

Flavor Characteristic Compounds Found in the Essential Oil of *Ocimum basilicum* L. with Sensory Evaluation and Statistical Analysis

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Oils obtained from various parts of the basil plant were categorized into five groups by preference ranking test of their aromas. Among them the aroma of fresh leaf-flower oil was the most preferable, while that of stem was the least. According to the odor assessment of each volatile compound by the sniffing method of gas chromatography, methylchavicol and 1-octen-3-ol were the most and the least preferable characteristic compounds, respectively. High statistical correlations were found among the volatile components of basil oil during sensory evaluation. A regression equation, which included four compounds, i.e., 1-octen-3-ol, α -muurolene, germacrene B, and methyleugenol, and which could explain the differences among the six kinds of oils about 70%, was obtained by SAS stepwise regression analysis. Also, this regression equation could be used to evaluate objectively the quality of basil oil.

INTRODUCTION

Basil essential oil has been widely used in all kinds of flavoring including those for confectionary, baked goods, seasonings, and spiced meats and sausages as well as in oral care products and certain perfumes (Guenther, 1952). A number of different types of basil oil have found their way into the world market over the years. European or sweet basil, which is distilled in France, Italy, Bulgaria, Egypt, Hungary, South Africa, and occasionally the United States, is an oil that contains approximately equal proportions of methylchavicol and linalool. Exotic or Reunion basil, which is distilled in the Comoro Islands, Malagasy Republic, Thailand, and occasionally in the Seychelles, is an oil rich in methylchavicol (Lawrence, 1978).

Many studies concerning basil oil have been carried out (Lawrence, 1978, 1980, 1986; Leung, 1980; Heath, 1981). Karawya et al. (1974) investigated the oils grown in Egypt and found linalool and methylchavicol the most prominent. Fleisher (1981) analyzed the oils grown in Israel, where linalool, methylchavicol, and eugenol are the main components. Vernin et al. (1984) analyzed the basil oils from Yugoslavia and Madagascar by GC-MS data bank, and 10 new compounds were identified. Brophy and Jogia (1986) studied the oils of which major components were methyl cinnamate, linalool, methyleugenol, and eugenol in Fiji. Nykänen (1986) investigated the oils, which were rich in methylchavicol and linalool, cultivated in Finland. Tsai and Sheen (1989) studied the oil cultivated in Taiwan. It was found to be rich in methylchavicol, possessing much similarity to the Reunion or Exotic basil oil.

With regard to the odor characteristic compounds in basil oil, nothing could be traced in the current literature. Accordingly, this work was carried out to find the odor characteristic compounds produced by *Ocimum basilicum* L. grown in Taiwan.

MATERIALS AND METHODS

Materials. Basil, which was harvested near the campus of the National Chung-Hsing University, Taichung, Taiwan, was divided into five portions: leaf, flower, stem, whole plant, and leaf-flower (fresh and stored 10 days at 4 °C). The ratio of the leaf-flower portion was 2.1:1 (w/w).

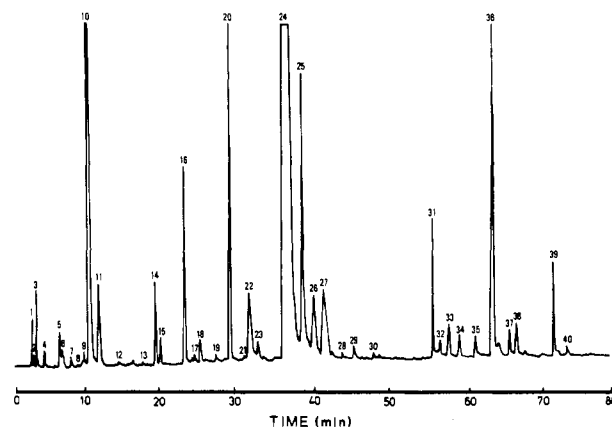


Figure 1. Gas chromatogram of the volatiles of basil stem oil. Conditions: 50 m \times 0.22 mm fused silica capillary column (Carbowax 20M); carrier gas, nitrogen; flow rate, 1.8 mL/min; split ratio, 80:1; injector and detector temperature, 250 °C; range, 10. Temperatures were as follows: initial temperature, 50 °C; program rate, 2 °C/min; final temperature, 200 °C. Shimadzu GC-9A and Shimadzu C-R3A Chromatopac were used in this analysis.

Sample Preparation. Essential oils from four portions of the basil plant were obtained by atmospheric steam distillation (Sakamura and Hayashi, 1978), while that from the stem was obtained by using a Likens-Nickerson apparatus (Nickerson and Likens, 1966). Redistilled pentane/ether (1:1 v/v) (Alps Chemical Co., Ltd., Taiwan) was used as the extracting solvent.

Gas Chromatographic Analysis. Gas chromatography was performed with a Shimadzu GC-9A instrument equipped with a flame ionization detector and a fused silica capillary column (Carbowax 20M; 50 m \times 0.22 mm i.d.). The column was temperature programmed from 50 to 200 °C at 2 °C/min and held at the final temperature until the chromatogram was complete. The other GC conditions were as follows: carrier gas (nitrogen) flow rate, 1.8 mL/min; temperature of the injection port and that of the detector, 250 °C. The samples were injected in the split mode with a split ratio of 80:1. A Shimadzu C-R3A Chromatopac integrator was used to determine the peak area for quantitative analysis.

Sensory Evaluation. Odor Assessment. The sniffing method of gas chromatography (Guadagni et al., 1966; Nursten and Sheen, 1974; Clark and Nursten, 1976; Chuyen and Kato, 1982) was used to describe the characteristic odor of each volatile compound of basil essential oil by well-trained panels. Samples were injected into a Varian 1400 GC equipped with Varian 4290 integrator. The instrument was equipped with a 3 m \times 2.2 mm i.d. stainless

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Table I

(A) Analysis of Variance for the Different Kinds of Basil Oil				
source of variance	df	ss	ms	F
among samples	5	143.84	28.77	112.96 ^a
among judges	49	0	0	0
error	245	62.41	0.2547	
total	299	206.25		

(B) Result of Tukey's Test for the Different Kinds of Basil Oil

leaf	flower	stem	whole	leaf-flower (fresh)	leaf-flower (stored 10 days at 4 °C)
d ^b	c	e	b	a	d

^a Significantly different at the 1% level. ^b Any two samples not having the same letter were significantly different at the 5% level.

Table II. Odor Assessment of Volatile Compounds of Basil Oil

peak no. ^a	compd ^b	odor description
4	α -pinene	fragrant, fresh
5	β -pinene	fragrant
6	sabinene	woody
7	myrcene	slightly green
8	limonene	fragrant, fresh, sweet
9	β -phellandrene	fragrant, fresh, sweet
10	1,8-cineole	fragrant, sweet, cooling, fresh, slightly green, minty
11	<i>cis</i> - β -ocimene	fragrant, sweet, cooling, fresh, slightly green, minty
12	terpinolene	fragrant, fresh, sweet
13	1-hexanol	sour
14	<i>cis</i> -3-hexanol	sour
15	3-octanol	sour
16	1-octen-3-ol	strongly green grasslike
17	sabinene hydrate	fragrant, fresh, sour
18	elixene	fragrant, fresh, sour
19	α -copaene	fragrant, fresh, sour
20	linalool	fragrant, sweet, fresh, woody
21	<i>trans</i> - α -bergamotene	fragrant, sweet, fresh
22	β -elemene	medium green
23	aromadendrene	medium green, sour
24	methylchavicol	fragrant, sweet, cooling, fresh minty
25	<i>trans</i> - β -farnesene	cooling, fresh, minty, slightly green
26	α -terpineol	cooling, fresh, minty, slightly green, fragrant, sweet
27	α -muurolene	cooling, fresh, minty, slightly green
28	germacrene B	sour
29	cardina-1,4-diene	fragrant, sweet
30	<i>trans</i> -anethole	fragrant, sour, sweet
31	methyleugenol	fragrant, sweet, fresh, slightly green
32	dibutyl octanedioate	fragrant
33	sesquiterpene	fragrant, fresh, woody
34	viridiflorol	fragrant
35	eugenol	fragrant, woody
36	T-cadinol	fragrant
37	α -cadinol	fragrant
38	β -eudesmol	fragrant, woody
39	chavicol	sour
40	ethyl linolenoate	sour

^a Peak no. refers to Figure 1. ^b Peak identifications are based on comparison with file spectra of known standards or published spectra and relative retention time.

steel column packed with 100–120-mesh Chromosorb W/AW coated with 15% Carbowax 20M. GC conditions were as follows: carrier gas (nitrogen) flow rate, 30 mL/min; air flow rate, 300 mL/min; hydrogen flow rate, 30 mL/min; injector and detector temperature, 250 °C; initial column temperature, 50 °C; final temperature, 200 °C; programmed rate, 4 °C/min; sample injection volume, 3 μ L. Effluent from the column was split at a ratio of 9:1; 10% of the sample volume went through the detector, while 90% went through the exit port for sniffing.

Preference Ranking Test. The six basil oils were ranked from

Table III. Percentage Compositions of Various Kinds of Basil Oil

peak no. ^a	leaf	flower	stem	whole	leaf-flower (fresh)	leaf-flower (stored 10 days at 4 °C)
1	– ^b	–	0.0814	–	–	–
2	–	–	0.0246	–	–	–
3	–	–	0.1410	–	–	–
4	0.1285	0.0374	0.1099	0.1377	0.1114	0.1112
5	0.3540	0.1009	0.3136	0.3108	0.2552	0.2989
6	0.2243	0.1171	0.1764	0.2458	0.1878	0.2437
7	0.1321	0.0456	0.1016	0.1159	0.0983	0.1083
8	0.0172	0.0075	0.0198	0.0189	0.0179	–
9	0.0804	0.0343	0.0791	0.0644	0.0578	0.0746
10	5.4740	3.2947	6.9673	6.0976	4.0837	4.0926
11	1.3331	2.6030	0.8731	2.2056	1.8652	1.4129
12	–	–	0.0449	–	–	–
13	0.0037	0.0046	0.0244	0.0040	0.0030	–
14	0.0281	0.0304	0.3904	0.0627	0.0475	0.0435
15	0.0174	0.0147	0.1944	0.0259	0.0189	0.0190
16	0.1698	0.0840	1.1421	0.2262	0.1519	0.0725
17	0.0112	0.0077	0.0290	0.0167	0.0119	0.0664
18	0.0044	0.0778	0.2277	0.0479	0.0359	0.0322
19	0.0179	0.0107	0.1313	0.0116	0.0136	–
20	0.3732	1.4787	2.2094	1.6790	1.3679	0.4540
21	0.0111	0.0129	0.0191	0.0061	0.0012	0.0208
22	0.9091	0.7343	1.5901	0.2731	0.6171	0.8127
23	–	0.0016	0.2744	0.0901	0.1095	0.0649
24	84.6845	85.5128	68.5023	86.5726	86.3960	85.0377
25	0.5397	2.5078	3.1799	0.2480	1.4445	2.0488
26	0.8976	0.7243	1.3667	0.2520	0.6656	1.1790
27	0.6600	0.7731	1.9549	0.2577	0.4868	1.1556
28	0.0098	0.0007	0.1256	0.0075	0.0946	0.0078
29	0.0759	0.0468	0.2819	0.0312	0.0701	0.1123
30	0.0048	0.0066	0.0389	–	0.0026	0.0119
31	2.4369	0.5587	0.7843	0.5980	0.9447	1.1979
32	–	0.0221	0.2975	0.0039	–	–
33	0.1272	0.1091	0.4866	0.0318	0.0850	0.1359
34	0.0147	0.0158	0.3068	–	0.0131	–
35	0.0232	0.0251	0.0452	0.0103	0.0194	0.0280
36	0.9044	0.7891	3.8199	0.2401	0.5202	0.7764
37	0.0198	0.0113	0.2920	0.0047	0.0223	0.0160
38	0.0799	0.0596	0.3928	0.0166	0.0462	0.0473
39	0.0177	0.0132	0.5286	0.0190	0.0297	0.0988
40	0.0025	0.0034	0.1794	–	–	0.0419

^a Numbers refer to Figure 1 and Table IV. ^b Not detected.

one to six according to the aroma preference of 50 panelists using the sniffing method (Larmond, 1977).

Statistical Analysis. The sensory rank for each sample was transformed by the Fisher and Yate method (Fisher and Yate, 1949) into a numerical score. The percentage composition for each compound of oil was transformed by arc-sine transformation for the following statistical analyses: analysis of variance (ANOVA), Tukey's test, correlation, and stepwise regression, which were all performed by using a SAS computer package.

RESULTS AND DISCUSSION

The transformed sensory scores from the preference ranking test for the six kinds of basil oil were analyzed by ANOVA. The result (Table IA) showed that the F value was significant at the 1% level. Since a significant difference was found among the samples, Tukey's test (Snedecor, 1956) was used to further elucidate the data. The result (Table IB) indicated that the aroma of leaf-flower (fresh) was ranked the best among the samples, while that of stem was the worst; any two samples not having the same letter were significantly different at the 5% level.

Figure 1 shows the GC profile of basil stem oil of which volatile compounds were identified by Tsai and Sheen (1989). Table II shows the aroma description of each volatile compound by GC odor assessment. Twenty-six

Table V. Correlation Coefficient (r) between Sensory Ranking Score and Quantity of Peak Area

peak no.	r^a	peak no.	r^a	peak no.	r^a
1	-0.755	15	-0.741	29	-0.842
2	-0.755	16	-0.702	30	-0.868
3	-0.755	17	-0.433	31	-0.219
4	0.003	18	-0.653	32	-0.752
5	-0.300	19	-0.723	33	-0.854
6	0.048	20	-0.069	34	-0.751
7	-0.131	21	-0.873	35	-0.889
8	0.097	22	-0.897	36	-0.836
9	-0.555	23	-0.477	37	-0.759
10	-0.481	24	0.828	38	-0.803
11	0.758	25	-0.599	39	-0.795
12	-0.755	26	-0.876	40	-0.830
13	-0.691	27	-0.909		
14	-0.717	28	-0.287		

^a If $r > 0.811$ or $r < -0.811$, then significant at $p = 0.05$.

Table VI. Summary of Computer Output of Stepwise Multiple Regression Analysis for Different Kinds of Basil Oil

compd (peak no.)	regression coeff	SE	partial R^2	partial F	P value
1-octen-3-ol (16)	-1.397 768	0.2670	0.028	27.41	<0.001
α -muurolene (27)	-1.843 625	0.1415	0.174	169.65	<0.001
germacrene B (28)	11.209 588	1.4660	0.060	58.46	<0.001
methyleugenol (31)	-0.515 951	0.0746	0.049	47.49	<0.001

$$Y = 1.24 - 1.40X_{16} - 1.84X_{27} + 11.21X_{28} - 0.52X_{31}$$

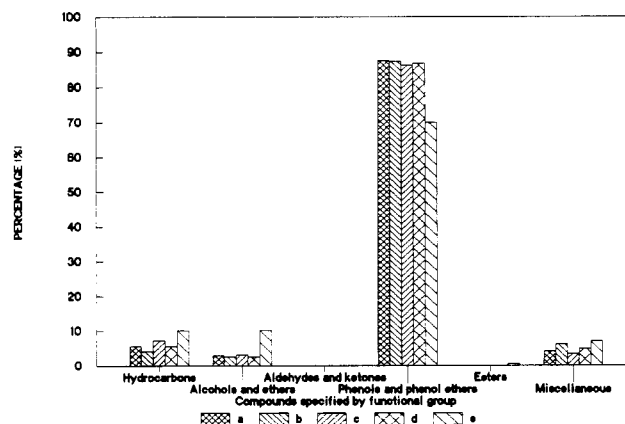
$$R^2 = 0.6974 \quad (R^2 = 0.9999)^a$$

^a Average transformed sensory scores were used in the analysis.

compounds, i.e., peaks 4, 5, 8–12, 17–21, 24–27, and 29–38, gave the preferable fragrant and fresh aroma. Among them, peak 24 (methylchavicol), being the major compound of Reunion type of basil oil, gave the best aroma. On the other hand, nine components, i.e., peaks 13–16, 22, 23, 28, 39, and 40, were found to possess a green and sour aroma and were not considered to be characteristic of basil oil. Of these compounds peak 16 (1-octen-3-ol) was the characteristic component possessing strongest green grass-like aroma. This compound also contributes significantly to the flavor of edible mushrooms such as *Agaricus campestris* (Tressl et al., 1982) and *Agaricus bisporus* (Chen and Wu, 1983). However, a comparison between odor assessment of peaks that have been identified by a spectroscopic method has one drawback. That is, it assumes that every peak is clean and that the aroma character assessed is that of the pure natural identified component of (in this case) basil oil. The fact that 1-octen-3-ol, a green, musty mushroom/fungal-smelling compound, was characterized as strongly green grasslike is indicative of more than one compound found under the same peak, of which 1-octen-3-ol could well be the major component yet a small component could well be contributing to the uncharacteristic aroma description. Similarly, *cis*-3-hexenol, which is characteristically strong smelling with a green character (it is known as leaf alcohol), was found to have a sour aroma.

Table III shows the percentage composition of the six kinds of basil oil, the results of which were the average of two determinations. The composition of basil stem oil was the most different compared with the others. This is not unexpected because the amount of methylchavicol (peak 24) that contributes to the preferable aroma was very low, while the amounts of peaks 13–16, 22, 23, 28, 39, and 40 which contribute to the green and sour aroma were very high. Consequently, the aroma of basil stem oil was the least acceptable.

Correlations among the volatile compounds of the oil

**Figure 2. Volatile compounds of five groups of basil oil according to Tukey's test.**

are shown in Table IV. The correlation between every two of the components was very high. For instance, methylchavicol (peak 24) had a significant negative correlation at the 5% level with peaks 1–3, 12–16, 18, 19, 22, 23, 27, 29, 30, and 32–40, respectively. This result indicates that there was a high level of mutual effect among volatile compounds during practicing sensory evaluation. As shown in Figure 2, the volatile components of basil oil were specified by their functional groups, and the six kinds of oil were divided into five groups according to the preference ranking test (Table IB). Concerning the "e" group, the percentages of phenols and phenol ethers decreased, while those of hydrocarbons, alcohols, and esters increased obviously, showing their mutual intimate correlations.

Table V shows the correlation between the sensory scores and the amounts of the volatile compounds of oil. Eleven compounds were found to be at the 5% significance level, i.e., peaks 21, 22, 26, 27, 29, 30, 33, 35, 36, and 40 with negative correlations but peak 24 with positive correlation. This result indicates that the richer the oil is in methylchavicol (peak 24), the more preferable the aroma is. However, there were some preferable aromas such as peaks 21, 26, 27, 29, 30, 33, 35, and 36 which had negative correlations. The reason for this was that when those preferable aromas were biosynthesized, the unacceptable ones such as peaks 13–16, 22, 39, and 40 were also produced coincidentally. However, the effect of the unacceptable aromas on sensory score was greater than that of the preferable ones. Thus, the biosynthesis of unacceptable aromas gave a critical contribution to preference.

To find the characteristic compounds that could be used to differentiate the six kinds of basil oil and evaluate the quality of basil oil, a stepwise regression method of analysis of the data was used. The percentage composition and the sensory score of the oils were transformed by arc-sine as independent variables and by the Fisher and Yates method as dependent variables, respectively. The result is shown in Table VI. Four components, i.e., 1-octen-3-ol (peak 16), α -muurolene (peak 27), germacrene B (peak 28), and methyleugenol (peak 31), were selected in the stepwise regression equation. The total R^2 was about 0.7, which means that this regression equation could explain ca. 70% of the differences between the six oils. The remaining 30% that cannot be explained by this equation was due to the differences among the panelists. The partial R^2 of these four compounds indicated that α -muurolene was the critical contributor to this equation followed by germacrene B, methyleugenol, and 1-octen-3-ol. The Y values of this regression equation for the basil oils obtained from various parts of the plant, such as leaf-flower (fresh),

whole plant, flower, leaf, leaf-flower (stored 10 days at 4 °C), and stem, were 0.939 (a), 0.652 (b), 0.196 (c), -0.255 (d), -0.343 (d), and -1.163 (e), respectively, cross referencing to Table IB. Therefore, the nearer the Y value to 0.939, the more preferable the quality of basil oil was or vice versa.

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Registry No. α -Pinene, 80-56-8; β -pinene, 127-91-3; sabinene, 3387-41-5; myrcene, 123-35-3; limonene, 138-86-3; β -phellandrene, 555-10-2; 1,8-cineole, 470-82-6; *cis*- β -ocimene, 3338-55-4; terpinolene, 586-62-9; 1-hexanol, 111-27-3; *cis*-3-hexenol, 928-96-1; 3-octanol, 589-98-0; 1-octen-3-ol, 3391-86-4; sabinene hydrate, 546-79-2; elixene, 3242-08-8; α -copaene, 3856-25-5; linalool, 78-70-6; *trans*- α -bergamotene, 13474-59-4; β -elemene, 515-13-9; aromadendrene, 72747-25-2; methylchavicol, 140-67-0; *trans*- β -farnesene, 18794-84-8; α -terpineol, 98-55-5; α -muurolene, 10208-80-7; germacrene B, 15423-57-1; cadina-1,4-diene, 29837-12-5; *trans*-anethole, 4180-23-8; methyleugenol, 93-15-2; dibutyl octanediote, 16090-77-0; viridiflorol, 552-02-3; eugenol, 97-53-0; T-cadinol, 5937-11-1; α -cadinol, 481-34-5; β -eudesmol, 473-15-4; chavicol, 501-92-8; ethyl linolenate, 1191-41-9.