

A Second Derivative Fourier-Transform Infrared Spectroscopy Method to Discriminate Perilla Oil Authenticity

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Abstract: The aim of this study was to discriminate the authenticity of perilla oils distributed in Korea using their Fourier-Transform infrared spectroscopy (FT-IR) spectra with attenuated total reflectance accessory. By using orthogonal projections for latent structures discriminant analysis (OPLS-DA) technique, the =C–H *cis*-double bond, –C–H asymmetric and –C–H symmetric stretching are determined to be the best variables for discriminating the perilla oil authenticity. Comparing the integral and the second derivative methods between authentic and adulterated perilla oil samples, the most obvious and significant differences among the three variables is =C–H *cis*-double bond stretching. The procedure for applying the second derivative range of variables found in authentic perilla oil samples correctly discriminated between the adulterated samples of perilla oils with soybean oils and/or corn oils added at concentrations of ≥ 5 vol%. These results showed that the second derivative FT-IR analysis can be used as a simple and alternative method for discriminating the authenticity of perilla oil.

Key words: authenticity, second derivative FT-IR, perilla oil, economically motivated adulteration, linolenic acid

1 Introduction

Perilla (*Perilla frutescens* var. *frutescens*) is a significant plant foods in Eastern Asian countries as well as Korea, Japan, and China. The seed of perilla has been widely used for cooking, medicinal, and industrial purposes, and used mostly as an unrefined oil after roasting process. Perilla oil is one of the most preferred and popular edible oils by Koreans for its characteristic nutty and roasted flavor to match with traditional Korean dishes^{1–3)}.

The worldwide concern about the economically motivated adulteration (EMA) of commercial products of food is grown because EMA represents economic fraud and threatens consumer health. Perilla oil is very expensive edible oil in Korea because of the limited production. Perilla oil is one of the targeted products for EMA in Korea because the adulterated perilla oils have been manufactured by intermingling with low-priced edible oils such as soybean or corn oils, and low grade ingredient (e.g., extracts from perilla cake) (Korea Ministry of Food and Drug Safety [http://www.mfds.go.kr]). Therefore, it is necessary

to develop a simple, rapid, inexpensive, precise analytical method to discriminate the authenticity of the perilla oil to help prevent the illegal distribution of adulterated oil products in the marketplace.

The authenticities of high-costly edible oil products such as roasted sesame oil and extra virgin olive oil have been discriminated by analyzing several different chemical components followed by multivariate statistical methods. The oil components analyzed include fatty acids (FAs)^{1–8)}, tocopherols^{1,9)}, sterols^{1,9)}, triacylglycerols^{8,9)}, specific functional groups¹⁰⁾ and carbon stable isotopes^{10,11)}. The techniques used to analyze oil components have involved spectrophotometry^{3–7)}, gas chromatography (GC)^{8,9,11–14)}, liquid chromatography (LC)^{1,9,15)}, mass spectrometry (MS)-based methods^{8,9,16)}, and nuclear magnetic resonance (NMR) spectroscopy^{10,17,18)}. However, few published studies have discriminated the authenticity of perilla oil using Fourier transform infrared (FT-IR) spectroscopy to the best of our knowledge^{4–8)}.

In the present study, FT-IR spectroscopy working with

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attenuated total reflectance (ATR) accessory was used as the analytical tool to discriminate the authenticity of perilla oil^{6, 12, 19–22}. FT-IR is much less expensive, and convenient instrument than other quantitative analytical instruments including GC, LC, MS, and NMR. FT-IR spectroscopy is a rapid, non-destructive technique with minimum sample preparation necessary^{19–24}. The application of this technique has expanded in food research and become particularly a powerful analytical tool in the study of edible oils and fats¹⁹. The raw FT-IR spectra is difficult to distinguish the overlapping peaks and low resolution due to error of baseline are generally less accurate, whereas, the application of second derivative to the raw spectra resulted a high degree of complexity of spectra and a clear separation of peaks^{3, 21}. The perilla oil sample with added soybean or corn oil was used as a model of adulterated perilla oil in this study, because many adulterated perilla oil products are produced in a similar way by blending other low-priced edible oil (Food news[<http://www.foodnews.co.kr>])^{4, 8}.

The combination of FT-IR spectroscopy and statistical analysis was conducted to develop the analytical method to discriminate the authenticity of perilla oils^{4–7, 12–15}. We have been working on the development of rapid, general-purpose quality control methods because FT-IR spectroscopy is a major advance over outstanding wavenumber reproducibility and accuracy, extensive data manipulation capabilities and advanced chemometric software to handle calibration development^{7, 19–24}. The amount of unsaturated FAs (USFAs) of perilla oils (18:3n-3; 60% of total FAs), is higher than other low-priced edible oils such as soybean or corn oil^{1–3, 11}. The adulterated perilla oils are distinguished using the difference in the constituents of USFAs. The FT-IR spectra of edible oils containing USFAs show *cis*-alkene (=C–H) absorption bands at around 3008–3010 cm^{–1} for the –C–H stretching. Among the functional group peaks of USFAs, the absorption band of *cis*-alkene (=C–H) is the most obvious difference between authentic and adulterated perilla oils.

Orthogonal projection to latent structure discriminant analysis (OPLS-DA), a supervised multivariate statistical method was used to select variables that most effectively discriminate between the authentic and adulterated perilla oils^{10, 11}. The predictive discrimination power of the selected variables was then evaluated using adulterated oil model samples consisting of soybean or corn oils which blended at different concentrations. An analysis of adulterated oil model samples is made to produce an accurate discriminating variables. The aim of the study was to investigate the possibility of determining the authenticity of the perilla oil using FT-IR spectroscopy and verify the second derivative method is more suitable for determination the authenticity of the perilla oils than the integral method.

2 Experimental Procedures

2.1 Samples

The samples of authentic and adulterated perilla oils described below were used to determine the best variables for discriminating authenticity of perilla oil using the OPLS-DA technique. Twenty-eight samples of authentic perilla oils were prepared by extraction from 19 samples of Korean perilla seeds and 9 samples of Chinese perilla seeds that were obtained from local grocery stores in Korea. The perilla seeds were toasted in a drum roaster (THDR-01, Taehwan Automation Industry Co., Seoul, Korea) at 200°C for 30 min. The perilla oil was extracted from the toasted seeds using an oil press (Oil Love, National ENG Co., Goyang, Korea). The extracted oil was centrifuged to remove the precipitated impurities at 9,600 × g for 10 min and then dried completely by flushing with nitrogen gas to protect the oils against the onset of oxidative rancidity and contamination. Ten samples of commercial perilla-flavored oils that a mixture of spices, flavoring and perilla seed extracts, such as perilla oil, soybean oil, corn oil as adulterated perilla oils were obtained from local grocery stores in Korea. Forty model samples of adulterated perilla oil samples were prepared by blending a sample of extracted authentic perilla oil with commercial soybean or corn oil at concentrations of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, and 100 vol%. These model samples were used to determine whether the authenticity of perilla oil could correctly be discriminated by applying the range of variables found in the authentic perilla oil samples. All oil samples were kept at 4°C prior to analysis.

2.2 Analysis of Fourier-transform infrared spectrometry

All spectra were acquired using FT-IR spectrometer (TENSOR-27; Bruker Optics GmbH, Karlsruhe, Germany) equipped with a diamond ATR system (A225/Q Platinum ATR; Bruker Optics GmbH, Karlsruhe, Germany). The spectra were collected by averaging 32 scans with 4 cm^{–1} resolution. Every sample was measured in absorbance unit within the wavelength range of 3800–400 cm^{–1} and averaged the triplicated data with the OMINC software (version 8.2, Thermo Fisher Scientific Inc. Waltham, MA, USA). The relative intensity value (I1–I16) of each peak was calculated using normalization by the intensity of peak 7 (1740 cm^{–1}, C=O stretching) as the internal standard obtained from triglycerides to reduce the sampling error. The range of the measured intensity (integral and/or second derivative) values of each sample was used for making a comparison between the authentic and adulterated perilla oils. Derivatizing spectral data by the Savitzky-Golay (SG) numerical algorithm are often used as a pre-processing step to resolve overlapping signals, enhancement of spectral significant difference, and to subdue unnecessary spectral characteristics caused by unusual equipment and sample properties. A second derivative spectra was calculated for each mea-

sured pixel by the Savitzky–Golay (SG) numerical algorithm at third-degree polynomial at 7 point^{25, 26}. The derivative values were utilized for quantitative measurement and data processing using the peak height of the second order spectra of the major spectral band.

2.3 Statistical analysis

A two-tailed Student's *t*-test was used to identify the differences between the authentic and adulterated perilla oil samples ($p < 0.05$, 0.01, or 0.001). Pearson's correlation test was conducted to establish whether a significant linear relationship existed between the two variables ($p < 0.01$). The statistical analysis was conducted using IBM SPSS Statistics (version 23) software (SPSS Inc., Chicago, IL, USA). Using the peak data of FA composition, OPLS-DA was conducted with Pareto scaling to determine if it was possible to differentiate authentic perilla oil from adulterated using the SIMCA-P+ (version 15.0) software (Umetrics, Umeå, Sweden). The best significant variables were selected using the S-plot generated by OPLS-DA to distinguish between the authentic and adulterated perilla oil samples.

3 Results

3.1 FT-IR spectra

The FT-IR spectra of an authentic perilla oil and soybean oil which is commonly used in the manufacture of adulterated oil products is shown in Fig. 1 within the 3800–400 cm^{-1} . Sixteen peaks, among many signals, which were strong and distinguishable intensity, were found in the FT-IR spectra. Table 1 shows the frequencies of the characteristic bands

or shoulders and functional group moieties of authentic and adulterated perilla oil on previously reported papers. Out of these, the triacylglycerol, which is a major component in perilla oils, was dominant in the spectra. The most important area in the spectra for discriminating the authenticity of the samples was the region 3100–2700 cm^{-1} that contains mainly the bands of triacylglycerol, unsaturated lipids, and carbohydrates. Ester carbonyl ($\text{C}=\text{O}$) groups produced strong FT-IR bands in the 1743 cm^{-1} range. Absorption bands at 3025 cm^{-1} and 3008–3010 cm^{-1} correspond to *trans*-double bond stretching vibrations and *cis*-double bond stretching vibration of FA bonds, respectively. The bands at 2962–2957 cm^{-1} , 1416 and 1377 cm^{-1} correspond to CH_3 stretching vibrations, rocking vibrations of FA bonds and bending vibrations of CH_3 groups, respectively. The bands at 1240, 1160, 1118 and 1098 cm^{-1} result from stretching or bending vibrations of the bonds which may be derived from fingerprint regions.

3.2 Integral values for the FT-IR

The FT-IR spectra shows that notable differences exist in the I2 (3008–3010 cm^{-1}), I4 (2923 cm^{-1}), and I6 (2853 cm^{-1}) assigned to the $=\text{C}-\text{H}$ stretching vibration of the *cis*-double bond, $-\text{C}-\text{H}$ asymmetric stretching, $-\text{C}-\text{H}$ symmetric stretching respectively, as seen in Fig. 1. Our analyses detected four different absorbance peaks between the samples of authentic perilla oils and the model samples of adulterated perilla oils. The Gaussian integral of each peak, which is commonly used for quantitative analysis of FT-IR spectrometer. The relative integral values of the three peaks for the oil samples are compared in Table 2.

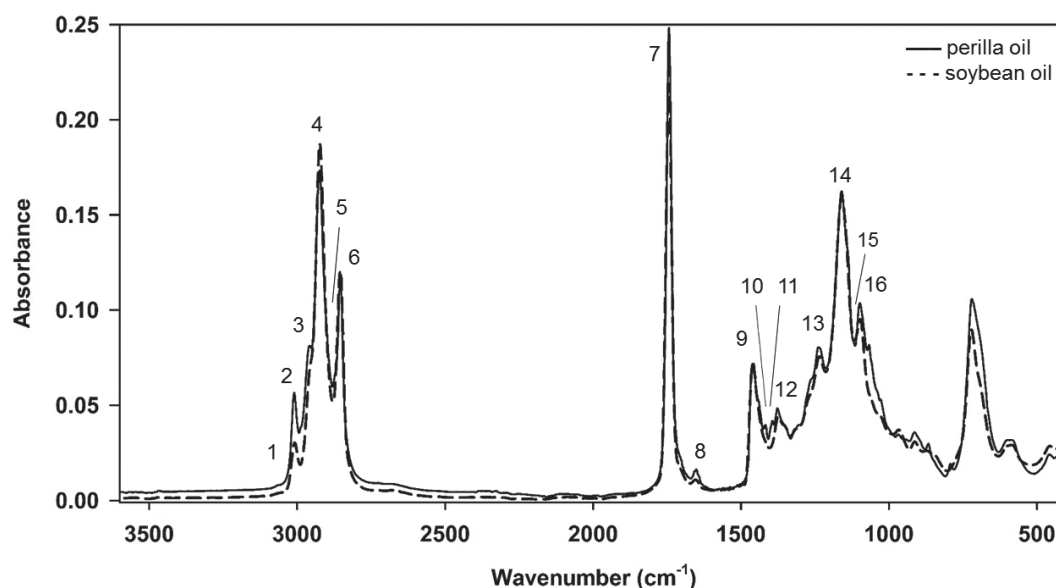


Fig. 1 Overlapped FT-IR spectra of authentic perilla oil samples and soybean oil samples in the region 3600 – 400 cm^{-1} . Labeled peaks in the FT-IR spectra are assigned in Table 1.

Table 1 Some Frequencies of bands of some edible oils in fourier transform infrared spectra, with the assigned classification, functional group, the mode of vibration^{5, 27)}.

No	Frequency (cm ⁻¹)	Classification	Functional group	Mode of vibration
1	3025		=C-H (<i>trans</i> -)	Stretching
2	3008-3010		=C-H (<i>cis</i> -)	Stretching
3	2959	Hydrogen's stretching	-C-H (CH ₃)	Stretching (asym)
4	2923		-C-H (CH ₂)	Stretching (asym)
5	2873		-C-H (CH ₃)	Stretching (sym)
6	2853		-C-H (CH ₂)	Stretching (sym)
7	1743	Double bond's stretching	-C=O (ester)	Stretching
8	1654		-C=C- (<i>cis</i> -)	Stretching
9	1466	Other bonds deformations and bendings	-C-H (CH ₂ , CH ₃)	Bending (scissoring)
10	1416		=C-H (<i>cis</i> -)	Bending (rocking)
11	1395		=C-H (<i>cis</i> -)	Bending
12	1377		-C-H (CH ₃)	Bending (sym)
13	1240	Fingerprint regions	-C-O, -CH ₂ -	Stretching, bending
14	1160		-C-O (ester), -CH ₂ -	Stretching, bending
15	1118		-C-O	Stretching
16	1098		-C-O	Stretching

Table 2 Relative integral values of FT-IR in authentic and adulterated perilla oil samples.

Variable ^{a)}		Authentic perilla oil ^{b)} (n = 28)	Adulterated perilla oil ^{c)} (n = 10)
I2	Range	0.081 – 0.104	0.054 – 0.060
	Mean	0.091	0.057***
I4	Range	0.809 – 0.862	1.006 – 1.037
	Mean	0.834	1.019***
I6	Range	0.231 – 0.279	0.329 – 0.347
	Mean	0.254	0.338***

^{a)} Relative integral value of FT-IR peaks. Numbers represent the peak numbers shown in Fig. 1.

^{b)} Prepared in the laboratory by extraction from perilla seeds after toasting.

^{c)} Consist of commercial perilla-flavored oils.

*** Significantly different from authentic perilla oil, $p < 0.001$.

The integrated area is calculated in the following region.

For =C-H stretching vibration of the *cis*-double bond group, 3033.9–2993.2 cm⁻¹.

For -C-H (asymmetric) stretching group, 2989.4–2881.6 cm⁻¹.

For -C-H (symmetric) stretching group, 2880.1–2819.9 cm⁻¹.

For C=O (ester carbonyl) group, 1830.9–1674.9 cm⁻¹.

Each integral value is normalized using ester carbonyl group (I7), which is proportional to the number of FAs. The =C-H stretching vibration of the *cis*-double bond (I2) is

the most abundant in FAs from authentic perilla oil with a mean of 0.091 a.u. (range of 0.081–0.104 a.u.), followed by -C-H asymmetric stretching group (I4; range of 0.809–0.862, mean = 0.834 a.u.), and -C-H symmetric stretching group (I6; range = 0.231–0.279 a.u., mean = 0.254 a.u.). The relative integral values of adulterated perilla oil samples consisted of commercial perilla-flavored oils are different from intensity of I2 (range = 0.054–0.060 a.u., mean = 0.057 a.u.) as compared with the authentic perilla oil samples, I4 (range = 1.006–1.037, mean = 1.019 a.u.) and I6 (range = 0.329–0.347 a.u., mean = 0.338 a.u.). The calculated values were different between the samples of authentic perilla oils

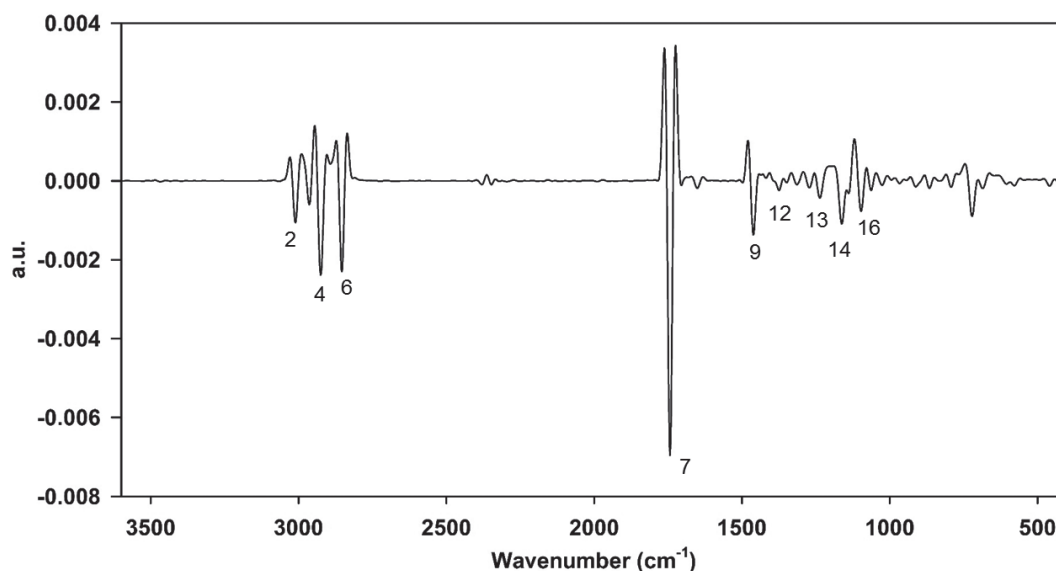


Fig. 2 Second derivative profiles of the FT-IR spectra of authentic perilla oil samples in the region 3600 – 400 cm^{-1} .

Table 3 Relative second derivative peak height values of FT-IR in authentic and other edible oil samples.

Variable ^{a)}		Authentic perilla oil ^{b)} (n = 28)	Adulterated perilla oil ^{c)} (n = 10)
I2	Range	0.170 – 0.190	0.108 – 0.118
	Mean	0.178	0.112***
I4	Range	0.239 – 0.294	0.397 – 0.418
	Mean	0.269	0.408***
I6	Range	0.430 – 0.511	0.583 – 0.603
	Mean	0.458	0.593***

^{a)} Relative second derivative value of FT-IR peaks. Numbers represent the peak numbers shown in Fig. 1.

^{b)} Prepared in the laboratory by extraction from perilla seeds after toasting.

^{c)} Consist of commercial perilla-flavored oils.

*** Significantly different from authentic perilla oil, $p < 0.001$.

and adulterated perilla oils at the $p = 0.001\%$ level. Particularly, the authentic perilla oil samples had a greater integral value for I2 than the adulterated perilla oil samples, whereas the I4 and I6 of the authentic perilla oil samples were smaller than those of adulterated perilla oil samples. This is because the two major plant oils, that is, soybean and corn oils contain less 18:3 n -3 but more 18:1 n -9 and 18:2 n -6 than perilla oil. The composition of edible oils affects the exact location of the band in FT-IR and the yields were shifted when the proportion of the FA changes. Authentic perilla oil contains USFAs much more than adulterated perilla oil, thus, leading to a reduction in the ratio of double bond stretching vibration of the FAs from authentic perilla oil as a result of actual inspection.

3.3 Second derivative peak height values for the FT-IR

Absorption peaks often overlap with each other is one of the reasons analysis of FT-IR spectroscopic data is complicated. For the more reliable construction of the calibration curve, we decided to use a pre-processing method with application of second derivative. Each FT-IR spectra was calculated second derivatives by the Savitzky-Golay (SG) numerical algorithm at 7 point and third polynomial. It allows for minimizing unwanted modification such as baseline shift, enhancement of signal properties, eliminating unwanted spectral features by broad band constituents. Each FT-IR spectra was identified from the second derivative spectra of 3600-400 cm^{-1} range to quantify the relative peak intensity. The second derivative peak intensity was calculated as the minimum intensity between 3002.6 and 3018.5 cm^{-1} for I2, the minimum intensity between 2913.9

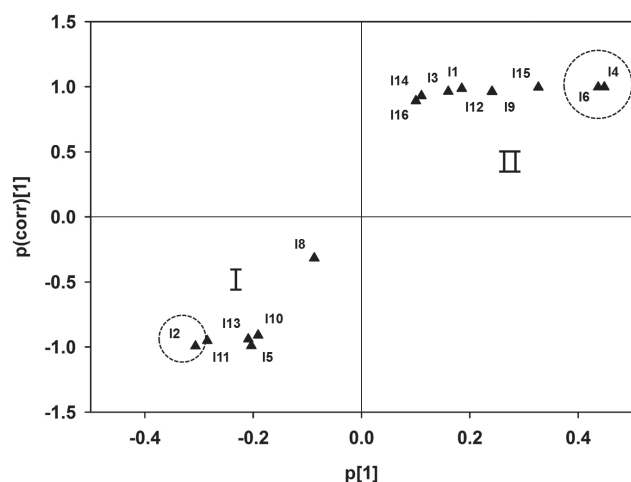


Fig. 3 S-plot generated by orthogonal partial least-squares discriminant analysis (OPLS-DA) of functional group stretching of the fatty acid data obtained from authentic and adulterated perilla oil samples. [I], authentic perilla oil; [II], adulterated perilla oil; I, variable from relative second derivative height value of FT-IR peaks.

and 2935.1 cm^{-1} for I4, the minimum intensity between 2846.4 and 2859.9 cm^{-1} for I6 and the minimum intensity in 1735.6 – 1751.1 range for I7 illustrated in Fig. 2. The calculated values were different between the authentic perilla oils and adulterated model samples at the $p = 0.001\%$ level. The relative second derivative values of the three peaks for the oil samples are compared in Table 3. The relative peak height values for the authentic and adulterated model samples clearly show that discernible differences exist in the intensity of I2, I4, and I6.

3.4 Variables for discriminating perilla oil authenticity

OPLS-DA is a kind of multivariate statistical method that maximizes variations between two comparison groups and minimizes variations between individual replicates, thereby being able to find important differences between the two groups.

OPLS-DA was performed to obtain information about differences in the FA composition data between the authentic and adulterated perilla oil samples. A cross-validated analysis of variance p -value of 3.036×10^{-34} has been worked out the OPLS-DA model derived from the data set. Total variance (R^2Y) and predictive ability (Q^2Y) values of the model were both 0.986, demonstrating that the model provides a very high level appropriateness and precision. From the established model, we produced an S-plot in which the X and Y-axes represents the contribution and confidence of each variable, respectively. A variable lies closer to the first quadrant corner or third quadrant corner of the rectangular coordinate system graph, the more strongly the variable contributes to the difference between

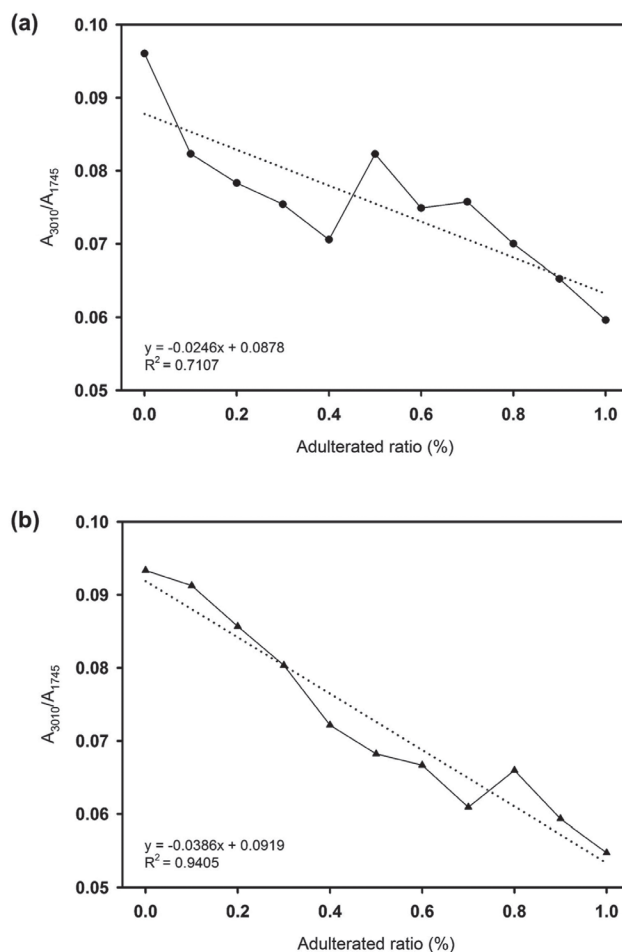


Fig. 4 Calculated integral value according to the adulterated ratio of perilla oils with soybean oil (a) and corn oil (b).

the two groups and the more significant its contribution is as seen in Fig. 3. Among the 16 different FT-IR peaks, the I4 and I6 in the upper-right corner, and the I2 in the lower-left corner, were evaluated to be appropriate variables that contribute to the differences between the authentic and adulterated perilla oil samples. Hence, I2 were selected as the best variable that identify the authenticities of perilla oils. Tables 2 and 3 summarize the range of relative integral values and relative second-derivative peak height values, respectively. The values were calculated from I2, I4, and I6 of the authentic and adulterated perilla oil samples used in this study. Using I2, one of the most accurate and simple discriminating variables, the application of the integral and secondary derivative analysis method was compared in Figs. 4 and 5. By the ratio of 10%, a sample of adulterated perilla oil models was created and compared by adding soybean or corn oil to the perilla oils. Comparing integral and second derivative method of perilla oil samples with added soybean oils in Fig. 4, in the case of integral method (a), the linearity of the content ratio = $C-H/C=O$

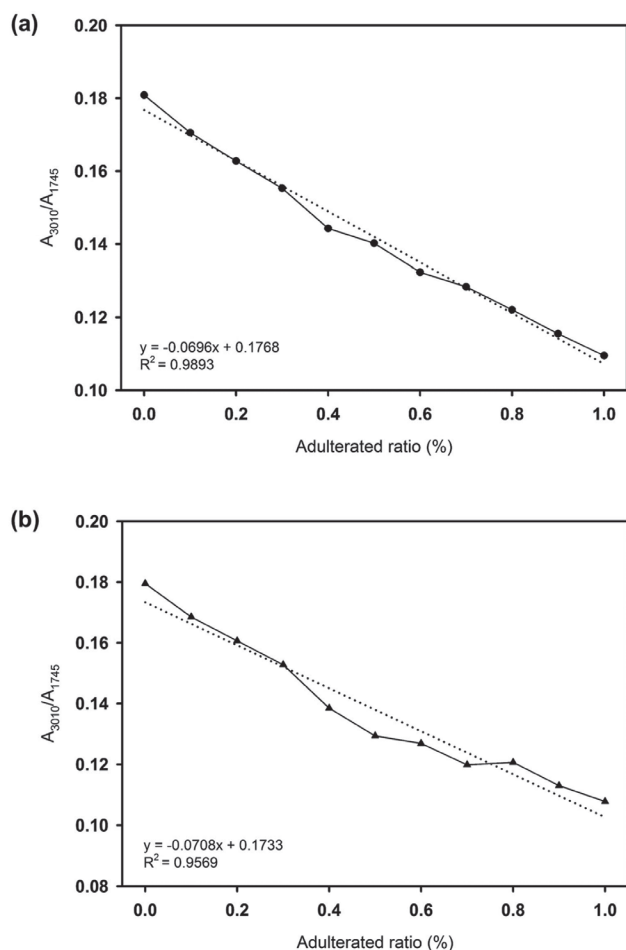


Fig. 5 Calculated second derivative peak height value according to the adulterated ratio of perilla oils with soybean oil (a) and corn oil (b).

value plot was measured low by $R^2 = 0.7107$, whereas the linearity of second derivative method (b) was measured higher as $R^2 = 0.9893$. In the samples with added corn oils, the linearity of the content ratio $=C-H/C=O$ value plot with integral method was measured low by $R^2 = 0.9405$, whereas the linearity of second derivative method (b) was measured higher as $R^2 = 0.9569$.

3.5 Discrimination of authenticity of model samples

In order to evaluate the predictive discrimination accuracies of the integral values and second derivative analysis methods of the 3 peaks as the variables for discriminating the perilla oil authenticity, we classified the 40 model samples with soybean or corn oils added at different concentrations by comparing the range of variables with the measured values as follows (Table 4).

If the measured values of the samples are included within the authenticated range for these variables, that means it is authentic perilla oil, whereas if the measured values are located outside of this range, then means the

sample may have been adulterated. Samples of perilla oils with soybean or corn oils added at concentrations were classified correctly using the $=C-H$ *cis*-double bond stretching values (I2), the $-C-H$ asymmetric stretching vibration values (I4), and the symmetric stretching vibration values (I6). We compared the accuracy of I2, I4 and I6 variable for the identification of the perilla oil authenticity using 40 model samples. The oil samples consisted of 20 samples of adulterated perilla oils with added soybean oils (nos. 1–20), and 20 samples of adulterated perilla oils with added soybean oils (nos. 21–40).

In the relative integral values, model samples added at concentrations between 5 and 35 vol% (nos. 1–7, 21–27) were not correctly classified as being adulterated in variable I2. Variable I4 nicely distinguished each sample of perilla oil with soybean or corn oils added at concentrations of 20–100 vol% (nos. 4–20), 15–100 vol% (nos. 23–40), respectively, because $=C-H$ *cis*-double bond stretching values was less the lower limit of this variable and $-C-H$ symmetric and asymmetric vibration values was greater the upper lower limit in authentic perilla oil samples as increasing the concentration. The addition of soybean or corn oil to perilla oil increased the relative values of $-C-H$ stretching vibration while decreasing the values of $=C-H$ *cis*-double stretching values in a similar fashion. Samples of perilla oils added at concentrations of equal to or more than 20 vol% using relative integral values were correctly discriminated as being adulterated.

In case of the second derivative values using variable I2, all samples of perilla oil with soybean or corn oils added at concentrations between 5 and 95 vol% (nos. 1–40) were correctly classified as being adulterated. Variable I4 distinguished each sample of perilla oil with soybean or corn oils added at concentrations of 25–100 vol% (nos. 5–20), 20–100 vol% (nos. 24–40). Variable I6 distinguished about half of each model sample with soybean or corn oils added at concentrations of 50–100 vol% (nos. 10–20), 55–100 vol% (nos. 31–40). The addition of soybean or corn oil to perilla oil decreased the relative values of $=C-H$ *cis*-double stretching while increasing the values of $-C-H$ stretching vibration in second derivative method. The limit of discrimination on second derivative method was determined to be 5 vol% for adulterated perilla oils with soybean and corn oils. Variable I2, I4 and I6 discriminated the authenticities of all, 33 and 19 samples of the 40 model samples, respectively. These results suggest that determining the $=C-H$ *cis*-double stretching vibration provides a more accurate variable for discriminating the perilla oil authenticity than $-C-H$ asymmetric and $-C-H$ symmetric vibration variables.

Using I2, the authenticity of all model samples were discriminated, while indicating that the use of the I4 ($-C-H$ asymmetric) and I6 variable ($-C-H$ symmetric vibration) has not increased the number of correctly classified samples.

Table 4 Classification of the authenticities of perila oil model samples using integral and second derivative method.

Sample no.	Ingredient	Integral			Second derivative			Classification ^{a)}	
		I2	I4	I6	I2	I4	I6	Integral	Second dev.
1	PO + SO (95:5, v/v)	0.0954	0.8471	0.2708	0.1694	0.2715	0.4357	I	C
2	PO + SO (90:10, v/v)	0.0925	0.8510	0.2714	0.1657	0.2795	0.4441	I	C
3	PO + SO (85:15, v/v)	0.0907	0.8548	0.2746	0.1624	0.2826	0.4512	I	C
4	PO + SO (80:20, v/v)	0.0887	0.8644	0.2771	0.1567	0.2906	0.4548	C	C
5	PO + SO (75:25, v/v)	0.0828	0.8781	0.2843	0.1550	0.3010	0.4753	C	C
6	PO + SO (70:30, v/v)	0.0818	0.8819	0.2844	0.1497	0.3113	0.4800	C	C
7	PO + SO (65:35, v/v)	0.0806	0.8970	0.2915	0.1476	0.3126	0.4859	C	C
8	PO + SO (60:40, v/v)	0.0796	0.8981	0.2925	0.1406	0.3240	0.4919	C	C
9	PO + SO (55:45, v/v)	0.0751	0.9139	0.2990	0.1365	0.3299	0.5062	C	C
10	PO + SO (50:50, v/v)	0.0729	0.9340	0.3059	0.1347	0.3410	0.5121	C	C
11	PO + SO (45:55, v/v)	0.0719	0.9449	0.3107	0.1345	0.3431	0.5172	C	C
12	PO + SO (40:60, v/v)	0.0716	0.9617	0.3125	0.1289	0.3463	0.5180	C	C
13	PO + SO (35:65, v/v)	0.0711	0.9689	0.3163	0.1238	0.3494	0.5195	C	C
14	PO + SO (30:70, v/v)	0.0684	0.9716	0.3211	0.1217	0.3574	0.5272	C	C
15	PO + SO (25:75, v/v)	0.0667	0.9791	0.3245	0.1193	0.3634	0.5387	C	C
16	PO + SO (20:80, v/v)	0.0648	0.9831	0.3268	0.1163	0.3704	0.5444	C	C
17	PO + SO (15:85, v/v)	0.0641	1.0012	0.3340	0.1132	0.3717	0.5453	C	C
18	PO + SO (10:90, v/v)	0.0618	1.0065	0.3358	0.1110	0.3795	0.5543	C	C
19	PO + SO (5:95, v/v)	0.0616	1.0155	0.3399	0.1095	0.3799	0.5535	C	C
20	SO	0.0606	1.0323	0.3471	0.1066	0.3859	0.5597	C	C
21	PO + CO (95:5, v/v)	0.0945	0.8421	0.2691	0.1692	0.2717	0.4358	I	C
22	PO + CO (90:10, v/v)	0.0914	0.8490	0.2698	0.1636	0.2820	0.4465	I	C
23	PO + CO (85:15, v/v)	0.0893	0.8671	0.2788	0.1602	0.2923	0.4649	C	C
24	PO + CO (80:20, v/v)	0.0866	0.8713	0.2788	0.1557	0.2984	0.4653	C	C
25	PO + CO (75:25, v/v)	0.0835	0.8950	0.2887	0.1518	0.3055	0.4787	C	C
26	PO + CO (70:30, v/v)	0.0831	0.9007	0.2914	0.1479	0.3129	0.4811	C	C
27	PO + CO (65:35, v/v)	0.0819	0.9173	0.2997	0.1435	0.3149	0.4876	C	C
28	PO + CO (60:40, v/v)	0.0805	0.9249	0.3008	0.1413	0.3202	0.4909	C	C
29	PO + CO (55:45, v/v)	0.0795	0.9341	0.3021	0.1385	0.3247	0.4977	C	C
30	PO + CO (50:50, v/v)	0.0760	0.9426	0.3102	0.1334	0.3360	0.5072	C	C
31	PO + CO (45:55, v/v)	0.0755	0.9633	0.3182	0.1294	0.3379	0.5123	C	C
32	PO + CO (40:60, v/v)	0.0716	0.9644	0.3190	0.1259	0.3501	0.5223	C	C
33	PO + CO (35:65, v/v)	0.0713	0.9831	0.3267	0.1216	0.3507	0.5273	C	C
34	PO + CO (30:70, v/v)	0.0677	0.9870	0.3282	0.1193	0.3620	0.5362	C	C
35	PO + CO (25:75, v/v)	0.0677	1.0092	0.3367	0.1155	0.3647	0.5405	C	C
36	PO + CO (20:80, v/v)	0.0644	1.0115	0.3383	0.1122	0.3733	0.5540	C	C
37	PO + CO (15:85, v/v)	0.0638	1.0255	0.3435	0.1109	0.3776	0.5475	C	C
38	PO + CO (10:90, v/v)	0.0632	1.0293	0.3448	0.1070	0.3829	0.5497	C	C
39	PO + CO (5:95, v/v)	0.0611	1.0359	0.3477	0.1034	0.3857	0.5625	C	C
40	CO	0.0586	1.0635	0.3592	0.1027	0.4038	0.5707	C	C
Total number of samples correctly classified								35	41

C, correctly classified; I, incorrectly classified; PO, perilla oil; SO, soybean oil, CO, corn oil; ^{a)} If measured values lie within the range of the variable listed in Table 2 and 3, then the sample is an authentic perilla oil; whereas, if any of the measured values lie outside this range, the sample has possibly been adulterated. ^{b)} Entries in boldface type lie outside the range for authentic perilla oils.

Among the three variables, =C–H *cis*-double stretching vibration (I2, 3010 cm⁻¹) variable contributed most to the discrimination of the authenticity of the oil samples, fol-

lowed by –C–H asymmetric (I4, 2922 cm⁻¹) and –C–H symmetric values (I6, 2850 cm⁻¹). Consequently, we determined =C–H *cis*-double bond stretching vibration were the

best variables for discriminating the authenticity of perilla oil.

4 Conclusion

This study has attempted to discriminate the authenticity of perilla oils using FT-IR with ATR accessory by comparing the integral or second derivative method of the perilla oils. From the functional group peaks of FT-IR, the =C–H *cis*-double bond, –C–H asymmetric and –C–H symmetric bond were selected as variables for discriminating the perilla oil authenticity using the S-plot of OPLS-DA technique. We confirmed that the analytical method using the range of relative calculated values of the 3 peaks of FT-IR spectra used in this study could discriminate the adulterated perilla oils with soybean or corn oil. Using integral method, this discrimination procedure discriminated perilla oils with soybean or corn oil added at concentration ≥ 20 vol%, whereas second derivative procedure discriminated perilla oils with soybean or corn oil added at concentrations ≥ 5 vol%. These results demonstrated that second derivative procedure is more accurate than integral measurements for discriminating the perilla oil authenticity. Comparing the 3 variables, the =C–H *cis*-double stretching (I_2 , 3008–3010 cm^{-1}) can be accurately classified. Therefore, we suggest that =C–H *cis*-double bond stretching values should become new variable for perilla oils distributed in Korea. This study suggests that the second derivative FT-IR spectroscopy can be used as a possible reliable, cost-effective and easy analytical method to discriminate the authenticity of perilla oils.

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Conflict of Interest

The authors have declared no conflict of interest.

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