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COMPARATIVE ANALYSIS OF VOLATILE COMPOUNDS FROM CORMS OF *Crocus sativus* AND *C. vernus*

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Crocus is a genus of cormous plants in the Iridaceae that grow naturally in southern Europe, the Mediterranean basin, and Asia Minor [1]. *Crocus sativus* L. is the source of saffron. The stigma is rich in carotenoids, such as crocin, and many carotenoid-derived components generated via cleavage, such as safranal, occur among the volatile oils (VO) [2–5]. Recent studies have shown that saffron performs many bioactivities, such as preventing PC-12 cell death, and has cancer chemopreventive and tumoricidal properties [6, 7]. The majority of *Crocus* species are grown for ornamental purposes only, and few studies of these species have investigated their chemical components [8]. One of the most well-known species, *Crocus vernus* (L.) Hill, which is commonly known as Dutch saffron or spring crocus, is an attractive spring-flowering species. Given optimum conditions, plants readily undergo vegetative reproduction by corm multiplication [9]. Although *Crocus* species are popular ornamentals, no analysis of the chemical components from any parts of the plant of most species has been reported.

In this study we analyzed the chemical components in the volatile fraction of *C. sativus* and *C. vernus*. We paid particular attention to the corm because that portion of the plant is available in all seasons. Previously, chemical components such as campesterol, stigmasterol, β-sitosterol, and ursolic, oleanolic, palmitic, palmitoleic, oleic, linoleic, and linolenic acids were extracted from *C. sativus* corms, and the volatile fraction was analyzed by TD-GC-MS and e-nose [10, 11]. However, previous studies did not detect carotenoid-derived components such as safranal from *C. sativus* corms. The present study used steam distillation (SD) because isolation of plant compounds by this method is relatively simple and a broad spectrum of temperature-sensitive compounds is isolated. In addition, we compared the VO components isolated from *C. vernus* and *C. sativus* corms and characterized the chemical composition of *C. vernus* flowers. This is the first reported analysis of VO isolated from *C. vernus*.

In terms of absolute abundance, higher yields of compounds were obtained from *C. vernus* corms than from *C. sativus* corms, and 15-fold higher yields were gained from *C. vernus* flowers than from corms. Identified components and their percentage contents are listed in Table 1. The VO components were identified by direct comparison of their mass spectral patterns and retention indices with those obtained in our previous works [12–15]. A total of 31 components was identified in the VO from *C. vernus* corms. The major components were (*Z,Z*)-9,12-octadecadienoic acid (51.21%), hexadecanoic acid (42.53%), octacosane (2.55%), and hexacosane (1.02%). Thirty-four components were extracted in the VO from *C. sativus* corms. The main compounds were 2(5*H*)-furanone (59.80%), hexadecanoic acid (15.65%), (*E*)-2-methyl-2-butenal (8.44%), and (*Z,Z*)-9,12-octadecadienoic acid (4.74%). Twenty-nine components were identified in the VO from flowers of *C. vernus*; the main constituents were nonadecane (28.09%), octacosane (10.46%), tetracosane (8.05%), and decanol (5.11%). No carotenoid-derived component was detected. These data indicated that the major components of the VO from corms were C_{14}–C_{18} fatty acids, whereas the main components in the VO from *C. vernus* flowers were C_{14}–C_{29} hydrocarbons.

With regard to the corms, *C. vernus* shared 91% compositional similarity with *C. sativus*. Compounds identified included (*E*)-2-methyl-2-butenal and sesquiterpenes of the bisabolene skeleton, *ar*-curcumene, *ar*-turmerone, α-zingiberene, and β-sesquiphellandrene. It is worthy of special mention that the VO from *C. sativus* corms included 0.04 μg/g safranal, which was calculated from the peak area in the GC-FID of safranal used as an internal standard and was not detected in previous reports [11]. It is hypothesized that safranal was derived from picrocrocin that was hydrolyzed by the action of heat during SD. This result indicated that picrocrocin was present in *C. sativus* corms.

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TABLE 1. Compositions of Volatile Oils from *C. vernus* and *S. sativus* (content, %[c])

Compound[a]	RIE-5[b]	RIE-W[b]	*C. vernus*		*C. sativus*
			corms	flowers	corms
(*E*)-2-Methyl-2-butenal	784	1050	Tr.	Tr.	**8.44**
Ethyl acetate	808	–	Tr.	Tr.	0.43
(*E*)-2-Hexanal	849	1191	Tr.	Tr.	0.17
Hexanol	862	1380	0.13	Tr.	Tr.
2(5*H*)-Furanone	915	1216	0.34	–	**59.80**
1-Octen-3-ol	964	1252	Tr.	–	0.26
2-Pentylfuran	990	–	Tr.	–	0.12
Phenylacetaldehyde	1043	1660	0.16	Tr.	0.58
Nonanal	1103	1365	0.15	Tr.	0.19
Pinocarvone	1162	–	Tr.	–	–
2,6,6-Trimethyl-1,3-cyclohexadiene-1-carboxaldehyde (safranal)	1199	–	Tr.	Tr.	1.21
(*E*)-*p*-Menthan-2-one	1212	–	–	2.96	–
Decanol	1269	1490	Tr.	**5.11**	Tr.
Tetradecane	1400	1400	Tr.	Tr.	Tr.
β-Caryophyllene	1420	–	–	–	0.15
ar-Curcumene	1482	1632	–	–	0.23
α-Zingiberene	1494	1670	–	–	0.72
Pentadecane	1500	–	Tr.	0.70	Tr.
β-Bisabolene	1507	–	–	–	Tr.
β-Sesquiphellandrene	1532	1701	–	–	0.49
ar-Turmerone	1665	1955	Tr.	3.35	–
Tetradecanoic acid	1760	2630	Tr.	Tr.	0.32
Octadecane	1800	1800	Tr.	2.39	Tr.
Pentadecanoic Acid	1862	–	0.36	Tr.	0.32
Nonadecane	1900	1900	Tr.	**28.09**	Tr.
Hexadecanoic acid	1987	2805	**42.53**	4.43	**15.65**
Heptadecanoic acid	2064	3044	0.22	Tr.	3.98
Eicosane	2100	2100	Tr.	1.05	Tr.
(*Z,Z*)-9,12-Octadecadienoic acid	2159	3164	**51.81**	2.11	**4.74**
(*Z*)-9-Octadecanoic acid	2164	3099	0.20	1.84	1.74
Docosane	2200	2200	Tr.	3.98	Tr.
1-Octadecanol acetate	2219	–	–	1.10	–
Tetracosane	2400	2400	0.13	**8.05**	Tr.
Pentacosane	2500	2500	0.16	7.04	Tr.
Hexacosane	2600	2600	1.02	6.91	Tr.
Heptacosane	2700	2700	0.12	6.23	0.46
Octacosane	2800	2800	**2.55**	**10.46**	Tr.
Nonacosane	2900	2900	Tr.	1.45	Tr.
Monoterpenoids			Tr.	2.96	1.21
Sesquiterpenoids			Tr.	3.35	1.59
$\Sigma_{Terpenoids}$			Tr.	6.31	2.80
Hydrocarbons			**3.98**	**76.35**	0.46
Alcohols			0.13	5.11	0.26
Aldehydes			0.27	Tr.	8.80
Esters			Tr.	1.10	0.43
Acids			**95.12**	**8.38**	**26.75**
$\Sigma_{Aliphatics}$			99.50	90.94	36.70
Aldehydes			0.16	Tr.	0.58
Frans			Tr.	–	0.12
Latones			0.34	–	**59.80**
$\Sigma_{Aromatics}$			0.50	Tr.	60.50
Total			100	97.25	100

Major compounds are shown in bold. [a]Compounds were identified by mass spectra and RI. [b]RIE-5 and RIE-W experimentally determined retention indices relative to C_8–C_{26} alkanes on HP-5MS and DB-WAX capillary column, respectively. [c]Content percentages are calculated in GC on HP-5MS column. Tr.: trace (< 0.1%).

The composition of the VO from both *Crocus* species is summarized in Table 1. The data indicate that compounds in *C. vernus* corms were mostly aliphatic acids (95.12%). These compounds comprised about 30% abundance in *C. sativus* corms, whereas lactones comprised about 60%. Additionally, *C. sativus* corms contained higher abundances of aliphatic aldehydes (8.93%) and terpenoids (2.80%). In *C. vernus* flowers, the major compounds present were aliphatic hydrocarbons (76.35%) and aliphatic acids (8.38%). Aromatic compounds were present as follows: *C. vernus* corms (0.50%), *C. sativus* corms (60.50%), and *C. vernus* flowers (Tr). These data indicate that considerable differences existed in the VO of *C. vernus* and *C. sativus* corms.

In summary, this study investigated the chemical components in the VO of corms and flowers of *C. vernus*, and characterized specific differences in the chemical constituents of the corms of *C. vernus* and *C. sativus*. In addition, safranal was detected in the VO isolated from *C. sativus* corms.

Plant Material. Corms of *C. sativus* were purchased from the Japan Agricultural Association in Oita Prefecture, Japan, in March 2009. The corms were purchased about 4 months after the flowering period (October–November 2008). Corms of *C. vernus* were purchased in August 2008 from a florists shop in Hokkaido Prefecture. Corms of *C. vernus* were planted and flowered in April 2009. The flowers were harvested at anthesis for chemical analysis.

Extraction and Analysis of the VO. The VO fraction was prepared by SD for 3 h. The VO yields were as follows: *C. vernus* corms, 12.4 mg (0.0034%); *C. vernus* flowers, 1.0 mg (0.0526%); and *C. sativus* corms, 13.2 mg (0.0021%). Compounds in the VO were determined from retention indices and mass spectra (MS). Gas chromatography was performed with an Agilent Technologies 5890A system equipped with a flame ionization detector. GC-MS analysis of the VO was carried out with an Agilent Technologies 6890N–5973A system. The GC was equipped with 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 280°C at 4°C/min and held at 280°C for 5 min. The injector and detector temperatures were 280°C 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 230°C, and the ionization voltage was 70 eV. The acquisition mass range was 30–450.

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