Quantitative Analysis of Blended Oils Based on Intensity Ratios of Marker Ions in MALDI-MS Spectra

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Abstract

Determination of quantitative compositions of blended oils is an essential but challenging step for the quality control and safety assurance of blended oils. We herein report a method for the quantitative analysis of blended oils based on the intensity ratio of triacylglycerol marker ions, which could be obtained from the highly reproducible spectra acquired by using MALDI-MS to directly analyze blended oils in their oily states. We demonstrated that this method could provide good quantitative results to binary, ternary and quaternary blended oils, with simultaneous quantitation of multiple compositions, and was applicable for quantitative analysis of commercial blended oil products. Moreover, the intensity ratio-based method could be used to rapidly measure the proportions of oil compositions in blended oils only based on the spectra of the blended oils and related pure oils, making the method as a high throughput method to meet the sharply growing analytical demands of blended oils.

Keywords: Blended oils; Intensity ratio; Mass spectrometry; Matrix-assisted laser desorption/ionization; Quantitative analysis.

Introduction

Blended oils are mixtures of pure edible oils for the purpose of improved properties, such as higher smoke points, increased oxidative stability, better nutrition and enhanced flavors, and they have become one of the most common edible oils in the market.[1-3] The properties and commercial values of blended oils are mainly determined by the species and abundances of pure oils. The replacing of expensive oil compositions, e.g., olive oil, with cheap oil compositions, e.g., palm oil, in blended oils for financial benefits has been frequently reported in recent years, which can also have negative impacts on human health due to the excessive intake of undesirable fatty acids, e.g., saturated fatty acids.[4-6] To deal with such food fraud, regulations have been promulgated across the world, such as in China, European Union and India, requiring suppliers to indicate the contained pure oils as well as their proportions on the labels of the blended oil products,[7-8] which is expected to become a trend in the world for the quality control of blended oils. Correspondingly, rapid and reliable methods for quantitative analysis of mass blended oil samples are highly desired.

The conventional method for analysis of edible oils is based on gas chromatography (GC), which requires conversion of triacylglycerols (TAGs), the dominant composition of edible oils, into fatty acids (FAs) and then their methyl esters, and measures the corresponding FA contents.[9] Different edible oils contain diverse FA contents and present various TAGs profiles, which could be detected by GC and other techniques for edible oil characterization.[10-12] For blended oils, the variations in the abundances of FAs or TAGs could also be detected by liquid chromatography (LC)[13-14] and spectroscopic techniques such as fluorescence,[15] UV[16] and IR,[17-18] typically with chemometric approaches used to interpret the obtained spectra for quantitative analysis. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is the technique of choice for analysis of edible oils due to its high sensitivity, short analysis time, high-throughput capacity, and the ability to directly profile the TAG patterns of edible oils.[19-21] Our team has previously developed a protocol for direct analysis of edible oils.[22] Unlike conventional MALDI-MS that typically analyzes solid crystalized samples and has significant signal variations, i.e., the hot-spot effect, our protocol directly analyzed oily and homogeneous samples and could produce highly reproducible spectra of edible oils, which allowed rapid and accurate classification of edible oils,[23] and provided the possibilities for quantitative analysis of blended oils based on a chemometric approach.[8]

In this study, the relationships between the intensity ratio of the TAG peaks in MALDI-MS spectra and the proportion of oil compositions in blended oils were mathematically derived and systematically investigated, allowing establishment of an intensity ratio-based method for the quantitative analysis of blended oils for the first time. The developed method showed powerful quantitative ability for rapid analysis of not only binary blends but also blends of multiple compositions, making the method efficient and easy to operate for the quantitative analysis of various blended oils.

Materials and Methods

Chemicals and oil samples

α-Cyano-4-hydroxycinnanic acid (CHCA) and 2, 5-dihydroxybenzoic acid (DHB) were purchased from Aldrich (St. Louis, USA). Acetonitrile (HPLC grade) and acetone (HPLC grade) were obtained from Anaqua Chemical Supply (Houston, USA) and Acros Organic (Waltham, USA), respectively. Polyethylene glycol (PEG) standards were purchased from Fluka (St. Louis, USA) and sodium iodide (NaI) was purchased from Panreac Química (Barcelona, Spain). All chemicals were used directly without further purification. Pure vegetable oils and commercial blended oil products were purchased from markets in Hong Kong and mainland China. Blended oil samples used for establishment of calibration relationships and validation of the obtained calibration relationships (Table S1) were prepared by manually mixing pure oils in different ratios (w/w). For each type of binary blended oils, 10 oil samples were used for calibration and 5 oil samples for validation. For ternary blended oils and quaternary blended oils, the calibration groups included 21 and 56 oil samples, respectively, and the validation groups had 12 and 9 oil samples, respectively. All blended oil samples were freshly prepared, sealed and stored in a dark and dry place, and analyzed within a few days.

MALDI-MS analysis

Sample preparation and spectral acquisition for MALDI-MS analysis were performed as reported previously.[8, 23] Briefly, oil samples were directly loaded onto the DHB layers pre-deposited on a MTP 384 target plate (polished steel BC) using medical cotton tips, and the MALDI-MS spectra were acquired in positive ion and reflectron mode using an UltrafleXtreme MALDI-TOF/TOF mass spectrometer (Bruker, Billerica, USA) equipped with a 355 nm smartbeam-II laser. The mass spectrometer was calibrated using the mixture of PEG standards with CHCA as the matrix. Mass spectra with a *m/z* range of 500–2000 Da were acquired automatically with the irradiation spot moved along a random path. The mass spectrum of each shot was summed up by 1000 laser pulses, and the spectra of eight shots with resolutions higher than 3000 in the *m/z* range of 850–920 Da were accumulated and saved as one MALDI-MS spectrum for further analysis. Each oil sample was analyzed in eight replicates.

Equations for the quantitative analysis

Considering the mass spectrum of oil mixture as a linear combination of the mass spectra of individual oils,[24-25] the intensity ratio of two selected peaks (e.g., peak A and peak B) shown in the spectrum of a binary blended oil could be expressed as

$$\frac{I_{\mathrm{B}}(blend)}{I_{\mathrm{A}}(blend)}=\frac{\frac{p_1}{M_1}I_{B,1}+\frac{p_2}{M_2}I_{B,2}}{\frac{p_1}{M_1}I_{A,1}+\frac{p_2}{M_2}I_{A,2}} \tag{1}$$

where $I_{A,1}$ and $I_{B,1}$, and $I_{A,2}$ and $I_{B,2}$ are the intensities of peak A and peak B in pure oil 1 and pure oil 2, respectively. M_1 and M_2 are the average molecular weights of pure oil 1 and pure oil 2, with p_1 and p_2 referring to the weight percentages of the two pure oils in their blends. After several steps of derivation (see Supporting Information for the details),[26-28] the above equation could be expressed as

$$\frac{1}{r-r_0}=\frac{K}{p_1}+E \tag{2}$$

where $r=\frac{I_B(blend)}{I_A(blend)}$, r_0 is the r value when $p_1=0$ (i.e., r value of pure oil 2), and K and E are constants that relate to the intensities of peak A and peak B shown in the spectra of pure oils.

For ternary blended oils

$$\frac{I_{\mathrm{B}}(blend)}{I_{\mathrm{A}}(blend)}=\frac{\frac{p_1}{M_1}I_{B,1}+\frac{p_2}{M_2}I_{B,2}+\frac{p_3}{M_3}I_{B,3}}{\frac{p_1}{M_1}I_{A,1}+\frac{p_2}{M_2}I_{A,2}+\frac{p_3}{M_3}I_{A,3}} \tag{3}$$

where $I_{A,1}$ and $I_{B,1}$, $I_{A,2}$ and $I_{B,2}$, and $I_{A,3}$ and $I_{B,3}$ are the intensities of peak A and peak B in pure oil 1, pure oil 2 and pure oil 3, respectively, with M_1, M_2 and M_3 as their corresponding average molecular weights. p_1, p_2 and p_3 are the weight percentages of the three pure oils in the ternary blends. Similar to the situation of binary blended oils, Equation 3 could be further derived as

$$(\frac{r}{Q}-C_1)^{-1}-C_2=\frac{Ap_1+a}{p_2+b} \tag{4}$$

where $r=\frac{I_B(blend)}{I_A(blend)}$, $Q=\frac{I_{B,1}}{I_{A,1}}$, and A, a, b, C_1 and C_2 are constants that relate to the intensities of peak A and peak B shown in the spectra of the pure oils (see Supporting Information for the details).

Substituting the weight ratios of pure oils with their weight percentages in blended oils, i.e., letting $R_{21}=\frac{p_2}{p_1}$ and $R_{31}=\frac{p_3}{p_1}$, Equation 3 could be expressed as

$$\frac{I_{\mathrm{B}}(blend)}{I_{\mathrm{A}}(blend)}=\frac{\frac{I_{B,1}}{M_1}+\frac{I_{B,2}}{M_2}R_{21}+\frac{I_{B,3}}{M_3}R_{31}}{\frac{I_{A,1}}{M_1}+\frac{I_{A,2}}{M_2}R_{21}+\frac{I_{A,3}}{M_3}R_{31}}$$

and derived as

$$\left(\frac{r}{Q}-C_1'\right)^{-1}-C_2'=\frac{A'R_{21}+a'}{R_{31}+b'} \tag{5}$$

where $r=\frac{I_B(blend)}{I_A(blend)}$, $Q=\frac{I_{B,1}}{I_{A,1}}$, and A', a', b', C_1' and C_2' are constants that relate to the intensities of peak A and peak B shown in the spectra of the pure oils (see Supporting Information for the details).

Data analysis

The mass spectra were processed by flexAnalysis (Bruker, Billerica, USA) under "centroid" peak detection algorithm, “TopHat” baseline subtraction algorithm and signal to noise ratio higher than 4. The quantitative analysis of binary blends was performed in Microsoft® Excel using the linear regression function, and for ternary and quaternary blends, the quantitative analysis was carried out using a nonlinear least squares approach (function nls in R) in RStudio Desktop (RStudio, Inc., Boston, USA). Grubbs test with detection level α = 0.05 was carried out to detect outliers of the measured results of validation samples.

Results and discussion

Quantitative analysis of binary blends

Validation of the quantitative method based on olive oil-sunflower seed oil blends

The binary olive oil and sunflower seed oil blend was selected as the initial research target due to its popularity in the market and the distinct FA contents of the pure oils. Olive oil is abundant (> 55%) with oleic acid (O),[29-30] while sunflower seed oil contains more linoleic acid (L) than oleic acid.[31-32] The difference in their FA contents leads to different TAG profiles of the two pure oils and thus significantly different MALDI-MS spectra (Figure S1). The major peaks shown in the spectra have been assigned in the previous studies.[8, 23] For pure olive oil and sunflower seed oil, the highest intensity peaks shown in the MALDI-MS spectra were *m/z* 881.8 (POO, P: palmitic acid) and *m/z* 907.8 (OOO), and *m/z* 901.7 (LLL), *m/z* 903.7 (LLO) and *m/z* 905.8 (LOO), respectively, and for the blends with different blending ratios, the main differences in their MALDI-MS spectra were the changes in the relative abundance of the five peaks. Therefore, for quantitative analysis of olive oil-sunflower seed oil blends, TAG peaks at *m/z* 881.8, *m/z* 901.7, *m/z* 903.7, *m/z* 905.8 and *m/z* 907.8 were chosen as potential marker ions and paired into four groups to calculate the intensity ratios, i.e., $I_{881.8}/I_{907.8}$, $I_{901.7}/I_{907.8}$, $I_{903.7}/I_{907.8}$ and $I_{905.8}/I_{907.8}$.

For these potential marker ions, except for the very low value of $I_{901.7}/I_{907.8}$ (0.029±0.004) causing the extreme RSD of 14.2%, the obtained intensity ratios for blended oils with olive oil from 0% to 100% showed excellent precision with RSD within 0.9-8.5% (Table S2), demonstrating the good reproducibility of intensity ratio for quantitative analysis. For all marker ion groups, significant differences were observed between the intensity ratios of samples with different blending ratios ($p <$

0.05 in ANOVA test), indicating the effect of proportion variation on TAG peak abundance. Plotting the obtained intensity ratio against the proportion of olive oil ($p_{(olive)}$) in blended oils, non-linear curves that were not directly suitable for quantitative analysis were observed (Figure S2). As described in Supporting Information, such non-linear relationships could be converted into the linear relationships between $\frac{1}{r-r_0}$ and $\frac{1}{p_1}$, as shown in Equation 2, where r is the intensity ratio of marker ions, p_1 is the weight percentage of pure oil 1 (e.g., olive oil in olive oil-sunflower seed oil blends) and r_0 is the r value of pure oil 2 (e.g., sunflower seed oil in olive oil-sunflower seed oil blends). For blends of olive oil and sunflower seed oil, considering olive oil as the measured composition (i.e., pure oil 1), calibration plots of $\frac{1}{r-r_0}$ against $\frac{1}{p_{(olive)}}$ showed excellent linearity for $I_{881.8}/I_{907.8}$, $I_{901.7}/I_{907.8}$, $I_{903.7}/I_{907.8}$ and $I_{905.8}/I_{907.8}$ with coefficients of determination (R^2) higher than 0.995 (Figure S3). To validate the quantitative ability of the obtained plots, the concentrations of olive oil in five validation samples were quantified based on their MALDI-MS spectra and the developed calibration plots. As shown in Table S3, the plot using $I_{905.8}/I_{907.8}$ as r showed the best quantitative ability with accuracy and precision in the range of (–7.8)-5.6% and 1.6-17.1%, respectively. When r represented $I_{901.7}/I_{907.8}$ and $I_{903.7}/I_{907.8}$, the measured results of olive oil were close to the actual concentrations as accuracy and precision within (–9.1)-6.1% and 0.2-12.6%, and (–10.8)-8.0% and 0.5-18.9%, respectively. The worst quantitative performance was observed in the results measured by the plot based on $I_{881.8}/I_{907.8}$ with poor accuracy (23.3%) and large RSD (40.6%).

Regarding sunflower seed oil as the measured composition in the olive oil-sunflower seed oil blends, a series of new calibration plots could be obtained based on r and the proportion of sunflower seed oil ($p_{(sunflower)}$) (Figure S3). For $I_{881.8}/I_{907.8}$, $I_{903.7}/I_{907.8}$ and $I_{905.8}/I_{907.8}$, the calibration plots showed strong linear relationships with R^2 higher than 0.995, while for the plot based on $I_{901.7}/I_{907.8}$, slightly poor linearity was observed (R^2=0.9798). The sum of $p_{(olive)}$ and $p_{(sunflower)}$ in the olive oil-sunflower seed oil blends was always 1, so the proportions of olive oil in the blends could be calculated after using the calibration plots based on $p_{(sunflower)}$ to measure the proportions of sunflower seed oil in the blends, and these results were summarized in Table S3. Comparing the results derived from the plots based on $p_{(olive)}$ and $p_{(sunflower)}$, it could be noted that the plots based on $p_{(sunflower)}$ provided poor quantitative results to samples with low-abundance olive oil ($\leqslant$30%), especially for the sample containing 7.7% olive oil. However, for samples with high-abundance olive oil (~92%), the quantitative results derived from the plots based on $p_{(sunflower)}$ were better than those derived from the plots based on $p_{(olive)}$. Such situation was observed for the plots based on different marker ions and was caused by the propagated error of regression analysis (see Supporting Information for the details).

A segmental strategy has been proposed to improve the quantitative results in this study, i.e., using the plot based on $p_{(olive)}$ to measure samples with low-abundance olive oil and using the plot based on $p_{(sunflower)}$ to measure samples with high-abundance olive oil. Root mean square error (RMSE) was used to summarize the differences between the actual concentrations and the measured results of segmental quantitation, which were 0.0788, 0.0241, 0.0164 and 0.0125 for the results derived from $I_{881.8}/I_{907.8}$, $I_{901.7}/I_{907.8}$, $I_{903.7}/I_{907.8}$ and $I_{905.8}/I_{907.8}$, respectively. Therefore, the peaks at *m/z* 905.8 and *m/z* 907.8 were recommended for the quantitative analysis of olive oil-sunflower seed oil blends and the improved results of segmental quantitation showed accuracy and precision within (–7.8)-3.0% and 1.5-17.1%, respectively (Table 1). The average molecular weights of olive oil and sunflower seed oil are similar,[33] i.e., $\frac{M_1}{M_2} \approx 1$, so the approximate values of K and E in Equation 2 can be estimated based on the spectra of pure olive oil and sunflower seed oil. For the calibration plot based on

$I_{905.8}/I_{907.8}$ and $p_{(olive)}$, the slope (K) and intercept (E) were –0.1165 and –0.3325 (Figure S3), which were estimated as –0.0918 and –0.3492, respectively, from the spectral data of pure oils. The estimated values of K and E were close to values of regression and were substituted into Equation 2 to calculate the concentrations of olive oil in validation samples. As shown in Table 1, the calculated results indicated the approximate concentrations of olive oil in the validation samples with absolute errors within ±7%, illustrating the successful semi-quantitation of oil compositions in binary blended oils.

Quantitative analysis of other binary blended oils

As described above, an intensity ratio-based method has been developed for the quantitative analysis of binary blended oils. To comprehensively validate the developed method, six types of binary blended oils were prepared, and they were the blends of pure oils with disparate TAG profiles (i.e., flaxseed oil-peanut oil blends and flaxseed oil-corn oil blends), the blends of pure oils with partly different TAG profiles (i.e., corn oil-canola oil blends and olive oil-peanut oil blends) and the blends of pure oils with similar TAG profiles (i.e., sunflower seed oil-soybean oil blends and corn oil-soybean oil blends). Based on the MALDI-MS spectra of each type of blended oils (Figure 1), TAG peaks that were characteristic for individual oils were selected as potential marker ions and applied to establish calibration plots, which were then utilized to quantify the oil compositions in validation samples with the implementation of the segmental strategy. Comparing the results measured by the plots based on different ions, the ions corresponding to the plots with the best quantitative performance were chosen as the marker ions of the blended oils and the detailed results were summarized in Table S4.

Flaxseed oil is rich in α-linolenic acid (Ln) [34], leading to strong peaks at *m/z* 895.7 (LnLnLn), *m/z* 897.7 (LnLnL) and *m/z* 899.7 (LnLL) in the MALDI-MS spectrum, which were very weak in the spectra of peanut oil and corn oil. Due to the high levels of oleic acid and linoleic acid in peanut oil and corn oil,[35-36] the spectrum of peanut oil had abundant peaks at *m/z* 907.8 and *m/z* 905.8, and the spectrum of corn oil showed high intensive peaks at *m/z* 903.7 and *m/z* 901.7. Therefore, the peaks at *m/z* 895.7 and *m/z* 899.7, *m/z* 905.8 and *m/z* 907.8, and *m/z* 901.7 and *m/z* 903.7 were selected to represent flaxseed oil, peanut oil and corn oil, respectively. For flaxseed oil-peanut oil blends, the calibration plots based on different ions (i.e., $I_{895.7}/I_{905.8}$, $I_{895.7}/I_{907.8}$, $I_{899.7}/I_{905.8}$ and $I_{899.7}/I_{907.8}$) provided similar quantitative results for flaxseed oil with RMSE varying from 0.0124 to 0.0145, indicating that these selected ions were powerful for quantitative analysis, and the best quantitative results were provided by the plot based on $I_{899.7}/I_{905.8}$ with accuracy and precision within (–3.6)-9.8% and 1.4-7.1%, respectively (Table S4). For flaxseed oil-corn oil blends, the RMSE of quantitative results provided by the plots based on $I_{895.7}/I_{901.7}$, $I_{895.7}/I_{903.7}$, $I_{899.7}/I_{901.7}$ and $I_{899.7}/I_{903.7}$ were 0.0295, 0.0241, 0.0233 and 0.0194, respectively, and the results measured by $I_{899.7}/I_{903.7}$ showed excellent accuracy and precision that were in the range of (–1.1)-4.0% and 1.1-8.5%, respectively.

The most abundant FA in canola oil was oleic acid, followed by linoleic acid,[37] while corn oil contained more linoleic acid than oleic acid.[35] Therefore, the main TAG peaks in the MALDI-MS spectra of corn oil and canola oil overlapped but with different relative intensities, and the peaks at *m/z* 901.7 and *m/z* 903.7 (abundant in corn oil) were paired with the peaks at *m/z* 905.8 and *m/z* 907.8 (abundant in canola oil) to calculate the intensity ratios. When the proportion of corn oil in the blends increased from 0% to 100%, the peak at *m/z* 901.7 showed a larger increase in the relative intensity than the peak at *m/z* 903.7, and the peak at *m/z* 907.8 had a greater decrement in the abundance than the peak at *m/z* 905.8. As a result, the calibration plot based on $I_{901.7}/I_{907.8}$ presented the best quantitative ability for the corn oil-canola oil blends. For olive oil-peanut oil blends with increasing proportion of olive oil (from 0% to 100%), the main changes in their MALDI-MS spectra were the decline of peaks at *m/z* 901.7, *m/z* 903.7 and *m/z* 905.8 with the peak at *m/z* 907.8 as the strongest peak, and the most significant variation in the intensity ratio was obtained from $I_{903.7}/I_{907.8}$, resulting in the best quantitative performance of the plot based on $I_{903.7}/I_{907.8}$.

The MALDI-MS spectra of sunflower seed oil, corn oil, and soybean oil showed similar TAG patterns due to their similar FA contents, and a characteristic peak at *m/z* 899.7 was observed for soybean oil because soybean oil contains a small amonut of α-linolenic acid which is deficient in sunflower seed oil and corn oil.[35] For sunflower seed oil-soybean oil blends, the peaks at *m/z* 901.7 and *m/z* 903.7 showed similar intensities that were stronger than other peaks, leading to the better quantitative performance of $I_{899.7}/I_{901.7}$ and $I_{899.7}/I_{903.7}$ (RMSE as 0.0225 and 0.0207), and the quantitative results derived from $I_{899.7}/I_{903.7}$ showed accuracy and precision within (–0.1)-4.4% and 0.8-23.6%, respectively (Table S4). Similarly, $I_{899.7}/I_{903.7}$ provided the best quantitative results for corn oil-soybean oil blends which were very close to the actual concentrations. For pure soybean oil and corn oil, comparable variation was noticed for $I_{899.7}/I_{879.7}$ and $I_{899.7}/I_{903.7}$ with $r_{(\text{soybean})}/r_{(\text{corn})}$ as 5.18 and 5.39, respectively, while the quantitative performance of $I_{899.7}/I_{879.7}$ (RMSE = 0.0377) was not as good as that of $I_{899.7}/I_{903.7}$ (RMSE = 0.0327). The peaks at *m/z* 879.7, *m/z* 899.7 and *m/z* 903.7 were related to three different TAGs, i.e., POO, LLLn and OLL, respectively. OLL (C54:5) had similar structure with LLLn (C54:7), while POO (C52:2) was quite different from LLLn, not only in the number of double bonds but also in the number of carbon atoms. Therefore, the difference between the response factors of POO and LLLn should be greater than that of OLL and LLLn, which might lead to a larger fluctuation of $I_{899.7}/I_{879.7}$ and deteriorated the quantitative ability.

Strategy for the selection of marker ions

The above comprehensive investigation about the quantitative analysis of binary blends has enabled us to propose a guideline for the selection of marker ions for the intensity-ratio based method. First, the characteristic peaks that are abundant in one pure oil and deficient in the other pure oil are the most potential markers. Second, common peaks with larger variations between the two pure oils are preferred for better quantitative capabilities. Third, for the peaks with similar variations, priority should be given to the peaks with higher abundances and closer *m/z* values for better accuracy and precision. This proposed guideline has been applied for various types of binary blended oils, and could be further extended to the analysis of blended oils with more oil compositions, such as ternary blends and quaternary blends. With this proposed guideline, the selection of marker ions for quantitative analysis can be simplified, making the intensity ratio-based method more convenient and acceptable for various analysis.

Quantitative analysis of ternary blends

In addition to binary blended oils, ternary blended oils are commonly available in the market. Comparisons of the MALDI-MS spectra of olive oil, sunflower seed oil, soybean oil and their ternary blends showed that the ternary blended oils had more complex spectral data with numerous TAG peaks and the abundances of TAG peaks were jointly determined by the multiple oil compositions (Figure S4), making it difficult to predict the changes in TAG profiles in ternary blended oils with different blending ratios. It was observed that the MALDI-MS spectrum of olive oil was abundant in the peaks at *m/z* 881.8, *m/z* 905.8 and *m/z* 907.8, and the spectra of sunflower seed oil and soybean oil showed high intensity peaks at *m/z* 901.7, *m/z* 903.7 and *m/z* 905.8 with a characteristic peak at *m/z* 899.7 for soybean oil. Therefore, these TAG peaks were chosen as potential marker ions and paired into different groups, i.e., $I_{899.7}/I_{881.8}$, $I_{899.7}/I_{903.7}$, $I_{903.7}/I_{901.7}$, $I_{905.8}/I_{903.7}$ and $I_{907.8}/I_{903.7}$, according to the proposed guideline, and nonlinear least squares (NLS) regression was utilized to develop the relationships between r and the proportions of sunflower seed oil (as p_1) and soybean oil (as p_2) based on Equation 4. As shown in Figure 2, by plotting r against the corresponding p_1 and p_2, curved surfaces with varied shapes and curvatures were observed for different potential marker ion groups, demonstrating the complex and changeable correlation between intensity ratio and oil compositions. For all the potential marker ion groups, the curved surfaces constructed by NLS regression showed good fitting abilities to the data points of the calibration samples, and the intensity ratios predicted by the developed nonlinear relationships were highly correlated with the intensity ratios obtained from the MALDI-MS spectra with Pearson correlation coefficients (PCC) higher than 0.996 (Table S5), illustrating that Equation 4 could be used to describe the changes in the intensity ratios of TAG peaks in ternary blended oils with varied compositions.

Equation 4 could be rewritten as

$$f(r) = \frac{Ap_1 + a}{p_2 + b} \tag{6}$$

where $f(r) = (\frac{r}{Q} - C_1)^{-1} - C_2$. For different groups of marker ions, the nonlinear relationship is different, so a system of equations could be constructed as below for the quantitative analysis of ternary blends.

$$\begin{cases} f(r_1) = \dfrac{A_1 p_1 + a_1}{p_2 + b_1} \\ f(r_2) = \dfrac{A_2 p_1 + a_2}{p_2 + b_2} \\ p_3 = 1 - p_1 - p_2 \end{cases} \tag{7}$$

where $f(r_1)$ and $f(r_2)$ are parameters related to the intensity ratios of different groups of marker ions (i.e., r_1 and r_2), respectively, and p_1, p_2 and p_3 are the proportions of the three oil compositions in the ternary blends. For the ternary blends of olive oil, sunflower seed oil and soybean oil, the combination of any two developed nonlinear relationships generated ten different NLS models that were applied for the quantitative analysis of validation samples. The difference and correlation between the actual concentrations and measured concentrations for each composition were described by RMSE and PCC, which were summarized in Table S6 except for the results provided by the model of $I_{899.7}/I_{881.8}$ and $I_{903.7}/I_{901.7}$ due to the poor quantitative performance (RMSE>0.5). Among all the NLS models, the models based on $I_{899.7}/I_{881.8}$ with $I_{899.7}/I_{903.7}$, $I_{899.7}/I_{903.7}$ with $I_{905.8}/I_{903.7}$ and $I_{899.7}/I_{903.7}$ with $I_{907.8}/I_{903.7}$ presented good quantitative ability, achieving accurate quantitation of the three oil compositions simultaneously, and the best quantitative results were provided by the model based on $I_{899.7}/I_{903.7}$ and $I_{907.8}/I_{903.7}$ with PCC as 0.9794, 0.9873 and 0.9971, and RMSE as 0.0526, 0.0475 and 0.0191 for sunflower seed oil, soybean oil and olive oil, respectively.

Applying $p_{(\text{soybean})}$ and $p_{(\text{olive})}$ as p_1 and p_2 respectively to develop nonlinear relationships (Table S5), the quantitative analysis of olive oil-sunflower seed oil-soybean oil blends was achieved, and the best quantitative performance was observed for the model using $I_{899.7}/I_{903.7}$ and $I_{907.8}/I_{903.7}$ as the indicators (Table S6), consistent with the results provided by the models based on $p_{(\text{sunflower})}$ and $p_{(\text{soybean})}$. The quantitative analysis of olive oil-sunflower seed oil-soybean oil blends could also be done by using $p_{(\text{olive})}$ and $p_{(\text{sunflower})}$ as p_1 and p_2, but the best quantitative results were provided by the model based on $I_{905.8}/I_{903.7}$ and $I_{907.8}/I_{903.7}$, because the absence of the peak at *m/z* 899.7 in the spectrum of pure olive oil made it impossible to establish the relationships of $I_{899.7}/I_{881.8}$ and $I_{899.7}/I_{903.7}$ (Figure S4). As shown in Table S6, for the same marker ions, the NLS models based on different oil compositions provided similar quantitative results since the RMSE and PCC values of the same oil compositions were very close or even the same, revealing that selecting different oil compositions for model establishment would not affect the quantitative ability of NLS models (see Table S7 for the details). Overall, for olive oil-sunflower seed oil-soybean oil blends, the most accurate quantitative results were provided by the NLS model based on $I_{899.7}/I_{903.7}$ and $I_{907.8}/I_{903.7}$. Excluding the results of sample T8 which had poor accuracy, the accuracy and precision of the measured results were within (–24.0)-23.9% and 0.2-17.6%, respectively, and for compositions not at low levels (>30%), most of the quantitative results showed excellent accuracy and precision which were within ±10% (Table S7).

For ternary blended oils, there are five parameters (i.e., C_1, C_2, A, b and a) in the equation describing the nonlinear relationship between r, p_1 and p_2, making it difficult and time-consuming to simultaneously estimate all the parameters using the NLS regression. Supposing similar average molecular weights for different vegetable oils,[33, 38] the approximate values of these five parameters could be calculated from the spectral data of pure oils (called theoretical values). Setting C_1 and C_2 as their theoretical values, the process of NLS regression to estimate A, b and a could be significantly simplified, and the obtained relationships were utilized to establish quantitative models (called simplified models). For olive oil-sunflower seed oil-soybean oil blends, the NLS model based on $I_{899.7}/I_{903.7}$ and $I_{907.8}/I_{903.7}$ had the best quantitative performance, and for peanut oil-corn oil-canola oil blends (Figure S5) and sunflower seed oil-canola oil-camellia oil blends (Figure S6), the NLS models based on $I_{881.8}/I_{905.8}$ and $I_{907.8}/I_{901.7}$, and $I_{901.7}/I_{903.7}$ and $I_{905.8}/I_{907.8}$ showed excellent quantitative ability, respectively. For the three types of ternary blended oils, the quantitative ability of the model based on the theoretical values (called theoretical model), the simplified model and the NLS model with five estimated parameters were compared, and the details of these models were summarized in Table S8. For olive oil-sunflower seed oil-soybean oil blends, the best quantitative performance (with the smallest RMSE) was observed from the NLS model, followed by the simplified model and the theoretical model, and the most significant reduction in RMSE was observed for the results of soybean oil with RMSE as 0.0655, 0.0583 and 0.0475 for the theoretical model, the simplified model and the NLS model, respectively (Table 2). A similar situation was observed for peanut oil-corn oil-canola oil blends, but the improvement in the measured results was not obvious since the variation in RMSE was within 0.005. On the other hand, the simplified model of sunflower seed oil-canola oil-camellia oil blends provided the best quantitative results. Increments in RMSE were observed for the results measured by the theoretical model and the NLS model, and compared with the theoretical model, the NLS model showed improvements in the quantitative results of sunflower seed oil and canola oil but deterioration in the quantitative results of camellia oil, indicating that overfitting might happen during the NLS regression process of estimating all the parameters.

As discussed above, the simplified model and the NLS model which were based on the same marker ions had similar quantitative capability. Generally, the NLS model tended to provide better quantitative results but the quantitative performance of the simplified model was also acceptable, and the improvement in the quantitative results was not obvious. When overfitting happened to the NLS model, the quantitative results provided by the simplified model were better. Therefore, for the quantitative analysis of ternary blended oils, the use of the simplified model should be considered first to strike a balance between the good quantitative ability and reasonable workload of data analysis, and the NLS model would be optional and could be applied for more accurate measurements. The theoretical model only required the spectra of pure oils to establish the quantitative model, and could approximate the proportions of compositions in blended oils, making the theoretical model more suitable for semi-quantitative analysis. Moreover, the quantitative performance of the theoretical model could be used to evaluate the quantitative capability of potential marker ions, facilitate the selection of marker ions and further simplify the processes of establishing calibration models. Hence, to quantify the oil compositions in ternary blended oils, potential marker ions were firstly selected by comparing the MALDI-MS spectra of pure oils, and then utilized to develop theoretical models using the spectra of pure oils. After evaluating the quantitative performance of different theoretical models, the potential marker ions that presented the best quantitative capability were applied to establish simplified model or NLS model using the spectra of calibration samples, and the established model was eventually used for quantitative analysis.

Quantitative analysis of quaternary blends

Compared with the quantitative analysis of ternary blends, the quantitative analysis of quaternary blends can be much more challenging due to the complicated relationship between the intensity ratio and the proportion of pure oils, and the miscellaneous possible combinations of oil compositions. As shown in Equation 5, the intensity ratio of marker ions in ternary blends can be expressed by the weight ratio of oil compositions, so the quantitative analysis of ternary blends can be realized as

$$\begin{cases} f(r_1)' = \dfrac{A_1' R_{21} + a_1'}{R_{31} + b_1'} \\ f(r_2)' = \dfrac{A_2' R_{21} + a_2'}{R_{31} + b_2'} \end{cases} \tag{8}$$

where $f(r)' = \left(\frac{r}{Q} - C_1'\right)^{-1} - C_2'$, $f(r_1)'$ and $f(r_2)'$ are parameters related to the intensity ratios of two groups of marker ions (r_1 and r_2), and R_{21} and R_{31} are the normalized ratios of pure oil 2 and pure oil 3, respectively, with pure oil 1 as the standard composition, i.e., $R_{21} = \frac{p_2}{p_1}$ and $R_{31} = \frac{p_3}{p_1}$. For quaternary blends of pure oils with distinct TAG profiles, the two pure oils showing the most different TAG profiles can be assigned to subgroup I and II, respectively, with the remaining two pure oils as common compositions in both subgroups, so that each subgroup contains three compositions. If both subgroups have characteristic peaks not observed or very weak in the other subgroup, the quantitative analysis of quaternary blended oils can be achieved by the combined analysis of subgroup I and II based on Equation 8.

For olive oil-flaxseed oil-rice bran oil-soybean oil blends, olive oil and flaxseed oil had strong peaks at *m/z* 881.8 and *m/z* 907.8, and *m/z* 895.7, *m/z* 897.7 and *m/z* 899.7, respectively, rice bran oil showed high intensity peaks at *m/z* 853.7 and *m/z* 879.7, and soybean oil had a characteristic peak at *m/z* 899.7 (Figure S7). Among the four pure oils, olive oil and flaxseed oil had significant different TAG patterns with most of the peaks not overlapped, hereby the two subgroups were the olive oil-rice bran oil-soybean oil subgroup and the flaxseed oil-rice bran oil-soybean oil subgroup, with the characteristic peaks at *m/z* 853.7, *m/z* 879.7, *m/z* 881.8, *m/z* 883.8 and *m/z* 909.8, and *m/z* 873.7, *m/z* 895.7, *m/z* 897.7, *m/z* 899.7 and *m/z* 901.7, respectively. Compared with binary and ternary blended oils, quaternary blended oils showed more complex spectral data, so each quaternary blended oil sample was analyzed in twenty-four replicates and the average data of eight replicates were used for further analysis to improve reproducibility of the quantitative results. Following the same procedure as ternary blended oils, simplified models were established for the two subgroups, and details of the models showing the best quantitative performance can be found in Table S9.

For the olive oil-rice bran oil-soybean oil subgroup, the model based on $I_{883.8}/I_{879.7}$ and $I_{909.8}/I_{853.7}$ provided the best quantitative results to $R_{(\text{olive/rice bran})}$ (RMSE = 0.3747), and for the flaxseed oil-rice bran oil-soybean oil subgroup, the model based on $I_{895.7}/I_{899.7}$ and $I_{895.7}/I_{901.7}$ provided excellent quantitative results to $R_{(\text{rice bran/flaxseed})}$ and $R_{(\text{soybean/flaxseed})}$ with RMSE as 0.3562 and 0.3865, respectively. Combining the measured results of $R_{(\text{olive/rice bran})}$, $R_{(\text{rice bran/flaxseed})}$ and $R_{(\text{soybean/flaxseed})}$, the weight percentages of individual oils were solved with flaxseed oil as the standard composition, and the RMSE of the measured and actual concentrations were 0.0437, 0.0289, 0.0251 and 0.0515 for olive oil, flaxseed oil, rice bran oil and soybean oil, respectively. As shown in Table S10, for most of the validation samples, the measured results were close to the actual concentrations with acceptable precision, and poorer accuracy (over ±15%) and larger RSD (>25%) were mainly observed for the results of low-abundance compositions (~10%). Overall, it was feasible to quantify the compositions of quaternary blended oils using the intensity ratio-based method, and similar strategy was applicable for blends of more oil compositions, such as quinary blends and senary blends. Briefly, for blended oils containing numerous oil compositions (⩾4), two or three subgroups were extracted according to the MALDI-MS spectra of pure oils. Each subgroup contained three oil compositions and showed some characteristic peaks, so the quantitative analysis of individual subgroup could be achieved using Equation 8 and following the same procedure as ternary blends. Combining the quantitative results of all the subgroups, the proportion of each oil composition in the blends could be obtained.

Analysis of commercial blended oil products

The developed intensity ratio-based method was applied in the quantitative analysis of commercial blended oil products collected from the market, i.e., sunflower seed oil-soybean oil blend, corn oil-soybean oil blend, olive oil-peanut oil blend, corn oil-canola oil blend, peanut oil-corn oil-canola oil blend and olive oil-corn oil-sunflower seed oil blend, with the results summarized in Table 3. For each binary blended oil product, the minor oil composition was selected as the measured composition according to the segmental strategy. The measured results of the first two products were close to the labeled proportions with absolute errors within ±4%, while large differences were observed between the measured results and labeled proportions of other two products with absolute errors exceeded ±10%. The proportions of all the compositions in each ternary blended oil product were quantified simultaneously by the simplified models. For the peanut oil-corn oil-canola oil blended product, balanced proportions were measured to the three oil compositions, and the highest proportion was corn oil (37.1%), followed by peanut oil (32.6%) and canola oil (30.2%). For the olive oil-corn oil-sunflower seed oil blended product, the measured result of olive oil was close to the labeled proportion with an absolute error of –3.7%. Compared with corn oil, the quantitative model detected a higher abundance of sunflower seed oil, while the label of the product indicated equal proportions for the two compositions.

Considering potentially varied TAG profiles of a type of oil from different origins, to minimize the effect of oil profile variations on the quantitative performance of models, it is suggested to use the corresponding pure oils that are used for the blended oil preparation to establish the models. Such pure oil products are typically from the same manufacturers, and our previous study[23] has demonstrated that the pure oil products of the same brand have very similar TAG profiles and thus should be able to provide similar quantitative results.

Conclusions

The MALDI-MS spectra of blended oils contain quantitative information of the comprised oil compositions. In this study, a rapid method applying the intensity ratio of marker ions, i.e., TAG peaks, for quantitation of oil compositions in the blends, as well as general guidelines for the selection of marker ions and the determination of quantitative models, were developed. The intensity ratio-based method significantly simplified the quantitative analysis of blended oils, especially those with multiple compositions, and was suitable for both accurate measurement and rough estimation, making this method advantageous in rapid screening of blended oils to promote quality control and safety assurance. The developed method can also be used for detection of adulterated blended oils, since the intensity ratios of the marker ions of the adulterated blended oils can be different from those of the authentic ones. The developed method could visually correlate the peak abundances in mass spectra with compositions of individual oils in blends in a straightforward way, and thus was convenient and user-friendly. Given the high analytical demand of blend oils, this developed method is expected to generate significant impact on the edible oil market, and can be extended to studies and quantitative analysis of other complex mixtures.

Supporting Information

Sample preparation and equation derivations for quantitative analysis; additional MALDI-MS spectra and quantitative results of blended oils (PDF).

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Figure caption

Figure 1. MALDI-MS spectra of pure oils and calibration plots. (a) Flaxseed oil-peanut oil blends; (b) flaxseed oil-corn oil blends; (c) corn oil-canola oil blends; (d) olive oil-peanut oil blends; (e) sunflower seed oil-soybean oil blends and (f) corn oil-soybean oil blends.

Figure 2. Plots of intensity ratios of marker ions against the proportions of sunflower seed oil ($p_{(sunflower)}$) and soybean oil ($p_{(soybean)}$) in olive oil-sunflower seed oil-soybean oil blends. (a) $I_{899.7}/I_{881.8}$; (b) $I_{899.7}/I_{903.7}$; (c) $I_{903.7}/I_{901.7}$; (d) $I_{905.8}/I_{903.7}$ and (e) $I_{907.8}/I_{903.7}$.

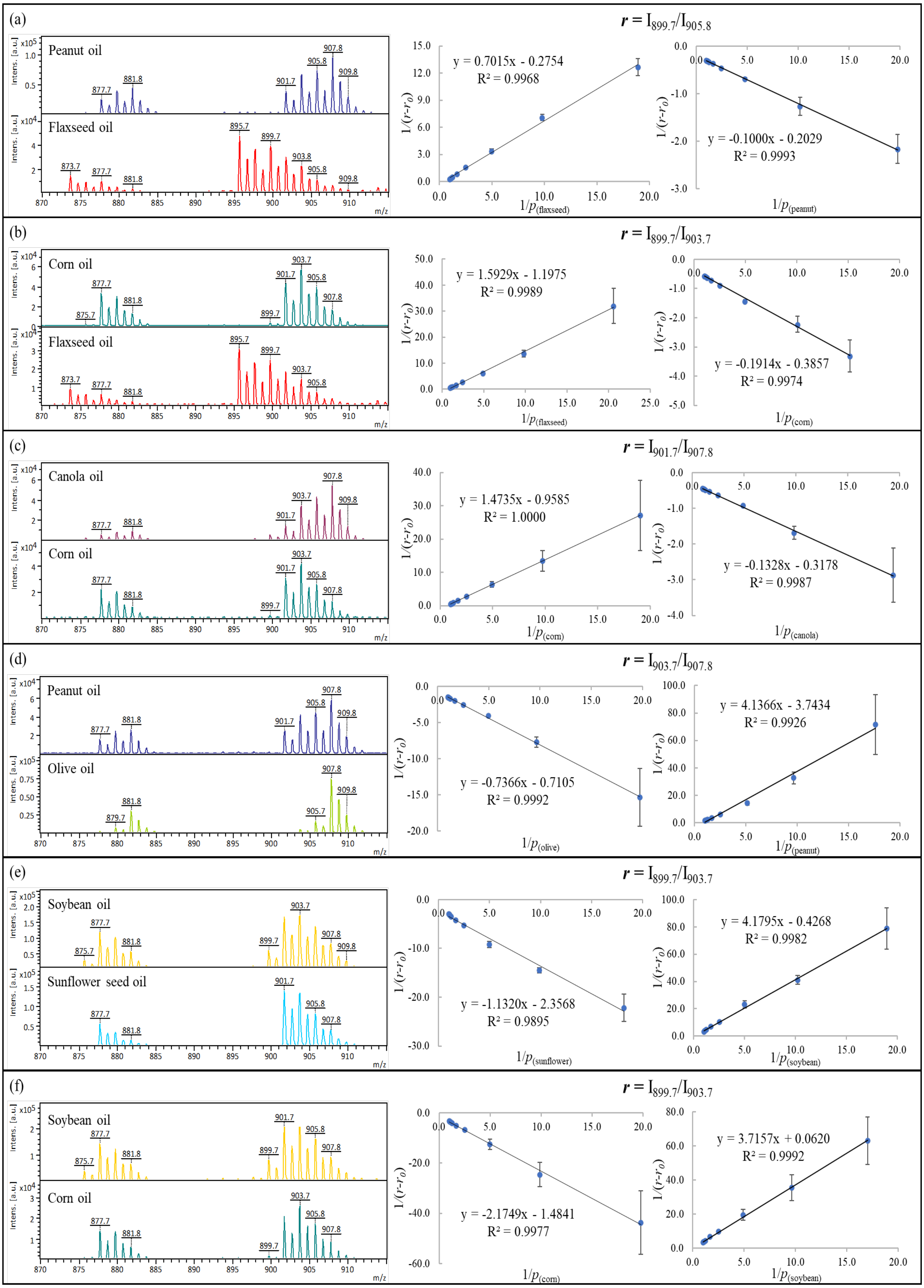
(a)
$r = I_{899.7}/I_{905.8}$
Peanut oil
Flaxseed oil
y = 0.7015x - 0.2754
R² = 0.9968
y = -0.1000x - 0.2029
R² = 0.9993
$1/p_{(flaxseed)}$
$1/p_{(peanut)}$
(b)
$r = I_{899.7}/I_{903.7}$
Corn oil
Flaxseed oil
y = 1.5929x - 1.1975
R² = 0.9989
y = -0.1914x - 0.3857
R² = 0.9974
$1/p_{(flaxseed)}$
$1/p_{(corn)}$
(c)
$r = I_{901.7}/I_{907.8}$
Canola oil
Corn oil
y = 1.4735x - 0.9585
R² = 1.0000
y = -0.1328x - 0.3178
R² = 0.9987
$1/p_{(corn)}$
$1/p_{(canola)}$
(d)
$r = I_{903.7}/I_{907.8}$
Peanut oil
Olive oil
y = -0.7366x - 0.7105
R² = 0.9992
y = 4.1366x - 3.7434
R² = 0.9926
$1/p_{(olive)}$
$1/p_{(peanut)}$
(e)
$r = I_{899.7}/I_{903.7}$
Soybean oil
Sunflower seed oil
y = -1.1320x - 2.3568
R² = 0.9895
y = 4.1795x - 0.4268
R² = 0.9982
$1/p_{(sunflower)}$
$1/p_{(soybean)}$
(f)
$r = I_{899.7}/I_{903.7}$
Soybean oil
Corn oil
y = -2.1749x - 1.4841
R² = 0.9977
y = 3.7157x + 0.0620
R² = 0.9992
$1/p_{(corn)}$
$1/p_{(soybean)}$
$1/(r-r_0)$
Intens. [a.u.]
m/z
870
875
880
885
890
895
900
905
910

Figure 1

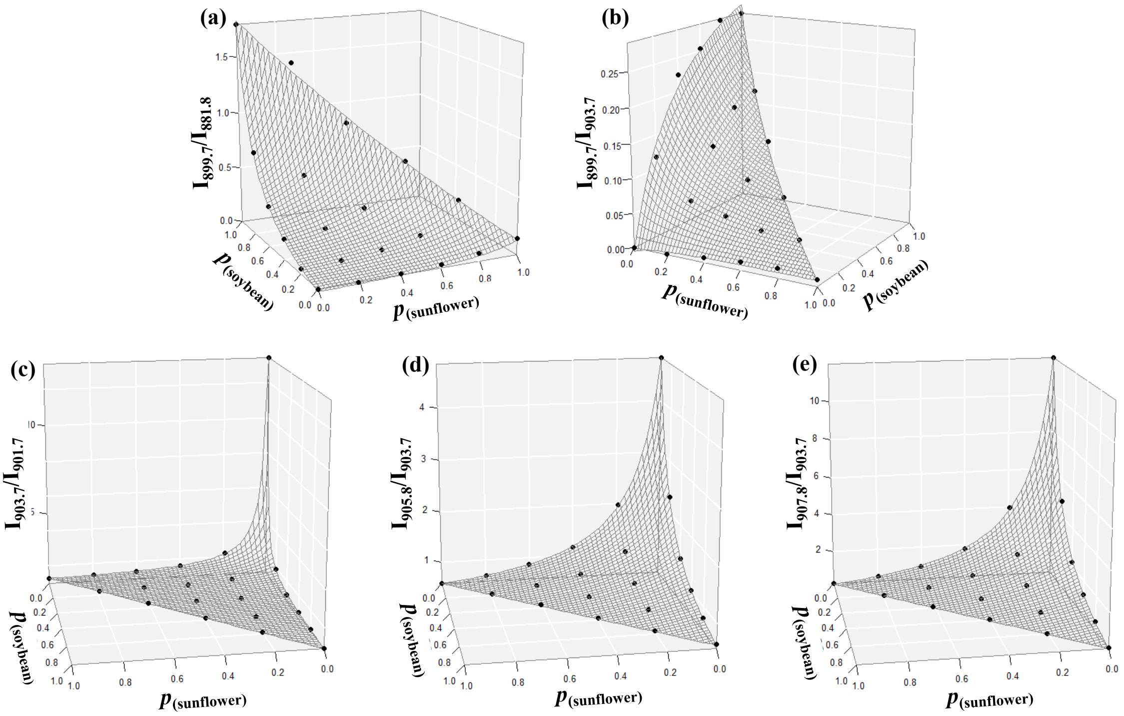
(a)
$I_{899.7}/I_{881.8}$
$p_{(soybean)}$
$p_{(sunflower)}$
(b)
$I_{899.7}/I_{903.7}$
$p_{(sunflower)}$
$p_{(soybean)}$
(c)
$I_{903.7}/I_{901.7}$
$p_{(soybean)}$
$p_{(sunflower)}$
(d)
$I_{905.8}/I_{903.7}$
$p_{(soybean)}$
$p_{(sunflower)}$
(e)
$I_{907.8}/I_{903.7}$
$p_{(soybean)}$
$p_{(sunflower)}$

Figure 2

Table 1. Quantitative results of olive oil in olive oil-sunflower seed oil blends measured by the calibration plots and calculated from the spectral data of pure oils with $I_{905.8}/I_{907.8}$ as *r*.

Actual conc. (%)	**Based on calibration plots**				**Based on pure oils**		
	Measured by $p_{(olive)}$ (%)	Measured by $p_{(sunflower)}$ (%)	Accuracy (%)	RSD (%)	Calculated conc. (%)	Accuracy (%)	RSD (%)
7.7	7.1±1.2	/	–7.8	17.1	5.7±1.0	–26.6	17.2
30.1	30.8±0.7	/	2.1	2.3	25.4±0.6	–15.8	2.4
49.7	51.3±1.0	/	3.0	1.9	43.6±0.9	–12.4	2.1
70.0	/	70.3±1.1	0.5	1.5	63.7±1.1	–8.9	1.7
92.4	/	92.3±1.5	–0.1	1.6	89.4±1.9	–3.2	2.2

Table 2. Quantitative performance of the theoretical models, simplified models and NLS models for ternary blended oils.

Blending type (A-B-C)	**Marker ions (r_1&r_2)**	**Model**	**Pure oil A**		**Pure oil B**		**Pure oil C**	
			PCC[a]	RMSE[b]	PCC	RMSE	PCC	RMSE
Olive oil-sunflower seed oil-soybean oil	$I_{899.7}/I_{903.7}$ & $I_{907.8}/I_{903.7}$	p_1: sunflower seed oil; p_2: soybean oil						
		Theo.	0.9975	0.0208	0.9861	0.0638	0.9826	0.0655
		Sim.	0.9977	0.0200	0.9775	0.0565	0.9854	0.0583
		NLS	0.9971	0.0191	0.9794	0.0526	0.9873	0.0475
Peanut oil-corn oil-canola oil	$I_{881.8}/I_{905.8}$ & $I_{907.8}/I_{901.7}$	p_1: peanut oil; p_2: corn oil						
		Theo.	0.9767	0.0554	0.9928	0.0316	0.9827	0.0513
		Sim.	0.9767	0.0540	0.9927	0.0313	0.9816	0.0480
		NLS	0.9768	0.0541	0.9930	0.0299	0.9826	0.0466
Sunflower seed oil-canola oil-camellia oil	$I_{901.7}/I_{903.7}$ & $I_{905.8}/I_{907.8}$	p_1: sunflower seed oil; p_2: canola oil						
		Theo.	0.9464	0.0473	0.9798	0.0619	0.9908	0.0344
		Sim.	0.9901	0.0369	0.9808	0.0504	0.9911	0.0337
		NLS	0.9899	0.0384	0.9791	0.0565	0.9883	0.0392

[a] PCC: Pearson correlation coefficients.

[b] RMSE: root mean square error.

Table 3. Quantitative results of the commercial blended oil products.

No.	**Blending type**	**Marker ions (*r*)**	**Measured oil**	**Labeled con. (%)**	**Measured con. (%)**	**RSD (%)**
1	Sunflower seed oil-soybean oil	$I_{899.7}/I_{903.7}$	Sunflower	6	9.4±2.5	26.5
2	Corn oil-soybean oil	$I_{899.7}/I_{903.7}$	Corn	10	10.5±4.4	41.3
3	Olive oil-peanut oil	$I_{903.7}/I_{907.8}$	Olive	10	21.4±1.7	8.1
4	Canola oil-corn oil	$I_{901.7}/I_{907.8}$	Canola	44	25.9±1.2	4.7
5	Peanut oil-corn oil-canola oil	$I_{881.8}/I_{905.8}$& $I_{907.8}/I_{901.7}$	Peanut	NA[a]	32.6±5.8	17.8
			Corn	NA[a]	37.1±1.7	4.5
			Canola	NA[a]	30.2±4.6	15.2
6	Olive oil-corn oil-sunflower seed oil	$I_{881.8}/I_{879.7}$ & $I_{901.7}/I_{905.8}$	Olive	10	6.3±0.7	11.1
			Corn	45	37.3±5.8	15.4
			Sunflower	45	56.4±5.9	10.4

[a] NA: Not available on the label.

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