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Volatile Oil Compounds from Corms and Flowers of *Crocus vernus* L. Hill and Corms of *C. sativus* L.

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Abstract: This paper was chemical components of volatile oils from flowers and corms of *Crocus vernus* L. Hill, and corms of *Crocus sativus* L. were analyzed by GC-MS. Furthermore, volatile flavor compounds of corms were investigated by using aroma extract dilution analysis (AEDA), GC-Olfactmetry-MS (GC-O-MS) and odor activity values (OAV; ratio of concentration to odor threshold). The GC-MS analysis results showed that the volatile oil from corms of *C. vernus* mainly contained C_{14}-C_{18} fatty acids, and shared 87% compositional similarity with corms of *C. sativus*. However, there was a difference between *C. vernus* and *C. sativus*, *ar*-turmerone was detected in *C. venus*. Flowers contented more *ar*-turmerone (3.55%) than corms (trace), and 1-octadecanol acetate (1.10%) was detected only this part. On the other hand, the corms of *C. sativus* composed comparatively more carotenoid-derived monoterpene: safranal (1.21%), 2-(5H)-furanone (59.80%) and sesquiterpenes of the bisabolene skeltone than those of *C. vernus*. AEDA, GC-O-MS and OVA showed that volatile components of corms were the predominant odor of *C. vernus* were phenylacetaldehyde, 2(5H)-furanone and nonanal. Beside, 2(5H)-furanone, (*E*)-2-methyl-2-butenal, 1-octen-3-ol, safranal and phenylacetaldehyde were strongly flavor in *C. sativus*. It was considered that 2(5H)-furanone and phenylacetaldehyde were typical flavor of *C. vernus* and *C. sativus*.

Key words: Corms of genus *Crocus* % *Crocus vernus* L. Hill % *Crocus sativus* L. % Volatile flavor components % AEDA % GC-O-MS % OVA

INTRODUCTION

The genus *Crocus* is a perennial herb in the family Iris (Iridaceous) which is grown in southern Europe, Mediterranean basin, and Asia Minors [1]. More than 80 species are known, of which approximately 30 are cultivated. *Crocus sativus* L. is commonly known as "Saffron" (dried long-dark-red stigma of the plant), blooming as a temperature autumn ephemeral. The saffron is used as a most expensive spice for seasoning and coloring agents or as folk medicine for various purposes such as antidepressant or menopausal symptoms [2]. Since the parts of stigmas are rich in carotenoids like crocin, a lot of carotenoid-derived components *via* cleavage as safranal were obtained from volatile oils [3-6]. In recent studies, it was known that saffron has many bioactivities like prevention of PC-12 cell death, cancer chemo preventive and tumoricidal properties [7, 8]. In contrast, few reports about chemical components of other series are presented [9]. Almost series are used only ornamental purpose. One of these series is *Crocus vernus* L. Hill, which is generally known as "Dutch saffron" or "Spring Crocus". This plant is a presentive spring bloomer and has an advantage that reduces variability between individuals among breeding and selection [10]. However, no volatile components from any parts of *C. vernus* were reported. It requires study on the *C. vernus* plant chemical components in order to determine the principal classes of compounds.

These plants are grown from corms. It is known that chemical components such as stigmasterol, palmitic, oleic and linolenic acids were extracted and TD-GC-MS and *e*-nose analysis detected several aliphatic acids and hydrocarbons in corms of *C. sativus*. Previous research did not detect volatile flavor components from corms of *C. sativus* [11-12]. However, almond like flavor has occurred when corms were boiled. Similar flavor was occurred in corms of *C. sativus*. This disadvantage of the static technique can be overcome, when the odor present in the plants are first screened by AEDA and are

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subsequently identified. The odor qualities of most of the components are known from the AEDA, these odors are easily recognized by GC-O-MS of the volatile oils. The concept of AEDA was applied to indicate the components that are mainly responsible for the odor of two *Crocus* species.

The objective of the present study was to investigate the volatile flavor components from corms of *C. vernus* and *C. sativus*, and characterized *C. vernus* flowers. To best of our knowledge, this is first report of volatile oil from *C. vernus*.

MATERIALS AND METHODS

Plant Material and Isolation Procedure: The corms of *C. vernus* and *C. sativus* were purchased from Oita prefecture in Japan on August 2008 and March 2009, respectively. Samples were aged about 4 months from flowering period (May-April 2008 and October-November 2007 respectively) before extraction. *C. vernus* flowers were purchased from Hokkaido prefecture in Japan on April 2009. Volatile oils were prepared using steam distillation for 3 hours. The yields (w/w) of volatile oils were as follow: *C. vernus* corms, 12 mg (0.0034%); *C. vernus* flower, 1.0 mg (0.053%); *C. sativus* corms, 13 mg (0.0021%). The oils from these plans were all colorless. On the absolute abundance, the corms of *C. vernus* produced more yields than corms of *C. sativus*, and flowers of *C. vernus* gained 15 times yields than the corms.

Chemicals: Nonanal, 1-octen-3-ol and alkans (C_8-C_{26}) were purchased from Sigma Aldrich (Tokyo, Japan). Safranal was given by Firmenich (Suitland).

AEDA: The flavor dilution (FD)-factor of the odor in the volatile oil was determined by AEDA of following dilution series. The highest dilution was defined as FD-factor 1 (10 mg/ml). The oil was stepwise diluted (1+1, v/v) by addition at which an individual component could be detected and was defined as the FD-factor for that odor.

GC: GC was performed on Agilent Technologies 6890N equipped with a flame ionization detector (FID). GC analysis was performed on a capillary column (HP-5MS, 30 m × 0.25 mm i.d., film thickness 0.25 μm). The column temperature was programmed from 60°C to 290°C at 5°C /min and held at 290°C for 5 min. The injector and detector temperatures were 280 and 290°C, respectively. The flow rate of the carrier gas (helium) was 3.0 ml/min.

GC-MS: GC-MS analysis of VO was carried out with an Agilent Technologies 6890N-Agilent Technologies 5973A. GC conditions were equipped on two capillary columns (HP-5MS, 30 m × 0.25 mm i.d., film thickness 0.25 μm and DB-WAX, 15 m × 0.25 mm i.d., film thickness 0.25 μm). The column temperature was programmed from 60 °C to 290°C at 5°C /min and held at 290°C for 5 min. The injector and detector temperatures were 270 and 290°C, respectively. The flow rate of the carrier gas (helium) was 3.0 ml/min with the actual temperature in the MS source reaching approximately 240°C and the ionization voltage 70 eV. Acquisition mass range was 30-450.

RI and Odor Quality: The identification of volatile compounds was done by matching odor descriptions and RI values on both polar and non-polar columns with those of retention indices experimentally (RIE) having the same/similar odor quality, their mass spectral patterned and RI with our previously reports [13-21]. Alkans (C_8-C_{26}) were analyzed under the same conditions to calculate RIE.

GC-O-MS: GC-O-MS analysis of volatile oil was carried out with an Agilent Technologies 6890N-Olfactory Detection port 2-Agilent Technologies 5973A. GC condition was equipped on a capillary column (HP-5MS, 30 m × 0.25 mm i.d., film thickness 0.25 μm). The column temperatures was programmed from 60°C to 290°C at 5°C /min and held at 290°C for 5 min. The injector and detector temperature were 270 and 290°C, respectively. The flow rate of the carrier gas (helium) was 3.0 ml/min with the actual temperature in the MS source reaching approximately 240°C and the ionization voltage 70 eV. Acquisition mass range was 30-450.

Quantification of Volatile Flavor Components: The quantitative analysis was performed on the basis of calibration for 1-octen-3-ol (peak number 6), nonanol (peak number 9) and safranal (peak number 11) within the six levels: 30, 60, 125, 250, 500 and 1000 μg/ml in diethyl ether.

RESULTS AND DISCUSSION

The volatile oils which were extracted by the steam distillation of flowers and corms of *C. vernus*, and corms of *C. sativus*, were analyzed by GC-MS. The identified components their RI and percentage of contents were compared and listed in Table 1. *C. vernus* shared 87% (rate of the same compounds) of compositional similarity

Table 1: Compositions of volatile oil from corms relative to total ion

No.	[a]	RIE-5	RIE-W	Content % *C. vernus* corms	*C. vernus* flowers	*C. sativus* corms
1	(*E*)-2-Methyl-2-	784	1050	Tr	Tr	**8.44**
2	Ethyl	808	-	Tr	Tr	0.43
3	(*E*)-2-	849	1191	Tr	Tr	0.17
4	1-	862	1380	0.13	Tr	Tr
5	2(5H)-	915	1216	0.34	-	**59.80**
6	1-Octen-3-	964	1252	Tr	-	0.26
7	2-	990	-	Tr	-	0.12
8	Phenylacetaldehyd	1043	1660	0.16	Tr	0.58
9	Nonanal	1103	1365	0.15	Tr	0.19
10	Pinocarvon	1162	-	Tr	-	-
11	2,6,6-Trimethyl-1,3- 1-carboxaldehyde	1199	-	Tr	Tr	1.21
12	(*E*)-*p*-Menthan-2-	1212	-	-	2.96	-
13	Decanol	1269	1490	Tr	**5.11**	Tr
14	Tetradecan	1400	1400	Tr	Tr	Tr
15	ب	1420	-	-	-	0.15
16	*ar*-	1482	1632	-	-	0.23
17	ب	1494	1670	-	-	0.72
18	Pentadecan	1500	-	Tr	0.70	Tr
19	ب Bisabolen	1507	-	-	-	Tr
20	ب	1523	1701	-	-	0.49
21	*ar*-Turmerone	1665	1955	Tr	3.35	-
22	Tetradecanoic	1760	2630	Tr	Tr	0.32
23	Octadecan	1800	1800	Tr	2.39	Tr
24	Pentadecanoic	1862	-	0.36	Tr	0.32
25	Nonadecan	1900	1900	Tr	**28.09**	Tr
26	Hexadecanoic	1987	2805	**42.53**	4.43	**15.52**
27	Heptadecanoic	2064	3044	0.22	Tr	3.98
28	Eicosane	2100	2100	Tr	1.05	Tr
29	(*Z, Z*)-9,12-Octadecadienoic	2159	3164	**51.81**	2.11	**4.74**
30	(*Z*)-9-Octadecanoic	2164	3099	0.20	1.84	1.74
31	Docosane	2200	2200	Tr	3.98	Tr
32	1-Octadecanol	2219	-	-	1.10	-
33	Tetracosane	2400	2400	0.13	**8.05**	Tr
34	Pentacosan	2500	2500	0.16	7.04	Tr
35	Hexacosan	2600	2600	1.02	6.91	Tr
36	Heptacosan	2700	2700	0.12	6.23	0.46
37	Octacosan	2800	2800	**2.55**	**10.46**	Tr
38	Nonacosan	2900	2900	Tr	1.45	Tr
	Total (%)			100	97.25	100

Major compounds were shown in

[a] Compounds were identfied Mass spectra and

[b] RIE-5 and RIE-W experimentally determined retention indices $_{8}$-C_{26} alkanes on HP-5M DB-WAX capillary column,

[c] Content percentages are calculated in GC on HP-5MS column. tr, trace

with *C. sativus* in corms. Total of 31 components were identified in the volatile oil of corms of *C. vernus*. The major components were (*Z,Z*)-9,12-octadecadienoic acid (51.21%); hexadecanoic acid (42.53%); octacosane (2.55%) and hexacosane (1.02%). On the other hand, total of 34 components were detected in the volatile oil of corms of *C. sativus*. The main compounds were 2-(5H)-furanone (59.80%); hexadecanoic acid (15.52%); (*E*)-2-methyl-2-butenal (8.44%) and (*Z,Z*)-9,12-octadecadienoic acid (4.74%). Volatile oil from flowers of *C. vernus* showed total of 29 components, in which nonadecane (28.09%), octacosane (10.46%), tetracosane (8.05%) and decanol (5.11%) were mainly constituents. These data indicated that the major components of the volatile oil from corms were C_{14}-C_{18} fatty acids. In the volatile oil from flowers of *C. vernus*, C_{14}-C_{29} hydrocarbons were found to be the main components. On the relative abundance, corms of *C. vernus* abounds (*Z,Z*)-9,12-octadecadienoic acid. In contrast, decanol, pentadecane (0.70%) and *ar*-turmerone (3.35%) were more abundant in flowers of *C. vernus*. 1-Octadecanol acetate (1.10%) was not found in the corms. On the other hand, several compounds like 2(5H)-

Table 2: Classifical analysis fo the volatile oils from *C. vernus* and *C. sativus*

Compounds		Content (%)[a]		
		C. vernus		*C. sativus*
		Corms	Flowers	Corms
Terpenoids	Subtotal	Tr	6.31	2.80
	Monoterpenoids	Tr	2.96	1.21
	Sesquiterpenoids	Tr	3.35	1.59
Aliphatics	Subtotal	99.84	90.94	96.62
	Hydrocarbons	3.98	50.62	0.33
	Alcohols	0.13	5.11	0.26
	Aldehydes	0.27	Tr	8.93
	Esters	Tr	1.10	0.43
	Acids	95.12	36.47	26.62
	Lactones	0.34	-	59.80
Aromatics	Subtotal	0.16	Tr	0.58
	Aldehydes	0.16	Tr	0.58
	Eters	Tr	-	0.12
Total		100	97.25	100

Table 3: Odors of corms of *C. vernus* and *C. sativus* identified on the basis of AEDA and OVA

No.	RI	Compounds	Odor description	FD-factor		Relative concentration (ng/g)[a]		Odor threshold (ng/g)[b]	OAV[c]	
				C. vernus	*C.sativus*	*C. vernus*	*C. sativus*		*C. vernus*	*C. sativus*
1	784	(*E*)-2-methyl-2-butenal	pungent	-	8	-	280	400[e]	-	<1
2	808	ethyl acetate	sweet	-	2	Tr	14	960[e]	-	<1
5	915	2(5h)-furanone	bitter	2	16	33.8	2000	Na[d]	-	-
6	964	1-octen-3-o1	mushroomy	1	2	Tr	9.3	1.0	-	9.3
7	990	2-pentyl furan	almond	1	2	Tr	3.9	6.0	-	<1
8	1043	phenylacetaldehyde	almond	4	8	18.9	20	4.0	4.7	5
9	1103	nonanal	fatty green	2	2	18.6	4.7	1.0	18.6	4.7
11	1199	safranal	herbaceous	-	4	Tr	40	Na	-	-

[a]Data were means of at least three assays.

[b]Threshold value were express as (ng/g) and unless otherwise stated, were measured as reported by Gengjun Chen, *et al* [22].

[c](OAV; conc. / odorthreshold) Odor active value, ratio of concentration to odor threshold.

[d]From G. Luna *et al* [23].

[e]Na: not available.

furanone were not detected in the flowers. In corms of *C. sativus*, 2-(5H)-furanone was rich and it is considered as a key agents for determination of specific difference since its contents reached over 59.80% in corms of *C. sativus* and accounted for 0.34% in the corms of *C. sativus*. Furthermore, *C. sativus* contained safranal, more amount of (*E*)-2-methyl-2-butenal and sesquiterpenes of the bisabolene skelton, *ar*-curcumene, *ar*-turmerone, "-zingibellene, and $-sesquiphellandrene. It is worthy of special mention that VO from *C. sativus* corms includes 0.04 µg/g (weight-safranal /weight-volatile oil) content of safranal, which was calculated from the peak area in the GC-FID, used internal standard (safranal). It was detcted in stigmas of *C. staivus*, however, not identified from past report [12]. This suggests safranal delivered from picrocrocin which was hydrolyzed by the action of heat by steam distillation in corms of *C. sativus*, and further identification studies are needed because of picrocrocin was not still detected in past report [11].

The classification analysis of the components of volatile oil from these plants was shown (Table 2). The data indicated that compounds in volatile oil of *C. vernus* corms were almost aliphatic acids (95.12%). These compounds were only about one third abundances; alternatively, lactones were occupied about 59.80% in corms of *C. sativus*. Additionally, corms of *C. sativus* contained more aliphatic aldehydes (8.93%) and terpenoids (2.80%). In flowers of *C. vernus*, major compounds were aliphatic hydrocarbons (50.62%) and aliphatic acids (36.47%). Aromatic compounds were the lowest materials in these plants: corms of *C. vernus*

(0.16%); corms of *C. sativus* (0.58%); flowers of *C. vernus* (trace). These data indicate considerable differences between *C. vernus* and *C. sativus* in corms from the constituents of volatile oil point of views.

The volatile flavor compounds (FD-factor of =1) from plants by AEDA, relative concentrations and OAVs were listed (Table 3). As a result, 5 and 8 compounds were identified from corms of *C. vernus* and *C. sativus*, respectively. However, no compound was detected in the flowers of *C. vernus*. The corms of *C. vernus* were covered a variety of odor properties, 2(5H)-furanone (bitter), 1-octen-3-ol (mushroom), 2-pentylfuran (almond), phenylacetaldehyde (almond) and nonanal (fattygreen). The highest FD-factor of 4 is phenylacetaldehyde. 2(5H)-furanone (FD=2) and nonanal (FD=2) were followed. The same odor compounds were identified in corms of *C. sativus*. Furthermore, (E)-2-methyl-butenal (pungent) and safranal (herbaceous) were detected. The high FD-factors were such as 2(5H)-furanone (FD=16), (E)-2-methyl-butenal (FD=8) and phenylacetaldehyde (FD=8). By comparison of the results from AEDA, it considered typical almond like odor from the corms of two *Crocus* species was caused by 2(5H)-furanone and phenylacetaldehyde. However, OVAs showed that nonanal and 1-octen-3-ol were more valueable compounds than phenylacetaldehyde in *C. vernus* and *C. vernus*, respectively. The disparity between OVAs and FD-factors cannot be readily explained, but might be due to errors relative quantification in AEDA as well in the odor thresholds values used for calculation of OVA [22]. It considered that nonanal and 1-octen-3-ol were also contributed in each species.

In summary, this paper identified the chemical component from the volatile oil of corms and flowers of *C. vernus*, and *C. sativus* using GC-MS. In corms of *C. sativus*, safranal which was obteined from stigma was detected (0.04 µg/g). Furthermore, volatile odor compositions were identified by AEDA, GC-O-MS and OAV. The results showd the predominant odors of *C. vernus* were phenylacetaldehyde, 2(5H)-furanone and nonanal. Beside, 2(5H)-furanone, (*E*)-2-methyl-2-butenal, 1-octen-3-ol, safranal and phenylacetaldehyde were strongly flavor in *C. sativus*. This suggested that 2(5H)-furanone and phenylacetaldehyde were the typical flavor of corms of two *Crocus* species.

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