18	-	-	-	16.44
91	Tetrapentacontane	43.40	-	-	1.23	-
92	1-Nonadecene	43.49	-	-	-	4.57
96	Pentacosane	44.20	-	5.22	-	6.66
97	Eicosane	44.43	2.81	0.75	-	-
99	Heptacosane	44.87	-	-	8.07	16.62
100	1,2-Benzisothiazole	44.92	0.58	0.60	-	0.48
102	10,18-Bisnorabieta-8,11,13-triene	45.58	-	-	6.19	-
103	Cyclotriacontane	45.64	-	0.86	-	1.51
105	1-Hexacosene	45.85	-	0.42	2.79	2.06
106	1-chloro-nonadecane	45.87	-	-	3.55	-
109	1-Bromo-11-iodoundecane	46.36	1.78	0.15	-	-
110	Cyclotetradecane	46.44	0.91	-	-	-
111	2-Methyl-7(E)-octadecene	46.61	0.84	-	-	-
112	Hexacosane	47.04	2.41	1.40	-	16.63
113	Docosane	47.85	4.11	0.25	2.55	-
114	Tetracosane	48.65	-	-	5.26	-
115	1-iodo-Octadecane	48.93	-	0.69	-	-
119	2-Methyl-4(Z)-tetradecene	49.88	0.05	-	-	-

[1]mean value; RT = Retention time

TABLE 2. Compounds of the bud and blossom of *C. grandis* flowers from different SPME fibre coatings as detected using GC-MS

Peak	Compounds	RT (min)[1]	Peak Area (%)					
			65 µm PDMS/DVB		75 µm CAR/PDMS		100 µm PDMS	
			Bud	Blos.	Bud	Blos.	Bud	Blos.
1	Toluene	6.59	-	-	-	0.01	-	-
3	Ethylbenzene	9.51	-	-	-	0.12	-	-
4	1,3-dimethyl-Benzene	9.80	-	-	-	0.09	-	4.25
5	Bicyclo hex-2-ene	12.03	-	-	-	-	0.16	-
6	α-Pinene	12.69	-	0.65	0.46	0.62	1.64	-
7	Camphene	12.90	-	-	-	-	0.53	-
8	β-Myrcene	14.02	1.11	3.99	1.72	9.60	1.72	10.56
9	β-Pinene	14.55	1.38	1.47	1.69	-	-	-
10	Hept-3-ene	15.25	1.90	-	7.36	-	9.19	-
11	Heptane	15.51	-	-	-	-	3.48	-
12	1,3-Cyclohexadiene	15.52	-	-	0.72	-	-	-
13	o-xylene	15.63	-	-	-	-	-	1.75
14	Hept-2-ene	15.75	-	0.59	-	-	-	-
15	Cyclotetrasiloxane	15.91	0.03	-	-	-	-	-
16	4-Carene	16.34	0.62	-	-	-	-	-
17	3-Carene	16.43	-	-	-	2.37	-	-
18	1,4-Cyclohexadiene	16.55	-	2.63	0.86	-	1.24	-
19	Limonene	16.67	38.17	63.27	83.01	68.07	61.61	72.79
21	Ocimene	16.87	8.20	14.24	2.35	5.65	4.40	7.81
22	2,6-dimethyl-Undecane	17.81	-	-	-	3.75	-	-
23	Cyclohexene	18.34	1.17	2.37	-	-	5.52	-
26	Linalool	19.95	10.90	5.91	-	5.44	0.15	-
29	2,6-dimethyl-2,4,6-Octatriene	20.33	0.64	1.36	0.88	1.75	0.60	-
30	3,4-dimethyl-2,4,6-Octatriene	20.39	-	-	-	-	0.75	-
44	Copaene	28.59	-	-	-	0.06	-	-
46	1-ethenyl-1-methyl-Cyclohexane	29.11	-	-	-	0.04	-	-
48	Methyl-2-amino-Benzoic acid	29.48	-	0.32	-	0.09	-	-
49	1H-Cyclopropa (a) naphthalene	30.30	-	-	-	0.06	-	-
51	Caryophyllene	30.62	2.78	0.97	0.72	1.10	3.27	1.48
52	Humulene	31.07	-	-	-	-	0.32	-
54	α- Caryophyllene	31.13	-	-	-	0.09	-	-
55	Azulene	31.36	-	-	-	-	0.14	-
56	1H-Cycloprop (e) azulene	31.38	0.35	0.29	-	0.16	0.29	-
57	2,5-Cyclohexadiene-1,4-dione	31.48	0.44	-	-	0.06	-	-
59	1,4,7-Cycloundecatriene	31.95	0.38	-	-	-	0.45	-
61	Pentadecane	32.33	0.73	-	-	-	-	-
62	1,6,10-Dodecatriene	32.64	0.18	-	-	-	-	-
63	β-Humulene	32.79	-	-	-	-	0.11	-
64	Butylated hydroxytoluene	32.95	-	-	-	-	0.05	-
65	Cycloheptasiloxane	33.11	1.49	0.23	-	-	0.25	-
66	Naphthalene	33.37	4.13	1.57	0.24	0.85	2.87	-
69	Heptasiloxane	34.65	2.14	-	-	-	-	-
70	Phenol	35.32	7.06	-	-	-	-	-
75	Benzene	37.33	3.93	-	-	-	-	-
77	Heptadecane	38.13	4.63	-	-	-	-	-
80	2,6-Bis (1,1-dimethylethyl)	39.34	-	-	-	0.03	1.26	-
83	2,5-Dimethyl-2-(3-methylbut-2-ene)	41.51	6.03	-	-	-	-	-
93	Androstane	43.73	1.35	-	-	-	-	-

[1]mean value; RT = Retention time; Blos. = Blossom

major fragrance compounds contained in the buds as well in the blossoms. Determination was also made based on the total compounds absorped and desorbed in the GC-MS analysis. The concentration of the major compound were taken as the percentage of the peak area in the GC-MS chromatogram.

Comparison of the constituents extracted based on types of SPME fibre coatings is presented in Table 2. It was found that β-myrcene, limonene, ocimene, linalool and caryophyllene were the compounds detected in almost all types of the fiber. Limonene was the most prominent compound found in highest concentration when using 100 μm PDMS fiber both in buds and blossoms. This observation is also true for the other compounds such as β-myrcene, ocimene, caryophyllene and several others.

The 65 μm PDMS/DVB fiber was found to be the most efficient adsorbent from the bud and blossoms which are respectively ampled the highest number of compounds was as the total numbers of compounds from the bud and blossom were 22 and 11 respectively. This fibre was therefore considered to be a suitable fiber for a wide range of compounds (Augusto & Valente 2002) Since the amount of absorbed components are the considered criteria in this study, then it was suggested that 100 μm PDMS fiber to be recommended for analyzing compounds in *C. grandis* flowers for future study. This was supported from several studies using 100 μm PDMS fiber which reported that it was the most efficient fiber for the sampling of volatiles compounds especially in flowers. Furthermore, the fiber has limited production of artifacts and good analytes recovery and produced highest recoveries with oxygenated sesquiterpenoids (Bicchi 2000; Kim & Lee 2002).

CONCLUSION

The use of different methods for the extraction of *C. grandis* flowers results in almost similar major compounds expected to contribute on the production of fragrance in the flowers. On the efficiency part, SPME coupled with GC-MS represents a simple, time saving, highly sensitive and solvent free extraction, which has great prospects for the future use as an alternative to conventional techniques for the analysis of volatile compounds. The most effective fiber for SPME sampling in *C. grandis* flowers was 100 μm PDMS fiber that is also recommended for the analysis of fragrance compounds from the flowers.

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