

Characterization by GC–MS of Vietnamese Citrus Species and Hybrids Based on the Isotope Ratio of Monoterpene Hydrocarbons

Nguyen Thi THAO,¹ Takehiro KASHIWAGI,² and Masayoshi SAWAMURA^{1,†}

¹Department of Bioresources Science, Faculty of Agriculture, Kochi University, B-200 Monobe, Nankoku, Kochi 783-8502, Japan

²JST Satellite Kochi, Japan Science and Technology Agency, 185 Miyanokuchi, Tosayamada, Kami, Kochi 782-8502, Japan

Received February 5, 2007; Accepted May 7, 2007; Online Publication, September 7, 2007

[doi:10.1271/bbb.70069]

The isotope ratio of monoterpene hydrocarbons was used to characterize the citrus essential oils from different species and hybrids. Citrus cold-pressed peel oils from Vietnam were analyzed for the composition and isotope ratio of monoterpene hydrocarbons by using gas chromatography-mass spectrometry. A profile of citrus essential oils on the basis of their isotope ratio values and levels of monoterpene hydrocarbons was developed for Vietnamese citrus. The molecular isotope ratios were lower than those calculated from natural abundance of ¹³C and ²H. In addition, the isotope ratio of the base peaks (*m/z* 94/93) was significantly different among the citrus essential oils from different species and hybrids. The results would be applicable for the characterization of citrus essential oils from different origins.

Key words: essential oil; citrus; isotope ratio; species characterization; GC–MS

Flavor authenticity control has been a concern of consumers, manufacturers and researchers in the past few years. The requirement of origin qualification for the flavor constituents has recently become important to the value of a product. An analysis of the isotope ratio is a powerful tool for assessing the authenticity of achiral flavor compounds.¹⁾ In this regard, highly sophisticated techniques are used in order to determine the authenticity of active compounds in a genuine aroma such as the site-specific natural isotope fraction detected by quantitative ²H-NMR and isotope ratio mass spectrometry online-coupled with an elemental analyzer or capillary gas chromatograph. Chemical element analysis and isotope ratio mass spectrometry have been conservatively used to measure the isotope ratio of carbon (¹³C/¹²C), hydrogen (²H/¹H) and oxygen (¹⁸O/¹⁶O).^{2–5)} The use of gas chromatography–pyrolysis–isotope ratio mass spectrometry (GC–P–IRMS) could determine the characteristic range of a flavor compound isotope values.⁶⁾

In the search for a common analytical method for flavor authentication, gas chromatography–mass spectrometry (GC–MS), which is widely used in analytical laboratories, was proposed to determine the isotope ratio of flavor compounds. Sawamura *et al.* have developed an analytical method by GC–MS for differentiating the quality of commercial yuzu fruits from various growing regions of Japan. The isotope fingerprint of Japanese yuzu (*Citrus junos* Tanaka) essential oil has also been established as a result of various studies.^{7–9)}

Citrus fruits are among the most easily hybridized species. It is natural that the composition of citrus essential oils often varies according to species, variety, ripening stage, growing place and other conditions.¹⁰⁾ However, the isotope ratio of compounds in a plant is a result of the isotope effect, genealogy and geological origin.⁷⁾ In addition to carbon isotope value $\delta^{13}\text{C}$, the hydrogen isotope plays an important role in the isotope analysis of a plant because ²H/¹H is a rich source of information. The isotope ratio of hydrogen varies over a wide range and depends on the isotopic variation of growing conditions.¹¹⁾ In addition, the isotope ratio, which is calculated on the basis of the intensities of the molecular peak (*m/z* 136) and its isotope peak (*m/z* 137) of monoterpene hydrocarbons, gives information about both carbon isotopes and hydrogen isotopes in the whole molecule of essential oil components. It is therefore reasonable to assume that this value would be characteristic for citrus species and that the distribution of isotope ratio in monoterpene hydrocarbon molecules among different citrus species would have a characteristic pattern.

Vietnam is located in a tropical area where the climatic conditions are favorable for the production of citrus fruits. Pummelo and orange are among the major fruits in Vietnam. Only compositional studies on citrus essential oils from Vietnam have so far been reported.¹²⁾ In addition, the fruits are now gaining reputation in international commercial markets, and the essential oils are also becoming an important source for foods,

† To whom correspondence should be addressed. Tel: +81-88-864-5184; Fax: +81-88-864-5200; E-mail: sawamura@cc.kochi-u.ac.jp

beverages and aroma therapy. It is therefore important to protect the genuineness of the products, and the isotope ratio is one of the criteria for determining their origins. The objective of this study is to examine the composition and isotope ratio of the monoterpene hydrocarbons in essential oils of Vietnamese pummelo (*C. grandis* Osbeck), lime (*C. aurantifolia* Swingle), orange (*C. sinensis* Osbeck), mandarin (*C. reticulata* Blanco) and their hybrids by using GC–MS. This is the first time the characterization of Vietnamese citrus essential oils has been reported on the basis of isotope ratio. The result will contribute to the authentication of Vietnamese citrus essential oils. In addition to evaluating the isotope ratio of the molecular peak, the isotope ratio of the base peak of monoterpene hydrocarbons is reported for the first time. This study will also illustrate the distribution of isotope ratio within monoterpene molecules and disclose the characterization of the citrus species and hybrids.

Materials and Method

Materials. Citrus fruits were collected from local wholesale markets from the southern to northern parts of Vietnam in November 2005. All of the samples were collected at the ripening stage and were of the best commercial quality. The sampling locations are shown in Fig. 1 and some specifications of samples are shown in Table 1. The first letter in the three-letter name of a sample is the abbreviated name of the species, for example, LDL represents lime from Dalat. Cold-pressed peel oil (CPO) was prepared as described in the previous paper.¹³ Each CPO sample was stored at -25°C until needed for analysis. Authentic chemicals for mass spectrometry were obtained from the commercial sources mentioned previously.¹³

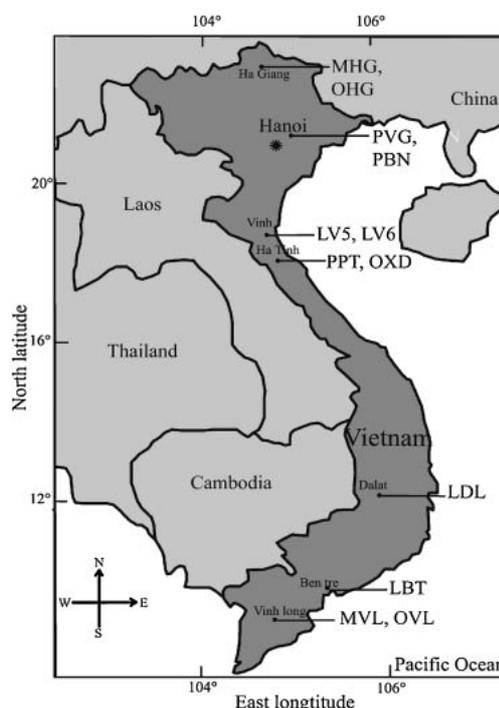


Fig. 1. Sampling Location for the Vietnamese Citrus Fruits.

Gas chromatography–mass spectrometry (GC–MS). The analysis was carried out with a GC-6890N instrument (Agilent Technologies) coupled with JMS-Q1000 GTA mass spectrometer (Jeol Datum) at an MS ionization voltage of 1500 V and ion source temperature of 250°C . The GC column was a DB-Wax fused-silica capillary type ($60\text{ m} \times 0.25\text{ mm i.d.}$, $0.25\text{ }\mu\text{m}$ film thickness; J & W Scientific, Folsom, CA, USA). The column temperature was programmed from 70°C (2-min hold) to 100°C at a rate of $2^{\circ}\text{C}/\text{min}$ and then heated to 230°C

Table 1. Citrus Samples

No.	Abbr. name	Local name	Scientific name	Place of sampling	Ave. fruit weight (g)	Yield of CPO (% fruit)
1	LDL	Da Lat lime	<i>Citrus aurantifolia</i> Swingle	Lam Dong province	53	0.03
2	LBT	Ben Tre lime	<i>C. aurantifolia</i> Swingle	Vinh Long province	33	0.07
3	LV5	Vinh Lime 2005	<i>C. aurantifolia</i> Swingle	Nghe An province	50	0.01
4	LV6	Vinh Lime 2006	<i>C. aurantifolia</i> Swingle	Nghe An province	50	0.01
5	PVG	Van Giang pummelo	<i>C. grandis</i> Osbeck	Hung Yen province	1400	0.07
6	PBN	Bac Ninh pummelo	<i>C. grandis</i> Osbeck	Bac Ninh province	900	0.09
7	PNR	Nam Roi pummelo	<i>C. grandis</i> Osbeck	Can Tho province	1200	0.07
8	PPT	Phuc Trach pummelo	<i>C. grandis</i> Osbeck	Nghe An province	1000	0.04
9	MVL	Vinh Long mandarin	<i>C. reticulata</i> Blanco	Vinh Long province	71	0.09
10	MHG	Ha Giang mandarin	<i>C. reticulata</i> × <i>C. sinensis</i>	Ha Giang province	94	0.07
11	OXD	Xa Doai orange	<i>C. sinensis</i> Osbeck	Ha Tinh province	192	0.04
12	OVL	Vinh Long orange	<i>C. reticulata</i> × <i>C. grandis</i>	Vinh Long province	156	0.19
13	OHG	Ha Giang orange	<i>C. reticulata</i> × <i>C. grandis</i>	Ha Giang province	208	0.12
14	ONR	Naruto orange	<i>C. medioglobosa</i> Hort. ex. Tanaka	Hyogo Prefecture, Japan	367	0.08

Abbr., abbreviated; Ave., average; CPO, cold-pressed oil

Table 2. Relative Peak Area Percentage of Monoterpene Hydrocarbons from Vietnamese Citrus Essential Oils

No.	Sample	α -Pinene	β -Pinene	Sabinene	Myrcene	α -Phellandrene	α -Terpinene	Limonene	β -Phellandrene	γ -Terpinene	Terpinolene
1	LDL	1.99	6.04	1.53	1.71	0.07	0.36	61.05	0.48	15.39	0.87
2	LBT	2.65	14.75	4.33	1.31	0.05	0.17	38.66	0.82	7.43	0.40
3	LV5	1.75	3.26	0.82	1.91	0.08	0.38	62.99	0.43	15.74	0.94
4	LV6	3.18	11.03	2.99	1.55	0.07	0.44	51.22	0.57	16.55	0.83
5	PVG	0.64	0.59	0.31	20.45	0.08	nd	70.73	0.51	0.45	nd
6	PBN	0.80	0.37	0.26	2.62	0.05	nd	93.14	0.46	0.25	nd
7	PNR	2.98	1.14	0.71	2.19	3.39	0.45	60.10	9.43	15.86	0.95
8	PPT	0.75	0.24	0.25	2.82	0.05	nd	92.00	0.50	0.70	nd
9	MHG	2.22	0.92	0.28	2.38	0.09	0.34	77.22	0.46	13.12	0.73
10	MVL	0.96	0.62	0.34	2.85	0.07	nd	93.61	0.55	0.04	0.15
11	OXD	0.83	0.05	1.01	0.31	2.87	0.10	92.26	0.60	0.07	0.11
12	OVL	1.03	nd	0.23	2.81	0.07	nd	94.99	0.57	nd	nd
13	OHG	0.95	nd	0.25	2.68	0.07	nd	94.93	0.54	0.06	nd

nd, not detected

(held for 15 min) for cleaning at the end of each run. The injector temperature was 250 °C, and helium was used as the carrier gas at a flow rate of 0.8 ml/min. An oil sample of 1 μ l, which had been diluted with acetone (1:5), was automatically injected at a split ratio of 1:100. To determine the oil composition, the total ion intensity in the ordinary scan mode was used. To determine the isotope ratio, on the other hand, the selected ion monitoring mode (SIM) was employed.

Identification of components. The individual components were initially identified by comparing Kovats retention indices, and then confirmed by the GC retention time of previously analyzed authentic compounds and by the mass spectra of the NIST library connected to the mass spectrometer.

Determination of the isotope ratio. The following ten monoterpene compounds were examined to determine the isotope ratio: α -pinene, β -pinene, sabinene, myrcene, α -phellandrene, α -terpinene, limonene, β -phellandrene, γ -terpinene and terpinolene. The isotope ratio was calculated on the basis of the intensity of molecular peak (m/z 136) and isotope peak (m/z 137). The isotope ratio of fragments which had been generated during mass bombardment was evaluated on the basis of the intensity of the fragment peak (m) and its isotope peak ($m+1$). In this study, the fragment having the highest intensity (base peak) was chosen so that the m/z 93 peak and its m/z 94 isotope peak were monitored. The SIM mode was employed to carry out the MS analysis in order to increase the sensitivity. The isotope ratio (Ir) was calculated by the following equation:

$$\text{Ir (\%)} = \left(\frac{\text{intensity of } m/z (m+1) \text{ isotope peak}}{\text{intensity of } m/z (m) \text{ peak}} \right) \times 100$$

where the intensity is the mean value from triplicate measurements.

Statistical analysis. All measurements were taken in

triplicate, the average value and standard deviation of the isotope value being calculated in order to evaluate the repeatability of the method. A principal component analysis (PCA) was conducted to treat the obtained data. All the statistical analyses were done by SPSS software for Windows (version 11.5, SPSS, Chicago, 2002).

Results and Discussion

Monoterpene hydrocarbon composition

Citrus essential oil is composed of a few hundred compounds¹⁰ in which monoterpene hydrocarbons are among the most abundant components. We investigated in this study the isotope ratio of ten monoterpene hydrocarbons which have the same molecular formula of $C_{10}H_{16}$. All the monoterpene hydrocarbons have a nominal molecular weight of 136; they were well separated, identified and quantified by GC-MS. Table 2 shows the concentrations of the monoterpene hydrocarbons from 13 essential oil samples collected in Vietnam. There was a variation in monoterpene hydrocarbon concentration among those samples. However, limonene was always present with the greatest level in all of the essential oils. The concentration of limonene varied from 38.66% in LBT to 94.99% in OVL. The essential oils of orange, mandarin and a hybrid of mandarin contained the highest proportion of limonene, while this proportion was the lowest in lime oils. This result is similar to the observation from previous studies.^{10,12}

Concerning the compositional variation within one species, we can see that lime oils had a considerably different distribution of monoterpene hydrocarbons. The variation in these compounds' concentrations from different regions was significant, especially in the level of β -pinene and γ -terpinene. On the other hand, the essential oils from the hybrids of pummelo and mandarin (OHG and OVL) had a similar composition, even though they originated from different areas. In this regard, compositional information cannot provide a

Table 3. Isotope Ratio of the Molecular Peak (m/z 137/136) of Monoterpene Hydrocarbons from Vietnamese Citrus Essential Oils

No. Samples	α -Pinene	β -Pinene	Sabinene	Myrcene	α -Phellandrene	α -Terpinene	Limonene	β -Phellandrene	γ -Terpinene	Terpinolene
1 LDL	10.50 ± 0.06	10.87 ± 0.09	10.72 ± 0.12	10.09 ± 0.14	9.21 ± 0.24	10.43 ± 0.01	11.06 ± 0.04	10.39 ± 0.04	10.98 ± 0.07	10.68 ± 0.05
2 LBT	10.61 ± 0.02	11.03 ± 0.02	10.91 ± 0.03	9.92 ± 0.12	8.90 ± 0.39	10.21 ± 0.05	11.03 ± 0.05	10.63 ± 0.04	10.90 ± 0.02	10.53 ± 0.03
3 LV5	10.60 ± 0.01	10.77 ± 0.05	10.60 ± 0.07	10.23 ± 0.13	9.30 ± 0.23	10.46 ± 0.06	11.16 ± 0.12	10.43 ± 0.13	10.99 ± 0.07	10.72 ± 0.08
4 LV6	10.67 ± 0.05	11.06 ± 0.13	10.88 ± 0.05	10.25 ± 0.24	9.53 ± 0.27	10.58 ± 0.09	11.17 ± 0.14	10.63 ± 0.14	11.05 ± 0.13	10.74 ± 0.10
5 PVG	10.14 ± 0.10	10.13 ± 0.07	9.97 ± 0.19	10.97 ± 0.06	9.14 ± 0.08	nd	11.13 ± 0.12	10.55 ± 0.11	nd	nd
6 PBN	10.19 ± 0.02	9.67 ± 0.19	9.80 ± 0.17	10.19 ± 0.08	8.12 ± 0.28	nd	11.18 ± 0.06	10.47 ± 0.09	nd	nd
7 PNR	10.63 ± 0.03	10.57 ± 0.09	10.63 ± 0.08	10.24 ± 0.18	10.92 ± 0.01	10.59 ± 0.07	11.23 ± 0.16	11.02 ± 0.04	11.11 ± 0.04	10.78 ± 0.05
8 PPT	10.31 ± 0.12	9.22 ± 0.26	9.79 ± 0.20	10.26 ± 0.11	8.41 ± 0.37	nd	11.10 ± 0.31	10.63 ± 0.14	9.18 ± 0.92	nd
9 MHG	10.55 ± 0.02	10.37 ± 0.10	10.01 ± 0.17	10.22 ± 0.17	9.17 ± 0.35	10.34 ± 0.10	11.21 ± 0.14	10.38 ± 0.15	11.00 ± 0.03	10.66 ± 0.06
10 MVL	10.41 ± 0.01	10.21 ± 0.13	10.20 ± 0.10	10.41 ± 0.11	10.60 ± 0.43	nd	11.30 ± 0.04	10.52 ± 0.06	nd	nd
11 OXD	10.31 ± 0.11	nd	10.67 ± 0.05	10.44 ± 0.13	9.48 ± 0.20	nd	11.25 ± 0.18	10.62 ± 0.00	nd	9.62 ± 0.25
12 OVL	10.47 ± 0.05	nd	9.66 ± 0.06	10.24 ± 0.03	8.60 ± 0.11	nd	11.14 ± 0.16	10.44 ± 0.03	nd	nd
13 OHG	10.26 ± 0.15	nd	9.93 ± 0.06	10.39 ± 0.00	9.12 ± 0.13	nd	11.27 ± 0.06	10.57 ± 0.04	nd	nd

nd, not detected; ±, standard deviation

definite differentiation of the origins of citrus essential oils.

Isotope ratio of monoterpene hydrocarbons in citrus essential oils

The relative abundance of ^{13}C and ^2H over ^{12}C and ^1H in certain monoterpene hydrocarbon molecules was determined by the ratio of the ion intensity of the two masses at m/z 137 and m/z 136. This ratio reflects the difference in distribution ratio within one molecule of a monoterpene hydrocarbon.⁷⁾ When analyzing in the SIM mode, we can estimate the difference of ion intensities from the molecular and isotope peaks to high accuracy. The isotope ratio of each molecular peak (m/z 137/136) of the monoterpene hydrocarbons and the standard deviation are shown in Table 3. The isotope values for α -pinene in lime essential oils were higher than the others and did not vary very much (from 10.50% to 10.67%). Orange and pummelo had lower values (from 10.26% to 10.49% for orange and 10.14% to 10.63% for pummelo). In addition, the isotope ratio of limonene was from 11.03% to 11.30%, which is lower than the theoretically calculated value from natural abundance of carbon and hydrogen (11.30%). This lower value (depleted heavier carbon and hydrogen isotopes) is a result of isotope fractionation and isotope effect which occurs along the biosynthetic pathway for plant materials.⁸⁾

It is interesting to note that the isotope ratio of monoterpene hydrocarbons varies in a narrow range, while the composition of essential oils from the same species varies substantially according to the location, growing stage, and variety. The concentration of limonene in the four lime samples, as shown in Table 2, was from 38.66% to 61.05% while the isotope ratio value for limonene varied from 11.06% to 11.17%. There was, in addition, little overlap in the isotope ratio value of one monoterpene hydrocarbon belonging to different species. On the other hand, it has been reported that the isotope ratio of monoterpene hydrocarbons was distinguishable among the species of lemon, yuzu and

lime.^{8,11)} Therefore, this result supports the conclusion that the isotope ratio of monoterpene hydrocarbons can be used for characterizing the essential oils.

Isotope ratio of the base peak (m/z 94/93)

For a given molecular species, significant differences have been observed as a function of the plant source.¹⁴⁾ There is a non-statistical distribution of ^{13}C and ^2H inside the monoterpene molecules. This results from genealogy and the isotope effect, so that there is a pattern that can be characteristic of the plant material. Consequently, we first investigated the isotope ratio which was calculated by the intensity of the base peak in order to differentiate the essential oils from different citrus species. Measurement of the base peak gives a greater sensitivity and more accurate results as well as better repeatability. For most of monoterpene hydrocarbons, the m/z 93 fragment is the base peak in their mass spectrum, accounting for 100% of relative intensity in the total ion chromatogram. Only limonene had a different base peak (m/z 68) in the mass spectrum. Figure 2 illustrates the fragmentation mechanism for limonene, myrcene and α -terpinene under the electron ionization condition in mass spectrometry to produce the $^{12}\text{C}_7^1\text{H}_9$ fragment (m/z 93). Similarly, the m/z 94 peak represents the $^{13}\text{C}^{12}\text{C}_6^1\text{H}_9$, $^{12}\text{C}_7^1\text{H}_8^2\text{H}$ and $^{12}\text{C}_7^1\text{H}_{10}$ fragments. Table 4 displays the isotope ratio of the base peak and its isotope peak (m/z 94/93), as well as the standard deviation of eight monoterpene hydrocarbons from citrus essential oils.

It can be seen from this result that there was a great difference in the isotope ratio values among the monoterpenes. The highest isotope ratio value of all the citrus essential oils can be seen in limonene (around 42%), and the lowest value in α -phellandrene (from 7.33% to 8.23%). On the other hand, the isotope ratio of the whole molecule (m/z 137/136) varied around 11% for all the samples (Table 3). The highest value which was observed for limonene might have resulted from not only the isotope ratio, but also the generation of the $^{12}\text{C}_7^1\text{H}_{10}$ fragment. Fortunately, the isotope ratio of the

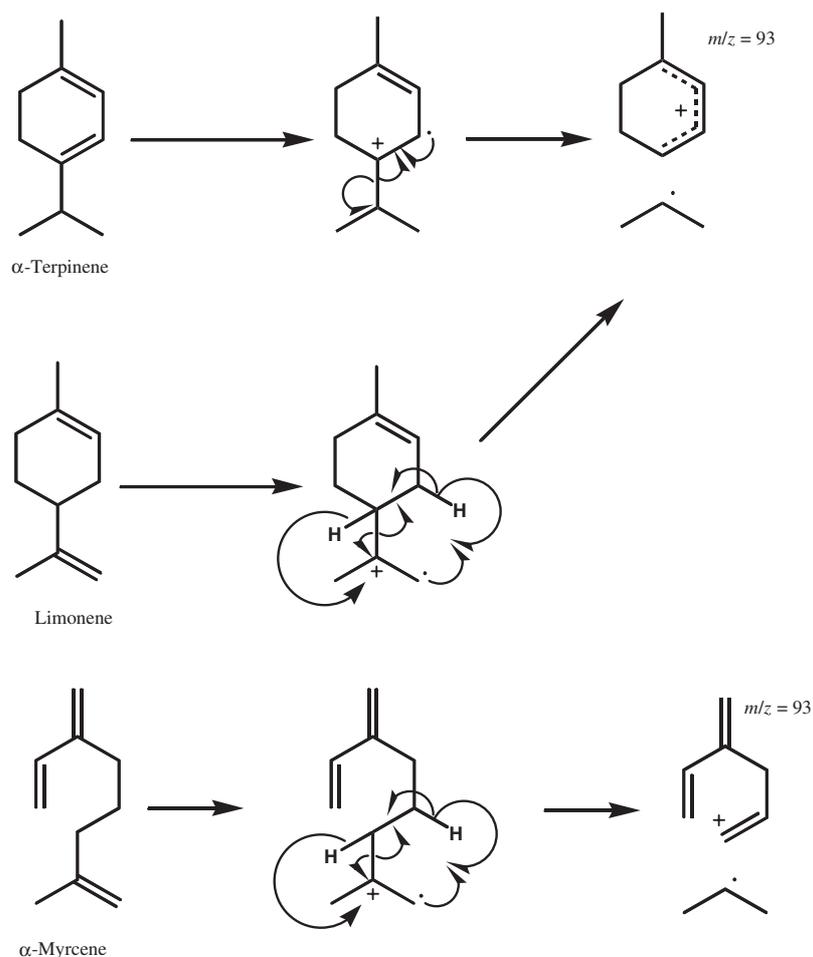


Fig. 2. Fragmentation Mechanism of Monoterpene Hydrocarbons for Producing the C_7H_9 Fragment (m/z 93) by Electron Ionization Mass Spectrometry.

Table 4. Isotope Ratio of Base Peak (m/z 94/93) of Monoterpene Hydrocarbons from Vietnamese Citrus Essential Oils

No.	Samples	α -Pinene	β -Pinene	Sabinene	Myrcene	α -Phellandrene	Limonene	β -Phellandrene	γ -Terpinene
1	LDL	9.43 \pm 0.02	13.76 \pm 0.26	13.96 \pm 0.58	10.11 \pm 0.05	7.66 \pm 0.13	41.67 \pm 0.47	14.63 \pm 0.58	9.36 \pm 0.04
2	LBT	9.43 \pm 0.04	13.99 \pm 0.07	14.11 \pm 0.43	10.08 \pm 0.06	7.33 \pm 0.40	42.15 \pm 0.67	14.64 \pm 0.52	9.42 \pm 0.04
3	LV5	9.27 \pm 0.10	13.79 \pm 0.27	14.04 \pm 0.49	10.14 \pm 0.03	7.65 \pm 0.16	41.54 \pm 1.21	14.79 \pm 0.09	9.46 \pm 0.13
4	LV6	9.36 \pm 0.04	14.15 \pm 0.17	14.18 \pm 0.40	10.14 \pm 0.07	7.84 \pm 0.23	41.24 \pm 1.02	14.79 \pm 0.03	9.68 \pm 0.17
5	PVG	9.57 \pm 0.01	13.35 \pm 0.03	13.39 \pm 0.03	11.84 \pm 0.01	7.91 \pm 0.59	42.62 \pm 0.38	14.18 \pm 0.06	11.24 \pm 0.02
6	PBN	9.61 \pm 0.06	13.31 \pm 0.02	13.31 \pm 0.02	10.16 \pm 0.02	7.77 \pm 0.42	42.83 \pm 0.42	14.19 \pm 0.02	11.16 \pm 0.03
7	PNR	9.47 \pm 0.07	13.40 \pm 0.02	13.48 \pm 0.04	10.17 \pm 0.01	8.23 \pm 0.03	42.40 \pm 0.32	14.39 \pm 0.39	9.55 \pm 0.25
8	PPT	9.59 \pm 0.12	13.26 \pm 0.06	13.31 \pm 0.05	10.17 \pm 0.02	8.10 \pm 0.43	42.65 \pm 0.01	14.42 \pm 0.13	11.34 \pm 0.04
9	MHG	9.35 \pm 0.11	13.35 \pm 0.02	13.35 \pm 0.04	9.82 \pm 0.67	7.76 \pm 0.17	42.45 \pm 0.13	14.13 \pm 0.01	9.38 \pm 0.08
10	MVL	9.61 \pm 0.06	13.35 \pm 0.02	13.40 \pm 0.03	10.17 \pm 0.02	7.58 \pm 0.25	42.72 \pm 0.43	14.28 \pm 0.12	11.09 \pm 0.78
11	OXD	9.59 \pm 0.02	13.07 \pm 0.07	13.45 \pm 0.03	10.20 \pm 0.00	8.06 \pm 0.27	43.07 \pm 0.24	14.19 \pm 0.02	11.93 \pm 0.13
12	OVL	9.63 \pm 0.12	12.65 \pm 0.12	13.28 \pm 0.02	10.17 \pm 0.02	7.58 \pm 0.02	42.53 \pm 0.05	14.14 \pm 0.04	12.75 \pm 0.33
13	OHG	9.57 \pm 0.11	12.79 \pm 0.08	13.34 \pm 0.02	10.18 \pm 0.01	7.68 \pm 0.06	43.07 \pm 0.04	14.18 \pm 0.00	11.35 \pm 0.59

other monoterpene hydrocarbons did not show the same behavior as limonene. Moreover, there was little variation in these values from one monoterpene of the same species. The isotope ratio for α -pinene in lime essential oils, for example, varied from 9.27% to 9.36%, whereas this value for pummelo was in the range of

9.47% to 9.59%. These results suggest the different distribution of ^{13}C and 2H at a different carbon position inside the monoterpene molecule. Considering the ratio of $^{13}C/^{12}C$ and $^2H/^1H$ at different sites in the monoterpene molecule, the isotope ratio values may be increased at certain carbon positions and depleted at

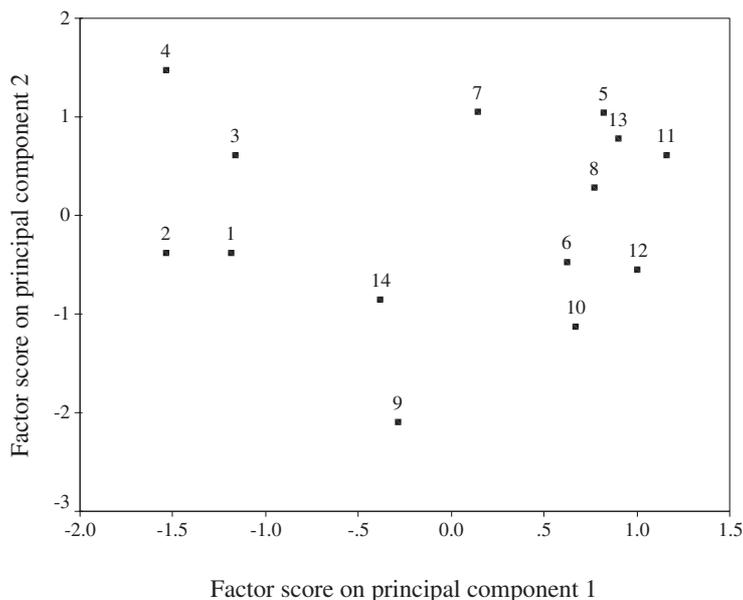


Fig. 3. Principal Component Analysis of Isotope Ratio m/z 94/93 of the Monoterpene Hydrocarbons. The numbers are the sample nos. shown in Table 1.

others. This notion is illustrated by the study on the fellowship of natural abundance ^2H -isotopomers of monoterpenes, for which Martin has reported that the value of $^2\text{H}/^1\text{H}$ differed at each carbon position.¹⁴⁾ On the other hand, going back to the biosynthetic pathway to monoterpenes, glucose is one of the main photosynthetic precursors, and there is evidence for a non-statistical carbon isotope distribution in natural glucose. The relative carbon isotope content at each position of glucose has been determined by revealing a defined and reproducible ^{13}C distribution in glucose from different origins.¹⁵⁾ Therefore, there should be different isotope ratios in monoterpenes from different citrus essential oils resulting from the characteristic distribution of an isotope within one molecule. In addition, variation in the form of a cleavage fragment according to individual compounds among monoterpene hydrocarbons will give more information for the differentiation of citrus essential oils.

Multivariate analysis

The isotope ratio values for the base peaks (m/z 94/93) with eight monoterpene hydrocarbons (Table 4) from 14 essential oils were subjected to a principal component analysis in order to find a relationship for these citrus essential oils. The result of the principal component analysis is displayed in Fig. 3 in which each point represents one citrus sample. The first two principal components accounted for 72% of the total variance of isotope ratio for the eight monoterpene hydrocarbons. In this analysis, lime oils could be clearly distinguished from the other oils by having a distinct location on the graph. Although orange and pummelos were placed in close proximity, they could be differentiated in this analysis. ONR was the only example

from Japan in this study and it is said to be a hybrid between pummelo and mandarin. The great distance between ONR and the same hybrid groups, OVL and OHG, from the PCA analysis might have been caused by the factor of geographical difference. In addition, mandarin sample MVL was located far from the other citrus samples, while the hybrid of mandarin (MHG) was at a location near to orange. This analysis for the base peak also gave a similar result to that from an analysis of the isotope ratio of the molecular peak.

In conclusion, characterization of the main Vietnamese citrus essential oils was established with regard to the concentration, and to the isotope ratios of the molecular and base peaks of each monoterpene hydrocarbon. In addition, we tried to find possible proof in recognizing the essential oils from different citrus species. The concentration of monoterpene hydrocarbons in citrus essential oils from the same species varied considerably, but the isotope ratio only varied in a narrow range. Measurement of the isotope ratio of the m/z 94/93 base peak gave a good indicator for differentiating citrus species and their hybrids. In addition to other techniques for the authenticity control of essential oils, GC-MS can be used to characterize the citrus species based on the isotope ratio of monoterpene hydrocarbons. In a further study, the isotope ratio of the essential oil components of the same species but from different geographical origins will be investigated. The outcome will contribute to analytical applications for the authenticity of essential oils.

Acknowledgments

The authors thank Dr. Do Dinh Ca, Research Institute of Fruits and Vegetables, Hanoi, for taxonomy of the

citrus samples and Ms. Nguyen Thi Lan Phi for collecting the samples in Vietnam. We are also indebted to Hanoi University of Technology for cooperating with the sample preparation.

References

- 1) Mosandl, A., Authenticity assessment: a permanent challenge in food flavor and essential oil analysis. *J. Chromatogr. Sci.*, **42**, 440–449 (2004).
- 2) Bilke, S., and Mosandl, A., $^2\text{H}/^1\text{H}$ and $^{13}\text{C}/^{12}\text{C}$ isotope ratios of *trans*-anethole using gas chromatography–isotope ratio mass spectrometry. *J. Agric. Food Chem.*, **50**, 3935–3937 (2002).
- 3) Bensaid, F. F., Wietzerbin, K., and Martin, G. J., Authentication of natural vanilla flavorings: isotopic characterization using degradation of vanillin into guaiacol. *J. Agric. Food Chem.*, **50**, 6271–6275 (2002).
- 4) Hor, K., Ruff, C., Weckerle, B., König, T., and Schreier, P., Flavor authenticity studies by $^2\text{H}/^1\text{H}$ ratio determination using on-line gas chromatography pyrolysis isotope ratio mass spectrometry. *J. Agric. Food Chem.*, **49**, 21–25 (2001).
- 5) Ruff, C., Hor, K., Weckerle, B., König, T., and Schreier, P., Authenticity assessment of estragole and methyl eugenol by on-line gas chromatography–isotope ratio mass spectrometry. *J. Agric. Food Chem.*, **50**, 1028–1031 (2002).
- 6) Nhu-Trang, T. T., Casabianca, H., and Grenier-Loustalot, M. F., Deuterium/hydrogen ratio analysis of thymol, carvacrol, γ -terpinene and *p*-cymene in thyme, savory and oregano essential oils by gas chromatography–pyrolysis–isotope ratio mass spectrometry. *J. Chromatogr. A*, **1132**, 219–227 (2006).
- 7) Sawamura, M., Ito, T., Une, A., Ukeda, H., and Yamasaki, Y., Isotope ratio by HRGC–MS of *Citrus junos* Tanaka (yuzu) essential oils: *m/z* 137/136 of terpene hydrocarbons. *Biosci. Biotechnol. Biochem.*, **65**, 2622–2629 (2001).
- 8) Satake, A., Une, A., Ueno, T., Ukeda, H., and Sawamura, M., Isotope ratio analysis by HRGC–MS of monoterpene hydrocarbons from citrus essential oils. *Biosci. Biotechnol. Biochem.*, **67**, 599–604 (2003).
- 9) Sawamura, M., Satake, A., Ueno, T., Une, A., and Ukeda, H., Effects of several variable factors on the isotope ratio by HRGC–MS. *Biosci. Biotechnol. Biochem.*, **68**, 215–217 (2004).
- 10) Sawamura, M., Volatile components of essential oils of the *Citrus* genus. In “Recent Res. Devel. Agricultural & Food Chem.” Vol. 4, ed. Pandalai, S. G., Research Signpost, Trivandrum, pp. 131–164 (2000).
- 11) Satake, A., Furukawa, K., Ueno, T., Ukeda, H., and Sawamura, M., MS fragment isotope ratio analysis for evaluation of citrus essential oils by HRGC–MS. *Biosci. Biotechnol. Biochem.*, **68**, 312–316 (2004).
- 12) Minh Tu, N. T., Thanh, L. X., Une, A., Ukeda, H., and Sawamura, M., Volatile constituents of Vietnamese pummelo, orange, tangerine and lime peel oils. *Flavour Fragr. J.*, **17**, 169–174 (2002).
- 13) Choi, H. S., and Sawamura, M., Composition of the essential oil of *Citrus tamurana* Hort. ex Tanaka (Hyuganatsu). *J. Agric. Food Chem.*, **48**, 4868–4873 (2000).
- 14) Martin, G. J., Lavoine-Hanneguelle, S., Mabon, F., and Martin, M. L., The fellowship of natural abundance ^2H -isotopomers of monoterpenes. *Phytochemistry*, **65**, 2815–2831 (2004).
- 15) Rossmann, A., Butzenlechner, M., and Schmidt, H. L., Evidence for a nonstatistical carbon isotope distribution in natural glucose. *Plant Physiol.*, **96**, 609–614 (1991).