Detection of Adulteration in Olive Oils Using Triacylglycerols Compositions by High Temperature Gas Chromatography

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Edible vegetable oils are a valuable component of a fully mature seed due to their relatively high caloric value, and are mainly the mixtures of triacylglycerols (TG), with different concentration levels.[1,2] The remaining nonglyceridic fraction consists of different compound classes such as hydrocarbons, tocopherols, phytosterols and sterol esters.[3-10]

The separation of TG in edible vegetable oils has been studied by a wide range of techniques such as gas chromatography (GC),[11-13] liquid chromatography (LC),[14,15] super- and sub-critical fluid chromatography (SFC and SubFC),[16,17] and capillary electrochromatography (CEC).[18] The most common approach to the analysis of TG is to release free fatty acid (FA) and perform GC or GC-mass spectrometry (MS) after derivatization, for example, methylation. However, this approach does not identify actual intact TG molecular species, but only determines the percentage of individual FA present in the total TG fraction.[1,2] The complete separation of TG is still very difficult because the edible vegetable oil samples are usually complex mixtures of TG which have very similar physico-chemical properties, and the number of possible molecular species is equal to the number of FA cubed. In addition, in some cases information about actual TG compositions is lost because TGs in oils and fats were formed more or less selectively.[8,16,19]

In spite of the chemical similarities of vegetable oils, small differences in their composition can lead to substantial differences in price and health properties. This consideration makes edible oils such as olive oil a primary candidate for adulteration by substitution with less expensive oils, due to their high market value.[1,5-8] The qualitative detection and especially the determination of low levels of adulteration remains an analytical problem.[20,21] Two general approaches can be taken in the detection of adulteration of foodstuff.[13] One is identification of a major constituent and its property, and the other is identification of the adulterant. Recently, various methods, coupled with a variety of analytical techniques, have been applied to the classification of edible vegetable oils and detection of their adulterations.[12-15,20,21] However, detection of adulterated oils based on TG compositions by high temperature (HT)-GC was not studied. In this study, TG profiles of selected vegetable oils were obtained by HT-GC. Then, detection of adulterated olive oil mixed with soybean oil was investigated by using TG profiles and principal components analysis (PCA).[6,13,15]

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Experimental Section

Materials. Sesame oils were obtained by compression method (60 kgf/cm^2 at 60 ^{o}C) from 1 kg of pan-roasted white seeds of *Sesamum indicum* Linne belongs to *Pedaliaceae*, which harvested in Andong County, Korea. Apricot oil, camella oil, castor oil, peanut oil (*Arachis hypogaea* L.), prikachberry oil, rape seed oil, red pepper seed oil, safflower oil, soybean oil, and walnut oil were purchased from a public market, as edible grades. Olive oil (*Olea europaea* L., 100%, Montolivo, Minerva, Italy) was purchased from a common market. TG reference working standards (analytical grade, purity 99%) were trimyristin (MMM, T_{42}, $C_{45}H_{86}O_6$, m.w. 723.14), 1,3-dipalmitoyl-2-oleoylglycerol (POP, T_{50}, $C_{53}H_{100}O_6$, m.w. 833.4), 1,2-dioleoyl-3-palmitoyl-*dl*-glycerol (OOP, T_{52}, $C_{55}H_{102}O_6$, m.w. 859.4), and triolein (OOO, T_{54}, $C_{57}H_{104}O_6$, m.w. 885.4) from Sigma (St. Louis, MO, USA). All other reagents of analytical grade were purchased from Sigma.

High temperature gas chromatography. All separations were performed on a HP-5890 series II gas chromatograph (Santa Clara, CA, USA) equipped with a split/splitless injector and a flame ionization detector (FID). The analytical column was a aluminum clad capillary column coated with 5% phenyl equiv. polysiloxane-carborane stationary phase (HT-5, SGE, Ringwood, Victoria, Australia; 25 m × 0.32 mm i.d. × 0.43 mm o.d. × 0.1 μm film thickness; maximum cycling temperature, 480 ^{o}C; conditioning temperature, 460 ^{o}C). The oven temperature program was 340 ^{o}C (1 min)-2 ^{o}C/min-370 ^{o}C (10 min). Both injector and FID temperatures were set at 380 ^{o}C. Hydrogen (99.995%) was the carrier gas, and its flow rate was 2.0 mL/min. Gas flow rates for FID were kept as follows: nitrogen (99.995%) make up gas, 33 mL/min; hydrogen (99.995%) fuel gas, 35 mL/min and air (99.995%), 350 mL/min. A split injection with a ratio of 1 : 100 was used. The sample volume injected was 2 μL. GC peak areas were computed with a HP3396A integrator (Santa Clara, CA, USA; chart speed, 0.3 cm/min; threshold, 3; peak width, 0.01; attenuation, 7). Peaks were identified tentatively by comparison of retention times of reference working standards with those of sample components spiked with standards.

Each 100 mg of reference working standard was

accurately weighed into a clean, dry 10 mL volumetric flask, and then was dissolved with chloroform (99%) to reach the final volume of 10 mL to prepare the stock solution (10 mg/mL) of reference working standard. Vegetable oil sample was dissolved in chloroform at the concentration of 50 mg/mL. The quantitative determination was carried out using MMM (T_{42}) as an internal standard (I.S.). The amount of each TG present in oil sample was calculated according to the following equation:

$$W_{TG} / W_{oil} = (A_{TG} / A_{IS}) \times (W_{IS} / W_{oil})$$

where W_{TG} is weight of TG, W_{oil} is weight of oil sample, A_{TG} is peak area of TG, and A_{IS} is peak area of I.S., respectively.

Principal Component Analysis (PCA). Chemometric analyses were accomplished with multivariate statistical analysis program (MVSAP, version 4.0) software developed in our laboratory and pre-validated by using known values and data sets in the literature.[6,13,15] From a multivariate data matrix having *p* variables and *n* samples, principal component scores were computed by using MVSAP.

Results and Discussion

Higher GC oven temperature up to 370 ºC and high temperature stable column stationary phase are required for analyses of relatively involatile intact TG. Figure 1 shows representative HT-GC FID chromatograms of the TG standards mixture and selected vegetable oils separated on a HT-5 column. It was possible to separate and to detect intact TG containing up to 54 carbon atoms in the acyl moieties directly without derivatization. Separation occurred according to carbon number in the acyl moieties. The advantage of determining intact acyl lipids is the ability to study individual molecular species. However, it is not yet possible to obtain complete structural information for intact TG molecule containing different FAs in the three acyl positions by only HT-GC-FID.

The reproducibility (defined as the relative standard deviation of retention times of 20 replicate runs) of separation for T_{42}, T_{50}, T_{52}, and T_{54} was less than 2.10%. The

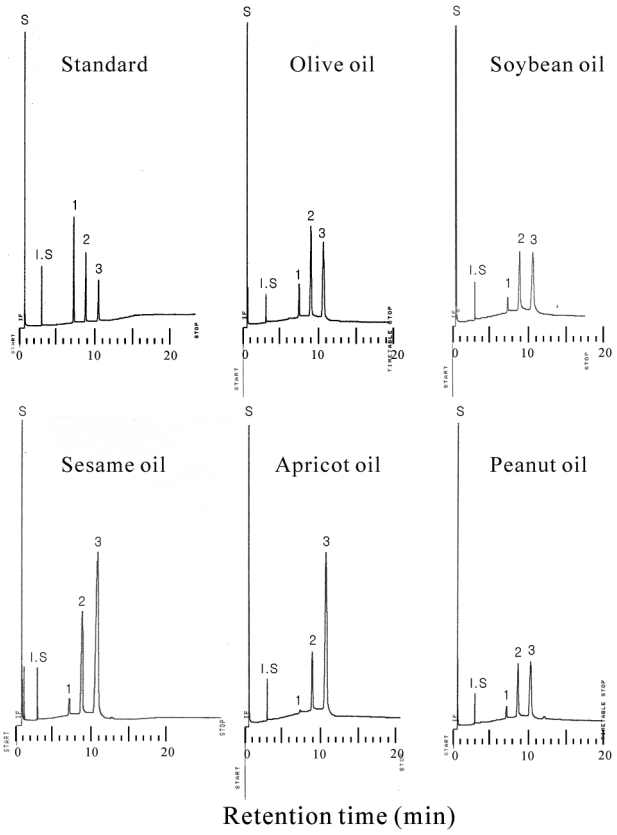

Figure 1. Separation of intact triacylglycerols in selected vegetable oils by high temperature gas chromatography with flame ionization detector. (S: solvent, I.S.: internal standard (T_{42}), 1: T_{50}, 2: T_{52}, 3: T_{54})

precision (defined as the relative standard deviation of peak areas of 20 replicate runs) of working standards was less than 4.72%. The limits of detection, defined by a signal to noise ratio of 3 : 1, of T_{50}, T_{52}, and T_{54} working standard solutions were found to be 8.3 ng/μL, 12.8 ng/μL, and 21.9 ng/μL, respectively. Linear calibration curves were obtained throughout the standard's concentration range (50 ng/μL~ 100 μg/μL).

Table 1 shows the TG composition of the selected vege-

Table 1. Quantitation of triacylglycerols in selected vegetable oils

Sample	Amount of triacylglycerols (unit : mg/g)			Ratio of triacylglycerols		
	T_{50}	T_{52}	T_{54}	T_{52}/T_{50}	T_{54}/T_{50}	T_{54}/T_{52}
Apricot	5.00 ± 10.58	131.67 ± 3.75	884.33 ± 0.46	26.23	176.87	6.72
Camella	15.83 ± 16.74	250.33 ± 20.63	734.33 ± 4.40	15.81	46.39	2.93
Castor Oil	1.33 ± 4.33	38.00 ± 2.63	255.67 ± 2.60	28.57	192.33	6.73
Olive	48.10 ± 4.22	300.33 ± 2.03	480.67 ± 2.54	6.24	9.99	1.60
Peanut	21.23 ± 3.06	201.00 ± 5.05	284.00 ± 0.00	9.47	13.38	1.41
Prickachberry	15.13 ± 2.12	104.33 ± 2.21	474.00 ± 0.00	6.90	31.33	4.54
Rape Seed	24.03 ± 2.64	149.67 ± 1.39	395.33 ± 2.80	6.23	16.45	2.64
Red Pepper	23.17 ± 1.25	222.00 ± 0.00	240.67 ± 1.20	9.58	10.39	1.08
Safflower	6.50 ± 11.62	144.00 ± 11.18	534.67 ± 8.96	22.15	82.26	3.71
Soybean	19.63 ± 3.96	208.67 ± 1.68	408.00 ± 7.88	10.63	20.78	1.96
Walnut	4.83 ± 3.16	142.00 ± 4.93	353.00 ± 4.42	29.40	73.08	2.49

Mean ± RSD% (*n* = 3).

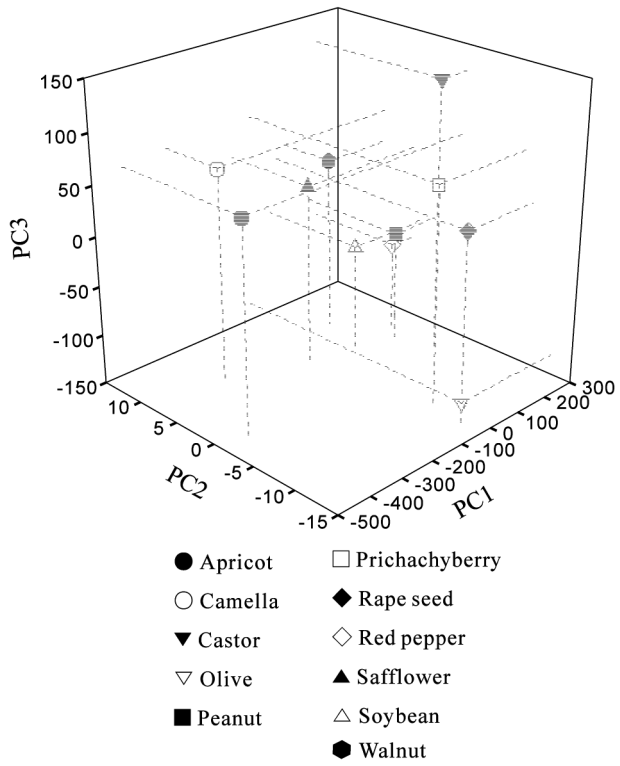

Figure 2. Principal components plot for intact triacylglycerols composition of selected vegetable oils listed in Table 1.

table oils. The feature of compositional patterns is that T_{54} is the most dominant TG and T_{50} is relatively minor TG in each sample. The detection of adulteration of oil is not easy, because vegetable oils have narrow intact TG compositional spectra. Therefore, adulteration of vegetable oils, involving the replacement of expensive oils with cheaper oils, could potentially be very lucrative for oil suppliers.[15]

PCA was applied to provide an overview of the capability to distinguish vegetable oils based on TG composition by HT-GC. The data set of TG composition for 11 vegetable oil samples in Table 1 was classified by using PCA. PCA is frequently employed for the purpose of generating a reduced set of varieties that account for the most of the variability in the original data, and the first principal component score (PC1) contains the most representative information in the data set. As shown in Figure 2, distributions of the data points of three PCA scores (cumulative proportions: 88.1%, 88.2%, and 100.0%, respectively) for 11 vegetable oils investigated in this study are different from each other. For example, it can be seen that the datum point of olive oil is far from that of soybean oil. PCA was useful for pattern recognition and detection of adulteration.

It should be noted, in Table 1, that relative ratios of T_{54}/T_{50} ranged from 9.99 to 192.33, while those of T_{52}/T_{50} and T_{54}/T_{52} ranged 6.23-29.40 and 1.08-6.73, respectively. These results suggest that relative ratio of T_{54}/T_{50} is useful to discriminate the authentic vegetable oil with the adulterated oils. Figure 3 shows graphically the mixing ratio of cheaper soybean oil added to expensive olive oil versus T_{54}/T_{50} ratio. In the case of adulterated olive oils blended with soybean oil,

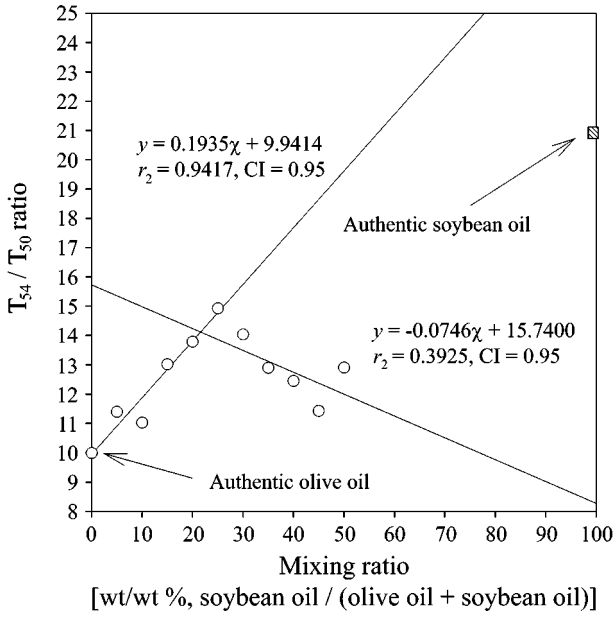

Figure 3. Relationship of mixing ratio of soybean oil in olive oil and relative ratio of T_{54}/T_{50}.

the T_{54}/T_{50} ratio is linearly increased as the mixing ratio approaches up to 25%, and then decreased. Based on this comparison the relative ratio of T_{54}/T_{50} looks promising as a detection index for adulterated olive oil blended with more than 5% soybean oil.

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