

MS Fragment Isotope Ratio Analysis for Evaluation of Citrus Essential Oils by HRGC-MS

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To evaluate the origin of citrus essential oils, the isotope ratio of fragment peaks on HRGC-MS of the volatile compounds from various citrus oils was measured. The MS fragment ratio was found by the ratio of fragment peak intensity, $m + 1/m$ (m/z). This ratio reflects the isotope effect of volatile compounds, that is, it provides information about locality, quality, and species for essential oils. Multivariate analysis based on the MS fragment ratio of monoterpene hydrocarbons clearly distinguished three citrus species, yuzu, lemon, and lime. The carbonyl fractions were also extracted from citrus essential oils by the sodium hydrogensulfite method. The isotope ratio of MS fragments of octanal, nonanal, and decanal was also examined. The results suggest that there was no significant difference in the individual fragment isotope ratios of the three aldehydes.

Key words: citrus essential oil; fragment isotope ratio; HRGC/MS; monoterpene hydrocarbon; aldehyde

Citrus fruits are widely cultivated in the tropical and the temperate zones in both the northern and southern hemispheres. Citrus is one of the most important commercial crops, since it provides us with pleasant tastes, flavors, and fragrances. Recently, it has become commercially important to evaluate the property, quality, origin, and genuineness of raw and processed citrus products.

The ratio of enantiomer or isotope is an important factor in the authenticity control of flavor compounds. Recently, the method of enantioselective analysis in which enantioselective multidimensional gas chromatography (enantio-MDGC) is used has been established.¹⁾ Analysis of the isotope ratio, on the other hand, is a powerful tool for the authenticity assessment of achiral flavor compounds.^{2–4)} Chemical element analysis and isotope ratio mass spectrometry (IRMS) have been used conservatively to measure the isotope ratio of C (¹³C/¹²C), H (²H/¹H) and O (¹⁸O/¹⁶O). Gas chromatography-

isotope ratio mass spectrometry (GC-IRMS) has been used for the measurement of isotope values of constituents in flavors. Hör *et al.*^{5,6)} and other researchers^{7–9)} described the isotope ratio analysis of citrus essential oils using GC-IRMS. However, GC-IRMS is not popular as a tool of routine work yet.

Recently, gas chromatography-mass spectrometry (GC-MS) has been the most essential and popular equipment for flavor analysis. It is used mainly for qualitative and quantitative analyses of volatile compounds. It has been thought that ordinary GC-MS is not proper for isotope analysis, because its sensitivity and accuracy is not sufficient for the analysis. It may be difficult to measure the stable isotope ratio based on the molecular ion peak and its isotope peak of ²H/¹H of which isotope ratio is low or ¹⁸O/¹⁶O of which proportion is relatively low in organic compounds. However, the measurement of the ¹³C/¹²C ratio is successful, because the isotope ratio and abundance in organic compounds are quite high.

In previous reports, the authors developed a new analytical method based on the ratio of the molecular ion peak and its isotope peak of monoterpene hydrocarbons with ordinary GC-MS, and applied this method to the differentiation of citrus oils from various origins.^{10,11)} Furthermore, it was statistically demonstrated that this method was not affected by such variations as sampling years, trees, and fruiting positions as long as the plants were grown under set conditions.¹²⁾

However, it is often difficult to measure the isotope ratio by ordinary GC-MS when the molecular ion peak is very small or not detected. In GC-IR-MS specialized for the measurement of isotope ratios, the value is determined as the whole molecule isotopic ratio. In ordinary GC-MS, on the other hand, the collectable data are not only the molecular ion peak but also various peak fragments resulting from the cleavage of molecules. Thus, the isotope ratio of the fragment ion peaks $m/z = m$ and $m/z = m + 1$ of a compound, $m + 1/m$ (m/z) (MS fragment ratio), can be measured in addition to that of the molecular ion peak. This paper deals with the

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Table 1. Origin of the Citrus Essential Oils

Sample	Origin
Lime A	Commercial product from Mexico
Lime B	Anonymous commercial product
Lime C	Commercial product from USA
Lime D	Anonymous commercial product
Lime E	Commercial product from Jamaica
Yuzu A	CPO ^a from Yamagata Prefecture
Yuzu B	CPO from Hiroshima Prefecture
Yuzu C	CPO from Kyoto Prefecture
Yuzu D	CPO from Saitama Prefecture
Yuzu E	CPO from Miyazaki Prefecture
Yuzu F	CPO from Kochi Prefecture
Yuzu G	CPO from Kagoshima Prefecture
Yuzu H	CPO from Tokushima Prefecture
Yuzu I	CPO from Shizuoka Prefecture
Lemon A	Commercial product from Italy (Sicily)
Lemon B	Commercial product from USA
Lemon C	Anonymous commercial product
Lemon D	CPO from Wakayama Prefecture
Lemon E	Artificial flavor based lemon essential oil

^a Cold-pressed oil.

application of fragment peaks to the HRGC-MS analysis in monoterpene hydrocarbons and aldehydes from various citrus essential oils.

Materials and Methods

Materials. The samples of lime and lemon essential oils were commercial products used as flavor materials. Yuzu fruits were collected from 9 different local wholesale markets from northern to southern Japan in November 1999, and their cold-pressed oils (CPO) were prepared as described in a previous paper.¹³⁾ These sources are shown in Table 1. Orange and grapefruit essential oil samples were also commercial products used as flavor materials. Authentic chemicals for mass spectrometry were obtained from the commercial sources mentioned previously.¹¹⁾ Authentic aldehydes were that of flavor material and reagent grade.

Gas chromatography-mass spectrometry (GC-MS). Gas chromatography combined with mass spectrometry were used for identifying the volatile components. The analysis was done with a Shimadzu GC-17A linked with a Shimadzu QP-5050 at an MS ionization voltage of 70 eV, accelerating voltage of 1500 V, and ion source temperature of 250°C. The GC column was DB-Wax fused-silica capillary type (60 m × 0.25 mm i.d., 0.25 μm film thickness; J & W Scientific, Folsom, CA, U.S.A). The column temperature was programmed from 70°C (2 min hold) to 100°C at a rate of 2°C/min. The column was cleaned by heating to 230°C before 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 0.2 μl was injected at a split ratio of 1:50. Each component was initially identified by the GC retention index and the NIST library connected to the

QP-5050 mass spectrometer, as described in previous papers.^{12,13)}

Measurement of the MS fragment ratio. The MS fragment ratio was measured using the isotope ratio measurement method described in previous papers.¹⁰⁻¹²⁾ Selective ion monitoring (SIM) of GC-MS was done in order to estimate the intensities of ion peaks of each major MS fragments ($m/z = m$) and of its +1 fragment peaks ($m/z = m + 1$). The MS fragment ratio of each peak was calculated using the following equation:

$$\begin{aligned} & [\text{MS fragment isotope ratio of } m/z = m] \\ & = (\text{intensity of a peak of } m/z = m + 1) \\ & \quad / (\text{intensity of a peak of } m/z = m) * 100 \end{aligned}$$

where the intensity is the mean value from quintuplicate measurements.

Extraction of carbonyl fractions. The carbonyl fractions from citrus oils were extracted by a conventional method with sodium hydrogen sulfite. A solution of 30% NaHSO₃ in water (50 g) was added to 50 g of citrus oil, and stirred for 2 hr. The synthesized sulphites were extracted with water and neutralized by Na₂CO₃. The liberated carbonyl compounds were extracted with diethyl ether (200 ml), and dried. The solvent was removed to obtain carbonyl fractions at a yield of 0.07–1.23% (w/w).

Results and Discussion

MS fragment isotope ratio analysis of monoterpene hydrocarbons

MS fragment isotope ratio analysis was done on various kinds of citrus essential oils. The MS fragment isotope ratio for α-pinene, β-pinene, myrcene, α-terpinene, β-phellandrene, γ-terpinene, and terpinolene are shown in Table 2. The ratio of $m/z = 137$, 122, and 108 isotope fragments corresponding to $m/z = 136$, 121, and 107 were measured for each compound. As can be seen in Table 2, the isotope effect was influenced by exogenous factors such as differences in location, climate, and cultivation conditions.^{2,6)} When the isotope ratio of monoterpene hydrocarbons is divided by the ratio of one compound, limonene, in each essential oil (so the MS fragment ratio of limonene is 1), the influence of isotope discrimination on CO₂ fixation is eliminated.⁶⁾ This calculation will result in an isotope ratio pattern based on secondary metabolites, showing the specific pattern of each plant species. In this study this calculation was applied to two MS fragment peaks, $m/z = 121$ and 107, cleaved from the molecule ($m/z = 136$).

The behavior of the molecular ion peak pattern agreed with that of the previous report.¹¹⁾ The fragment ratio patterns for citrus species were similar, but those for monoterpene hydrocarbons were quite different. The

Table 2. MS Fragment Isotope Ratio of the Monoterpene Hydrocarbons from Citrus Oils

Citrus oil	MS fragment isotope ratio																				
	α -Pinene			β -Pinene			Myrcene			α -Terpinene			β -Phellandrene			γ -Terpinene			Terpinolene		
	107	121	136	107	121	136	107	121	136	107	121	136	107	121	136	107	121	136	107	121	136
Lime A	0.835	0.991	0.999	0.824	1.094	1.036	0.921	1.012	0.919	1.644	1.006	1.012	1.725	0.908	0.975	0.751	1.011	0.993	1.097	1.023	0.994
Lime B	0.816	0.991	0.992	0.762	1.070	1.026	0.956	1.000	0.966	1.643	1.001	1.005	1.767	0.933	0.984	0.747	1.010	0.994	1.085	1.019	0.992
Lime C	0.839	0.995	0.995	0.854	1.003	1.048	1.011	1.000	1.007	1.648	0.998	1.012	1.724	1.002	0.975	0.748	1.009	0.996	1.086	1.020	0.992
Lime D	0.858	1.007	0.988	0.909	1.078	1.018	1.006	1.080	0.891	1.627	0.999	1.008	1.491	0.936	0.986	0.752	1.023	0.989	1.091	1.026	0.986
Lime E	0.816	0.988	0.995	0.835	1.082	1.018	0.994	0.978	0.992	1.633	1.007	1.009	1.712	0.942	0.995	0.750	1.010	0.995	1.097	1.023	0.990
Yuzu A	0.794	0.993	0.978	0.888	0.969	0.991	0.989	0.987	0.986	1.571	0.994	0.979	1.438	1.018	0.987	0.745	1.025	0.991	1.133	1.038	0.985
Yuzu B	0.785	0.989	0.969	0.928	0.968	0.993	1.006	0.991	0.998	1.662	0.992	0.972	1.405	1.019	0.986	0.758	1.030	0.990	1.170	1.030	0.983
Yuzu C	0.786	0.997	0.984	0.894	0.953	0.989	0.993	0.994	0.955	1.527	1.015	0.868	1.432	1.029	0.985	0.761	1.031	0.990	1.163	1.044	0.987
Yuzu D	0.795	1.004	0.985	0.821	0.982	1.017	1.016	0.987	0.991	1.583	0.989	0.968	1.530	1.012	0.985	0.741	1.025	0.988	1.130	1.031	0.994
Yuzu E	0.793	0.992	0.980	0.915	0.969	1.005	1.016	0.981	0.985	1.667	0.980	0.988	1.403	1.029	0.984	0.741	1.018	0.988	1.117	1.037	0.987
Yuzu F	0.788	0.995	0.974	0.860	0.960	1.011	0.996	0.992	0.973	2.016	0.992	0.990	1.523	1.032	0.982	0.751	1.041	0.991	1.121	1.029	0.982
Yuzu G	0.800	0.992	0.983	0.873	0.977	1.003	1.017	0.990	0.987	1.754	1.005	0.976	1.482	1.013	0.982	0.753	1.033	0.987	1.143	1.038	0.974
Yuzu H	0.808	0.989	0.979	0.889	0.961	0.993	1.024	1.000	0.990	1.679	0.978	0.956	1.469	1.030	0.983	0.751	1.030	0.986	1.143	1.035	0.983
Yuzu I	0.796	0.992	0.974	0.883	0.975	1.008	1.018	0.993	0.986	1.596	0.994	0.985	1.471	1.013	0.984	0.763	1.038	0.985	1.151	1.037	0.975
Lemon A	0.791	0.998	0.982	0.660	0.965	1.023	1.016	0.980	0.992	1.498	1.029	0.967	1.866	1.035	0.973	0.765	1.033	0.989	1.158	1.026	0.987
Lemon B	0.805	0.999	0.980	0.694	0.979	1.017	1.025	0.996	0.981	1.641	0.986	0.990	1.775	1.023	0.978	0.761	1.024	0.988	1.172	1.027	0.979
Lemon C	0.809	1.004	0.981	0.655	0.949	1.007	1.039	0.999	1.007	1.615	0.983	0.983	1.891	1.048	0.993	0.751	1.017	0.989	1.142	1.029	0.981
Lemon D	0.822	0.988	0.988	0.684	1.001	1.010	1.061	0.990	0.978	1.475	0.991	0.950	1.808	1.002	0.988	0.757	1.013	0.990	1.161	1.037	0.979
Lemon E	0.807	0.994	0.994	0.721	0.980	1.019	1.033	1.008	0.979	1.724	1.012	1.016	1.710	1.028	0.975	0.755	1.024	0.987	1.138	1.011	0.978

The MS fragment isotope ratio of $m/z = 108, 122,$ and 137 fragments was measured for each compound. The isotope ratio of each compound was divided by the isotope ratio of one compound, limonene, of each essential oils. Thus, the isotope ratio of limonene comes to 1.000.

lime samples were characterized by MS fragment ratios of $m/z = 107$ for β -phellandrene, $m/z = 121$ for γ -terpinene and $m/z = 107$ for terpinolene. Regarding yuzu, the MS fragment ratios of $m/z = 107$ for α -pinene, $m/z = 121$ for β -pinene, $m/z = 107$ for myrcene, and $m/z = 121$ for β -phellandrene were noteworthy. The lemon samples were also characterized by MS fragment ratios of $m/z = 107$ for β -pinene, $m/z = 107$ for myrcene and $m/z = 107$ for β -phellandrene. Multivariate analysis was done in order to obtain more distinct information.

Multivariate analysis of monoterpene hydrocarbons

The MS fragment ratios for monoterpene hydrocarbons from the three species of citrus oils were subjected to principal component (PC) analysis. The PC number with an eigenvalue greater than 1 was six. The contribution ratio was 36.8%, 14.9%, and 11.4% for PC1, PC2, and PC3, respectively, thus both PC1 and PC2 explained 51.7% of the information regarding those essential oils. PC analysis clearly discriminated among the citrus essential oils, as shown in Fig. 1. The lemon essential oils were distinguished from the other essential oils by PC1, and the lime essential oils were distinguished from the other oils by PC2.

The cluster analysis is shown in Fig. 2: all of the essential oils were classified according to species. In the cases of lemon and lime, the result based on the MS fragment ratio agreed almost perfectly with the result based on the ratio of the intensity of isotope peak to its molecular peak.¹¹⁾ In the case of yuzu, these two results were different and four clusters of locality (A–C, D–G, H, and I) in which the isotope ratio was common were

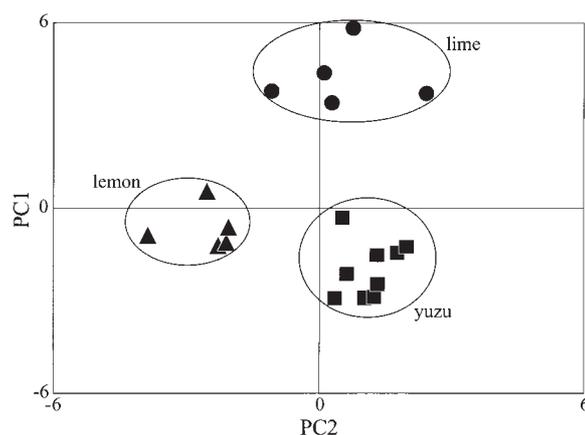


Fig. 1. Principal Component Analysis of MS Fragment Isotope Ratios of the Monoterpene Hydrocarbons from Citrus Oils.

●, lime; ■, yuzu; ▲, lemon.

obtained. The lemon CPO from Wakayama Prefecture in Japan (Lemon D) and artificial lemon flavor essence (Lemon E) were classified into the lemon species cluster including the commercial essential oils. This suggests that the MS fragment isotope ratio analysis method can be also applied to the evaluation of monoterpene hydrocarbons from essential oils in addition to the isotope ratio analysis of molecular ion peaks.

MS fragment isotope ratio analysis of carbonyl fractions

It is thought that the oxygenated compounds make a greater contribution to flavor than the hydrocarbons, thus there is some interest in applying the isotope ratio

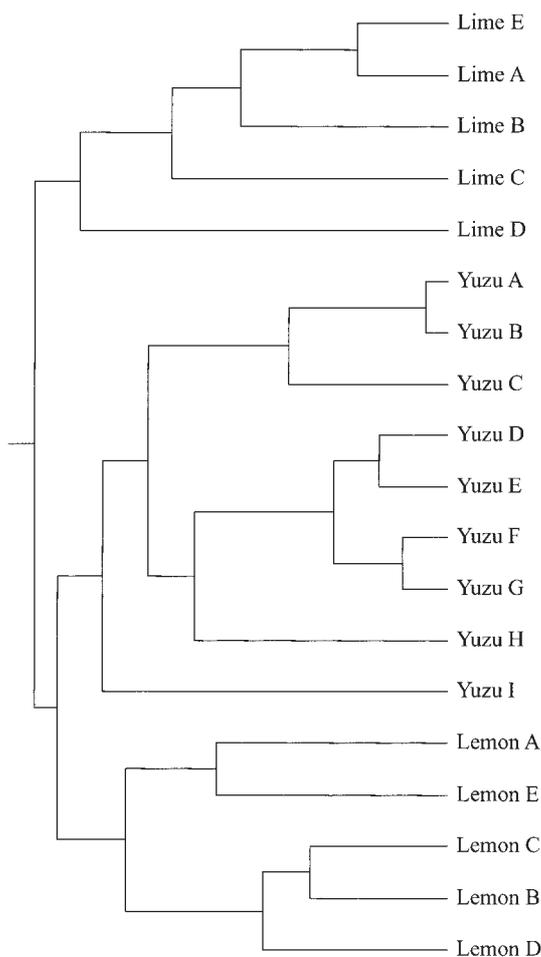


Fig. 2. Cluster Analysis of MS Fragment Isotope Ratios of the Monoterpene Hydrocarbons from Citrus Oils.

analysis to the evaluation of flavor of citrus essential oils. However, the molecular ion peaks of the oxygenated compounds were too small for measurement of the isotope ratios of the molecular ion peaks. To examine the isotope ratios with oxygenated compounds, their MS fragment ratio analysis was done in addition to

Table 3. Percentage Composition of the Aldehydes in Carbonyl Fractions from Citrus Oils

Aldehydes	Concentration (%)				
	Yuzu	Lime	Lemon	Orange	Grapefruit
Octanal	24.7	9.5	23.7	32.6	25.0
Nonanal	11.4	5.9	36.7	10.1	14.6
Decanal	24.5	28.0	18.4	20.0	18.3

the analysis of monoterpene compounds.

Three aldehydes of decanal, nonanal, and octanal were found by GC and GC-MS as main and common components in the carbonyl fractions from lime, yuzu, lemon, orange, and grapefruit essential oils, as shown in Table 3. The concentrations were 9–32%, 6–37%, and 18–28% for octanal, nonanal, and decanal, respectively. The MS fragment isotope ratios of $m/z = 95$, 100, and 110 for octanal, $m/z = 94$, 114, and 124 for nonanal and $m/z = 123$, 128, and 138 for decanal were measured. It is thought that those fragment peaks resulted from M-(H₂O, CH₃), M-(CO), and M-(H₂O). The results of MS fragment isotope ratio measurement are shown in Table 4. These results indicate that the measurement of the isotopic ratio for oxygenated compounds could be done, even when the molecular ion peak was too small or not detectable. The behavior of the MS fragment ratios were similar between citrus species. For all three aldehydes the fragment isotope ratios ($m/z = 100$, 114, and 128 for octanal, nonanal, and decanal, respectively) were the lowest of the three fragment peaks. These results suggest that the difference in isotope ratios seems to be small for straight-chain aldehydes, unlike monoterpene hydrocarbons.

Multivariate analysis of carbonyl fractions

The results of the PC analysis of the carbonyl fractions are shown in Fig. 3. The PC number with an eigenvalue greater than 1 was four. The contribution ratios were 32.3%, 25.7%, and 16.6% for PC1, PC2, and

Table 4. MS Fragment Ratio of the Aldehydes in Carbonyl Fractions from Citrus Oils

Citrus oil	MS fragment ratio								
	Octanal			Nonanal			Decanal		
	95	100	110	109	114	124	123	128	138
Aldehyde standard A	8.71	6.46	10.13	9.88	7.88	7.53	14.72	8.15	13.69
Aldehyde standard B	9.10	6.48	11.15	9.45	8.70	7.60	15.59	8.76	14.32
Lemon A	8.76	6.76	11.23	9.34	8.67	8.11	15.41	8.52	13.57
Lemon C	8.93	6.53	10.48	9.21	8.11	7.88	15.67	7.98	13.44
Lemon D	9.02	6.47	10.96	10.62	8.61	7.57	17.37	8.07	20.30
Lime C	8.73	6.24	10.91	10.61	8.47	8.64	15.86	8.82	13.97
Lime E	8.41	6.52	10.83	9.64	7.73	9.19	13.87	8.59	13.52
Yuzu A	9.08	6.60	10.90	9.34	11.35	7.41	15.31	10.05	14.22
Yuzu B	8.79	6.36	10.26	9.90	8.73	8.09	15.35	10.05	12.99
Yuzu D	8.79	6.59	10.39	9.94	8.51	7.50	16.00	8.94	14.01

The MS fragment ratio of $m/z = 95$, 100, and 110 in octanal, $m/z = 94$, 114, and 124 in nonanal and $m/z = 123$, 128, and 138 in decanal were measured on each carbonyl fraction.

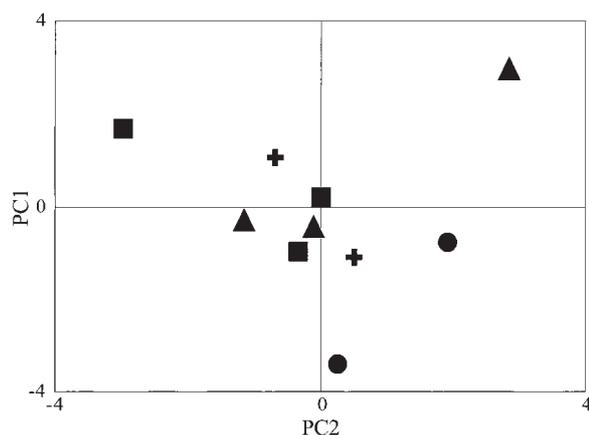


Fig. 3. Principal Component Analysis of MS Fragment Isotope Ratios of the Aldehydes in Carbonyl Fractions from Citrus Oils.

●, lime; ■, yuzu; ▲, lemon; +, synthesized aldehyde standard.

PC3, respectively. This result, together with PC1 and PC2, explained 58% of the information regarding those essential oils. No correlation was found among the samples of lime, yuzu, and lemon or synthetic products and natural compounds on the basis of these MS fragment isotope ratios. There was no significant difference in individual fragment isotope ratios (Table 4). This may result from the simple straight-chain structure of these aldehydes. However, Yuzu A, Lemon D, and Lime C and D, as shown in Fig. 3, presented some distinction from other corresponding samples. It is suggested that this discrimination would imply the difference of locality or processing of these essential oils.

In this study, the isotope ratio of monoterpene hydrocarbons and aldehydes from citrus essential oils as natural food materials were measured by ordinary GC-MS on the basis of MS fragment ratio. Then, those data obtained were significant, as given in Table 2 and Table 3. Regarding the volatile compounds from food materials, this fragmentation analysis has been first done. These results suggest that in addition to enantiomeric analysis^{14,15)} and isotope ratio analysis of the molecular ion peaks on mass spectra,^{10–12)} this technique for MS fragment isotope ratio analysis can provide diverse information on the evaluation of natural essential oils. This technique is also applicable to the compounds of which peaks on gas chromatograms are very small or a molecular ion peak on a mass spectrum is not detectable. Furthermore, it will compensate ordinary GC-MS for the lower sensitivity and accuracy than those of GC-IRMS on the basis of the analysis for mass fragments by the mode of selected-ion monitor of GC-MS.

References

1) Mosandl, A., Enantioselective capillary gas chromatography and stable isotope ratio mass spectrometry in the

authenticity control of flavors and essential oils. *Food Rev. Int.*, **11**, 597–664 (1995).

- 2) Werner, R. A., Kornexl, B. E., Rossman, A., and Schmidt, H. L., On-line determination of $\delta^{18}\text{O}$ values of organic substances. *Anal. Chim. Acta*, **319**, 159–164 (1996).
- 3) Koziat, J., Isotope ratio mass spectrometric method for the on-line determination of oxygen-18 in organic matter. *J. Mass Spectrom.*, **32**, 103–108 (1997).
- 4) Begley, I. S., and Scrimgeour, C. M., High-precision $\delta^2\text{H}$ and $\delta^{18}\text{O}$ measurement for water and volatile organic compounds by continuous-flow pyrolysis isotope ratio mass spectrometry. *Anal. Chem.*, **69**, 1530–1535 (1997).
- 5) Hör, 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 isotope ratio mass spectrometry. *J. Agric. Food Chem.*, **49**, 21–25 (2001).
- 6) Hör, K., Ruff, C., Weckerle, B., König, T., and Schreier, P., $^2\text{H}/^1\text{H}$ ratio analysis of flavor compounds by on-line gas chromatography-pyrolysis-isotope ratio mass spectrometry (HRGC-P-IRMS): citral. *Flavour Fragr. J.*, **16**, 344–348 (2001).
- 7) Faulhaber, S., Hener, U., and Mosandl, A., GC/IRMS analysis of mandarin essential oils. 2. $\delta^{13}\text{C}_{\text{PDB}}$ values of characteristic flavor components. *J. Agric. Food Chem.*, **45**, 4719–4725 (1997).
- 8) Mosandl, A., and Juchelka, D., Advances in the authenticity assessment of citrus oils. *J. Essent. Oil Res.*, **9**, 5–12 (1997).
- 9) Hanneguelle, S., Thibault, J. N., Naulet, N., and Martin, G. J., Authentication of essential oils containing linalool and linalyl acetate by isotopic methods. *J. Agric. Food Chem.*, **40**, 81–87 (1992).
- 10) Sawamura, M., Ito, T., Une, A., Ukeda, H., and Yamazaki, 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).
- 11) 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).
- 12) 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).
- 13) Choi, H. S., and Sawamura, M., Volatile flavor components of ripe and overripe ki-mikans (*Citrus flaviculpus* Hort. ex Tanaka) in comparison with Hyuganatsu (*Citrus tamurana* Hort. ex Tanaka). *Biosci. Biotechnol. Biochem.*, **65**, 48–55 (2001).
- 14) Cotroneo, A., d'Alcontres, I. S., and Trozzi, A., On the genuineness of citrus essential oils. Part XXXIV. Detection of added reconstituted bergamot oil in genuine bergamot essential oil by high-resolution gas chromatography with chiral capillary columns. *Flavour Fragr. J.*, **7**, 15–17 (1992).
- 15) Mitiku, S. B., Sawamura, M., Njoroge, S. M., and Koaze, H., Analytical discrimination of the cold-pressed oils of mandarins and their hybrids. *J. Essent. Oil Res.*, **14**, 196–202 (2002).