RAPID QUANTITATION OF OIL COMPOSITIONS IN BLENDED OILS BY MATRIX-ASSISTED LASER DESORPTION/IONIZATION MASS SPECTROMETRY

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Rapid Quantitation of Oil Compositions in Blended Oils by Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry

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Li Suying

Abstract

Blended oils are becoming popular due to their advantageous physical and chemical properties, better nutritional values and enhanced flavors. However, deliberate mislabeling of oil compositions to mislead consumers is a problem frequently encountered in the market of blended oils. To better control the quality of blended oils and ensure food safety, quantitative labeling of oil compositions has become a trend while there is still a lack of rapid and reliable methods for quantitative analysis of blended oils, particularly for blended oils with multiple compositions. Conventional gas chromatography (GC) method for edible oil analysis needs chemical derivatization and column separation which are laborious and time-consuming. In this study, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) was applied to profile triacylglycerols (TAGs) in blended oils with minimal requirement of sample preparation and short analysis time. The MALDI-MS technique was advantageous for high-throughput analysis and provided high quality spectra with good reproducibility for blended oils.

Based on the MALDI-MS spectra of blended oils, relationships between the intensity ratio of marker TAG peaks and the concentration of oil compositions have been investigated and employed to establish calibration curves and models for binary and ternary blended oils, respectively, from which quantitative results with good accuracy and precision were obtained after the optimization of marker ions. Quantitative analysis

of quaternary blended oils has been demonstrated to be feasible using the intensity ratio of marker ions, but further study is required due to the complexity and challenge of data analysis. The developed intensity ratio-based method could approximate the abundance of oil compositions in blended oils based on the spectral data of pure oils, making this method powerful for rapid semi-quantitative analysis of blended oils

A chemometric approach, partial least squares regression (PLS-R), was employed to establish multivariate calibration models based on the acquired MALDI-MS spectra. It was demonstrated that the PLS-R approach provided good quantitative results for binary, ternary and quaternary blended oils with good limit of detection, i.e., detectability of 1.5% olive oil in sunflower seed oil, and allowed simultaneous quantitation of multiple compositions. Compared with the conventional GC method, the MALDI-MS method showed comparable quantitative performance, while allowed direct analysis of blended oils, analysis of one blended oil sample within minutes, and accurate quantitation of low-abundance oil compositions and blended oils with similar fatty acid (FA) contents. The compositions of several commercial blended oil products were successfully quantified by the developed method and some mislabeled products were determined, indicating the coupling of MALDI-MS and PLS-R was rapid, efficient and powerful for quantitative analysis of blended oils, especially for multi-compositions blended oils.

To further simplify the quantitative analysis of blended oils, a framework was constructed to provide semi-quantitation of oil compositions of blended oils using the MALDI-MS spectra of pure oils as reference. Preliminary results demonstrated that the developed framework based on spectral comparison and spectral simulation could determine the compositions of blended oils and then quantify the determined compositions without the establishment of calibration relationships, which would significantly reduce the time required for quantitative analysis and be potential for rapid identification and approximate quantitation of unknown blended oils. However, further improvement and optimization of the framework was needed to decrease the false positive rate of determination and increase the accuracy of quantitation.

List of publications

Journal papers

1. Ng, T. T.; Li, S.; Ng, C. C. A.; So, P. K.; Wong, T. F.; Li, Z. Y.; Chan, S. T.; Yao, Z. P., Establishment of a spectral database for classification of edible oils using matrix-assisted laser desorption/ionization mass spectrometry. *Food Chem.* **2018,** *252*, 335-342.
2. Li, S.; Ng, T. T.; Yao, Z. P., Quantitative analysis of blended oils by matrix-assisted laser desorption/ionization mass spectrometry and partial least squares regression. *Food Chem.* **2021,** *334*, 127601.
3. Li, S.; Lin, X.; Ng, T. T.; Yao, Z. P., Quantitative analysis of blended oils based on intensity ratios of marker ions in MALDI-MS spectra (under preparation).

Conference papers

1. Li, S.; Ng, T. T.; Yao, Z. P., Rapid detection and quantitation of mixed edible oils by MALDI-MS. The 24th Symposium on Chemistry Postgraduate Research in Hong Kong. Hong Kong. 6 May **2017**. (Poster)
2. Li, S.; Ng, T. T.; Yao, Z. P., Rapid detection and quantitation of mixed edible oils by MALDI-MS. HKSMS Symposium 2017. Hong Kong. 24 June **2017**. (Poster)
3. Li, S.; Ng, T. T.; Yao, Z. P., Rapid detection and quantitation of mixed edible oils by MALDI-MS. 7th Asia-Oceania Mass Spectrometry Conference 2017. Singapore. 10-13 December **2017**. (Poster)
4. Li, S.; Ng, T. T.; Yao, Z. P., Rapid quantitation of blended edible oils by matrix-assisted laser desorption/ionization mass spectrometry and partial least squares

regression. The 25th Symposium on Chemistry Postgraduate Research in Hong Kong. Hong Kong. 28 April **2018**. (Poster)

5. Li, S.; Ng, T. T.; Yao, Z. P., Rapid quantitation of blended edible oils by matrix-assisted laser desorption/ionization mass spectrometry and partial least squares regression. HKSMS Symposium 2018. Hong Kong. 30 June **2018**. (Oral, HKSMS Conference Award)
6. Li, S.; Ng, T. T.; Yao, Z. P., Rapid determination of compositions of blended oils by matrix-assisted laser desorption/ionization mass spectrometry and partial least squares regression. China Mass Spectrometry Conference 2018. Guangzhou, China. 23-26 November **2018**. (Poster)
7. Li, S.; Ng, T. T.; Yao, Z. P., Rapid quantitation of compositions of blended oil by matrix-assisted laser desorption/ionization mass spectrometry and partial least squares regression. The 26th Symposium on Chemistry Postgraduate Research in Hong Kong. Hong Kong. 4 May **2019**. (Poster)
8. Li, S.; Ng, T. T.; Yao, Z. P., Quantitative analysis of blended oils with multiple components. HKSMS Symposium 2019. 22 June **2019**. (Poster)
9. Li, S.; Ng, T. T.; Yao, Z. P., Quantitative analysis of blended oils by matrix-assisted laser desorption/ionization mass spectrometry and multivariate regression. 8th Asia-Oceania Mass Spectrometry Conference **2020**. Macau. 3-7 January **2020**. (Oral for the Young Scientist Forum and poster for the main conference)

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List of abbreviations

Abbreviation	Full form
BIC	Bayesian information criterion
CSS	Cosine similarity score
DHB	2, 5-Dihydroxybenzoic acid
ESI	Electrospray ionization
FA	Fatty acid
FAME	Fatty acid methyl ester
FID	Flame ionization detector
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatography
HPLC	High-performance liquid chromatography
L	Linoleic acid (C18:2)
Ln	α-Linolenic acid (C18:3)
LOD	Limit of detection
MALDI	Matrix-assisted laser desorption/ionization
MS	Mass Spectrometry
MUFA	Monounsaturated fatty acid
NLS	Nonlinear least squares
NNLS	Non-negative least squares

O	Oleic acid (C18:1)
P	Palmitic acid (C16:0)
PC	Phosphatidylcholine
PCA	Principle component analysis
PCC	Pearson correlation coefficient
PLS	Partial least squares
PLS-R	Partial least squares regression
PNNLS	Penalized non-negative least squares
PRESS	Predicted error sum of squares
PUFA	Polyunsaturated fatty acid
R^2	Coefficient of determination
R.I.	Relative intensities
RMSE	Root mean square error
RMSEcv	Root mean square error of cross-validation
RMSEE	Root mean square error of estimation
RMSEP	Root mean square error of prediction
RSD	Relative standard deviation
RSS	Residual sum of squares
SD	standard deviation
SFA	Saturated fatty acid
TAG	Triacylglycerol

TSS	Total sum of squares
UHC	Unsupervised hierarchical clustering
UV	Ultraviolet
VIP	Variable importance for the projection

Introduction

1.1 Blended oils

Vegetable oils and animal fats are two major types of edible oil commonly used in cooking methods, such as frying, roasting and baking. The average consumption of vegetable oils per capita per year was reported to be 17.3 kg in India, 25.3 kg in China, and 25.8 kg in Europe in 2015-2017[1]. Therefore, the nutrition of edible oils attracts more and more attention with the increasing awareness of public health. Triacylglycerols (TAGs), the esters derived from glycerol and fatty acids (FAs), are the predominant compounds (95-98%) of edible oils, while other minor compounds are represented by different varieties of molecules, such as diglycerides, free fatty acids, phospholipids, phytosterols and tocopherols[2-4].

The presence of different FAs could affect the structures of TAGs and cause differences in the physical properties, chemical properties and nutritional values of edible oils. FAs could be categorized into saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA), which have different effects on human health, according to the number of double bonds they have. Intake of suitable FAs was recommended by the World Health Organization (WHO) for a healthy diet. The total intake of SFA should not be higher than 10% of total energy intake and SFA should be replaced with PUFA to decrease the risk of coronary heart disease. To prevent deficiency of essential FAs (linoleic acid and α-linolenic acid), the intake of PUFA

should be higher than 6 % of total energy intake and lower than 11 % of total energy intake as high PUFA intake could increase the risk of lipid peroxidation. How much MUFA one should get would depend on one's intakes of total fat, SFA, PUFA and trans fatty acids, which could vary a lot with the different dietary patterns of individuals[5-7]. However, the FA profiles of most edible oils do not meet all the requirements of a healthy diet, so the blending of several pure oils for improved FA profiles and enhanced nutritional values of edible oil products has been practiced by many manufacturers.

Both the FA and TAG profiles of edible blended oils could be easily modified by changing the oil compositions and blending ratios, which allows the changing of the physical and chemical properties of blended oils to meet the demands of customers, such as higher smoke points for frying and improved oxidative stability for storage[8-10]. Blending is a simple and cost-effective way to create edible oil products with desired properties and blended oils are gradually becoming popular in the market due to their advantageous physical and chemical properties, higher nutritional values and enhanced flavors. The market price of pure edible oils can vary enormously, thus deliberate mislabeling of the contents of oil compositions to confuse customers for financial gains frequently encountered in the market of blended oils. Some blended oil products may highlight the presence of the more expensive or nutritious compositions, such as olive oil and flaxseed oil, but exaggerate their actual concentrations[11-12]. To better inform consumers, China has passed a regulation that requires all blended oil products to label

the percentages of all the contained pure oil compositions, and there are similar regulations in India too[13-14]. In the European Union, if olive oil-containing blended oil products highlight the presence of olive oil, the percentage of olive oil in the product must be indicated[15]. However, there is still a lack of reliable method for quantitative analysis of blended oils, which is highly required to meet the numerous analytical demands for quantitative labeling and quality control of blended oil products.

Gas chromatography (GC) is the conventional method for analyzing edible oils. After the conversion of TAGs to fatty acid methyl esters (FAMEs) by chemical derivatization, the FAs contents of edible oils could be determined using GC coupled with flame ionization detector (FID) or mass spectrometry (MS). Pure edible oils could be identified by matching the obtained FAs contents with the Codex standards[16], and the concentrations of the individual oils in blended oils were determined using chemometric tools to process the obtained FAs contents[17-18]. TAGs can be directly analyzed using high-temperature gas chromatography and were applied as indicators for the quantitative analysis of blended oils[19-20]. High-performance liquid chromatography (HPLC) is the most widely used technique for TAGs analysis with good reproducibility and high resolution. A linear relationship between the abundance of selected TAG peaks and the concentrations of olive oil in blends of olive oil and soybean oil was observed[21]. Chemometric methods could be used to achieve the quantitation of olive oil in blended oils based on their HPLC chromatograms[22]. Both

the GC and HPLC approaches require sample pretreatment and column separation which could be laborious and time-consuming[23]. Direct analysis techniques for quantitative analysis of blended oils have been developed, including those based on fluorescence[24-25], UV-visible[26-27], Raman[28-29] and Fourier transform infrared spectroscopy (FTIR)[30-31], and electrospray ionization-mass spectrometry (ESI-MS)[32-33]. However, these methods have some limitations in the analysis of oil samples, such as the poor analytical accuracy of Raman spectroscopy and the low analytical efficiency of ESI-MS. Although quantitative analysis of blended oils has been extensively investigated using various analytical techniques, most of the previous studies on oil quantitation focused on binary blended oils with very few on blended oils with multiple compositions, the latter of which are very common in the market[34-36]. For blended oils with more compositions, the possible combinations of oil compositions become more complicated, resulting in a significantly increase in the number of samples for establishing the calibration relationship, more complex spectral data and consequently more challenging quantitative analysis.

1.2 MALDI-MS for analysis of edible oils

Matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS) was first introduced by Karas et al. in 1985 and was developed in 1988[37-38]. MALDI is based on the utilization of an UV-absorbing matrix which was mixed with the analyte. Upon irradiation by an UV-laser, the ionization of the analyte was enabled by the matrix. The

matrix should be able to absorb the laser energy and separate the analyte molecules from each other to avoid cluster formation. Transferring energy from the matrix to the analyte avoided the direct hitting of laser on the analyte which might result the intense fragmentation. An ideal matrix should have absorption at the used laser wavelength (typically fixed at 337nm for the common N_2 laser) and have good mixing properties with the analyte to form homogeneous co-crystallization[39].

MALDI-MS is outstanding for its simple sample preparation, rapid analysis and high tolerance to impurities, and showed high reproducibility for lipid analysis[23]. Both lipids and matrix are readily soluble in organic solvents, resulting in extremely homogeneous co-crystals of lipids and matrix and excellent reproducibility of the MALDI-MS spectra[39]. Generally, the physicochemical and nutritional properties of edible oils are mainly determined by the major component, i.e., TAGs, thus TAGs are considered as the principle parameter for analysis of edible oils[40]. It has been shown that MALDI-MS could detect the TAG contents of edible oils[41-43], and the chemical structure of TAG could be confirmed using MS/MS fragmentation and calculating the losing weight of fragments[44-46]. The TAG profiles of various vegetable oils[47-49], such as olive oil, sunflower seed oil, soybean oil and corn oil, and animal fats[50-51], such as lard, butter and cod liver oil, have been identified by MALDI-MS, which allowed the classification of edible oils in different species. Moreover, the TAG profiles could be useful for discrimination of vegetable oils of the same type but from different regions[52-53] or

different varieties[54-55].

Deliberate adulteration has been used to increase economic benefits by adding vegetable oils of low commercial values, e.g., soybean oil and palm oil, to high price oils, e.g., olive oil and almond oil, which might have important health implications for consumers due to the introduction of allergens[56]. The adulteration of expensive edible oils could be detected using MALDI-MS[57] and an accurate and sensitive investigation of the common adulterants was achieved using statistical analysis, such as unsupervised hierarchical clustering (UHC) and principal component analysis (PCA)[58]. The inappropriate use of gutter oils has become an important problem for food safety in many counties. To solve such problem, a spectral database has been established using MALDI-MS for authentication of pure edible oils and screening of adulterated oils and used oils[59-60].

Edible oils are oxidized during processing and storage, and the oxidative progress would be accelerated with exposure to heat, humidity, and the presence of transition metals[61]. To characterize the effects of thermal stressing on unsaturated vegetable oils, MALDI-MS was applied to directly detect the changes of oils upon heating and provide reliable information of the formation of oxidation products[62]. Comparing the MALDI-MS spectra of coconut oil, a saturated edible oil, before and after heating, a 14 Da increasement was shown in the *m/z* value of TAG peaks, which was caused by the

conversion of one methylene group into a carbonyl group. Moreover, clear changes that highly depended on the heating temperature and heating time were observed from the TAG profiles[63].

Using MALDI-MS to analyze edible oils can simplify the sample preparation without requiring of chemical derivatization and column separation, and analysis of one oil sample could be finished within several minutes[64-65]. Meanwhile, MALDI-MS allows loading of several hundreds of samples on a target plate and data acquisition could be processed automatically, making it advantageous for high-throughput analysis. Therefore, MALDI-MS is a powerful technique for rapid analysis of edible oils and has strong potential for various applications.

1.3 Outline of this thesis

In the whole study, we aimed to develop a method for rapid quantitation of oil compositions of blended oils using MALDI-MS. We hypothesized that the MALDI-MS spectrum of a blend oil contained the quantitative information of its compositions and tried to solve such information from the spectrum using different approaches.

In Chapter 1, the basic background of blended oils has been introduced as well as the quantitative analysis of blended oils using various techniques. The fundamental of MALDI-MS, the main technique used in the whole study, was introduced together with

the current application of MALDI-MS on the analysis of edible oils.

In Chapter 2, the development of MALDI-MS technique for quantitative analysis of blended oils was discussed. Based on the MALDI-MS spectra of blended oils, relationships between the intensity ratio of TAG peaks and the concentration of oil compositions in blended oils were investigated for binary blended oils and ternary blended oils, and these relationships were established using linear regression and nonlinear regression, respectively. The developed intensity ratio-based method was applied to the quantitative analysis of various types of blended oils for comprehensive investigation and validation, and then the developed method was optimized based on the obtained results. Quantitative analysis of quaternary blended oils using the intensity ratio-based method was preliminarily explored.

In Chapter 3, a chemometric approach (i.e., PLS-R) was applied to process the normalized TAG profiles of blended oils provided by the MALDI-MS spectra for quantitative analysis. Quantitative analysis of blended oils involving binary, ternary and quaternary blends has been investigated, especially for the multi-composition blended oils and oil compositions with similar TAG profiles. Based on the PLS-R approach, the quantitative performances of MALDI-MS and GC-FID, the conventional technique for analyzing edible oils, were compared. Finally, the quantitative results of commercial blended oil products provided by the MALDI-MS-based method were discussed.

In Chapter 4, an initial framework has been constructed for quantitative analysis of blended oils based on spectral comparison and spectral simulation. The preliminary results revealed the possibilities of determining and quantifying the oil compositions of blended oils, which only using the reference MALDI-MS spectra of pure oils. With further investigation and optimization, this new method would achieve the rapid semi-quantitation of oil compositions of blended oils based on a MALDI-MS spectral database of edible oils, where the conventional procedures of quantitative analysis are not required.

In Chapter 5, the results of the whole research were summarized and some general conclusions were obtained. The properties of the intensity ratio- and the PLS-R-based approached were compared to figure out their advantages and limitations. The prospects of this study were also discussed in this chapter.

Chapter 2: Quantitative analysis of blended oils by MALDI-MS and intensity ratios of marker ions

2.1 Introduction

MALDI-MS has been applied for quantitation of FA contents of pure edible oils, and the quantitative results obtained from MALDI-MS and GC-MS showed large agreement with some minor statistical variations. Furthermore, MALDI-MS appeared to be more sensitive to detect minor FAs which were not observed from the GC-MS chromatograms[66-67]. With the prior deposition of nitrocellulose layer onto the target plate, the MALDI-MS spectra of edible oils showed improved shot-to-shot and sample-to-sample reproducibility, enabling the consistent quantitation of TAGs[68]. Improved shot-to-shot reproducibility was also observed from the TAG profiles determined by laser desorption/ionization time-of-flight mass spectrometry without the use of a matrix[69]. It has been demonstrated that MALDI-MS is sensitive to detect FAs, TAGs and phospholipids[70] contained in edible oils for qualitative analysis.

Compared with the individual pure oils, blended oils would show more complex TAG patterns[71], and the complexity of spectra would increase as the number of oil compositions increases[72], resulting in the difficulty of quantitative analysis of blended oils. The intensity ratio of marker ions shown in MS spectra has been widely used in quantitative analysis, such as the quantitation of diacylglycerol in biological samples[73], the quantitation of phosphorylation of peptides[74] and the relative quantitation of

disaccharide isomers[75], and the intensity ratio of TAG peaks has been demonstrated to be a good indicator of the adulteration of pure edible oils[76-78]. We have previously developed a protocol for MALDI-MS analysis of edible oils with high reproducibility and demonstrated the TAG patterns of blended oils are determined by the contained pure oils[60]. Different blending ratios of pure oils generate varied TAG patterns of blended oils, which show stronger intensities for the peaks associated with the high-abundance pure oils and weaker intensities for the peaks contributed by the low-abundance pure oils, resulting in fluctuations in the intensity ratios of the TAG peaks. Hence, the quantitative information of blended oils should be able to be deduced from the intensity ratios of the TAG peaks in their MALDI-MS spectra.

In this chapter, we applied the MALD-MS technique to rapidly profile the TAG profiles of blended oils, and the relationships between the intensity ratios of TAG peaks and the proportions of oil compositions were systematically investigated. After extensive validation and optimization, an intensity ratio-based method was established for the quantitative analysis of blended oils, which showed powerful quantitative ability for various types of blended oils. The intensity ratio-based method allowed rapid analysis of not only binary blended oils but also blended oils of multiple compositions, i.e., ternary blended oils and quaternary blended oils, as well as simultaneous quantitation of multiple oil compositions, making the method simple, efficient and straightforward for the quantitative analysis of complex oil mixtures.

2.2 Materials and methods

2.2.1 Chemicals

2, 5-Dihydroxybenzoic acid (DHB) and α-cyano-4-hydroxycinnanic acid (CHCA) were purchased from Aldrich (St. Louis, MO, USA). HPLC grade acetone and HPLC grade acetonitrile (ACN) were purchased from Acros Organic (Waltham, MA, USA) and Anaqua Chemical Supply (Houston, TX, USA), respectively. Polyethylene glycol (PEG) standards were purchased from Fluka (St. Louis, MO, USA) and sodium iodide (NaI) was purchased from Panreac Química (Barcelona, Spain). All chemicals were used directly without further purification.

2.2.2 Oil samples

Pure vegetable oils and commercial blend 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 were prepared by manually mixing pure oils in different ratios (w/w). For each type of binary blended oils, 10 samples were used for calibration with 5 samples for validation (Table 2-1). For ternary blended oils, 21 samples and 12 samples were prepared for calibration and validation, respectively (Table 2-2). For quaternary blended oils, 56 samples and 9 samples were prepared for calibration and validation, respectively (Table 2-3). All the oil samples were sealed and stored in a dark and dry place before analysis.

Table 2-1. Blended oil samples prepared for binary blended oils.

Type	Calibration (10)		Validation (5)
Pure	0%:100%	100%:0%	8%:92%
Binary blends	5%:95% 10%:90% 20%:80% 40%:60%	60%:40% 80%:20% 90%:10% 95%:5%	30%:70% 50%:50% 70%:30% 92%:8%

Table 2-2. Blended oil samples prepared for ternary blended oils.

Type	Calibration (21)			Validation (12)
Pure	0%:0%:100%	0%:100%:0%	100%:0%:0%	0%:50%:50% 50%:0%:50% 50%:50%:0%
Binary blends	0%:20%:80% 0%:40%:60% 0%:60%:40% 0%:80%:20%	20%:80%:0% 40%:60%:0% 60%:40%:0% 80%:20%:0%	20%:0%:80% 40%:0%:60% 60%:0%:40% 80%:0%:20%	10%:10%:80% 10%:30%:60% 10%:60%:30% 10%:80%:10%
Ternary blends	20%:20%:60% 20%:40%:40%	20%:60%:20% 40%:20%:40%	40%:40%:20% 60%:20%:20%	30%:10%:60% 30%:60%:10% 60%:10%:30% 60%:30%:10% 80%:10%:10%

Table 2-3. Blended oil samples prepared for quaternary blended oils.

Type	Calibration (56)		Validation (9)
Pure	0%:0%:0%:100%	0%:100%:0%:0%	
	0%:0%:100%:0%	100%:0%:0%:0%	
Binary blends	0%:0%:20%:80%	0%:20%:0%:80%	
	0%:0%:40%:60%	0%:40%:0%:60%	
	0%:0%:60%:40%	0%:60%:0%:40%	
	0%:0%:80%:20%	0%:80%:0%:20%	
	20%:0%:0%:80%	20%:0%:80%:0%	
	40%:0%:0%:60%	40%:0%:60%:0%	
	60%:0%:0%:40%	60%:0%:40%:0%	
	80%:0%:0%:20%	80%:0%:20%:0%	
	20%:80%:0%:0%	0%:20%:80%:0%	10%:10%:10%:70%
	40%:60%:0%:0%	0%:40%:60%:0%	10%:10%:70%:10%
	60%:40%:0%:0%	0%:60%:40%:0%	10%:70%:10%:10%
	80%:20%:0%:0%	0%:80%:20%:0%	70%:10%:10%:10%
Ternary blends	0%:20%:20%:60%	20%:20%:0%:60%	20%:20%:20%:40%
	0%:20%:40%:40%	20%:40%:0%:40%	20%:20%:40%:20%
	0%:20%:60%:20%	20%:60%:0%:20%	20%:40%:20%:20%
	0%:40%:20%:40%	40%:20%:0%:40%	40%:20%:20%:20%
	0%:40%:40%:20%	40%:40%:0%:20%	25%:25%:25%:25%
	0%:60%:20%:20%	60%:20%:0%:20%	
	20%:0%:20%:60%	20%:20%:60%:0%	
	20%:0%:40%:40%	20%:40%:40%:0%	
	20%:0%:60%:20%	20%:60%:20%:0%	
	40%:0%:20%:40%	40%:20%:40%:0%	
	40%:0%:40%:20%	40%:40%:20%:0%	
	60%:0%:20%:20%	60%:20%:20%:0%	
Quaternary blends	20%:20%:20%:40%	20%:40%:20%:20%	
	20%:20%:40%:20%	40%:20%:20%:20%	

2.2.3 MALDI-MS analysis

Sample preparation for MALDI-MS analysis was performed using a previously reported protocol[59]. Briefly, aliquots of 0.5 μL of 100 mg mL^{-1} DHB in acetone were loaded onto spots of the MADLI plate and air-dried. Each oil sample was directly applied as a thin layer on the DHB by using a medical cotton tip. PEG solution mixture (PEG600/PEG1000/PEG2000/NaI = 1/2/2/5 (v/v)) was mixed with 10 mg mL^{-1} CHCA solution (ACN/H_2O = 7/3 (v/v)) and then loaded onto the MALDI plate for calibration of the mass spectrometer.

An UltrafleXtreme MALDI-TOF/TOF mass spectrometer (Bruker, Billerica, MA, USA) equipped with a 355 nm smartbeam-II laser was operated in positive ion and reflectron mode for the MALDI-MS analysis. The ion source voltage 1, ion source voltage 2, lens voltage, reflector voltage 1 and reflector voltage 2 were set to 20.00 kV, 18.00 kV, 8.50 kV, 21.10 kV and 10.85 kV, respectively. The ion pulse excitation was set to 140 ns and each shot included 1000 laser pulses. Mass spectra with a *m/z* range of 500–2000 Da were acquired automatically with the irradiation spot moved along a random path and the laser intensity varied in the limited range. 8 single spectra with resolutions higher than 3000 in the TAG range (typically *m/z* 850–920) were accumulated and saved as one spectrum for further analysis. Each sample was analyzed in eight replicates.

2.2.4 Equations for quantitative analysis

Considering the mass spectrum of an oil blend as a linear combination of the mass spectra of individual oils, the intensity of a selected peak shown in the spectrum of the oil blend can be expressed as

$$I(blend) = \sum_{i=1}^{k} \alpha_i I_i \tag{2-1}$$

where I_i is the intensity of the selected peak shown in the spectrum of the individual oil i, α_i is the contribution of individual oil i in the oil blend (e.g., molecular quantity) and k is the number of individual oils contained in the oil blend. Therefore, the intensity ratio of two selected peaks (i.e., peak A and peak B) shown in the spectrum of the oil blend can be obtained as

$$\frac{I_{\mathrm{B}}(blend)}{I_{\mathrm{A}}(blend)} = \frac{\sum_{i=1}^{k} \alpha_i I_{B,i}}{\sum_{i=1}^{k} \alpha_i I_{A,i}} = \frac{\sum_{i=1}^{k} \frac{p_i}{M_i} I_{B,i}}{\sum_{i=1}^{k} \frac{p_i}{M_i} I_{A,i}} \tag{2-2}$$

where p_i is the weight percentage of individual oil i in the oil blend and M_i is the average molecular weight of individual oil i.

2.2.4.1 *Equations for binary blended oils*

For binary blended oils, Equation (2-2) can be expressed as

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

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 2, with p_1 and p_2 referring to the weight percentages of the two pure

oils in their blends. Thus, $p_2 = 1 - p_1$.

Let $r = \frac{I_B(blend)}{I_A(blend)}$, Equation (2-3) can be rewritten as

$$r = \frac{\frac{p_1}{M_1} I_{B,1} + \frac{1-p_1}{M_2} I_{B,2}}{\frac{p_1}{M_1} I_{A,1} + \frac{1-p_1}{M_2} I_{A,2}} = \frac{I_{B,1}}{I_{A,1}} \times \frac{p_1 + \frac{M_1 I_{B,2}}{M_2 I_{B,1}}(1-p_1)}{p_1 + \frac{M_1 I_{A,2}}{M_2 I_{A,1}}(1-p_1)} \tag{2-4}$$

Let $m = \frac{M_1 I_{A,2}}{M_2 I_{A,1}}$, $n = \frac{M_1 I_{B,2}}{M_2 I_{B,1}}$ and $Q = \frac{I_{B,1}}{I_{A,1}}$, the above equation can be simplified as

$$r = Q \times \frac{p_1 + (1-p_1)n}{p_1 + (1-p_1)m} = Q \times \frac{(1-n)p_1 + n}{(1-m)p_1 + m} \tag{2-5}$$

If $1 - m \neq 0$, Equation (2-5) can be derived as

$$\frac{r}{Q} = \frac{\frac{1-n}{1-m}[(1-m)p_1 + m] + n - \frac{m(1-n)}{1-m}}{(1-m)p_1 + m}$$

$$= \frac{1-n}{1-m} + \frac{\frac{n-m}{1-m}}{(1-m)p_1 + m}$$

or

$$\frac{r}{Q} = \frac{1-n}{1-m} + \frac{n-m}{(1-m)^2 p_1 + m(1-m)} \tag{2-6}$$

As previously described by Yao et al.[79-80], when $p_1 = 0$, Equation (2-6) can be expressed as

$$\frac{r_0}{Q} = \frac{1-n}{1-m} + \frac{n-m}{m(1-m)}$$

and $\frac{1-n}{1-m}$ can be solved as

$$\frac{1-n}{1-m} = \frac{r_0}{Q} - \frac{n-m}{m(1-m)} \tag{2-7}$$

Substituting Equation (2-7) into Equation (2-6) yields

$$\begin{aligned}\frac{r-r_0}{Q} &= \frac{n-m}{(1-m)^2 p_1 + m(1-m)} - \frac{n-m}{m(1-m)} \\ &= \frac{n-m}{1-m} \times \left[\frac{1}{(1-m)p_1 + m} - \frac{1}{m}\right] \\ &= \frac{(m-n)p_1}{m(1-m)p_1 + m^2}\end{aligned}$$

which can be rewritten as

$$\frac{1}{r-r_0} = \frac{m(1-m)p_1 + m^2}{Q(m-n)p_1} = \frac{m^2}{Q(m-n)p_1} + \frac{m(1-m)}{Q(m-n)}$$

or

$$\frac{1}{r-r_0} = \frac{K}{p_1} + E \tag{2-8}$$

where $K = \frac{m^2}{Q(m-n)}$ and $E = \frac{m(1-m)}{Q(m-n)}$.

If $1 - m = 0$, Equation (2-5) can be expressed as

$$\frac{r}{Q} = (1-n)p_1 + n \tag{2-9}$$

When $p_1 = 0$, the above equation can be simplified as

$$\frac{r_0}{Q} = n \tag{2-10}$$

Substituting Equation (2-10) into Equation (2-9) yields

$$\frac{r - r_0}{Q} = (1 - n)p_1$$

and the above equation can be rewritten as

$$\frac{1}{r - r_0} = \frac{1}{Q(1 - n)p_1} \tag{2-11}$$

which is the same form as Equation (2-8) with $K = \frac{1}{Q(1-n)}$ and $E = 0$.

2.2.4.2 *Equations for ternary blended oils based on weight percentage*

For ternary blended oils, Equation (2-2) can be expressed as

$$\frac{I_{\mathrm{B}}(blend)}{I_{\mathrm{A}}(blend)} = \frac{\frac{p_1}{M_1} I_{B,1} + \frac{p_2}{M_2} I_{B,2} + \frac{p_3}{M_3} I_{B,3}}{\frac{p_1}{M_1} I_{A,1} + \frac{p_2}{M_2} I_{A,2} + \frac{p_3}{M_3} I_{A,3}} \tag{2-12}$$

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, so $p_3 = 1 - p_1 - p_2$.

Let $r = \frac{I_B(blend)}{I_A(blend)}$, Equation (2-12) can be expressed as

$$r = \frac{I_{B,1}}{I_{A,1}} \times \frac{p_1 + \frac{M_1 I_{B,2}}{M_2 I_{B,1}} p_2 + \frac{M_1 I_{B,3}}{M_3 I_{B,1}} (1 - p_1 - p_2)}{p_1 + \frac{M_1 I_{A,2}}{M_2 I_{A,1}} p_2 + \frac{M_1 I_{A,3}}{M_3 I_{A,1}} (1 - p_1 - p_2)}$$

$$= \frac{I_{B,1}}{I_{A,1}} \times \frac{\left(1 - \frac{M_1 I_{B,3}}{M_3 I_{B,1}}\right) p_1 + \left(\frac{M_1 I_{B,2}}{M_2 I_{B,1}} - \frac{M_1 I_{B,3}}{M_3 I_{B,1}}\right) p_2 + \frac{M_1 I_{B,3}}{M_3 I_{B,1}}}{\left(1 - \frac{M_1 I_{A,3}}{M_3 I_{A,1}}\right) p_1 + \left(\frac{M_1 I_{A,2}}{M_2 I_{A,1}} - \frac{M_1 I_{A,3}}{M_3 I_{A,1}}\right) p_2 + \frac{M_1 I_{A,3}}{M_3 I_{A,1}}}$$

which can be simplified as

$$r = Q \times \frac{(1-n_2)p_1 + (n_1-n_2)p_2 + n_2}{(1-m_2)p_1 + (m_1-m_2)p_2 + m_2} \tag{2-13}$$

where $m_1 = \frac{M_1 I_{A,2}}{M_2 I_{A,1}}$, $m_2 = \frac{M_1 I_{A,3}}{M_3 I_{A,1}}$, $n_1 = \frac{M_1 I_{B,2}}{M_2 I_{B,1}}$, $n_2 = \frac{M_1 I_{B,3}}{M_3 I_{B,1}}$ and $Q = \frac{I_{B,1}}{I_{A,1}}$.

If $1 - m_2 \neq 0$, Equation (2-13) can be expressed as

$$\frac{r}{Q} = \frac{1-n_2}{1-m_2} + \frac{(n_1-n_2)p_2 + n_2 - \frac{1-n_2}{1-m_2}[(m_1-m_2)p_2 + m_2]}{(1-m_2)p_1 + (m_1-m_2)p_2 + m_2}$$

which can be rewritten as

$$\left(\frac{r}{Q} - \frac{1-n_2}{1-m_2}\right)^{-1} = \frac{(1-m_2)^2 p_1 + (1-m_2)(m_1-m_2)p_2 + m_2(1-m_2)}{(n_1-n_2-m_2n_1-m_1+m_1n_2+m_2)p_2 + (n_2-m_2)} \tag{2-14}$$

Let $C_1 = \frac{1-n_2}{1-m_2}$ and $H = n_1 - n_2 - m_2 n_1 - m_1 + m_1 n_2 + m_2$, Equation (2-14) can be derived as

$$\left(\frac{r}{Q} - C_1\right)^{-1} = \frac{(1-m_2)^2 p_1 + m_2(1-m_2) - \frac{(1-m_2)(m_1-m_2)(n_2-m_2)}{H}}{Hp_2 + (n_2-m_2)} + \frac{(1-m_2)(m_1-m_2)}{H} \tag{2-15}$$

Let $C_2 = \frac{(1-m_2)(m_1-m_2)}{H}$, Equation (2-15) can be expressed as

$$\left(\frac{r}{Q} - C_1\right)^{-1} - C_2 = \frac{\frac{(1-m_2)^2}{H} p_1 + \frac{m_2(1-m_2)}{H} - \frac{(1-m_2)(m_1-m_2)(n_2-m_2)}{H^2}}{p_2 + \frac{n_2-m_2}{H}}$$

and further simplified as

$$\left(\frac{r}{Q} - C_1\right)^{-1} - C_2 = \frac{Ap_1 + a}{p_2 + b} \tag{2-16}$$

where $A = \frac{(1-m_2)^2}{H}$, $a = \frac{m_2(1-m_2)}{H} - \frac{(1-m_2)(m_1-m_2)(n_2-m_2)}{H^2}$ and $b = \frac{n_2-m_2}{H}$.

If $1 - m_2 = 0$ and $m_1 - 1 \neq 0$, Equation (2-13) can be expressed as

$$r = Q \times \frac{(n_1 - n_2)p_2 + (1 - n_2)p_1 + n_2}{(m_1 - 1)p_2 + 1}$$

$$= Q \times \frac{\frac{n_1 - n_2}{m_1 - 1}[(m_1 - 1)p_2 + 1] + (1 - n_2)p_1 + n_2 - \frac{n_1 - n_2}{m_1 - 1}}{(m_1 - 1)p_2 + 1}$$

and further rewritten as

$$\frac{r}{Q} = \frac{n_1 - n_2}{m_1 - 1} + \frac{(1 - n_2)p_1 + \frac{n_2 m_1 - n_1}{m_1 - 1}}{(m_1 - 1)p_2 + 1} \tag{2-17}$$

Let $C_1 = \frac{n_1 - n_2}{m_1 - 1}$, Equation (2-17) can be expressed as

$$\frac{r}{Q} - C_1 = \frac{\frac{1 - n_2}{m_1 - 1}p_1 + \frac{n_2 m_1 - n_1}{(m_1 - 1)^2}}{p_2 + \frac{1}{m_1 - 1}} \tag{2-18}$$

which is similar to Equation (2-16) with $A = \frac{1 - n_2}{m_1 - 1}$, $a = \frac{n_2 m_1 - n_1}{(m_1 - 1)^2}$ and $b = \frac{1}{m_1 - 1}$.

2.2.4.3 *Equations for ternary blended oils based on weight ratio*

Substituting the weight ratios of pure oils with their weight percentages in blended oils, i.e., let $R_{21} = \frac{p_2}{p_1}$ and $R_{31} = \frac{p_3}{p_1}$, Equation (2-12) can be expressed as

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

and simplified as

$$r = Q \times \frac{n_1 R_{21} + n_2 R_{31} + 1}{m_1 R_{21} + m_2 R_{31} + 1} \tag{2-19}$$

where $m_1 = \frac{M_1 I_{A,2}}{M_2 I_{A,1}}$, $m_2 = \frac{M_1 I_{A,3}}{M_3 I_{A,1}}$, $n_1 = \frac{M_1 I_{B,2}}{M_2 I_{B,1}}$, $n_2 = \frac{M_1 I_{B,3}}{M_3 I_{B,1}}$ and $Q = \frac{I_{B,1}}{I_{A,1}}$.

If $m_1 \neq 0$, Equation (2-19) can be rewritten as

$$\frac{r}{Q} = \frac{n_1}{m_1} + \frac{\left(n_2 - \frac{n_1 m_2}{m_1}\right) R_{31} + \frac{m_1 - n_1}{m_1}}{m_1 R_{21} + m_2 R_{31} + 1}$$

or

$$\frac{r}{Q} = \frac{n_1}{m_1} + \frac{(m_1 n_2 - m_2 n_1) R_{31} + (m_1 - n_1)}{m_1^2 R_{21} + m_1 m_2 R_{31} + m_1} \tag{2-20}$$

If $m_1 n_2 - m_2 n_1 \neq 0$, Equation (2-20) can be derived as

$$\left(\frac{r}{Q} - \frac{n_1}{m_1}\right)^{-1} = \frac{\frac{m_1^2}{m_1 n_2 - m_2 n_1} R_{21} + \frac{m_1 m_2}{m_1 n_2 - m_2 n_1} R_{31} + \frac{m_1}{m_1 n_2 - m_2 n_1}}{R_{31} + \frac{m_1 - n_1}{m_1 n_2 - m_2 n_1}}$$

$$= \frac{m_1 m_2}{m_1 n_2 - m_2 n_1} + \frac{\frac{m_1^2}{m_1 n_2 - m_2 n_1} R_{21} + \frac{m_1^2 (n_2 - m_2)}{(m_1 n_2 - m_2 n_1)^2}}{R_{31} + \frac{m_1 - n_1}{m_1 n_2 - m_2 n_1}}$$

and rewritten as

$$\left(\frac{r}{Q} - \frac{n_1}{m_1}\right)^{-1} - \frac{m_1 m_2}{m_1 n_2 - m_2 n_1} = \frac{\frac{m_1^2}{m_1 n_2 - m_2 n_1} R_{21} + \frac{m_1^2 (n_2 - m_2)}{(m_1 n_2 - m_2 n_1)^2}}{R_{31} + \frac{m_1 - n_1}{m_1 n_2 - m_2 n_1}}$$

or

$$\left(\frac{r}{Q} - C_1'\right)^{-1} - C_2' = \frac{A' R_{21} + a'}{R_{31} + b'} \tag{2-21}$$

where $C_1' = \frac{n_1}{m_1}$, $C_2' = \frac{m_1 m_2}{m_1 n_2 - m_2 n_1}$, $A' = \frac{m_1^2}{m_1 n_2 - m_2 n_1}$, $a' = \frac{m_1^2 (n_2 - m_2)}{(m_1 n_2 - m_2 n_1)^2}$ and $b' = \frac{m_1 - n_1}{m_1 n_2 - m_2 n_1}$.

If $m_1 = 0$ and $m_2 \neq 0$, Equation (2-19) can be expressed as

$$\frac{r}{Q} = \frac{\frac{n_1}{m_2}R_{21} + \frac{n_2}{m_2}\left(R_{31} + \frac{1}{m_2}\right) + \frac{1}{m_2} - \frac{n_2}{m_2^2}}{R_{31} + \frac{1}{m_2}}$$

$$= \frac{n_2}{m_2} + \frac{\frac{n_1}{m_2}R_{21} + \frac{m_2 - n_2}{m_2^2}}{R_{31} + \frac{1}{m_2}}$$

and rewritten as

$$\frac{r}{Q} - \frac{n_2}{m_2} = \frac{\frac{n_1}{m_2}R_{21} + \frac{m_2 - n_2}{m_2^2}}{R_{31} + \frac{1}{m_2}} \quad (2\text{-}22)$$

which is similar to Equation (2-21) with $C_1' = \frac{n_2}{m_2}$, $A' = \frac{n_1}{m_2}$, $a' = \frac{m_2 - n_2}{m_2^2}$ and $b' = \frac{1}{m_2}$.

2.2.5 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 blended oils was performed in Microsoft® Excel using the linear regression function, and for ternary and quaternary blended oils, 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.

2.3 Results and discussion

2.3.1 Quantitative analysis of binary blended oils

2.3.1.1 *Establishment and validation of the quantitative method*

Olive oil and sunflower seed oil blends are one of the most common blended oil products in the market due to their improved quality and high nutritional value[81-82], and the MALDI-MS spectra of pure olive oil and sunflower seed oil showed differentiated TAG patterns, so these two oils were chosen as reference oils to investigate the quantitative analysis of blended oils at the beginning of the study.

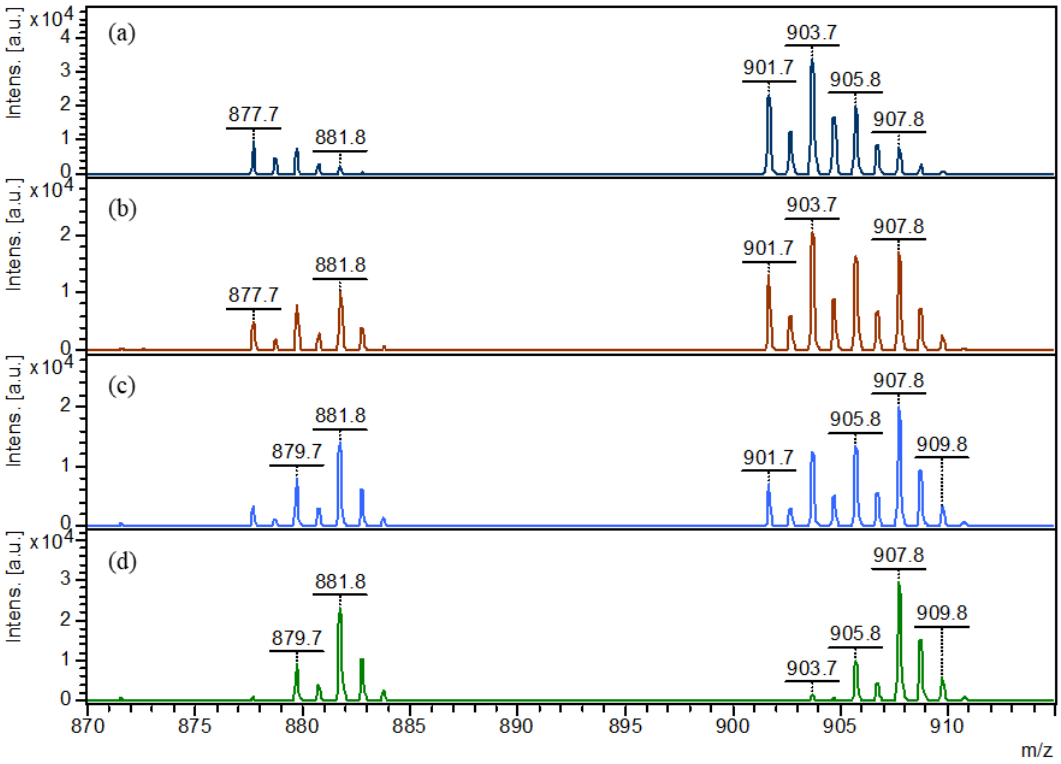

Figure 2-1. The TAG region of the MALDI-MS spectra for (a) 100% sunflower seed oil, (b) 40% olive oil – 60% sunflower seed oil blend, (c) 60% olive oil – 40% sunflower seed oil blend and (d) 100% olive oil.

Figure 2-1 showed the MALDI-MS spectra of pure olive oil, pure sunflower seed oil and blends of olive oil and sunflower seed oil. Compared with pure olive oil and pure sunflower seed oil, blended oils showed more complex spectral data in the TAG region where obvious changes of peak abundances were observed for blended oils with different blending ratios. The peaks shown in the MALDI-MS spectra were assigned according to the literatures[42, 69] and our previous MS/MS analysis[59]. Olive oil was abundant (> 55%) with oleic acid (C18:1)[83], a MUFA contained 18 carbon atoms and a double bond, so the highest intensity peaks shown in the spectrum of pure olive oil were *m/z* 881.8 (POO, P: palmitic acid; O: oleic acid) and *m/z* 907.8 (OOO). Sunflower seed oil contains more linoleic acid (C18:2) than oleic acid[84], leading to strong peaks at *m/z* 901.7 (LLL, L: linoleic acid), *m/z* 903.7 (OLL) and *m/z* 905.8 (OOL) in the spectrum. With increased concentration of olive oil in the oil blends, the relative abundances of *m/z* 881.8 and *m/z* 907.8 increased and the peaks at *m/z* 901.7, *m/z* 903.7 and *m/z* 905.8 became lower, indicating a correlation between the oil compositions of the blended oils and their MALDI-MS spectra.

To investigate the quantitative analysis of blends of olive oil and sunflower seed oil, 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 their intensity ratios, i.e., intensity ratio of peaks at *m/z* 881.8 and *m/z* 907.8 ($I_{881.8}/I_{907.8}$), intensity ratio of peaks at *m/z* 901.7 and *m/z* 907.8 ($I_{901.7}/I_{907.8}$), intensity ratio of peaks at *m/z* 903.7 and *m/z*

907.8 ($I_{903.7}/I_{907.8}$), and intensity ratio of peaks at *m/z* 905.8 and *m/z* 907.8 ($I_{905.8}/I_{907.8}$). Good spectral reproducibility of the MALDI-MS method for edible oil analysis has been demonstrated for inter-day and intra-day measurements[60]. In this study, eight replicate measurements were performed for each oil sample to further improve the reproducibility of spectral data. For the olive oil – sunflower seed oil blends with olive oil varied from 0% to 100%, the intensity ratios of the selected four groups of marker ions showed excellent precision with RSD lower than 10% (Table 2-4), except for an extreme RSD of 14.2% caused by the very low value of $I_{901.7}/I_{907.8}$ (0.029±0.004), illustrating the intensity ratio had good reproducibility and was suitable for quantitative analysis.

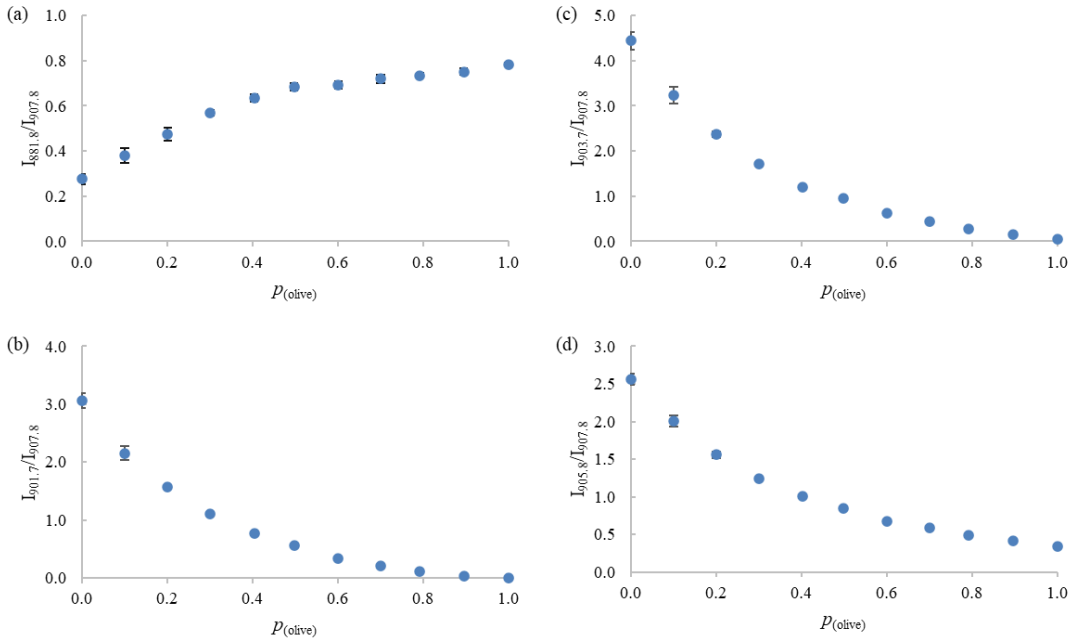

Figure 2-2. Plots of (a) $I_{881.8}/I_{907.8}$, (b) $I_{901.7}/I_{907.8}$, (c) $I_{903.7}/I_{907.8}$ and (d) $I_{905.8}/I_{907.8}$ against the concentration of olive oil ($p_{(olive)}$) in olive oil – sunflower seed oil blends.

Table 2-4. The intensity ratios of selected TAG peaks shown in the MALDI-MS spectra of olive oil – sunflower seed oil blends with different olive oil concentrations.

Olive oil con. (%)	**$I_{881.8}/I_{907.8}$**		**$I_{901.7}/I_{907.8}$**		**$I_{903.7}/I_{907.8}$**		**$I_{905.8}/I_{907.8}$**	
	Mean±SD	RSD (%)	Mean±SD	RSD (%)	Mean±SD	RSD (%)	Mean±SD	RSD (%)
0	0.275±0.022	7.9	3.059±0.134	4.4	4.360±0.203	4.7	2.610±0.074	2.8
10	0.380±0.032	8.5	2.151±0.117	5.5	3.087±0.182	5.9	1.959±0.071	3.6
20	0.476±0.029	6.2	1.570±0.025	1.6	2.296±0.068	3.0	1.535±0.041	2.7
30	0.570±0.012	2.1	1.104±0.031	2.8	1.637±0.036	2.2	1.204±0.017	1.4
40	0.634±0.015	2.4	0.769±0.013	1.6	1.198±0.014	1.2	0.980±0.015	1.5
50	0.684±0.018	2.7	0.561±0.017	3.0	0.916±0.017	1.9	0.824±0.014	1.7
60	0.693±0.017	2.5	0.342±0.011	3.2	0.632±0.006	0.9	0.680±0.010	1.5
70	0.719±0.018	2.6	0.211±0.013	6.1	0.433±0.013	2.9	0.583±0.010	1.8
80	0.735±0.010	1.3	0.103±0.008	7.6	0.278±0.009	3.1	0.490±0.007	1.5
90	0.752±0.015	2.0	0.029±0.004	14.2	0.142±0.007	4.8	0.413±0.010	2.5
100	0.783±0.007	0.9	0.000±0.000	/	0.047±0.003	6.4	0.342±0.006	1.7

The obtained intensity ratios of TAG peaks were plotted against the concentrations of olive oil ($p_{(olive)}$) in blended oil samples to investigate the correlated relationships, where non-linear curves were observed for all the four groups of marker ions (Figure 2-2). Such non-linear curves (i.e., hyperbolic curve) were not suitable for quantitative analysis. As discussed in Section 2.2.4, the non-linear relationships could be mathematically derived to linear relationships as shown in Equation (2-8)

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

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), r_0 is the r value of pure oil 2 (e.g., sunflower seed oil in olive oi l– sunflower seed oil blends), and K and E are constants.

For the binary blends of olive oil and sunflower seed oil, considering olive oil as the measured oil composition (i.e., pure oil 1), calibration curves developed by plotting $\frac{1}{r-r_0}$ against $\frac{1}{p_{(olive)}}$ for the selected groups of marker ions (i.e., $I_{881.8}/I_{907.8}$, $I_{901.7}/I_{907.8}$, $I_{903.7}/I_{907.8}$ and $I_{905.8}/I_{907.8}$) showed excellent linearities with coefficients of determination (R^2) higher than 0.995 when $p_{(olive)}$ varied from 5% to 100% (Figure 2-3). To validate the quantitative abilities and reliabilities of the obtained calibration curves, five olive oil – sunflower seed oil blended oil samples were prepared with the ratio of olive oil varied from low to high levels. These five samples were directly analyzed by MALDI-MS and the concentrations of olive oil in these samples were

quantified based on their MALDI-MS spectra and the developed calibration curves. As shown in Table 2-5, the calibration curves using $I_{905.8}/I_{907.8}$ as *r* showed the best quantitative ability among the developed calibration curves 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 concentrations of olive oil were close to the actual concentrations and the corresponding accuracy and precision were 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 from the measured results provided by the curve based on $I_{881.8}/I_{907.8}$ with poor accuracy (–12.7-23.3%) and large RSD (5.4-40.6%).

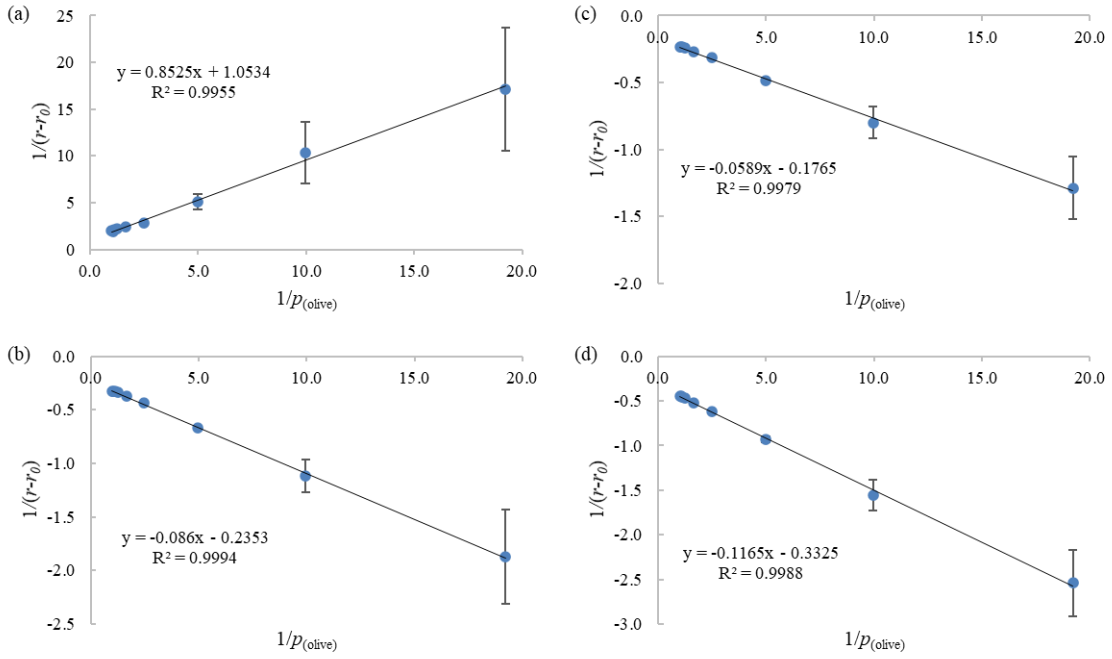

Figure 2-3. Calibration curves based on (a) $I_{881.8}/I_{907.8}$, (b) $I_{901.7}/I_{907.8}$, (c) $I_{903.7}/I_{907.8}$ and (d) $I_{905.8}/I_{907.8}$, and the concentration of olive oil ($p_{(olive)}$) in olive oil – sunflower seed oil blends.

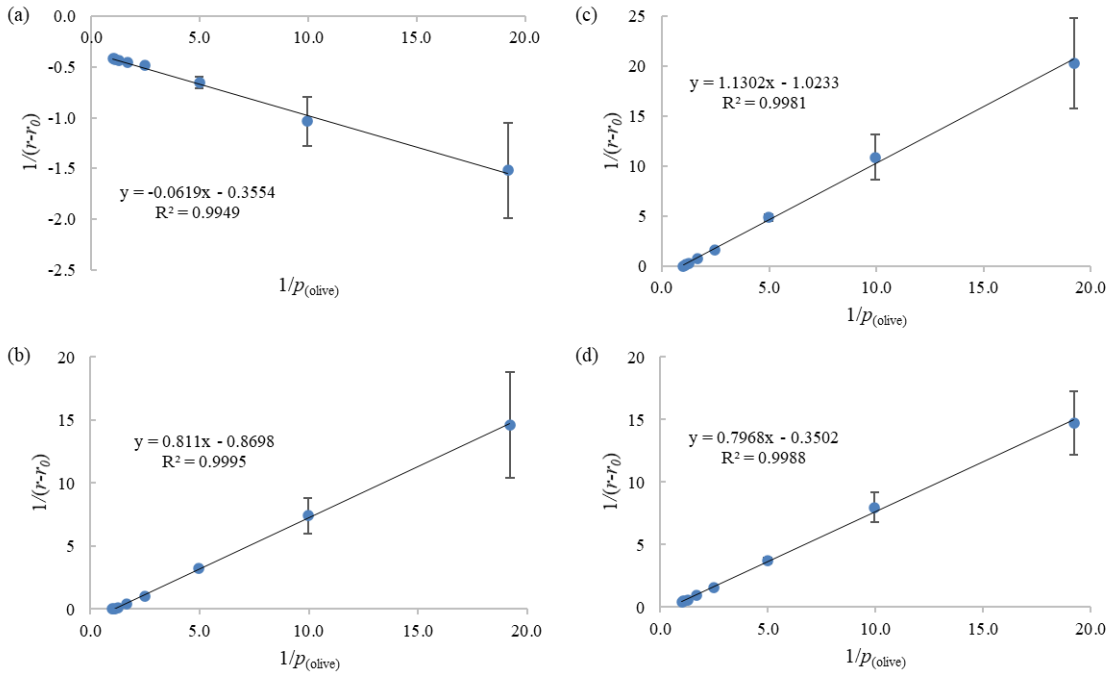

Figure 2-4. Calibration curves based on (a) $I_{907.8}/I_{881.8}$, (b) $I_{907.8}/I_{901.7}$, (c) $I_{907.8}/I_{903.7}$ and (d) $I_{907.8}/I_{905.8}$, and the concentration of olive oil ($p_{(olive)}$) in olive oil – sunflower seed oil blends.

Table 2-5. Quantitative results of olive oil in olive oil – sunflower seed oil blends using olive oil as the measured oil composition.

I_a & I_b	Actual conc. (%)	Based on I_a/I_b			Based on I_b/I_a		
		Measured conc. (%)	Accuracy (%)	RSD (%)	Measured conc. (%)	Accuracy (%)	RSD (%)
$I_{881.8}$ & $I_{907.8}$	7.7	7.4±3.0	–4.3	40.6	7.3±2.9	–4.9	40.0
	30.1	36.5±2.1	21.1	5.8	36.2±2.1	20.3	5.9
	49.7	61.4±4.8	23.3	7.9	61.6±5.0	23.9	8.1
	70.0	71.3±5.4	1.8	7.6	71.9±5.6	2.7	7.8
	92.4	80.7±4.4	–12.7	5.4	81.8±4.6	–11.5	5.6
$I_{901.7}$ & $I_{907.8}$	7.7	7.0±0.9	–9.1	12.6	7.0±0.9	–9.0	12.7
	30.1	31.1±0.9	3.4	2.9	31.2±0.9	3.7	2.9
	49.7	52.1±0.9	4.8	1.7	52.1±0.9	4.8	1.6
	70.0	74.3±1.0	6.1	1.4	74.0±1.0	5.7	1.4
	92.4	91.9±0.2	–0.5	0.2	91.3±0.2	–1.2	0.2
$I_{903.7}$ & $I_{907.8}$	7.7	6.9±1.3	–10.8	18.9	6.9±1.3	–10.6	19.0
	30.1	30.9±0.8	2.5	2.5	31.0±0.8	2.9	2.5
	49.7	51.7±0.7	4.0	1.3	51.8±0.7	4.0	1.3
	70.0	75.3±0.8	7.6	1.0	75.1±0.8	7.3	1.0
	92.4	99.8±0.5	8.0	0.5	99.1±0.5	7.3	0.5
$I_{905.8}$ & $I_{907.8}$	7.7	7.1±1.2	–7.8	17.1	7.1±1.2	–7.7	17.1
	30.1	30.8±0.7	2.1	2.3	30.8±0.7	2.3	2.3
	49.7	51.3±1.0	3.0	1.9	51.3±1.0	3.0	1.9
	70.0	72.5±1.1	3.6	1.6	72.4±1.1	3.4	1.6
	92.4	97.6±1.8	5.6	1.9	97.3±1.8	5.3	1.8

When TAG peaks with close *m/z* values (e.g., *m/z* 905.8 and *m/z* 907.8) are selected as marker ions, the isotopic distribution in mass spectrum should be discussed. Supposing there are two peaks ($[M]^+$ and $[M+2]^+$) shown in the MALDI-MS spectrum which are related to TAG *a* and TAG *b*, respectively, thus the $[M]^+$ peak has isotopic effect on the $[M+2]^+$ peak with a fixed contribution rate (*F*). If the intensity ratio of marker ions is calculated as the intensity of $[M+2]^+$ peak divided by the intensity of $[M]^+$ peak, r can be expressed as

$$r = \frac{I_{\mathrm{M+2}}}{I_{\mathrm{M}}} = \frac{I_b + I_{a,isotopic}}{I_a} = \frac{I_b}{I_a} + F \tag{2-23}$$

where I_a and I_b are the absolute intensities of peaks related to TAG *a* and TAG *b*, respectively, and $I_{a,isotopic}$ is the absolute intensity of the isotopic peak of TAG *a*.

Therefore, $r_0 = \left(\frac{I_b}{I_a}\right)_0 + F$ and $\frac{1}{r-r_0}$ can be derived as

$$\frac{1}{r - r_0} = \frac{1}{\left(\frac{I_b}{I_a} + F\right) - \left(\left(\frac{I_b}{I_a}\right)_0 + F\right)} = \frac{1}{\frac{I_b}{I_a} - \left(\frac{I_b}{I_a}\right)_0} \tag{2-24}$$

Therefore, Equation (2-8) can be rewritten as

$$\frac{1}{\frac{I_b}{I_a} - \left(\frac{I_b}{I_a}\right)_0} = \frac{K}{p_1} + E \tag{2-25}$$

If $r = \frac{I_{\mathrm{M}}}{I_{\mathrm{M+2}}}$, it is obtained that

$$r = \frac{I_{\mathrm{M}}}{I_{\mathrm{M+2}}} = \frac{I_a}{I_b + I_{a,isotopic}} = \frac{1}{\frac{I_b}{I_a} + F} \tag{2-26}$$

and $\frac{1}{r-r_0}$ can be expressed as

$$\frac{1}{r-r_0}=\frac{1}{\frac{1}{\frac{I_b}{I_a}+F}-\frac{1}{\left(\frac{I_b}{I_a}\right)_0+F}}=\frac{\left(\left(\frac{I_b}{I_a}\right)_0+F\right)^2}{\frac{I_b}{I_a}-\left(\frac{I_b}{I_a}\right)_0}-\left(\frac{I_b}{I_a}\right)_0-F \qquad (2\text{-}27)$$

Substituting Equation (2-27) into Equation (2-8) yields

$$\frac{1}{\frac{I_b}{I_a}-\left(\frac{I_b}{I_a}\right)_0}=\frac{K\left(\left(\frac{I_b}{I_a}\right)_0+F\right)^{-2}}{p_1}+\frac{E+\left(\frac{I_b}{I_a}\right)_0+F}{\left(\left(\frac{I_b}{I_a}\right)_0+F\right)^2}$$

which can be rewritten as

$$\frac{1}{\frac{I_b}{I_a}-\left(\frac{I_b}{I_a}\right)_0}=\frac{K'}{p_1}+E' \qquad (2\text{-}28)$$

where $K'=K\left(\left(\frac{I_b}{I_a}\right)_0+E\right)^{-2}$ and $E'=\frac{E+\left(\frac{I_b}{I_a}\right)_0+F}{\left(\left(\frac{I_b}{I_a}\right)_0+F\right)^2}$.

F is constant for specific TAG *a* and TAG *b*, and $\left(\frac{I_b}{I_a}\right)_0$ is also fixed for a selected pure oil. Therefore, Equation (2-25) and Equation (2-28) have the same linear relationships but different parameters, indicating the presence of isotopic ions would not change the quantitative relationship.

For further validation, four other calibration curves were established based on $I_{907.8}/I_{881.8}$, $I_{907.8}/I_{901.7}$, $I_{907.8}/I_{903.7}$ and $I_{907.8}/I_{905.8}$ with olive oil as the measured oil composition (Figure 2-4), and the new developed calibration curves were used to measure the concentration of olive oil in the same five validation samples. It could be

observed from Table 2-5 that for the same group of marker ions (I_a and I_b), the quantitative results provided by the curves based on I_a/I_b and I_b/I_a were almost the same regardless of whether the *m/z* values of the marked ions were very close (i.e., *m/z* 905.8 and *m/z* 907.8) or quite different (i.e., *m/z* 881.8 and *m/z* 907.8). The above discussion and results demonstrated that the quantitative analysis based on the intensity ratio of marker ions would not be affected by the isotopic distribution in the mass spectrum.

Regarding sunflower seed oil as the measured oil composition in the olive oil – sunflower seed oil blends, a series of new calibration curves could be obtained based on the intensity ratio of marker ions (i.e., $I_{881.8}/I_{907.8}$, $I_{901.7}/I_{907.8}$, $I_{903.7}/I_{907.8}$ and $I_{905.8}/I_{907.8}$) and the concentration of sunflower seed oil ($p_{(sunflower)}$) in blended samples (Figure 2-5). Curves using $I_{881.8}/I_{907.8}$, $I_{903.7}/I_{907.8}$ and $I_{905.8}/I_{907.8}$ as r showed strong linear relationships with R^2 higher than 0.995, while for the curve based on $I_{901.7}/I_{907.8}$, slightly poor linearity was observed with R^2 as 0.9798. The linear range of the calibration curve based on $p_{(sunflower)}$ was 5-100% sunflower seed oil, which was equivalent to 0-95% olive oil in the blends and thus was complimentary to the linear range of the calibration curve based on $p_{(olive)}$ (i.e., 5-100% olive oil).

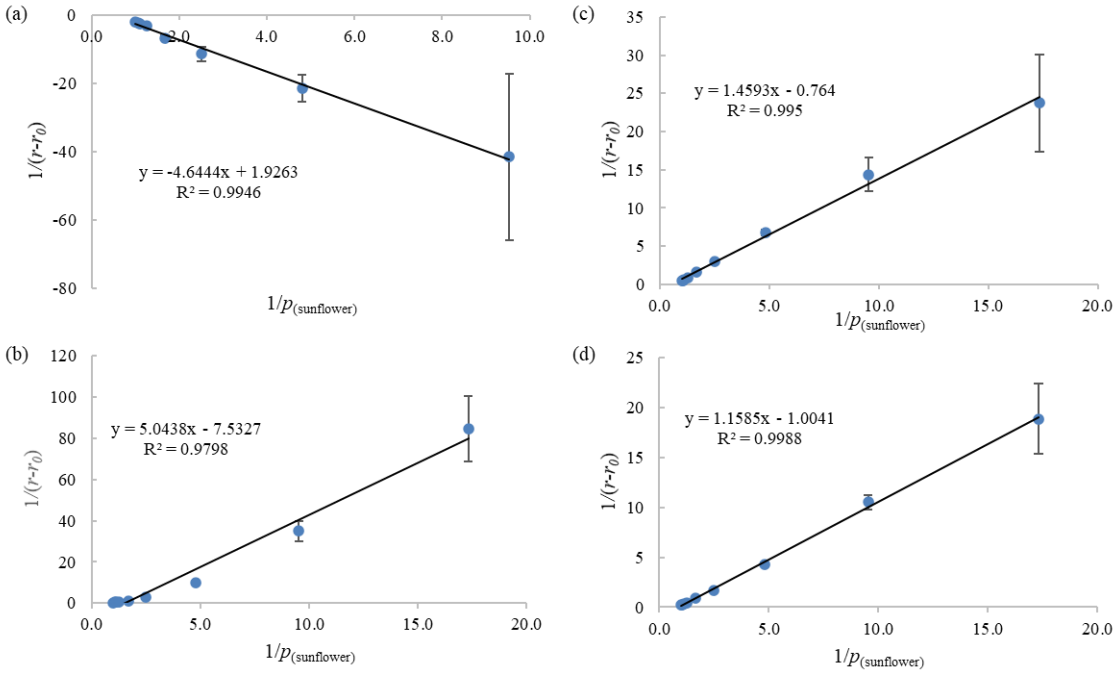

Figure 2-5. Calibration curves based on (a) $I_{881.8}/I_{907.8}$, (b) $I_{901.7}/I_{907.8}$, (c) $I_{903.7}/I_{907.8}$ and (d) $I_{905.8}/I_{907.8}$, and the concentration of sunflower seed oil ($p_{(sunflower)}$) in olive oil – sunflower seed oil blends.

The total concentration of olive oil and sunflower seed oil in their binary blend was always 1, so the quantitative results of olive oil in the olive oil – sunflower seed oil blends could be obtained after using the calibration curve based on $p_{(sunflower)}$ to measure the ratio of sunflower seed oil in the blends, which were summarized in Table 2-6. Comparing the results measured by the curves based on $p_{(olive)}$ and $p_{(sunflower)}$, it was noticed that the curves based on $p_{(sunflower)}$showed poor quantitative abilities to samples with low-abundance olive oil (⩽30%), especially the sample contained only 7.7% olive oil (Table 2-6). However, for samples with olive oil at high levels (~92%), the quantitative results provided by the curves based on $p_{(sunflower)}$ were better than those provided by the curves based on $p_{(olive)}$.

Table 2-6. Quantitative results of olive oil in olive oil – sunflower seed oil blends based on the intensity ratio of different marker ions.

Marker ions (*r*)	**Actual conc. (%)**	**Based on $p_{(olive)}$**			**Based on $p_{(sunflower)}$**		
		Measured conc. (%)	Accuracy (%)	RSD (%)	Measured conc. (%)	Accuracy (%)	RSD (%)
$I_{881.8}/I_{907.8}$	7.7	7.4±3.0	–4.3	40.6	–9.0±4.1	–217.6	–45.3
	30.1	36.5±2.1	21.1	5.8	29.9±2.8	–0.8	9.3
	49.7	61.4±4.8	23.3	7.9	61.6±6.0	23.7	9.8
	70.0	71.3±5.4	1.8	7.6	73.8±6.6	5.4	9.0
	92.4	80.7±4.4	–12.7	5.4	85.2±5.3	–7.8	6.2
$I_{901.7}/I_{907.8}$	7.7	7.0±0.9	–9.1	12.6	36.6±0.1	375.5	0.3
	30.1	31.1±0.9	3.4	2.9	40.2±0.2	33.6	0.4
	49.7	52.1±0.9	4.8	1.7	45.9±0.3	–7.8	0.7
	70.0	74.3±1.0	6.1	1.4	58.9±1.0	–15.8	1.7
	92.4	91.9±0.2	–0.5	0.2	91.9±0.8	–0.5	0.9
$I_{903.7}/I_{907.8}$	7.7	6.9±1.3	–10.8	18.9	11.1±0.9	44.1	8.3
	30.1	30.9±0.8	2.5	2.5	29.1±0.6	–3.5	2.1
	49.7	51.7±0.7	4.0	1.3	46.2±0.6	–7.1	1.2
	70.0	75.3±0.8	7.6	1.0	67.8±0.8	–3.2	1.1
	92.4	99.8±0.5	8.0	0.5	92.7±0.5	0.3	0.5
$I_{905.8}/I_{907.8}$	7.7	7.1±1.2	–7.8	17.1	–9.6±1.9	–224.7	–20.0
	30.1	30.8±0.7	2.1	2.3	24.1±0.9	–19.8	3.8
	49.7	51.3±1.0	3.0	1.9	48.6±1.1	–2.3	2.3
	70.0	72.5±1.1	3.6	1.6	70.3±1.1	0.5	1.5
	92.4	97.6±1.8	5.6	1.9	92.3±1.5	–0.1	1.6

To quantify the oil compositions of a binary blended oil sample, the $\frac{1}{r-r_0}$ value of the sample is substituted into the calibration plot of $\frac{1}{r-r_0}$ against $\frac{1}{p}$, and based on the obtained $\frac{1}{p}$ (marked as *U*), the ratio of the measured oil composition could be calculated as Equation (2-29) shows.

$$p = \frac{1}{U} \tag{2-29}$$

According to the propagation of error, the propagated error of *p* caused by the error of *U* can be expressed as

$$\Delta p = \frac{d\left(\frac{1}{U}\right)}{dU}\Delta U = \frac{\Delta U}{U^2} \tag{2-30}$$

where Δp is the absolute propagated error and ΔU is the absolute error caused by the regression. For the same ΔU, Δp will become larger when *U* becomes smaller (equal to larger p). In other words, when the concentration of the measured oil composition increases from low to high level, the quantitative performance of the calibration curve based on the measured oil composition deteriorates due to the increased propagated error.

As discussed above, for the quantitative analysis of olive oil – sunflower seed oil blends, the calibration curves based on $p_{(olive)}$ were more suitable to quantify olive oil at low levels in the blended samples, and the calibration curves based on $p_{(sunflower)}$ provided better quantitative results to the samples with high-abundance olive oil (i.e.,

low-abundance sunflower seed oil). Therefore, a segmental strategy has been proposed for more accurate quantitative measurements, i.e., using the curve based on $p_{(olive)}$ to measure samples with low-abundance olive oil and using the curve based on $p_{(sunflower)}$ to measure samples with high-abundance olive oil. As shown in Table 2-6, the critical concentrations of segmental quantitation for curves based on $I_{881.8}/I_{907.8}$, $I_{901.7}/I_{907.8}$, $I_{903.7}/I_{907.8}$ and $I_{905.8}/I_{907.8}$ were 92% olive oil, 92% olive oil, 70% olive oil and 70% olive oil, respectively. To evaluate the quantitative ability of different groups of marker ions, the root mean square error (RMSE) was applied to summary the difference between the actual concentrations and the measured concentrations of olive oil after implementing the segmental strategy for quantitation. For the quantitative results based on $I_{881.8}/I_{907.8}$, $I_{901.7}/I_{907.8}$, $I_{903.7}/I_{907.8}$ and $I_{905.8}/I_{907.8}$, the RMSE values were 0.0788, 0.0241, 0.0164 and 0.0125, respectively, indicating the calibration curves using $I_{905.8}/I_{907.8}$ as r provided excellent quantitative results with accuracy and precision within –7.8-3.0% and 1.5-17.1%, respectively (Table 2-7). Therefore, the group of peaks at *m/z* 903.7 and *m/z* 907.8 was recommended as marker ions for the quantitative analysis of the olive oil – sunflower seed oil blends.

The average molecular weights of olive oil and sunflower seed oil are similar[85], i.e., $\frac{M_1}{M_2} \approx 1$, so the approximate values of K and E in Equation (2-8) can be estimated based on the spectra of pure olive oil and sunflower seed oil. For the calibration curve based on $I_{905.8}/I_{907.8}$ and $p_{(olive)}$, the slope (K) and intercept (E) were –0.1165 and –

0.3325 (Figure 2-3), 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 (2-8) to calculate the concentration of olive oil in validation samples. As shown in Table 2-7, the calculated results indicated the approximate concentration of olive oil in the validation samples with absolute error within 7%, illustrating the successful semi-quantitation of oil compositions in binary blended oils.

Table 2-7. Quantitative results of olive oil in olive oil – sunflower seed oil blends measured by the calibration curves and calculated from the spectral data of pure oils with $I_{905.8}/I_{907.8}$ as r.

Actual conc. (%)	**Results based on calibration curves**				**Results based on pure oils**		
	Based on $p_{(olive)}$ (%)	Based on $p_{(sunflower)}$ (%)	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

2.3.1.2 *Selection of marker ions for quantitative analysis*

An intensity ratio-based method has been developed for the quantitative analysis of binary blended oils, but blended oils showed numerous TAG peaks in the MALDI-MS spectra, making it difficult to select marker ions for accurate quantitative analysis. To comprehensively validate the developed method and investigate the selection of marker ions for quantitative analysis, six types of binary blended oils were prepared for analysis, i.e., flaxseed oil – peanut oil blends, flaxseed oil – corn oil blends, olive oil – peanut oil blends, corn oil – canola oil blends, sunflower seed oil – soybean oil blends and corn oil – soybean oil blends, which could be divided into three groups, i.e., blends of pure oils with disparate TAG profiles, blends of pure oils with partly different TAG profiles and blends of pure oils with similar TAG profiles.

Flaxseed oil is a new type of vegetable oils that have emerged in the market due to its special nutritional characteristics. Flaxseed oil is rich in α-linolenic acid (C18:3), lignans and tocopherol[86-87], and have many potential health benefits such as lowering blood pressure, decreasing cardiovascular diseases and protecting against Alzheimer's disease[88-90]. Therefore, flaxseed oil has been regarded as a healthy oil and added into blended oils for improved nutritional values. The high percentage of α-linolenic acid in flaxseed oil caused strong peaks at *m/z* 895.7 (LnLnLn, Ln: α-linolenic acid), *m/z* 897.7 (LnLnL) and *m/z* 899.7 (LnLL) in the spectrum (Figure 2-6 d), which were usually very weak in common edible oils, such as peanut oil and corn oil. The main FA in peanut oil

is the oleic acid, followed by the linoleic acid and the palmitic acid (C16:0), thus the MALDI-MS spectrum of peanut oil showed strong peaks at *m/z* 907.8 (OOO), *m/z* 905.8 (OOL), *m/z* 903.7 (OLL), *m/z* 881.8 (POO) and *m/z* 901.7 (LLL) (Figure 2-6 a), which were consistent with those previously reported[91-92]. On the other hand, the level of linoleic acid contained in corn oil was higher than that of oleic acid, resulting in the highest peak at *m/z* 903.7 (OLL), followed by the peaks at *m/z* 901.7 (LLL) and *m/z* 905.8 (OOL) (Figure 2-7 a), as reported previously[77, 93]. The major FAs of flaxseed oil were disparate from those of peanut oil and corn oil, making the MALDI-MS spectrum of flaxseed oil significantly different from the spectra of peanut oil and corn oil in the TAG region.

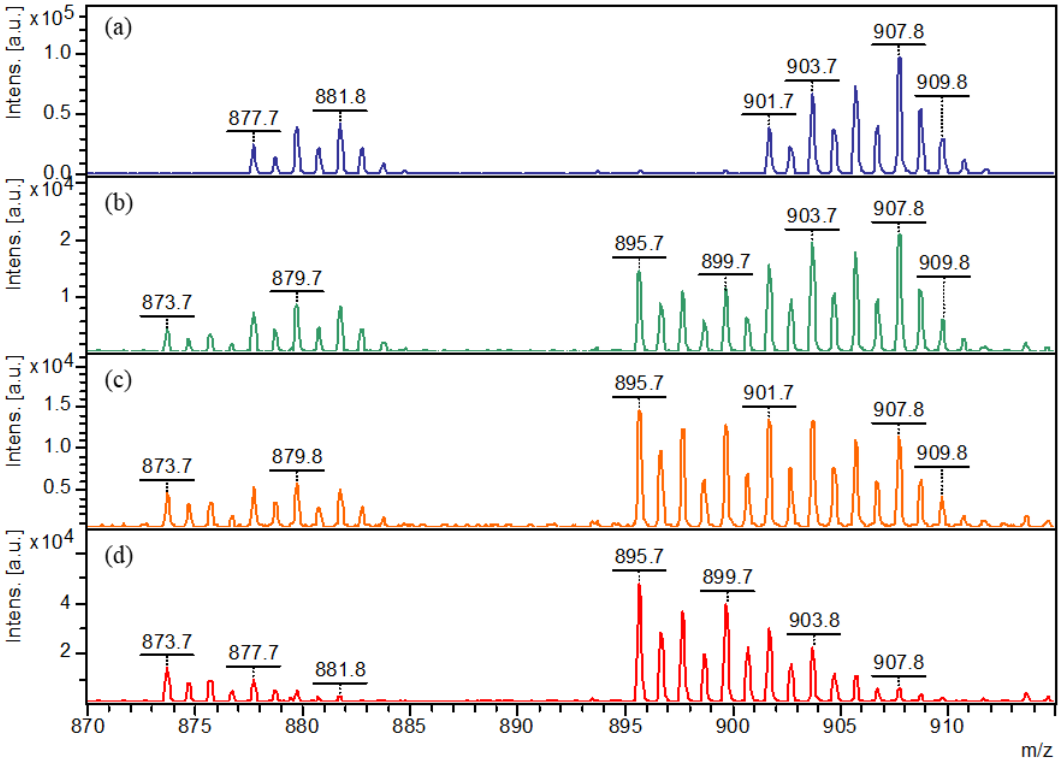

Figure 2-6. The TAG region of the MALDI-MS spectra for (a) 100% peanut oil, (b) 40% flaxseed oil – 60% peanut oil blend, (c) 60% flaxseed oil – 40% peanut oil blend and (d) 100% flaxseed oil.

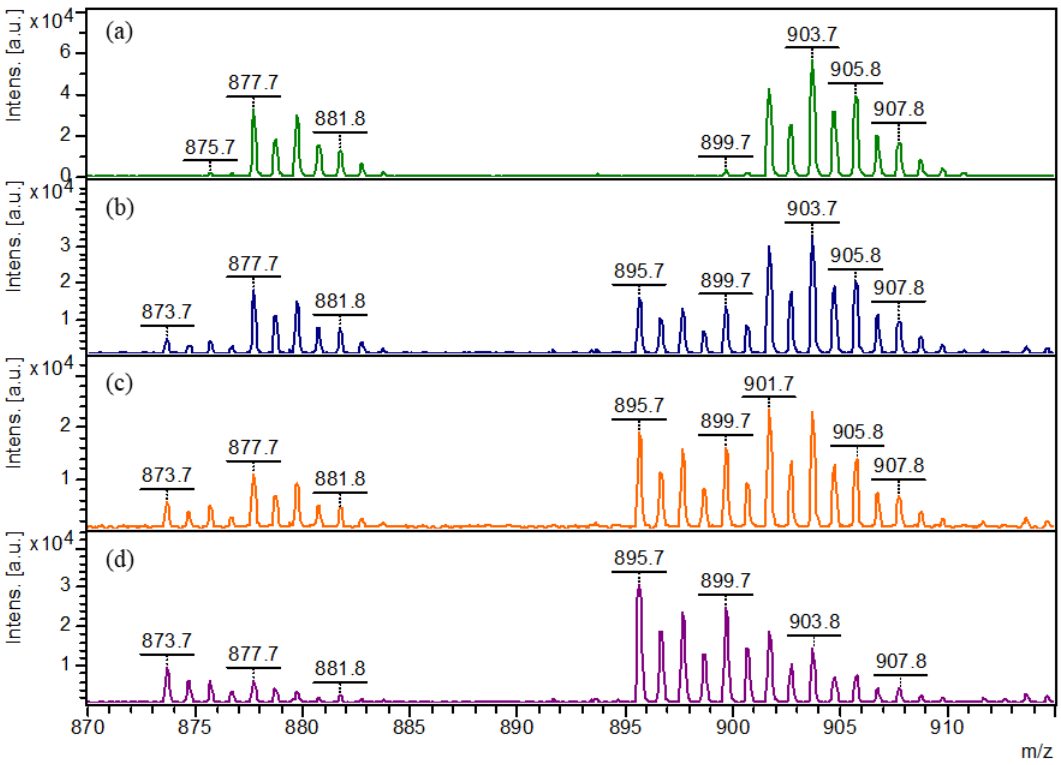

Figure 2-7. The TAG region of the MALDI-MS spectra for (a) 100% corn oil, (b) 40% flaxseed oil – 60% corn oil blend, (c) 60% flaxseed oil – 40% corn oil blend and (d) 100% flaxseed oil.

According to the obtained spectra of pure oils, peaks at *m/z* 895.7 and *m/z* 899.7 were selected as the characteristic peaks of flaxseed oil, while peaks at *m/z* 905.8 and *m/z* 907.8, and peaks at *m/z* 901.7 and *m/z* 903.7 were chosen as the potential marker ions of peanut oil and corn oil, respectively. Calibration curves based on the selected peaks were established for the quantitative analysis of flaxseed oil – peanut oil blends and flaxseed oil – corn oil blends, which showed strong linearity with R^2 higher than 0.995 (Table 2-8). The established curves were applied to quantify the concentration of flaxseed oil in validation samples and the optimized results provided by segmental quantitation were summarized in Table 2-9 and Table 2-10.

For flaxseed oil – peanut oil blends, the four groups of marker ions provided similar quantitative results and the RMSE values varied from 0.0124 to 0.0145, indicating all the selected marker ions were powerful for quantitative analysis (Table 2-9). The most excellent quantitative results were provided by the group of peaks at *m/z* 895.7 and *m/z* 905.8, and the group of peaks at *m/z* 899.7 and *m/z* 905.8 with accuracy and precision within –1.2-3.2% and 1.3-10.0%, and –3.6-9.8% and 1.4-7.1%, respectively. For flaxseed oil – corn oil blends, the RMSE of quantitative results provided by the curves 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 (Table 2-10), and the results measured by $I_{899.7}/I_{903.7}$ showed the most excellent accuracy and precision within –1.1-4.0% and 1.1-8.5%, respectively.

Table 2-8. Calibration curves for quantitative analysis of flaxseed oil – peanut oil blends and flaxseed oil – corn oil blends.

Type	**Marker ions (*r*)**	**Curve based on $p_{(flaxseed)}$**	**R^2**	**Curve based on $p_{(peanut)}$ or $p_{(corn)}$**	**R^2**
Flaxseed oil – peanut oil blends	$I_{895.7}/I_{905.8}$	y = 0.5957x – 0.2789	0.9982	y = –0.0808x – 0.1774	0.9970
	$I_{895.7}/I_{907.8}$	y =0.7801x – 0.5410	0.9975	y = –0.0227x – 0.1077	0.9948
	$I_{899.7}/I_{905.8}$	y = 0.7015x – 0.2754	0.9968	y = –0.1000x – 0.2029	0.9993
	$I_{899.7}/I_{907.8}$	y =0.9176x – 0.5713	0.9956	y = –0.0267x – 0.1306	0.9954
Flaxseed oil – corn oil blends	$I_{895.7}/I_{901.7}$	y = 0.6455x + 0.1609	0.9957	y = –0.4176x – 0.2170	0.9990
	$I_{895.7}/I_{903.7}$	y = 0.8613x – 0.1736	0.9962	y = –0.1519x – 0.3255	0.9977
	$I_{899.7}/I_{901.7}$	y = 1.2539x – 0.6052	0.9986	y = –0.5552x – 0.1939	0.9965
	$I_{899.7}/I_{903.7}$	y = 1.5929x – 1.1975	0.9989	y = –0.1914x – 0.3857	0.9974

Table 2-9. Quantitative results of flaxseed oil in flaxseed oil – peanut oil blends based on the intensity ratio of marker ions.

Marker ions (*r*)	**Actual conc. (%)**	**Measured conc. (%)**		**Accuracy (%)**	**RSD (%)**	**RMSE**
		Based on $p_{(\text{flaxseed})}$	Based on $p_{(\text{peanut})}$			
$I_{895.7}/I_{905.8}$	7.6	7.9±0.8	/	3.2	10.0	0.0127
	30.1	29.7±0.9	/	–1.2	2.9	
	50.0	49.9±0.9	/	–0.2	1.8	
	70.1	/	70.7±1.8	0.9	2.6	
	92.4	/	91.3±1.2	–1.2	1.3	
$I_{895.7}/I_{907.8}$	7.6	7.9±0.9	/	3.9	11.6	0.0138
	30.1	29.5±2.2	/	–2.0	7.5	
	50.0	50.8±1.0	/	1.6	2.0	
	70.1	/	69.6±1.1	–0.7	1.6	
	92.4	/	91.6±0.8	–0.9	0.9	
$I_{899.7}/I_{905.8}$	7.6	8.4±0.6	/	9.8	7.1	0.0124
	30.1	29.0±1.0	/	–3.6	3.6	
	50.0	/	50.1±0.9	0.3	1.7	
	70.1	/	70.0±1.5	–0.2	2.1	
	92.4	/	91.6±1.2	–0.9	1.4	
$I_{899.7}/ I_{907.8}$	7.6	8.4±0.6	/	10.1	7.5	0.0145
	30.1	28.8±1.4	/	–4.4	4.7	
	50.0	51.6±1.0	/	3.2	1.9	
	70.1	/	68.9±0.9	–1.6	1.3	
	92.4	/	92.0±0.9	–0.5	1.0	

Table 2-10. Quantitative results of flaxseed oil in flaxseed oil – corn oil blends based on the intensity ratio of marker ions.

Marker ions (*r*)	**Actual conc. (%)**	**Measured conc. (%)**		**Accuracy (%)**	**RSD (%)**	**RMSE**
		Based on $p_{(\text{flaxseed})}$	Based on $p_{(\text{corn})}$			
$I_{895.7}/I_{901.7}$	6.5	6.5±0.7	/	–0.9	10.9	0.0295
	30.3	27.1±1.6	/	–10.6	6.0	
	50.1	51.0±3.4	/	1.7	6.8	
	69.7	/	72.5±1.9	3.9	2.7	
	92.3	/	93.7±2.7	1.5	2.9	
$I_{895.7}/I_{903.7}$	6.5	6.6±0.5	/	0.6	8.3	0.0241
	30.3	27.8±1.4	/	–8.3	5.2	
	50.1	51.0±2.0	/	1.8	3.9	
	69.7	/	73.1±1.3	4.8	1.7	
	92.3	/	93.8±1.3	1.7	1.4	
$I_{899.7}/I_{901.7}$	6.5	6.5±0.9	/	–0.4	14.2	0.0233
	30.3	30.1±1.5	/	–0.7	5.1	
	50.1	49.1±2.2	/	–2.0	4.6	
	69.7	/	71.4±2.0	2.3	2.8	
	92.3	/	91.3±3.7	–1.0	4.0	
$I_{899.7}/I_{903.7}$	6.5	6.7±0.6	/	2.3	8.5	0.0194
	30.3	31.2±0.5	/	2.8	1.7	
	50.1	49.6±2.0	/	–1.1	4.1	
	69.7	/	72.6±0.8	4.0	1.1	
	92.3	/	92.5±2.3	0.3	2.5	

Canola oil is commnoly used in cooking and considered as a healthy vegetable oil due to the high contents of unsatrated FAs, e.g., oleic acid and linoleic acid, with a roughly ratio of 2:1 for the two FAs[94]. As mentioned previous, corn oil is also abundant in oleic acid and linoleic acid while the level of linoleic acid is higher than the level of oleic acid. Therefore, the main TAG peaks shown in the MALDI-MS spectra of canola oil and corn oil were overlapped with major difference in their relative intensities (Figure 2-8). Canola oil showed the strongest peak at *m/z* 907.8 (OOO) with the second highest peak at *m/z* 905.8 (OOL), while corn oil had the strongest peak at *m/z* 903.7 (OLL), followed by the peak at *m/z* 901.7 (LLL). To quantify the concentration of corn oil in the corn oil – canola oil blends, the peaks at *m/z* 901.7 and *m/z* 903.7 were paired with the peaks at *m/z* 905.8 and *m/z* 907.8 as marker ions and then applied to establish calibration curves (Table 2-11). Compared with the quantitative results provided by $I_{901.7}/I_{905.8}$ and $I_{903.7}/I_{905.8}$, the results provided by $I_{901.7}/I_{907.8}$ and $I_{903.7}/I_{907.8}$ showed much smaller RMSE values (Table 2-12), indicating the peak at *m/z* 907.8 was more powerful for quantitative analysis than the peak at *m/z* 905.8, because the variation in the R.I. of the peak at *m/z* 907.8 from canola oil to corn oil was larger than that of the peak at *m/z* 905.8, which were from 0.178 to 0.047 and from 0.145 to 0.102, respectively. Similarly, the peak at *m/z* 901.7 showed a greater increment in the abundance (from 0.051 to 0.117) than the peak at *m/z* 903.7 (from 0.117 to 0.153) when the concentration of corn oil in the corn oil – canola oil blends increased from 0% to 100% (Figure 2-8), thus the RMSE values of $I_{901.7}/I_{905.8}$ and $I_{901.7}/I_{907.8}$ were smaller than that of $I_{903.7}/I_{905.8}$ and $I_{903.7}/I_{907.8}$,

respectively. Overall, the combination of peaks at *m/ z* 901.7 and *m/z* 907.8 presented the best quantitative ability with the smallest RMSE at 0.0158 and accuracy and precision of measured results within –2.1-0.5% and 1.1-4.0%, respectively, except for an extreme RSD (30.8%) caused by the low-abundance corn oil sample (Table 2-12).

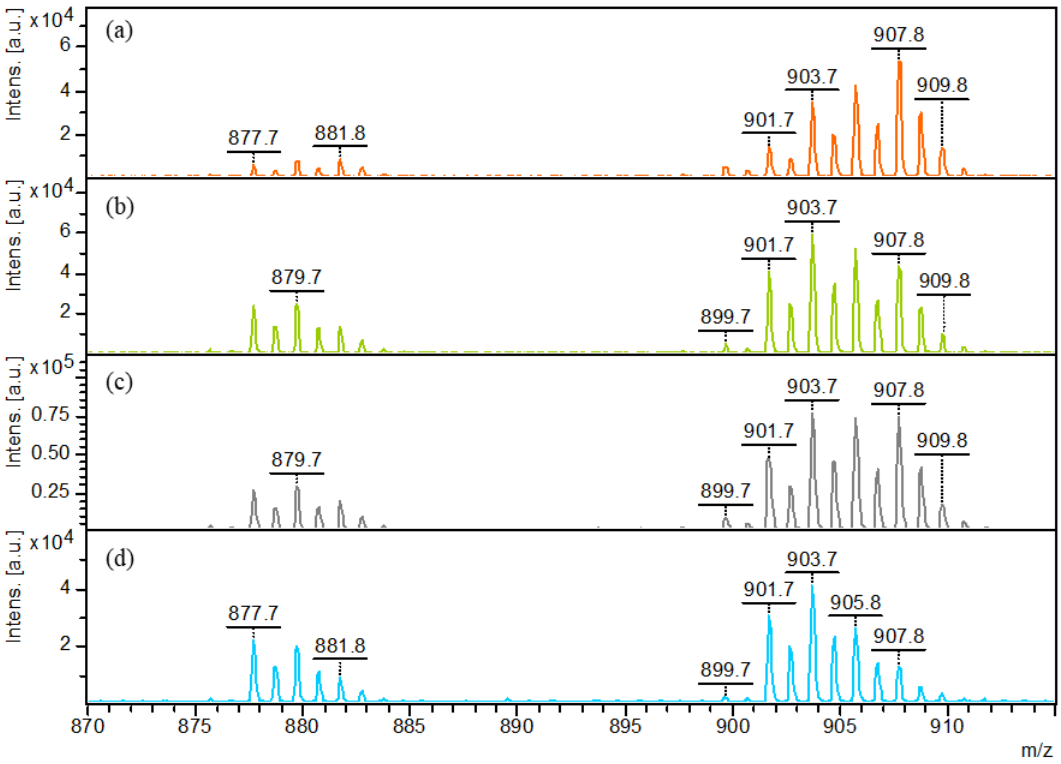

Figure 2-8. The TAG region of the MALDI-MS spectra for (a) 100% canola oil, (b) 40% corn oil – 60% canola oil blend, (c) 60% corn oil – 40% canola oil blend and (d) 100% corn oil.

Table 2-11. Calibration curves for quantitative analysis of corn oil – canola oil blends.

Type	**Marker ions (*r*)**	**Curve based on $p_{(corn)}$**	**R^2**	**Curve based on $p_{(canola)}$**	**R^2**
Corn oil – canola oil blends	$I_{901.7}/I_{905.8}$	y = 1.2440x + 0.3765	0.9944	y = –0.9747x – 0.1698	0.9971
	$I_{903.7}/I_{905.8}$	y = 1.9353x – 0.5896	0.9960	y = –0.8414x – 0.6816	0.9990
	$I_{901.7}/I_{907.8}$	y = 1.4735x – 0.9585	1.0000	y = –0.1328x – 0.3178	0.9987
	$I_{903.7}/I_{907.8}$	y = 1.6850x – 1.5680	0.9983	y = –0.1110x – 0.2727	0.9983

Table 2-12. Quantitative results of corn oil in corn oil – canola oil blends based on the intensity ratio of marker ions.

Marker ions (*r*)	**Actual conc. (%)**	**Measured conc. (%)**		**Accuracy (%)**	**RSD (%)**	**RMSE**
		Based on $p_{(corn)}$	Based on $p_{(canola)}$			
$I_{901.7}/I_{905.8}$	7.6	6.8±1.7	/	–10.3	25.8	0.0348
	30.1	27.5±2.3	/	–8.7	8.5	
	50.1	52.3±5.2	/	4.3	9.9	
	70.3	/	70.2±2.6	–0.1	3.8	
	92.2	/	91.7±3.3	–0.6	3.6	
$I_{903.7}/I_{905.8}$	7.6	8.5±3.8	/	13.0	43.9	0.0465
	30.1	30.3±3.8	/	0.6	12.5	
	50.1	47.9±5.4	/	–4.5	11.3	
	70.3	/	73.8±5.3	4.9	7.1	
	92.2	/	92.9±4.0	0.8	4.4	
$I_{901.7}/I_{907.8}$	7.6	7.4±2.3	/	–1.5	30.8	0.0158
	30.1	/	30.1±1.2	–0.1	4.0	
	50.1	/	49.2±1.3	–1.8	2.7	
	70.3	/	68.8±1.1	–2.1	1.6	
	92.2	/	92.7±1.0	0.5	1.1	
$I_{903.7}/I_{907.8}$	7.6	7.4±2.3	/	–1.8	30.5	0.0181
	30.1	/	30.6±2.0	1.7	6.5	
	50.1	/	48.2±1.0	–3.8	2.0	
	70.3	/	69.1±1.1	–1.8	1.6	
	92.2	/	92.3±0.9	0.2	1.0	

Both olive oil and peanut oil are abundant in oleic acid, followed by the linoleic acid and the palmitic acid, so the main TAGs contained in olive oil and peanut oil were POO, OOL and OOO, resulting in the strong peaks at *m/z* 881.8, *m/z* 905.7 and *m/z* 907.8 in the MALDI-MS spectra (Figure 2-9). Compared with the spectrum of olive oil, the spectrum of peanut oil showed more TAG peaks at *m/z* 879.7 (POL), *m/z* 901.7 (LLL) and *m/z* 903.7 (OLL) since peanut oil contained more linoleic acid than olive oil. As shown in Figure 2-9, when the concentration of olive oil in the olive oil – peanut oil blends increased from 0% to 100%, the major changes observed in the spectra of blended oils were the decline of peaks at *m/z* 879.7, *m/z* 901.7, *m/z* 903.7 and *m/z* 905.7. Therefore, $I_{879.7}/I_{881.7}$, $I_{901.7}/I_{907.8}$, $I_{903.7}/I_{907.8}$ and $I_{905.8}/I_{907.8}$ were used to develop calibration curves (Table 2-13), and the quantitative results of validation samples provided by the developed curves were summarized in Table 2-14. For peanut oil and olive oil, $I_{879.7}/I_{881.7}$, $I_{901.7}/I_{907.8}$, $I_{903.7}/I_{907.8}$ and $I_{905.8}/I_{907.8}$ were 0.957, 0.421, 0.730 and 0.767, and 0.242, 0.000, 0.064 and 0.240, respectively, where the most significant variation was observed from $I_{903.7}/I_{907.8}$ ($r_{(peanut)}/r_{(olive)} = 11.5$). According to the previous discussion, $I_{903.7}/I_{907.8}$ should present the best quantitative ability for olive oil – peanut oil blends, which was consistent to the results shown in Table 2-14 because $I_{903.7}/I_{907.8}$ had the smallest RMSE (0.0252). For $I_{879.7}/I_{881.7}$ and $I_{905.8}/I_{907.8}$, the values of $r_{(peanut)}/r_{(olive)}$ were 3.958 and 3.196, respectively, while the quantitative performance of $I_{879.7}/I_{881.7}$ was worse than that of $I_{905.8}/I_{907.8}$ with RMSE as 0.0433 and 0.0375, respectively. Although $I_{879.7}/I_{881.7}$ showed larger variation than $I_{905.8}/I_{907.8}$, the peaks at

m/z 879.7 and *m/z* 881.8 were much weaker than the peaks at *m/z* 905.8 and *m/z* 907.8 (Figure 2-9), leading to the poorer accuracy (–6.4-1.9%) and precision (5.6-48.1%) of $I_{879.7}/I_{881.7}$.

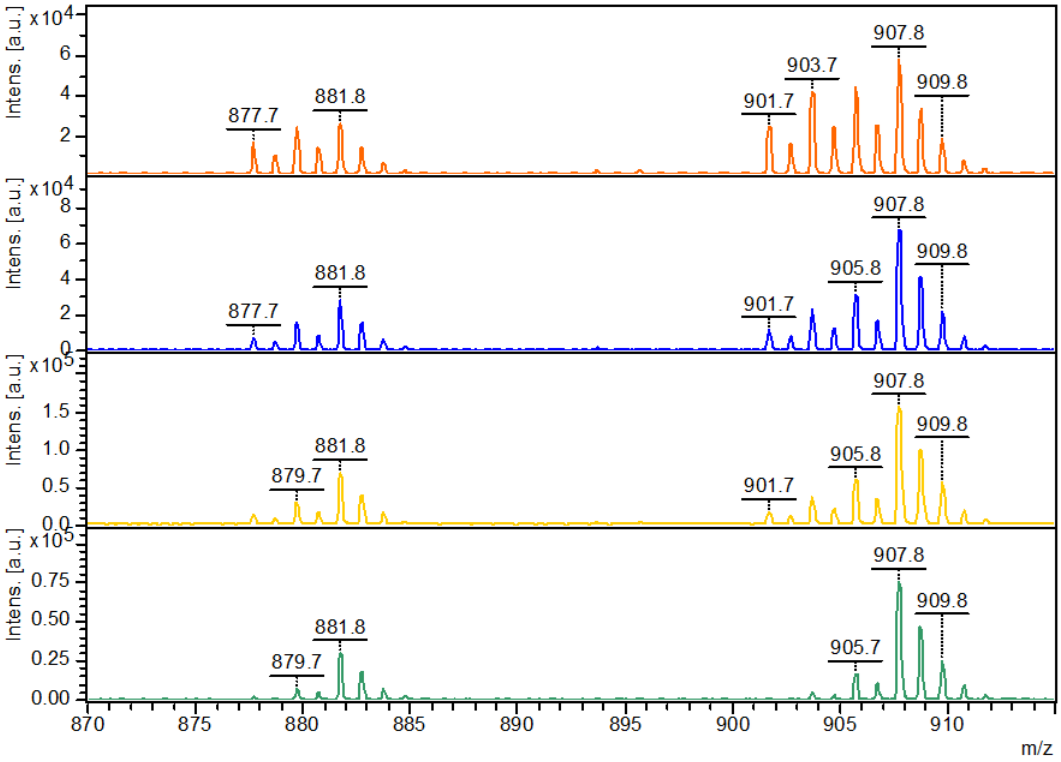

Figure 2-9. The TAG region of the MALDI-MS spectra for (a) 100% peanut oil, (b) 40% olive oil – 60% peanut oil blend, (c) 60% olive oil – 40% peanut oil blend and (d) 100% olive oil.

Table 2-13. Calibration curves for quantitative analysis of olive oil – peanut oil blends.

Type	**Marker ions (*r*)**	**Curve based on $p_{(olive)}$**	**R^2**	**Curve based on $p_{(peanut)}$**	**R^2**
Olive oil – peanut oil blends	$I_{879.7}/I_{881.7}$	y = –0.6654x – 0.8053	0.9971	y = 3.8460x – 2.7590	0.9943
	$I_{901.7}/I_{907.8}$	y = –1.0983x – 1.4243	0.9993	y = 2.9490x + 0.1568	0.9903
	$I_{903.7}/I_{907.8}$	y = –0.7366x – 0.7105	0.9992	y = 4.1366x – 3.7434	0.9926
	$I_{905.8}/I_{907.8}$	y = –0.8593x – 1.1223	0.9998	y = 3.2529x – 1.1193	0.9999

Table 2-14. Quantitative results of olive oil in olive oil – peanut oil blends based on the intensity ratio of marker ions.

Marker ions (*r*)	**Actual conc. (%)**	**Measured conc. (%)**		**Accuracy (%)**	**RSD (%)**	**RMSE**
		Based on $p_{(olive)}$	Based on $p_{(peanut)}$			
	7.5	7.7±3.7	/	1.9	48.1	
	29.7	28.6±3.3	/	–3.6	11.4	
$I_{879.7}/I_{881.7}$	49.8	/	46.6±3.5	–6.4	7.4	0.0433
	70.1	/	68.2±5.5	–2.6	8.0	
	92.1	/	93.5±5.3	1.5	5.6	
	7.5	6.5±1.4	/	–14.1	20.9	
	29.7	29.0±1.8	/	–2.3	6.2	
$I_{901.7}/I_{907.8}$	49.8	48.9±1.9	/	–1.7	3.9	0.0407
	70.1	72.5±1.5	/	3.4	2.0	
	92.1	/	89.7±0.8	–2.6	0.9	
	7.5	9.3±0.7	/	24.2	7.9	
	29.7	31.4±2.3	/	5.6	7.2	
$I_{903.7}/I_{907.8}$	49.8	/	49.4±2.1	–0.8	4.3	0.0252
	70.1	/	67.1±2.3	–4.3	3.4	
	92.1	/	91.4±1.7	–0.7	1.8	
	7.5	7.5±1.8	/	–0.3	24.5	
	29.7	30.7±3.2	/	3.3	10.5	
$I_{905.8}/I_{907.8}$	49.8	/	49.6±4.9	–0.3	9.9	0.0375
	70.1	/	71.5±4.6	2.0	6.4	
	92.1	/	95.7±2.0	3.9	2.1	

Sunflower seed oil, corn oil and soybean oil showed similar TAG profiles in the MALDI-MS spectra due to their similar FA contents, where the presence of TAG peaks was almost the same and the abundances of individual TAG peak were quite close, e.g., peaks at *m/z* 901.7, *m/z* 903.7, *m/z* 905.8 and *m/z* 907.8 (Figure 2-10 and Figure 2-11). Compared with sunflower seed oil and corn oil, soybean oil contained a small amonut of α-linolenic acid (4.5-11.0%) which was deficient in sunflower seed oil and corn oil[16], resulting in a characteristic peak at *m/z* 899.7 (LLLn) for soybean oil. For sunflower seed oil – soybean oil blends and corn oil – soybean oil blends with different blending ratios, the difference between their MALDI-MS spectra was mainly observed from the intensity of the peak at *m/z* 899.7, which should be a good indicator for the quantitative analysis of blended oils containing soybean oil. Therefore, the peak at *m/z* 899.7 was paired with other abundant peaks shown in the MALDI-MS spectra, i.e., peaks at *m/z* 877.7, *m/z* 879.7, *m/z* 901.7, *m/z* 903.7, and *m/z* 905.8, to establish calibration curves for the quantitative analysis of sunflower seed oil – soybean oil blends and corn oil – soybean oil blends (Table 2-15).

Table 2-15. Calibration curves for quantitative analysis of sunflower seed oil – soybean oil blends and corn oil – soybean oil blends.

Type	Marker ions (r)	Curve based on $p_{(sunflower)}$ or $p_{(corn)}$	R^2	Curve based on $p_{(soybean)}$	R^2
Sunflower seed oil – soybean oil blends	$I_{899.7}/I_{877.7}$	y = –1.7937x – 0.5814	0.9982	y = 1.8566x + 0.4485	0.9980
	$I_{899.7}/I_{901.7}$	y = –1.0973x – 2.2623	0.9906	y = 4.3660x – 0.6451	0.9986
	$I_{899.7}/I_{903.7}$	y = –1.3593x – 1.4238	0.9965	y = 2.6754x + 0.1583	0.9983
	$I_{899.7}/I_{905.8}$	y = –1.3378x – 0.2376	0.9986	y = 1.3253x + 0.3167	0.9977
Corn seed oil – soybean oil blends	$I_{899.7}/I_{879.7}$	y = –2.7313x + 1.8770	0.9859	y = 2.2305x – 0.5226	0.9927
	$I_{899.7}/I_{901.7}$	y = –3.1992x – 1.0200	0.9890	y = 3.8241x – 0.6635	0.9923
	$I_{899.7}/I_{903.7}$	y = –2.1749x – 1.4841	0.9977	y = 3.7157x + 0.0620	0.9992
	$I_{899.7}/I_{905.8}$	y = –2.5993x + 0.2976	0.9977	y = 3.5302x – 1.3455	0.9914

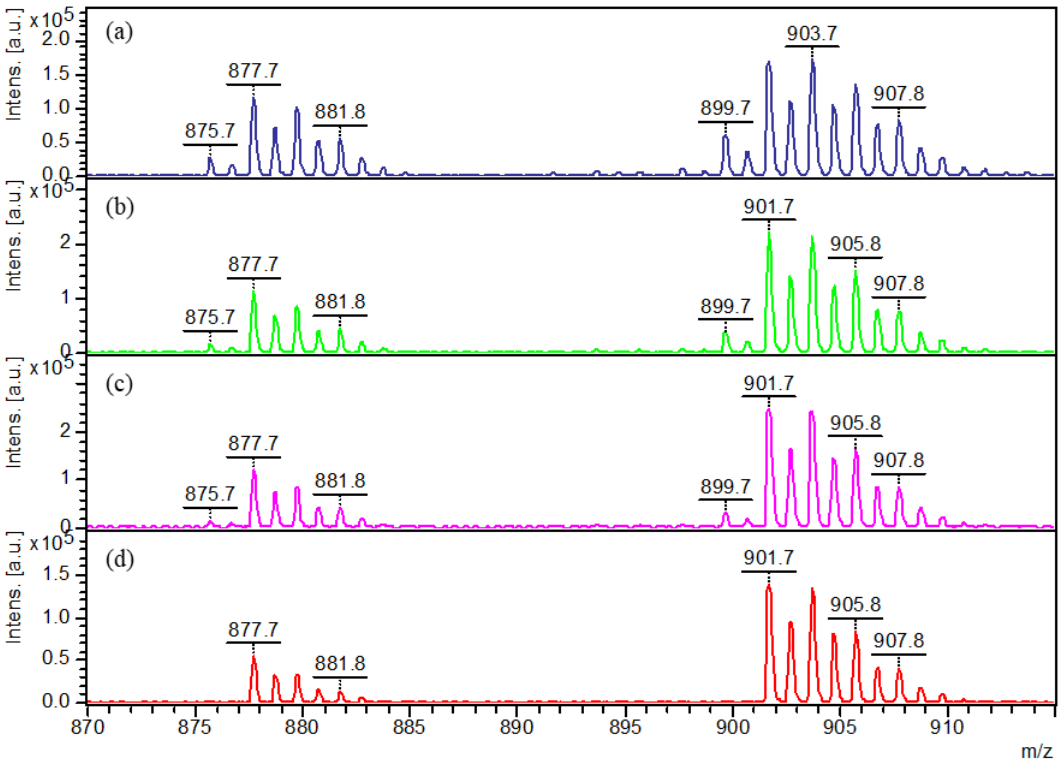

Figure 2-10. The TAG region of the MALDI-MS spectra for (a) 100% soybean oil, (b) 40% sunflower seed oil – 60% soybean oil blend, (c) 60% sunflower seed oil – 40% soybean oil blend and (d) 100% sunflower seed oil.

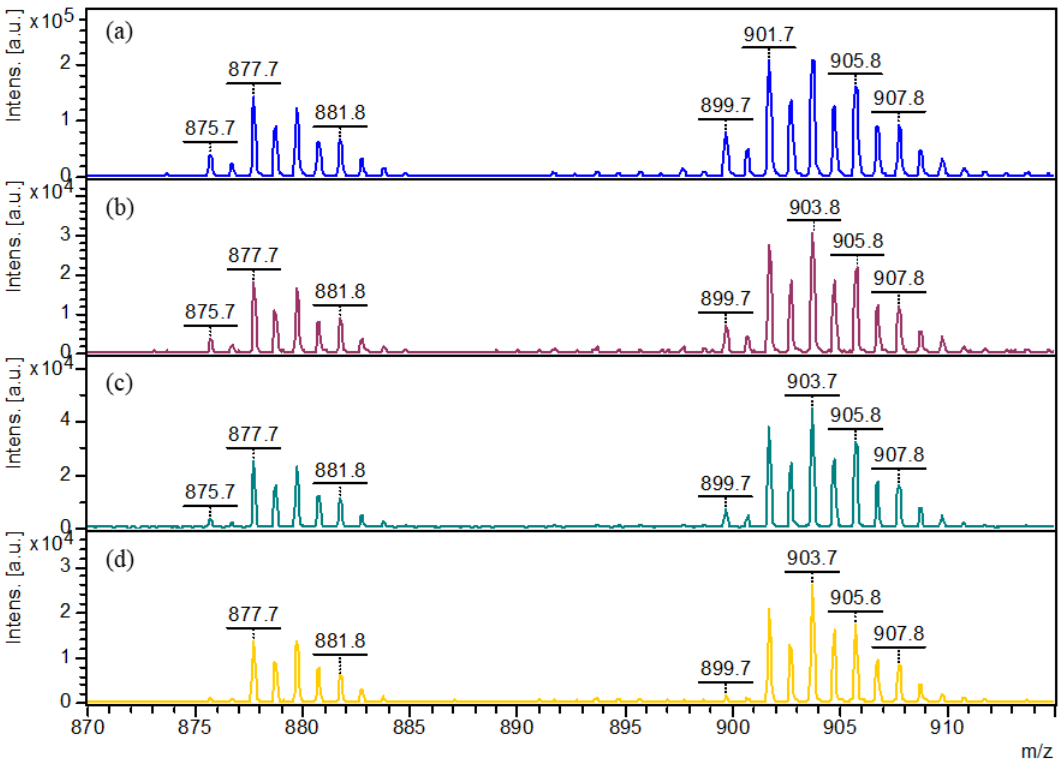

Figure 2-11. The TAG region of the MALDI-MS spectra for (a) 100% soybean oil, (b) 40% corn oil – 60% soybean oil blend, (c) 60% corn oil – 40% soybean oil blend and (d) 100% corn oil.

For sunflower seed oil – soybean oil blends, $I_{899.7}/I_{901.7}$ and $I_{899.7}/I_{903.7}$ showed comparable quantitative ability with RMSE as 0.0225 and 0.0207, respectively (Table 2-16), which was better than that of $I_{899.7}/I_{877.7}$ and $I_{899.7}/I_{905.8}$. The peaks at *m/z* 877.7, and *m/z* 905.8 were weaker than the peaks at *m/z* 901.7 and *m/z* 903.8, so the variations of $I_{899.7}/I_{877.7}$ and $I_{899.7}/I_{905.8}$ were smaller between sunflower seed oil and soybean oil, leading to slightly worse acurracy and precision of the quantitative results but they were still acceptable. For corn oil – soybean oil blends, $I_{899.7}/I_{903.7}$ provided the best quantitative results (RMSE = 0.0327) with the accuracy and precision within –16.6-0.2% and 1.8-17.2%, respectively, followed by $I_{899.7}/I_{879.7}$, $I_{899.7}/I_{905.8}$ and $I_{899.7}/I_{901.7}$ (Table

2-17). Between pure soybean oil and pure corn oil, similar variations were observed for $I_{899.7}/I_{879.7}$ and $I_{899.7}/I_{903.7}$ with $r_{(soybean)}/r_{(corn)}$ as 5.18 and 5.39, respectively. However, the quantitative performance of $I_{899.7}/I_{879.7}$ was not as good as that of $I_{899.7}/I_{903.7}$ which was mainly caused by the poor accuracy (20.1%) and the huge variation (57.4%) obtained for the measured results of the low-abundance corn oil samples (≤30%) (Table 2-17). 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.

Table 2-16. Quantitative results of sunflower seed oil in sunflower seed oil – soybean oil blends based on the intensity ratio of marker ions.

Marker ions (*r*)	**Actual conc. (%)**	**Measured conc. (%)**		**Accuracy (%)**	**RSD (%)**	**RMSE**
		Based on $p_{(\text{sunflower})}$	Based on $p_{(\text{soybean})}$			
$I_{899.7}/I_{877.7}$	7.5	6.9±2.6	/	–8.8	38.3	0.0288
	30.1	/	29.3±4.6	–2.6	15.6	
	50.0	/	51.8±1.9	3.6	3.7	
	70.0	/	72.9±1.0	4.1	1.3	
	92.4	/	92.5±0.8	0.1	0.9	
$I_{899.7}/I_{901.7}$	7.5	7.7±2.1	/	2.2	27.8	0.0225
	30.1	/	30.6±3.1	1.5	10.3	
	50.0	/	49.6±3.2	–0.7	6.5	
	70.0	/	71.5±0.9	2.1	1.3	
	92.4	/	92.5±0.7	0.1	0.7	
$I_{899.7}/I_{903.7}$	7.5	7.8±1.9	/	4.4	23.6	0.0207
	30.1	/	30.5±3.1	1.3	10.3	
	50.0	/	49.9±2.7	0.0	5.4	
	70.0	/	71.5±0.8	2.2	1.2	
	92.4	/	92.3±0.7	–0.1	0.8	
$I_{899.7}/I_{905.8}$	7.5	8.0±2.3	/	6.4	28.2	0.0278
	30.1	/	29.7±5.2	–1.3	17.5	
	50.0	/	50.9±2.2	1.9	4.3	
	70.0	/	72.0±1.1	2.8	1.5	
	92.4	/	92.4±0.7	0.0	0.7	

Table 2-17. Quantitative results of corn oil in corn oil – soybean oil blends based on the intensity ratio of marker ions.

Marker ions (*r*)	**Actual conc. (%)**	**Measured conc. (%)**		**Accuracy (%)**	**RSD (%)**	**RMSE**
		Based on $p_{(corn)}$	Based on $p_{(soybean)}$			
$I_{899.7}/I_{879.7}$	7.5	7.3±4.2	/	–2.8	57.4	0.0377
	30.1	36.1±3.3	/	20.1	9.0	
	50.0	50.8±1.4	/	1.5	2.8	
	70.0	/	70.5±1.7	0.8	2.4	
	92.1	/	91.5±2.3	–0.7	2.5	
$I_{899.7}/I_{901.7}$	7.5	4.6±2.1	/	–38.6	46.0	0.0496
	30.1	32.0±4.7	/	6.2	14.7	
	50.0	50.2±7.5	/	0.4	15.0	
	70.0	/	69.0±4.0	–1.4	5.7	
	92.1	/	90.9±3.8	–1.4	4.1	
$I_{899.7}/I_{903.7}$	7.5	6.3±0.5	/	–16.6	8.3	0.0327
	30.1	30.1±5.2	/	0.2	17.2	
	50.0	47.7±1.8	/	–4.7	3.7	
	70.0	/	69.3±3.9	–0.9	5.6	
	92.1	/	92.0±1.6	–0.2	1.8	
$I_{899.7}/I_{905.8}$	7.5	/	10.5±3.1	39.7	29.8	0.0417
	30.1	/	34.3±4.4	14.0	12.8	
	50.0	/	49.1±3.9	–1.8	7.9	
	70.0	/	67.7±2.8	–3.2	4.1	
	92.1	/	90.6±2.9	–1.6	3.2	

Together the results discussed above, a comprehensive investigation about the quantitative analysis of binary blended oils based on the intensity ratio of marker ions has been conducted and a guideline for the selection of marker ions was proposed with the priority of the points decreased in order. (1) The characteristic peaks abundant in one pure oil and deficient in the other pure oil are the most potential marker ions. (2) The common peaks with larger variations between the two pure oils are preferred for better quantitative capabilities. (3) For the peaks with similar variations, priority should be given to peaks with higher abundance and closer *m/z* values for improved accuracy and precision. The proposed guideline was applicable to most types of binary blended oils, including blended oils of pure oils with obvious different, partly different and similar TAG profiles, and could be referenced for the analysis of ternary blended oils. Moreover, the proposed guideline could significantly simplify the selection of marker ions with powerful quantitative ability, thereby making the intensity ratio-based method straightforward and efficient.

2.3.2 Quantitative analysis of ternary blended oils

2.3.2.1 *Establishment of the quantitative method*

Blended oil products could contain various oil compositions. Not only binary blended oils but also ternary and quaternary blended oils are commonly available in the market, but such multiple oil compositions make quantitative analysis of their compositions much more complicated. Figure 2-12 showed the MALDI-MS spectra of pure olive oil, pure sunflower seed oil, pure soybean oil and their ternary blends. Compared with binary blended oils, ternary blended oils presented more peaks in the MALDI-MS spectra because all the peaks shown in the spectra of individual pure oils were observed, resulting in more complex spectral data. On the other hand, for ternary blended oils with varied blending ratios, the abundances of TAG peaks were determined by the three compositions together, especially for TAGs that were not unique for a specific oil composition. For example, the increased intensities of *m/z* 901.7 and *m/z* 903.7 could be caused by the increased percentage of sunflower seed oil or soybean oil or even both (Figure 2-12). Such joint effects made it difficult to predict the changes in TAG patterns of ternary blended oils with different blending ratios. Therefore, Equation (2-8) was not applicable for the quantitative analysis of ternary blended oils since the relationship between the intensity ratio of marker ions and the concentration of individual oil composition could not be developed.

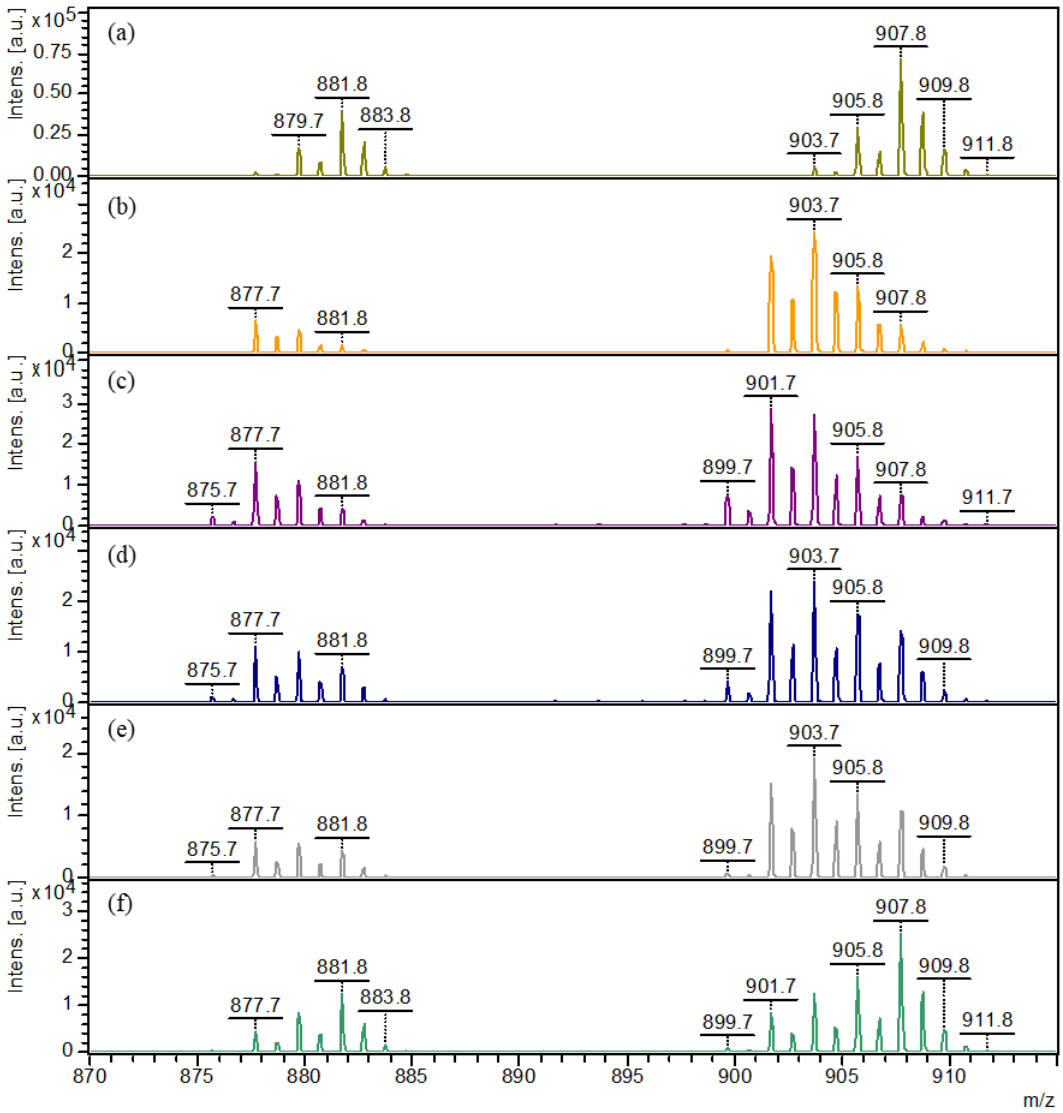

Figure 2-12.The TAG region of the MALDI-MS spectra for (a) 100% olive oil, (b) 100% sunflower seed oil, (c) 100% soybean oil, (d) 20% olive oil – 20% sunflower seed oil – 60% soybean oil blend, (e) 20% olive oil – 60% sunflower seed oil – 20% soybean oil blend and (f) 60% olive oil – 20% sunflower seed oil – 20% soybean oil blend.

For ternary blends of olive oil, sunflower seed oil and soybean oil, the MALDI-MS spectra showed major peaks at *m/z* 881.8 (POO), *m/z* 899.7 (LLLn), *m/z* 901.7 (LLL), *m/z* 903.7 (OLL), *m/z* 905.8 (OOL) and *m/z* 907.8 (OOO) (Figure 2-12). Among these peaks, pure olive oil was abundant in *m/z* 881.8, *m/z* 905.8 and *m/z* 907.8, and sunflower seed oil and soybean oil were abundant in *m/z* 901.7, *m/z* 903.7 and *m/z* 905.8.

Compared with olive oil and sunflower seed oil, soybean oil had a characteristic peak at *m/z* 899.7. To investigate the quantitative analysis of olive oil – sunflower seed oil – soybean oil blends, the six major peaks were chosen as potential marker ions and were paired into different groups to obtain the intensity ratios, 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 $I_{907.8}/I_{903.7}$.

As Equation (2-16) showed, there was a nonlinear relationship between the intensity ratio of marker ions (r) and the concentrations of two oil compositions (p_1 and p_2) contained in the ternary blended oils. All the parameters (i.e., C_1, C_2, A, b and a) involved in the nonlinear relationship could be calculated based on the spectral data of pure oils (see Equation (2-12) to Equation (2-18) for the details), which called theoretical values, and nonlinear least squares (NLS) regression was available for the estimation of parameters and the development of nonlinear relationship. The nonlinear relationship involved five parameters, making it difficult and time-consuming to estimate all the parameters simultaneously. Therefore, the NLS regression was performed in three steps. In the 1st step, C_1 and C_2 were set as the theoretical values, and A, b and a were estimated by NLS regression. In the 2nd step, C_1 was set as the theoretical value, while C_2, A, b and a were estimated based on the results provided by the 1st NLS regression. In the 3rd step, NLS regression was performed again based on the results obtained from the 2nd step, and all the five parameters were estimated at the same time.

For olive oil – sunflower seed oil – soybean oil blends, both theoretical calculation and NLS regression were used to develop the nonlinear relationships between the intensity ratios of marker ions and the concentrations of sunflower seed oil (p_1) and soybean oil (p_2) in the ternary blended oils, at the beginning. As Table 2-18 shown, for the selected groups of marker ions, theoretical calculation and NLS regression provided similar parameters for the nonlinear relationships and the intensity ratios predicted by the developed nonlinear relationships were highly correlated with the intensity ratios obtained from the experiment with Pearson correlation coefficient (PCC) higher than 0.98, indicating the algorithms discussed in Section 2.2.4.2 was available to describe the changes in the intensity ratio of TAG peaks when the compositions of ternary blended oils varied. Compared with the nonlinear relationships established by theoretical calculation, the nonlinear relationships established using NLS regression were more powerful and accurate, showing higher PCC between the actual intensity ratios and predicted intensity ratios. Hence, the nonlinear relationships obtained from NLS regression were further applied to quantify the oil compositions of olive oil – sunflower seed oil – soybean oil blends.

Table 2-18. Parameters of nonlinear relationships obtained from theoretical calculation and NLS regression for olive oil – sunflower seed oil – soybean oil blends with the concentrations of sunflower seed oil and soybean oil as p_1 and p_2, respectively.

Marker ions (r)	**Model**	A	a	b	C_1	C_2	**PCC***
$I_{899.7}/I_{881.8}$	Theoretical	–0.466	0.533	0.050	–0.099	–0.426	0.9818
	NLS	–0.415	0.540	0.065	/	/	0.9977
		–0.458	0.600	0.083	/	–0.473	0.9980
		–0.424	0.601	0.149	–0.430	–0.445	0.9984
$I_{899.7}/I_{903.7}$	Theoretical	0.041	0.005	–0.005	1.118	0.027	0.9817
	NLS	0.067	0.006	–0.014	/	/	0.9925
		0.066	0.010	–0.016	/	0.021	0.9967
		0.067	0.010	–0.016	1.142	0.021	0.9967
$I_{903.7}/I_{901.7}$	Theoretical	–4.319	–1.713	–0.413	0.906	–4.021	0.9992
	NLS	–3.766	–2.538	–0.616	/	/	0.9998
		–3.249	–1.422	–0.530	/	–2.581	0.9999
		–3.777	–2.813	–0.752	0.814	–3.641	1.0000
$I_{905.8}/I_{903.7}$	Theoretical	–5.326	–19.436	–5.229	0.122	–3.597	0.9994
	NLS	–5.256	–19.842	–5.340	/	/	0.9995
		–3.408	–7.950	–3.486	/	–2.162	0.9996
		–4.185	–13.279	–4.538	0.038	–2.808	0.9997
$I_{907.8}/I_{903.7}$	Theoretical	–0.564	–1.460	–3.659	–4.805	–0.381	0.9997
	NLS	–0.562	–1.453	–3.642	/	/	0.9997
		–0.464	–0.961	–3.016	/	–0.301	0.9997
		–0.526	–1.333	–3.571	–5.205	–0.355	0.9997

* Pearson correlation coefficient between the intensity ratios that were measured experimentally and predicted by nonlinear relationships.

Equation (2-16) could be rewritten as

$$f(r) = \frac{Ap_1 + a}{p_2 + b} \tag{2-31}$$

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 (called quantitative model) could be constructed as

$$\begin{cases} f(r_1) = \dfrac{A_1 p_1 + a_1}{p_2 + b_1} \\ f(r_2) = \dfrac{A_2 p_1 + a_2}{p_2 + b_2} \\ p_3 = 1 - p_1 - p_2 \end{cases} \tag{2-32}$$

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 blended oils. For an unknown ternary blended oil sample, the corresponding $f(r_1)$ and $f(r_2)$ could be obtained from its MALDI-MS spectrum and substituted into the established equation system to quantify the contained oil compositions.

For the olive oil – sunflower seed oil – soybean oil blends, the five groups of marker ions generated ten different NLS quantitative models, and for each model, the relationships obtained in the same step of NLS regression were paired, leading to three parallel models, i.e., 1st step model, 2nd step model and 3rd step model. 12 testing samples of olive oil – sunflower seed oil – soybean oil blends were prepared and the proportions of the three oil compositions in these sample were measured by the

established quantitative models. For each composition, the difference and correlation between the accurate concentrations and measured concentrations were described by RMSE and PCC, which were summarized in Table 2-19. The results provided by the group of $I_{899.7}/I_{881.8}$ and $I_{903.7}/I_{901.7}$ were not included in Table 2-19 because of the poor quantitative performance (RMSE>0.5).

The models based on $I_{905.8}/I_{903.7}$ and $I_{907.8}/I_{903.7}$ showed large RMSE (>0.2) and small R^2 (<0.8) for sunflower seed oil and soybean oil (Table 2-19), indicating the model was not powerful for quantitative analysis of olive oil – sunflower seed oil – soybean oil blends. Similar results were observed for the 1st and 2nd models based on $I_{899.7}/I_{903.7}$ and $I_{903.7}/I_{901.7}$ for the measurements of sunflower seed oil and olive oil. Among the remaining seven 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 3rd 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 (Table 2-19). For olive oil, sunflower seed oil and soybean oil, olive oil showed the most different TAG profile, so that the established NLS models had the best quantitative ability for olive oil with comparable quantitative ability for sunflower seed oil and soybean oil due to their similar TAG patterns.

Table 2-19. Quantitative results of olive oil – sunflower seed oil – soybean oil blends by NLS models based on different groups of marker ions with the concentrations of sunflower seed oil and soybean oil as p_1 and p_2, respectively.

Marker ions	**NLS model**	**Oil species**					
		Sunflower seed oil		Soybean oil		Olive oil	
		PCC	RMSE	PCC	RMSE	PCC	RMSE
$I_{899.7}/I_{881.8}$& $I_{899.7}/I_{903.7}$	1st	0.9608	0.0898	0.9811	0.0619	0.9915	0.0881
	2nd	0.9170	0.1298	0.9829	0.0582	0.9737	0.1338
	3rd	0.9797	0.0512	0.9857	0.0511	0.9947	0.0256
$I_{899.7}/I_{881.8}$& $I_{905.8}/I_{903.7}$	1st	0.9834	0.0556	0.9837	0.0577	0.9973	0.0268
	2nd	0.9614	0.0749	0.9815	0.0741	0.9962	0.0249
	3rd	0.9691	0.0649	0.9823	0.0602	0.9971	0.0190
$I_{899.7}/I_{881.8}$& $I_{907.8}/I_{903.7}$	1st	0.9739	0.0601	0.9803	0.0638	0.9976	0.0213
	2nd	0.9479	0.0856	0.9786	0.0828	0.9966	0.0221
	3rd	0.9598	0.0753	0.9795	0.0684	0.9975	0.0178
$I_{899.7}/I_{903.7}$& $I_{903.7}/I_{901.7}$	1st	0.9392	0.2853	0.9592	0.2132	0.9133	0.3860
	2nd	0.9320	0.2598	0.9771	0.0660	0.8199	0.2819
	3rd	0.9568	0.0886	0.9849	0.0523	0.9436	0.0880
$I_{899.7}/I_{903.7}$& $I_{905.8}/I_{903.7}$	1st	0.9792	0.0551	0.9833	0.0653	0.9970	0.0288
	2nd	0.9799	0.0534	0.9860	0.0500	0.9959	0.0237
	3rd	0.9799	0.0519	0.9862	0.0498	0.9963	0.0212
$I_{899.7}/I_{903.7}$& $I_{907.8}/I_{903.7}$	1st	0.9775	0.0565	0.9854	0.0583	0.9977	0.0200
	2nd	0.9795	0.0533	0.9871	0.0476	0.9968	0.0199
	3rd	0.9794	0.0526	0.9873	0.0475	0.9971	0.0191
$I_{903.7}/I_{901.7}$& $I_{905.8}/I_{903.7}$	1st	0.9310	0.1395	0.9486	0.1770	0.9964	0.0443
	2nd	0.9739	0.0889	0.9476	0.1056	0.9943	0.0296
	3rd	0.9759	0.0617	0.9718	0.0679	0.9957	0.0234
$I_{903.7}/I_{901.7}$& $I_{907.8}/I_{903.7}$	1st	0.9164	0.1562	0.9470	0.1750	0.9963	0.0283
	2nd	0.9746	0.0969	0.9467	0.1071	0.9956	0.0238
	3rd	0.9773	0.0622	0.9720	0.0683	0.9964	0.0212
$I_{905.8}/I_{903.7}$& $I_{907.8}/I_{903.7}$	1st	0.7757	0.2388	0.6303	0.2811	0.9773	0.0555
	2nd	0.7778	0.2569	0.6974	0.2846	0.9828	0.0479
	3rd	0.7514	0.2267	0.7009	0.2694	0.9784	0.0534

For binary blended oils, the quantitative ability of developed calibration curve will be affected by the measured oil composition. To investigate whether the quantitative performance of the NLS model for different oil compositions will be affected by the concern oil compositions, the concentrations of soybean oil and olive oil, and the concentrations of olive oil and sunflower seed oil in the ternary blended oils were regarded as p_1 and p_2, respectively. The corresponding nonlinear relationships between r, p_1 and p_2 based on the selected groups of marker ions were developed by NLS regression and the estimated parameters were shown in Table 2-20 and Table 2-21. When the concentration of olive oil was considered as p_1, the nonlinear relationships of $I_{899.7}/I_{881.8}$ and $I_{899.7}/I_{903.7}$ could not be established since the peak at *m/z* 899.7 was lacking in the spectrum of pure olive oil (Figure 2-12). Similar to the previous results, the nonlinear relationships established by NLS regression showed better correlation (higher PCC) between the actual intensity ratios and predicted intensity ratios compared with the relationships established by theoretical values.

Table 2-20. Parameters of nonlinear relationships obtained from theoretical calculation and NLS regression for olive oil – sunflower seed oil – soybean oil blends with the concentrations of soybean oil and olive oil as p_1 and p_2, respectively.

Marker ions (*r*)	**Model**	A	a	b	C_1	C_2	**PCC***
$I_{899.7}/I_{881.8}$	Theoretical	–0.041	–0.002	0.094	2.065	–0.482	0.9818
	NLS	–0.045	–0.001	0.122	/	/	0.9872
		–0.044	–0.001	0.121	/	–0.483	0.9872
		–0.003	–0.001	0.269	–15.070	0.067	0.9984
$I_{899.7}/I_{903.7}$	Theoretical	0.137	–0.001	–1.116	–2.335	0.422	0.9817
	NLS	0.147	–0.004	–1.210	/	/	0.9836
		0.163	0.002	–1.238	/	0.435	0.9867
		0.984	–0.016	–1.127	–0.656	1.446	0.9967
$I_{903.7}/I_{901.7}$	Theoretical	–0.016	0.006	–0.984	5.598	–0.227	0.9992
	NLS	–0.022	0.011	–0.967	/	/	0.9999
		–0.017	0.013	–0.963	/	–0.221	1.0000
		–0.004	0.003	–0.993	10.720	–0.104	1.0000
$I_{905.8}/I_{903.7}$	Theoretical	–0.630	3.296	0.580	0.623	–1.942	0.9994
	NLS	–0.648	3.373	0.618	/	/	0.9995
		–0.525	2.661	0.483	/	–1.651	0.9997
		–0.508	2.308	0.365	0.681	–1.546	0.9997
$I_{907.8}/I_{903.7}$	Theoretical	–0.068	0.248	0.070	0.575	–0.209	0.9997
	NLS	–0.072	0.246	0.061	/	/	0.9997
		–0.063	0.225	0.037	/	–0.194	0.9997
		–0.063	0.224	0.035	0.589	–0.194	0.9997

* Pearson correlation coefficient between the intensity ratios that were measured experimentally and predicted by nonlinear relationships.

Table 2-21. Parameters of nonlinear relationships obtained from theoretical calculation and NLS regression for olive oil – sunflower seed oil – soybean oil blends with the concentrations of olive oil and sunflower seed oil as p_1 and p_2, respectively.

Marker ions (r)	Model	A	a	b	C_1	C_2	PCC*
$I_{903.7}/I_{901.7}$	Theoretical	–33.242	32.694	0.397	0.074	2.463	0.9992
	NLS	–42.982	41.619	0.857	/	/	0.9997
		–82.315	110.297	1.532	/	–17.395	0.9999
		–31.158	30.933	0.745	0.061	1.165	1.0000
$I_{905.8}/I_{903.7}$	Theoretical	–20.575	–11.936	3.649	–0.018	9.891	0.9994
	NLS	–23.243	–14.115	4.188	/	/	0.9995
		–36.605	–47.954	6.648	/	13.692	0.9996
		–15.963	–5.832	3.173	–0.038	7.824	0.9997
$I_{907.8}/I_{903.7}$	Theoretical	–12.955	–0.911	2.589	–0.148	6.228	0.9997
	NLS	–14.381	–0.882	2.845	/	/	0.9997
		–16.744	–3.422	3.337	/	6.907	0.9997
		–12.104	–0.421	2.536	–0.159	5.794	0.9997

* Pearson correlation coefficient between the intensity ratios that were measured experimentally and predicted by nonlinear relationships.

The obtained nonlinear relationships based on the same p_1 and p_2 were paired for the establishment of NLS models. For the quantitation based on with $p_{(soybean)}$ and $p_{(olive)}$, the group of $I_{905.8}/I_{903.7}$ and $I_{907.8}/I_{903.7}$ showed poor quantitative ability for soybean oil and sunflower seed oil with RMSE higher than 0.2 (Table 2-23), consistent with the results based on $p_{(olive)}$ and $p_{(sunflower)}$ (Table 2-22), and $p_{(sunflower)}$ and $p_{(soybean)}$(Table 2-19), indicating this group of marker ions could not distinguish soybean oil from sunflower seed oil. The NLS models using $I_{899.7}$ as an indicator provided better quantitative results for soybean oil and sunflower seed oil compared

with the models without $I_{899.7}$, demonstrating the characteristic peak at *m/z* 899.7 should be selected as a marker ion to achieve accurate quantitation of soybean oil, although the peak was not a main peak. Together the results shown in Table 2-19, Table 2-22 and Table 2-23, it was noticed that for the same group of marker ions, the NLS models based on different oil compositions presented comparable quantitative performance to the same oil compositions. For example, when the group of $I_{903.7}/I_{901.7}$ and $I_{905.8}/I_{903.7}$ were applied as marker ions, for the same composition, the RMSE provided by the 1st and 2nd NLS models with $p_{(\text{sunflower})}$, $p_{(\text{soybean})}$ or $p_{(\text{olive})}$ as p_1 were similar, and the RMSE provided by the 3rd models were exactly the same, which were 0.0234, 0.0617 and 0.0679 for olive oil, sunflower seed oil and soybean oil, respectively. These results revealed that selecting different oil compositions for model establishment would not affect the quantitative ability of NLS models. Therefore, using NLS regression to establish an intensity ratio-based model for the quantitative analysis of ternary blended oils, the selection of marker ions is more important for the improvement of quantitative ability.

Overall, for the olive oil – sunflower seed oil – soybean oil blends, the most accurate quantitative results were provided by the 3rd model based on the group of $I_{899.7}/I_{903.7}$ and $I_{907.8}/I_{903.7}$ with $p_{(soybean)}$ or $p_{(sunflower)}$ as p_1, and the detailed results were summarized in Table 2-24. As discussed above, the results measured by the models with $p_{(\text{soybean})}$ or $p_{(\text{olive})}$ as p_1 were almost the same with very slight differences that only a

few of the differences could be differentiated by one decimal place (T6 and T9). 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%, demonstrating the NLS models based on $I_{899.7}/I_{903.7}$ and $I_{907.8}/I_{903.7}$ were powerful for quantitative analysis.

Table 2-22. Quantitative results of olive oil – sunflower seed oil – soybean oil blends by NLS models based on different groups of marker ions with the concentrations of olive oil and sunflower seed oil as p_1 and p_2, respectively.

Marker ions	**NLS model**	**Oil species**					
		Olive oil		Sunflower seed oil		Soybean oil	
		PCC	RMSE	PCC	RMSE	PCC	RMSE
$I_{903.7}/I_{901.7}$& $I_{905.8}/I_{903.7}$	1st	0.9956	0.0455	0.9110	0.1748	0.9700	0.2128
	2nd	0.9917	0.0419	0.9306	0.1467	0.9614	0.1823
	3rd	0.9957	0.0234	0.9759	0.0617	0.9718	0.0679
$I_{903.7}/I_{901.7}$& $I_{907.8}/I_{903.7}$	1st	0.9959	0.0283	0.8920	0.1947	0.9694	0.2121
	2nd	0.9947	0.0284	0.9183	0.1647	0.9619	0.1845
	3rd	0.9964	0.0212	0.9773	0.0622	0.9720	0.0683
$I_{905.8}/I_{903.7}$& $I_{907.8}/I_{903.7}$	1st	0.9820	0.0481	0.7908	0.2347	0.6370	0.2705
	2nd	0.9520	0.0830	0.6135	0.2958	0.5338	0.3738
	3rd	0.9784	0.0534	0.7514	0.2267	0.7009	0.2694

Table 2-23. Quantitative results of olive oil – sunflower seed oil – soybean oil blends by NLS models based on different groups of marker ions with the concentrations of soybean oil and olive oil as p_1 and p_2, respectively.

Marker ions	**NLS model**	**Oil species**					
		Soybean oil		Olive oil		Sunflower seed oil	
		PCC	RMSE	PCC	RMSE	PCC	RMSE
$I_{899.7}/I_{881.8}$& $I_{899.7}/I_{903.7}$	1st	0.9837	0.0512	0.9777	0.0606	0.9838	0.0757
	2nd	0.9834	0.0472	0.9952	0.0386	0.9788	0.0562
	3rd	0.9588	0.1594	0.9147	0.3171	0.9545	0.1955
$I_{899.7}/I_{881.8}$& $I_{905.8}/I_{903.7}$	1st	0.9831	0.0486	0.9971	0.0241	0.9851	0.0478
	2nd	0.9793	0.0626	0.9964	0.0213	0.9704	0.0615
	3rd	0.9400	0.2993	0.9904	0.0513	0.8323	0.2534
$I_{899.7}/I_{881.8}$& $I_{907.8}/I_{903.7}$	1st	0.9797	0.0592	0.9976	0.0187	0.9768	0.0551
	2nd	0.9752	0.0771	0.9969	0.0197	0.9553	0.0790
	3rd	0.9282	0.3112	0.9950	0.0347	0.7888	0.2827
$I_{899.7}/I_{903.7}$& $I_{903.7}/I_{901.7}$	1st	0.9728	0.0831	0.8482	0.4119	0.9405	0.3861
	2nd	0.9791	0.0554	0.9660	0.0779	0.9738	0.0656
	3rd	0.9849	0.0523	0.9436	0.0880	0.9568	0.0886
$I_{899.7}/I_{903.7}$& $I_{905.8}/I_{903.7}$	1st	0.9819	0.0547	0.9963	0.0259	0.9876	0.0487
	2nd	0.9826	0.0470	0.9954	0.0238	0.9869	0.0402
	3rd	0.9862	0.0498	0.9963	0.0212	0.9799	0.0519
$I_{899.7}/I_{903.7}$& $I_{907.8}/I_{903.7}$	1st	0.9829	0.0520	0.9974	0.0187	0.9867	0.0487
	2nd	0.9836	0.0453	0.9965	0.0209	0.9863	0.0411
	3rd	0.9873	0.0475	0.9971	0.0191	0.9794	0.0526
$I_{903.7}/I_{901.7}$& $I_{905.8}/I_{903.7}$	1st	0.9483	0.1099	0.9964	0.0344	0.9764	0.0847
	2nd	0.9720	0.0677	0.9958	0.0231	0.9763	0.0615
	3rd	0.9718	0.0679	0.9957	0.0234	0.9759	0.0617
$I_{903.7}/I_{901.7}$& $I_{907.8}/I_{903.7}$	1st	0.9463	0.1109	0.9966	0.0234	0.9754	0.0971
	2nd	0.9724	0.0678	0.9964	0.0212	0.9778	0.0619
	3rd	0.9720	0.0683	0.9964	0.0212	0.9773	0.0622
$I_{905.8}/I_{903.7}$& $I_{907.8}/I_{903.7}$	1st	0.6086	0.2722	0.9741	0.0582	0.7740	0.2253
	2nd	0.6573	0.2848	0.9712	0.0630	0.6992	0.2302
	3rd	0.7009	0.2694	0.9784	0.0534	0.7514	0.2267

Table 2-24. Quantitative results of olive oil (OO) – sunflower seed oil (SF) – soybean oil (SO) blends based on the group of $I_{899.7}/I_{903.7}$ and $I_{907.8}/I_{903.7}$.

No.	Oil species	Actual con. (%)	Based on $p_{(sunflower)}$ and $p_{(soybean)}$			Based on $p_{(soybean)}$ and $p_{(olive)}$		
			Measured con. (%)	Accuracy (%)	RSD (%)	Measured con. (%)	Accuracy (%)	RSD (%)
T1	OO	79.6	82.5±1.1	3.7	1.3	82.5±1.1	3.7	1.3
	SF	10.3	9.5±0.4	–7.6	4.7	9.5±0.4	–7.6	4.7
	SO	10.2	7.7±0.6	–24.0	8.2	7.7±0.6	–24.0	8.2
T2	OO	59.1	58.4±0.4	–1.1	0.6	58.4±0.4	–1.1	0.6
	SF	30.4	32.3±1.3	6.2	4.0	32.3±1.3	6.2	4.0
	SO	10.5	9.1±1.4	–13.4	15.9	9.1±1.4	–13.4	15.9
T3	OO	59.6	60.6±1.0	1.6	1.6	60.6±1.0	1.6	1.6
	SF	10.3	12.2±1.3	18.5	10.7	12.2±1.3	18.5	10.7
	SO	30.1	27.3±2.2	–9.4	7.9	27.3±2.2	–9.4	7.9
T4	OO	29.6	27.4±0.5	–7.4	1.8	27.4±0.5	–7.4	1.8
	SF	60.5	64.6±0.9	6.6	1.4	64.6±0.9	6.6	1.4
	SO	9.8	8.0±0.6	–18.5	6.9	8.0±0.6	–18.5	6.9
T5	OO	30.4	29.8±0.3	–1.9	0.9	29.8±0.3	–1.9	0.9
	SF	9.9	10.0±1.8	0.4	17.6	10.0±1.8	0.4	17.6
	SO	59.7	60.1±1.7	0.7	2.8	60.1±1.7	0.7	2.8
T6	OO	10.2	10.6±0.2	4.8	1.6	10.7±0.2	5.3	1.9
	SF	79.2	80.3±1.1	1.4	1.4	80.3±1.1	1.4	1.4
	SO	10.6	9.1±1.1	–14.5	11.9	9.1±1.1	–14.5	11.9
T7	OO	10.2	10.7±0.6	4.6	5.3	10.7±0.6	4.6	5.3
	SF	60.0	60.8±1.7	1.3	2.8	60.8±1.7	1.3	2.8
	SO	29.8	28.1±0.9	–5.6	3.3	28.1±0.9	–5.6	3.3
T8	OO	10.2	10.5±0.5	2.9	4.3	10.5±0.5	2.9	4.3
	SF	30.4	15.9±0.9	–47.6	5.9	15.9±0.9	–47.6	5.9
	SO	59.4	73.6±0.7	23.9	1.0	73.6±0.7	23.9	1.0

(To be continued)

Table 2-24-*continued*. Quantitative results of olive oil – sunflower seed oil – soybean oil blends based on the group of $I_{899.7}/I_{903.7}$ and $I_{907.8}/I_{903.7}$.

No.	Oil species	Actual con. (%)	Based on $p_{(sunflower)}$ and $p_{(soybean)}$			Based on $p_{(soybean)}$ and $p_{(olive)}$		
			Measured con. (%)	Accuracy (%)	RSD (%)	Measured con. (%)	Accuracy (%)	RSD (%)
T9	OO	10.0	10.6±0.5	6.7	4.7	10.6±0.5	6.6	4.7
	SF	10.7	11.2±1.6	4.7	14.5	11.2±1.6	4.7	14.5
	SO	79.4	78.2±1.6	–1.5	2.0	78.2±1.6	–1.5	2.0
T10	OO	0.0	4.0±0.8	/	/	4.0±0.8	/	/
	SF	50.1	41.4±2.3	–17.4	5.5	41.4±2.3	–17.4	5.5
	SO	49.9	54.7±1.7	9.5	3.1	54.7±1.7	9.5	3.1
T11	OO	50.0	48.9±0.6	–2.3	1.2	48.9±0.6	–2.3	1.2
	SF	0.0	–0.2±0.9	/	/	–0.2±0.9	/	/
	SO	50.0	51.3±1.1	2.7	2.2	51.3±1.1	2.7	2.2
T12	OO	50.1	47.4±0.1	–5.3	0.2	47.4±0.1	–5.3	0.2
	SF	49.9	51.0±0.5	2.2	1.0	51.0±0.5	2.2	1.0
	SO	0.0	1.4±0.3	/	/	1.4±0.3	/	/

2.3.2.2 *Validation and optimization of the quantitative method*

The intensity ratio-based method for quantitative analysis of ternary blended oils has been preliminarily developed, but the selection of marker ions and the establishment of NLS models was complicated and time-consuming. To further validate and optimize of the developed method, three types of ternary blended oils, i.e., peanut oil – corn oil – canola oil blends, sunflower seed oil – camellia oil – canola oil blends and olive oil – corn oil – sunflower seed oil blends, were prepared and the quantitative analysis of these blended oils was explored. The MALDI-MS spectra of the types of ternary

blended oils were shown in Figure 2-13, Figure 2-14 and Figure 2-15.

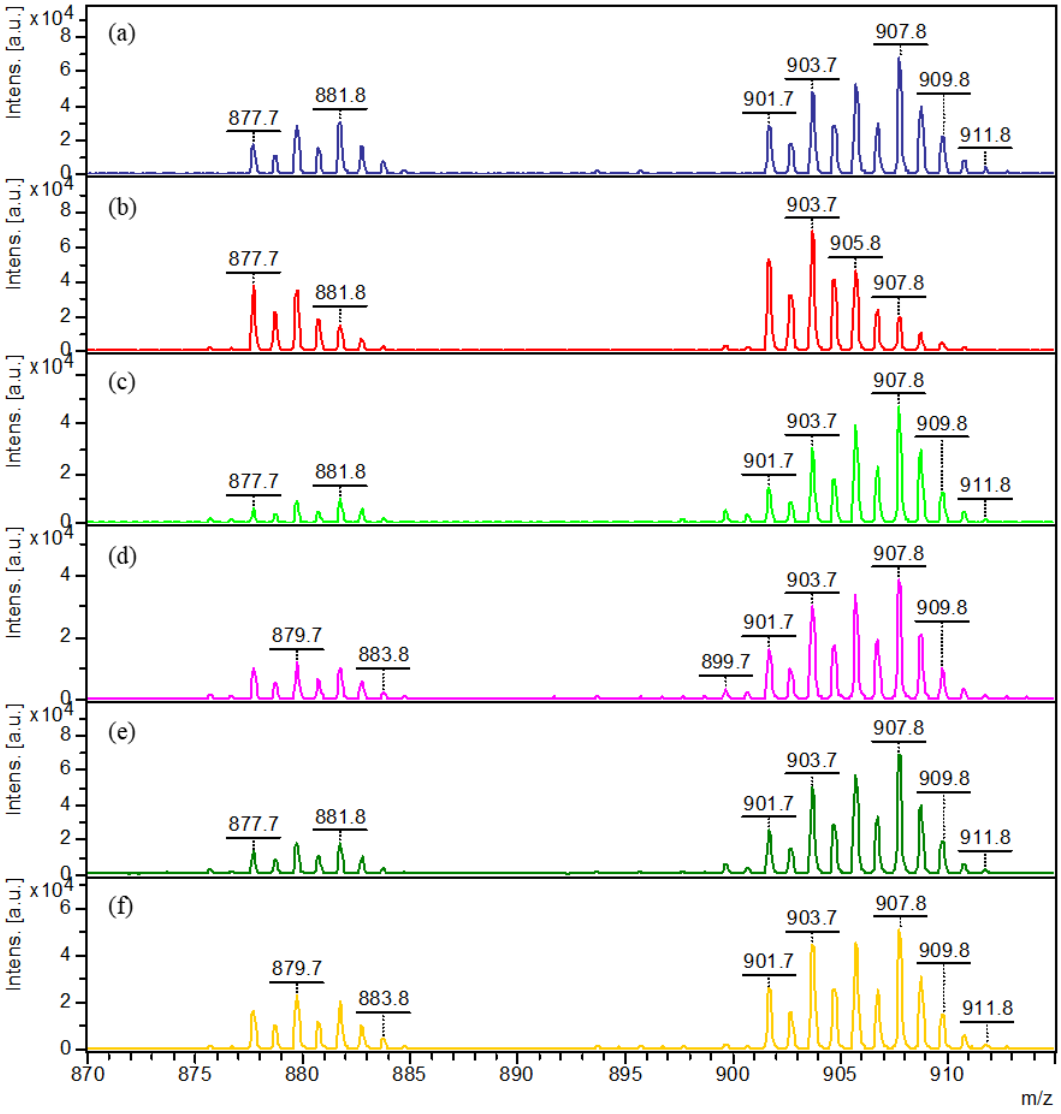

Figure 2-13. The TAG region of the MALDI-MS spectra for (a) 100% peanut oil, (b) 100% corn oil, (c) 100% canola oil, (d) 20% peanut oil – 20% corn oil – 60% canola oil blend, (e) 20% peanut oil – 60% corn oil – 20% canola oil blend and (f) 60% peanut oil – 20% corn oil – 20% canola oil blend.

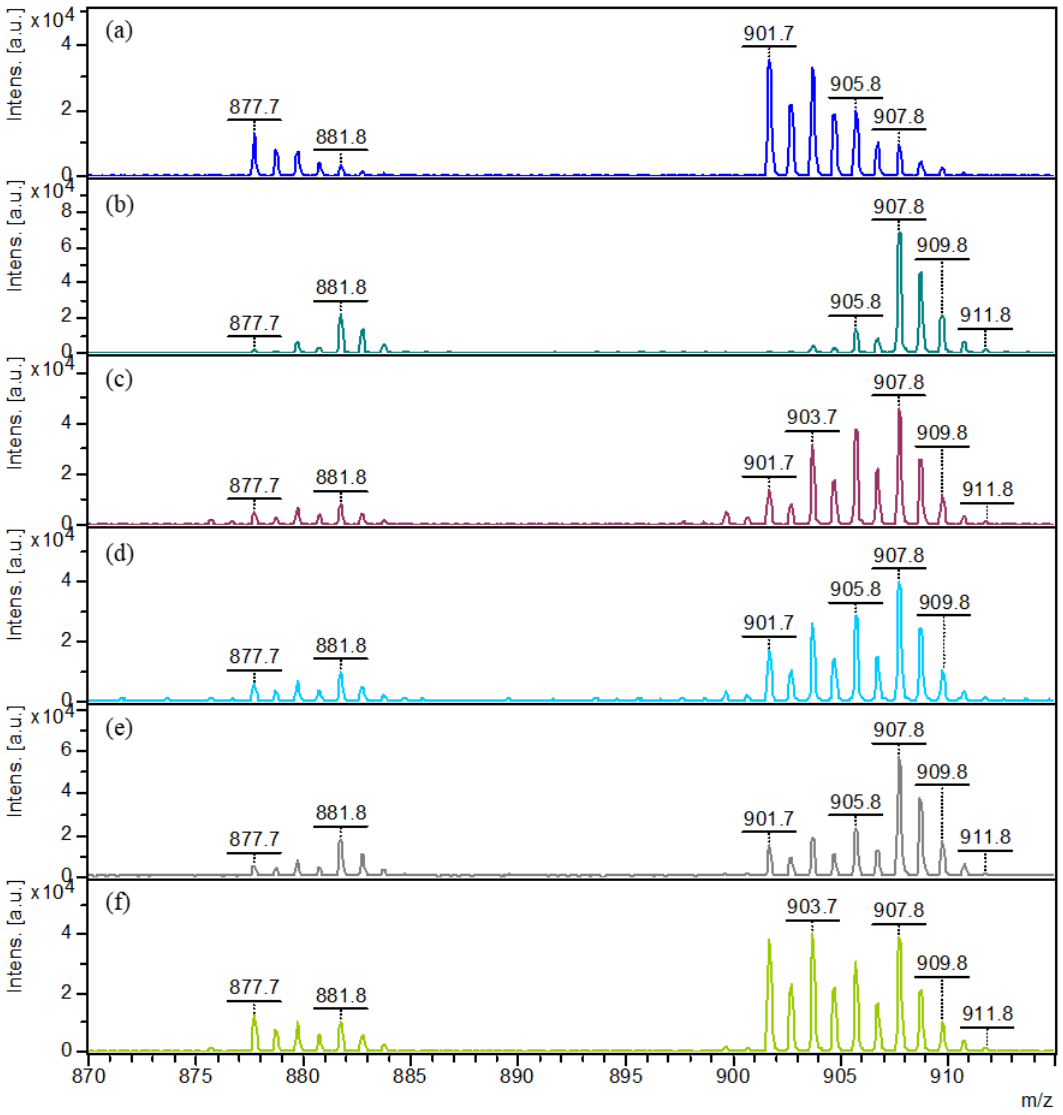

Figure 2-14. The TAG region of the MALDI-MS spectra for (a) 100% sunflower seed oil, (b) 100% camellia oil, (c) 100% canola oil, (d) 20% sunflower seed oil – 20% camellia oil – 60% canola oil blend, (e) 20% sunflower seed oil – 60% camellia oil – 20% canola oil blend and (f) 60% sunflower seed oil – 20% camellia oil – 20% canola oil blend.

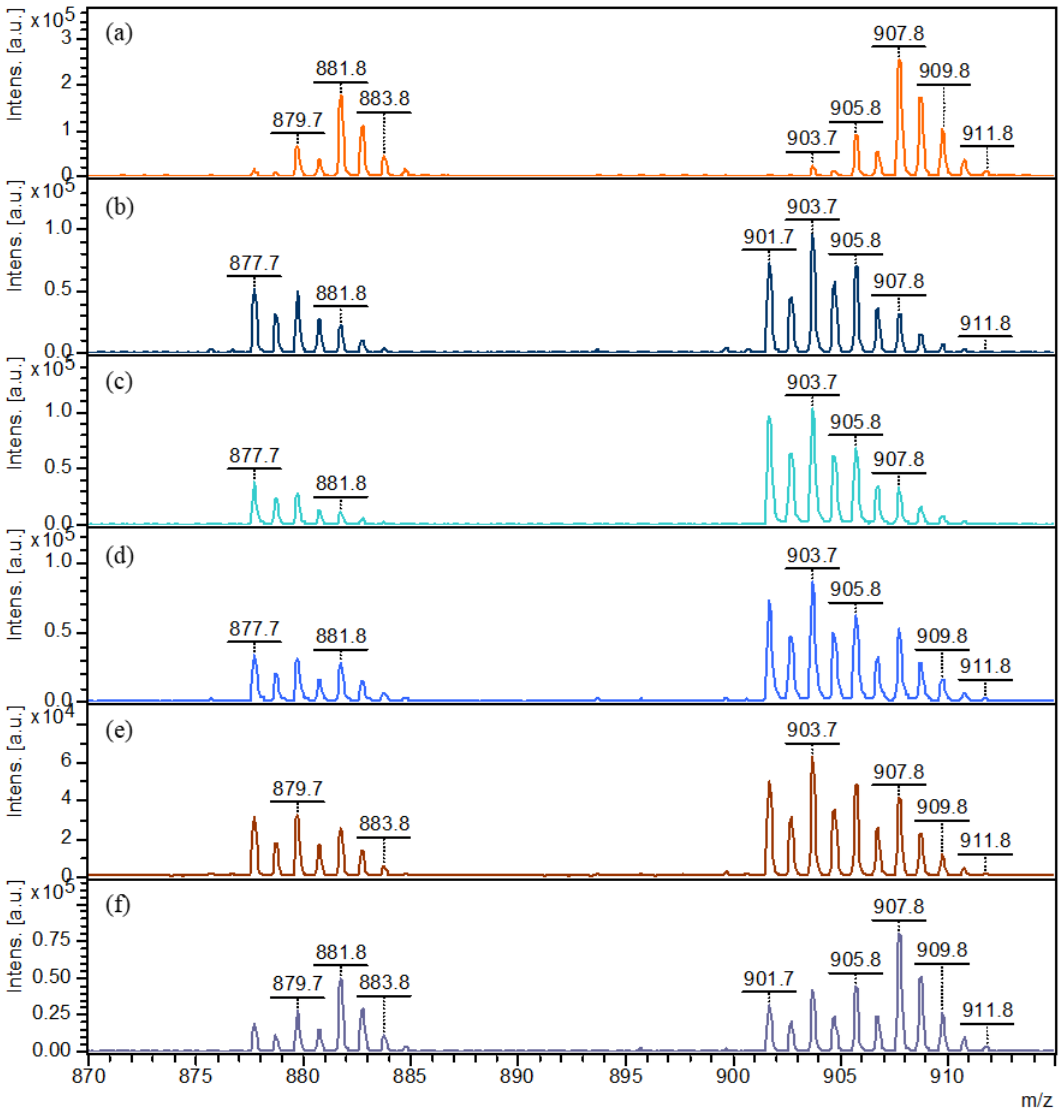

Figure 2-15. The TAG region of the MALDI-MS spectra for (a) 100% olive oil, (b) 100% corn oil, (c) 100% sunflower seed oil, (d) 20% olive oil – 20% corn oil – 60% sunflower seed oil blend, (e) 20% olive oil – 60% corn oil – 20% sunflower seed oil blend and (f) 60% olive oil – 20% corn oil – 20% sunflower seed oil blend.

For the peanut oil – corn oil – canola oil blends, the MALDI-MS spectra of peanut oil, corn oil and canola oil showed main differences in the relative intensities of peaks at *m/z* 881.8, *m/z* 901.7, *m/z* 903.7, and *m/z* 907.8 (Figure 2-13), leading to the best quantitative performance of the NLS model based on the group of $I_{881.8}/I_{905.8}$ and $I_{907.8}/I_{901.7}$, followed by the quantitative performance of the model based on the group of $I_{881.8}/I_{905.8}$ and $I_{905.8}/I_{901.7}$. Both models were regarding $p_{(peanut)}$ as p_1 and $p_{(corn)}$ as p_2 and the parameters obtained from the theoretical calculation, 1st, 2nd and 3rd NLS regression were shown in Table 2-25.

For the models using the group of $I_{881.8}/I_{905.8}$ and $I_{907.8}/I_{901.7}$, and the group of $I_{881.8}/I_{905.8}$ and $I_{905.8}/I_{901.7}$ as indicators, the best quantitative performances (smallest RMSE) were observed from the 3rd NLS models, followed by the 2nd models, 1st models and the models based on theoretical values, but the improvement in the quantitative results was not significant. For the group of $I_{881.8}/I_{905.8}$ and $I_{907.8}/I_{901.7}$, the 3rd NLS model provided better quantitative results for the three oil compositions compared with the model based on theoretical values, and the most powerful improvement was observed from the results of canola oil with RMSE decreased from 0.0513 to 0.0466 (Table 2-26). For the models obtained from NLS regression, RMSE of peanut oil, corn oil and canola oil varied within 0.0539-0.0541, 0.0299-0.0314, and 0.0466-0.0480, respectively, demonstrating the three models had comparable quantitative ability. Such situation was also observed from the quantitative results measured by the group of $I_{881.8}/I_{905.8}$ and

$I_{905.8}/I_{901.7}$.

Table 2-27 showed the detailed quantitative results of the models based on the group of $I_{881.8}/I_{905.8}$ and $I_{907.8}/I_{901.7}$. Among the three models, peanut oil and canola oil had similar TAG profiles, making it difficult to differential these two oil compositions in the blended oils, resulting in poor precision of the quantitative results. However, the large RSD (>20%) were only obtained from the samples with low-abundance compositions (~10%) and the measured results showed good accuracy for the three oil compositions even for the low-abundance compositions. For the 3rd and 1st NLS models, the accuracy of measured results was within –18.0-21.5% and –22.6-23.0%, respectively, which was in the range of –21.9-25.6% for the model based on theoretical values except for an extreme accuracy as –48.3% (Table 2-27).

Table 2-25. Parameters of nonlinear relationships obtained from theoretical calculation and NLS regression for peanut oil – corn oil – canola oil blends with the concentrations of peanut oil and corn oil as p_1 and p_2, respectively.

Marker ions (r)	Model	A	a	b	C_1	C_2	PCC*
$I_{881.8}/I_{905.8}$	Theoretical	0.547	0.839	–4.835	–1.521	0.683	0.9979
	NLS	0.538	0.815	–4.692	/	/	0.9979
		0.412	0.439	–3.638	/	0.629	0.9981
		0.418	0.444	–3.630	–1.502	0.636	0.9981
$I_{907.8}/I_{901.7}$	Theoretical	0.340	–7.387	4.143	–1.242	2.147	0.9989
	NLS	0.321	–7.103	3.984	/	/	0.9991
		0.216	–3.389	2.663	/	1.636	0.9994
		0.009	–0.056	0.912	–5.609	0.203	0.9996
$I_{905.8}/I_{901.7}$	Theoretical	0.127	–0.854	1.765	–1.551	0.804	0.9984
	NLS	0.121	–0.809	1.671	/	/	0.9987
		0.093	–0.511	1.272	/	0.721	0.9991
		0.007	–0.024	0.751	–6.774	0.152	0.9994

Table 2-26. Quantitative results of peanut oil – corn oil – canola oil blends by NLS models with the concentrations of peanut oil and corn oil as p_1 and p_2, respectively.

Marker ions	NLS model	Oil species					
		Peanut oil		Corn oil		Canola oil	
		PCC	RMSE	PCC	RMSE	PCC	RMSE
$I_{881.8}/I_{905.8}$& $I_{907.8}/I_{901.7}$	Theo.	0.9767	0.0554	0.9928	0.0316	0.9827	0.0513
	1st	0.9767	0.0540	0.9927	0.0313	0.9816	0.0480
	2nd	0.9767	0.0539	0.9921	0.0314	0.9822	0.0474
	3rd	0.9768	0.0541	0.9930	0.0299	0.9826	0.0466
$I_{881.8}/I_{905.8}$& $I_{905.8}/I_{901.7}$	Theo.	0.9763	0.0555	0.9904	0.0397	0.9821	0.0544
	1st	0.9762	0.0545	0.9904	0.0351	0.9809	0.0491
	2nd	0.9762	0.0543	0.9896	0.0359	0.9814	0.0490
	3rd	0.9764	0.0544	0.9906	0.0340	0.9817	0.0482

Table 2-27. Quantitative results of peanut oil (PA) – corn oil (CO) – canola oil (CA) blends based on $I_{881.8}/I_{905.8}$ and $I_{907.8}/I_{901.7}$.

No.	Oil species	Actual con. (%)	Based on theoretical values			Based on 1st NLS model			Based on 3rd NLS model		
			Measured con. (%)	Accuracy (%)	RSD (%)	Measured con. (%)	Accuracy (%)	RSD (%)	Measured con. (%)	Accuracy (%)	RSD (%)
T1	PA	80.0	81.2±5.7	1.5	7.0	79.6±5.6	–0.5	7.0	80.1±5.6	0.1	7.0
	CO	9.9	10.7±2.6	7.7	24.5	10.8±2.5	9.4	22.9	10.2±2.4	2.8	23.3
	CA	10.1	8.2±4.0	–19.1	48.6	9.5±4.0	–5.4	41.4	9.7±4.0	–3.8	41.2
T2	PA	60.1	61.2±7.7	1.7	12.6	60.0±7.5	–0.1	12.6	60.5±7.7	0.6	12.6
	CO	29.7	30.8±0.9	3.5	3.0	30.0±0.9	1.0	3.0	28.7±0.9	–3.6	3.1
	CA	10.2	8.1±7.7	–20.5	95.9	9.9±7.7	–2.2	77.1	10.9±7.5	6.8	69.0
T3	PA	60.0	64.4±5.3	7.2	8.2	63.1±5.2	5.1	8.2	63.3±5.2	5.5	8.2
	CO	10.1	10.1±3.1	–0.3	30.7	10.2±3.0	0.4	29.0	9.9±2.8	–2.4	28.1
	CA	29.9	25.5±3.4	–14.4	13.2	26.7±3.4	–10.5	12.6	26.8±3.4	–10.2	12.7
T4	PA	30.2	34.3±5.7	13.6	16.6	33.7±5.6	11.8	16.6	33.5±5.6	11.1	16.8
	CO	59.6	60.5±2.3	1.4	3.8	58.4±2.2	–2.1	3.8	58.1±2.5	–2.5	4.3
	CA	10.2	5.3±4.1	–48.3	77.3	7.9±4.1	–22.6	51.6	8.4±3.8	–18.0	45.3
T5	PA	29.8	31.4±2.4	5.2	7.6	30.7±2.3	2.9	7.6	30.7±2.4	2.7	7.7
	CO	9.8	12.3±2.1	25.6	17.0	12.1±2.0	23.2	16.7	11.9±1.9	21.5	16.2
	CA	60.3	56.3±3.3	–6.7	5.8	57.2±3.2	–5.2	5.6	57.4±3.2	–4.8	5.5
T6	PA	10.1	12.1±3.3	20.3	27.6	12.0±3.3	19.1	27.4	11.3±3.2	11.8	28.7
	CO	79.7	79.9±1.6	0.3	2.0	76.9±1.6	–3.5	2.0	79.0±1.8	–0.9	2.3
	CA	10.2	8.0±2.9	–21.9	35.7	11.1±2.8	8.5	25.5	9.8±2.6	–4.7	26.8

Table 2-27-*continued*. Quantitative results of peanut oil (PA) – corn oil (CO) – canola oil (CA) blends based on $I_{881.8}/I_{905.8}$ and $I_{907.8}/I_{901.7}$.

No.	Oil species	Actual con. (%)	Based on theoretical values			Based on 1st NLS model			Based on 3rd NLS model		
			Measured con. (%)	Accuracy (%)	RSD (%)	Measured con. (%)	Accuracy (%)	RSD (%)	Measured con. (%)	Accuracy (%)	RSD (%)
T7	PA	10.1	9.0±7.6	–11.1	85.0	8.8±7.5	–12.6	85.1	8.9±7.4	–11.7	82.8
	CO	60.1	62.2±2.1	3.5	3.4	59.9±2.0	–0.4	3.4	60.6±2.3	0.9	3.7
	CA	29.8	28.8±7.1	–3.3	24.6	31.3±7.0	5.0	22.5	30.4±6.7	2.1	21.9
T8	PA	10.2	11.0±4.1	8.6	37.2	10.7±4.0	5.7	37.6	11.0±4.0	8.0	36.5
	CO	30.0	31.1±2.8	3.9	8.9	30.0±2.6	0.2	8.8	29.7±2.7	–0.9	9.0
	CA	59.9	57.8±3.6	–3.4	6.3	59.2±3.6	–1.1	6.0	59.3±3.5	–0.9	6.0
T9	PA	10.3	10.4±6.0	0.9	57.2	10.1±5.9	–2.5	58.3	9.9±5.9	–4.5	59.4
	CO	9.7	11.5±1.8	18.5	15.9	11.1±1.7	15.0	15.5	10.9±1.6	12.7	15.1
	CA	80.0	78.1±4.8	–2.4	6.1	78.8±4.7	–1.5	6.0	79.2±4.8	–1.0	6.1
T10	PA	50.0	49±5.6	–2.0	11.5	47.9±5.5	–4.1	11.6	47.7±5.6	–4.5	11.7
	CO	0.0	0.9±2.0	/	/	1.2±1.9	/	/	1.6±1.8	/	/
	CA	50.0	50.2±5.5	0.3	11.1	50.9±5.5	1.7	10.8	50.7±5.5	1.3	10.9
T11	PA	50.0	46.0±4.8	–8.0	10.4	45.2±4.7	–9.6	10.4	45.1±4.8	–9.7	10.5
	CO	50.0	54.9±2.2	9.7	4.0	53.1±2.1	6.2	4.0	52.2±2.3	4.3	4.4
	CA	0.0	–0.9±3.9	/	/	1.7±3.9	/	/	2.7±3.7	/	/
T12	PA	0.0	1.4±3.6	/	/	1.3±3.5	/	/	1.7±3.5	/	/
	CO	50.3	46.3±4.1	–7.8	8.8	44.6±3.9	–11.3	8.8	44.7±4.1	–11.0	9.2
	CA	49.7	52.3±2.4	5.1	4.6	54.1±2.3	8.8	4.3	53.6±2.4	7.7	4.5

For the sunflower seed oil – camellia oil – canola oil blends, the main differences between the MALDI-MS spectra of three pure oils were observed from the peaks at *m/z* 881.8, *m/z* 901.7, *m/z* 903.7, *m/z* 905.8 and *m/z* 907.8 (Figure 2-14), and the NLS modes based on the group of $I_{881.8}/I_{877.7}$ and $I_{901.7}/I_{905.8}$, the group of $I_{901.7}/I_{903.7}$ and $I_{905.8}/I_{907.8}$ and the group of $I_{901.7}/I_{905.8}$ and $I_{903.7}/I_{907.8}$ provided better quantitative results compared with other models. The 2nd and 3rd NLS regressions of $I_{903.7}/I_{907.8}$ were not successful (Table 2-28), thus the combination of $I_{901.7}/I_{905.8}$ and $I_{905.8}/I_{907.8}$ only had two parallel calibration models, i.e., the model based on theoretical values and the model based on the 1st regression. For the models based on the group of $I_{881.8}/I_{877.7}$ and $I_{901.7}/I_{905.8}$, and the group of $I_{901.7}/I_{903.7}$ and $I_{905.8}/I_{907.8}$, the 1st NLS models provided the best quantitative results for the overall evaluation of the three oil compositions. Improvement in the quantitative results of some oil compositions were observed for the 2nd and 3rd NLS models while deterioration in the quantitative results of other compositions was also observed (Table 2-29). For the same group of marker ions, the quantitative ability of the NLS models was similar and better than that of the model based theoretical values. Although the combination of $I_{901.7}/I_{905.8}$ and $I_{903.7}/I_{907.8}$ only had the 1st NLS model, the model had the smallest RMSE as 0.0229, 0.0314 and 0.0445 for sunflower seed oil, camellia oil and canola oil, respectively (Table 2-29), indicating the model had the best quantitative ability compared with the models based on other marker ions.

For olive oil – corn oil – sunflower seed oil blends, the MALDI-MS spectra of corn oil

and sunflower seed oil showed main differences at *m/z* 877.7, *m/z* 881.8, *m/z* 901.7 and *m/z* 905.8 (Figure 2-15), so $I_{881.8}/I_{877.7}$, $I_{881.8}/I_{879.7}$ and $I_{901.7}/I_{905.8}$ could differentiate corn oil with sunflower seed oil. Combining $I_{881.8}/I_{877.7}$ and $I_{881.8}/I_{879.7}$ with $I_{901.7}/I_{905.8}$, the developed NLS models presented good quantitative performance for validation samples, and the 3rd NLS models were lacking due to the failed 3rd regression of $I_{901.7}/I_{905.8}$ (Table 2-28). Similar to the previous results, for the same group of marker ions, the RMSE values obtained from the 1st and 2nd NLS models were quite close with slightly variations for different oil composition, and were smaller than those of the models based theoretical values (Table 2-29). The best quantitative results were provided by the 1st NLS model based on the group of $I_{881.8}/I_{879.7}$ and $I_{901.7}/I_{905.8}$, and the RMSE of the measured results for olive oil, corn oil and sunflower seed oil was 0.0159, 0.0560 and 0.0551, respectively.

Table 2-28. Parameters of nonlinear relationships obtained from theoretical calculation and NLS regression for sunflower seed oil – camellia oil – canola oil blends and olive oil – corn oil – sunflower seed oil blends.

Marker ions (r)	**Model**	A	a	b	C_1	C_2	**PCC***
Sunflower seed oil – camellia oil – canola oil blends (p_1: sunflower seed oil; p_2: canola oil)							
$I_{881.8}/I_{877.7}$	Theoretical	–0.277	–0.177	–2.179	–5.855	–0.062	0.9985
	NLS	–0.281	–0.175	–2.146	/	/	0.9985
		–0.394	–0.376	–2.948	/	–0.109	0.9991
		–0.388	–0.344	–2.805	–5.527	–0.104	0.9991
$I_{901.7}/I_{903.7}$	Theoretical	–2.489	0.031	0.213	1.076	–1.591	0.9980
	NLS	–2.869	0.023	0.200	/	/	0.9989
		–2.840	0.028	0.198	/	–1.609	0.9989
		–2.975	0.027	0.180	1.061	–1.646	0.9990
$I_{901.7}/I_{905.8}$	Theoretical	–0.257	0.046	0.844	2.344	–0.495	0.9975
	NLS	–0.349	0.055	1.083	/	/	0.9983
		–0.354	0.063	1.096	/	–0.500	0.9984
		–0.475	0.077	0.919	1.981	–0.607	0.9989
$I_{905.8}/I_{907.8}$	Theoretical	–8.184	17.046	1.831	–0.084	–3.918	0.9989
	NLS	–8.082	16.592	1.762	/	/	0.9989
		–12.244	33.427	2.716	/	–6.882	0.9993
		–13.656	39.051	2.873	–0.072	–7.912	0.9993
$I_{903.7}/I_{907.8}$	Theoretical	–33.953	175.727	8.290	–0.184	–16.256	0.9985
	NLS	–32.563	168.786	7.972	/	/	0.9987
Olive oil – corn oil – sunflower seed oil blends (p_1: olive oil; p_2: corn oil)							
$I_{881.8}/I_{877.7}$	Theoretical	–10.249	1.847	2.578	–0.184	4.104	1.0000
	NLS	–11.510	2.105	2.884	/	/	1.0000
		–11.724	1.940	2.943	/	4.168	1.0000
		–11.210	1.982	2.836	–0.186	4.095	1.0000
$I_{881.8}/I_{879.7}$	Theoretical	–0.026	0.000	1.246	6.944	–0.147	0.9996
	NLS	–0.025	0.000	1.182	/	/	0.9996
		–0.026	0.000	1.218	/	–0.147	0.9996
		–0.022	0.000	1.244	7.517	–0.136	0.9996
$I_{901.7}/I_{905.8}$	Theoretical	0.028	–0.105	10.063	221.191	0.003	0.9962
	NLS	0.025	–0.091	8.527	/	/	0.9971
		0.018	–0.051	6.216	/	0.001	0.9972

Table 2-29. Quantitative results of sunflower seed oil – camellia oil – canola oil blends and olive oil – corn oil – sunflower seed oil blends.

Blending type	**Marker ions**	**NLS model**	**Oil species**					
			Sunflower seed oil		Camellia oil		Canola oil	
			PCC	RMSE	PCC	RMSE	PCC	RMSE
Sunflower seed oil – camellia oil – canola oil blends (p_1: sunflower seed oil; p_2: canola oil)	$I_{881.8}/I_{877.7}$ & $I_{901.7}/I_{905.8}$	Theo.	0.9970	0.0447	0.9803	0.0498	0.9707	0.0702
		1st	0.9963	0.0232	0.9803	0.0493	0.9728	0.0605
		2nd	0.9966	0.0223	0.9757	0.0577	0.9740	0.0691
		3rd	0.9967	0.0229	0.9773	0.0570	0.9739	0.0672
	$I_{901.7}/I_{903.7}$ & $I_{905.8}/I_{907.8}$	Theo.	0.9464	0.0473	0.9908	0.0344	0.9798	0.0619
		1st	0.9901	0.0369	0.9911	0.0337	0.9808	0.0504
		2nd	0.9901	0.0366	0.9887	0.0391	0.9797	0.0548
		3rd	0.9899	0.0384	0.9883	0.0392	0.9791	0.0565
	$I_{901.7}/I_{905.8}$ & $I_{903.7}/I_{907.8}$	Theo.	0.9975	0.0410	0.9932	0.0296	0.9844	0.0601
		1st	0.9966	0.0229	0.9924	0.0314	0.9849	0.0445
			Olive oil		Corn oil		Sunflower seed oil	
Olive oil – corn oil – sunflower seed oil blends (p_1: olive oil; p_2: corn oil)	$I_{881.8}/I_{877.7}$ & $I_{901.7}/I_{905.8}$	Theo.	0.9989	0.0151	0.9670	0.1324	0.9677	0.1385
		1st	0.9990	0.0112	0.9660	0.0681	0.9662	0.0650
		2nd	0.9990	0.0113	0.9671	0.0659	0.9684	0.0630
	$I_{881.8}/I_{879.7}$ & $I_{901.7}/I_{905.8}$	Theo.	0.9958	0.0257	0.9759	0.1000	0.9768	0.1157
		1st	0.9981	0.0157	0.9751	0.0569	0.9752	0.0562
		2nd	0.9980	0.0159	0.9753	0.0560	0.9764	0.0551

Together the results of different types of ternary blended oils, it has been demonstrated that the quantitative analysis of ternary blended oils could be achieved using the intensity ratio-based method with simultaneous quantitation of multiple compositions. The theoretical values obtained from the spectral data of pure oils could be used to develop quantitative models and provide approximate measurements for unknown samples. Meanwhile, the capability of selected peaks for quantitative analysis could be evaluated according to the quantitative performance of the models based on theoretical values, thus the selection of marker ions could be optimized without performing the NLS regression. For the optimized marker ions, the quantitative models based on the 1st, 2nd and 3rd NLS regression presented similar quantitative performance with slight differences observed, and the improvement of quantitative results was not obvious or even worse. Therefore, the 2nd and 3rd NLS regression might not be necessary and could be waived since the model based the 1st NLS regression was able to provide good quantitative results, which could significantly simplify the establishment of quantitative models.

2.3.3 Quantitative analysis of quaternary blended oils

To investigate whether the intensity ratio-based method was available for quantitative analysis of quaternary blended oils, olive oil, flaxseed oil, rice bran oil and soybean oil were applied to prepared blended oils because they showed characteristic peaks in the MALDI-MS spectra. As shown in Figure 2-16, olive oil had abundant peaks at *m/z*

881.8 and *m/z* 907.8, flaxseed oil was rich in the peaks at *m/z* 895.7, *m/z* 897.7 and *m/z* 899.7, 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. Among the four pure oils, olive oil and flaxseed oil had the most different MALDI-MS spectra with most of the TAG peaks non overlapped. Therefore, the four pure oils were divided into two groups, i.e., the group of olive oil, rice bran oil and soybean oil, and the group of flaxseed oil, rice bran oil and soybean oil, and each group had characteristic peaks that were not observed or very weak in the other group. Peaks at *m/z* 853.7, *m/z* 855.7, *m/z* 879.7, *m/z* 881.8, *m/z* 883.8, *m/z* 907.8 and *m/z* 909.8 were specific for the group of olive oil, rice bran oil and soybean, and peaks at *m/z* 873.7, *m/z* 875.7, *m/z* 895.7, *m/z* 897.7, *m/z* 899.7 and *m/z* 901.7 were distinctive for the group of flaxseed oil, rice bran oil and soybean oil. For these characteristic peaks, their intensities were determined by the abundances of oil compositions in the corresponding group and would not be affected by the oil composition not in the group. Hence, regarding these characteristic peaks as marker ions, the quantitative analysis of quaternary blended oils could be converted into the quantitative analysis of ternary blended oils, and the quantitative results of quaternary blended oils could be obtained by combining the results of the two groups of ternary blended oils.

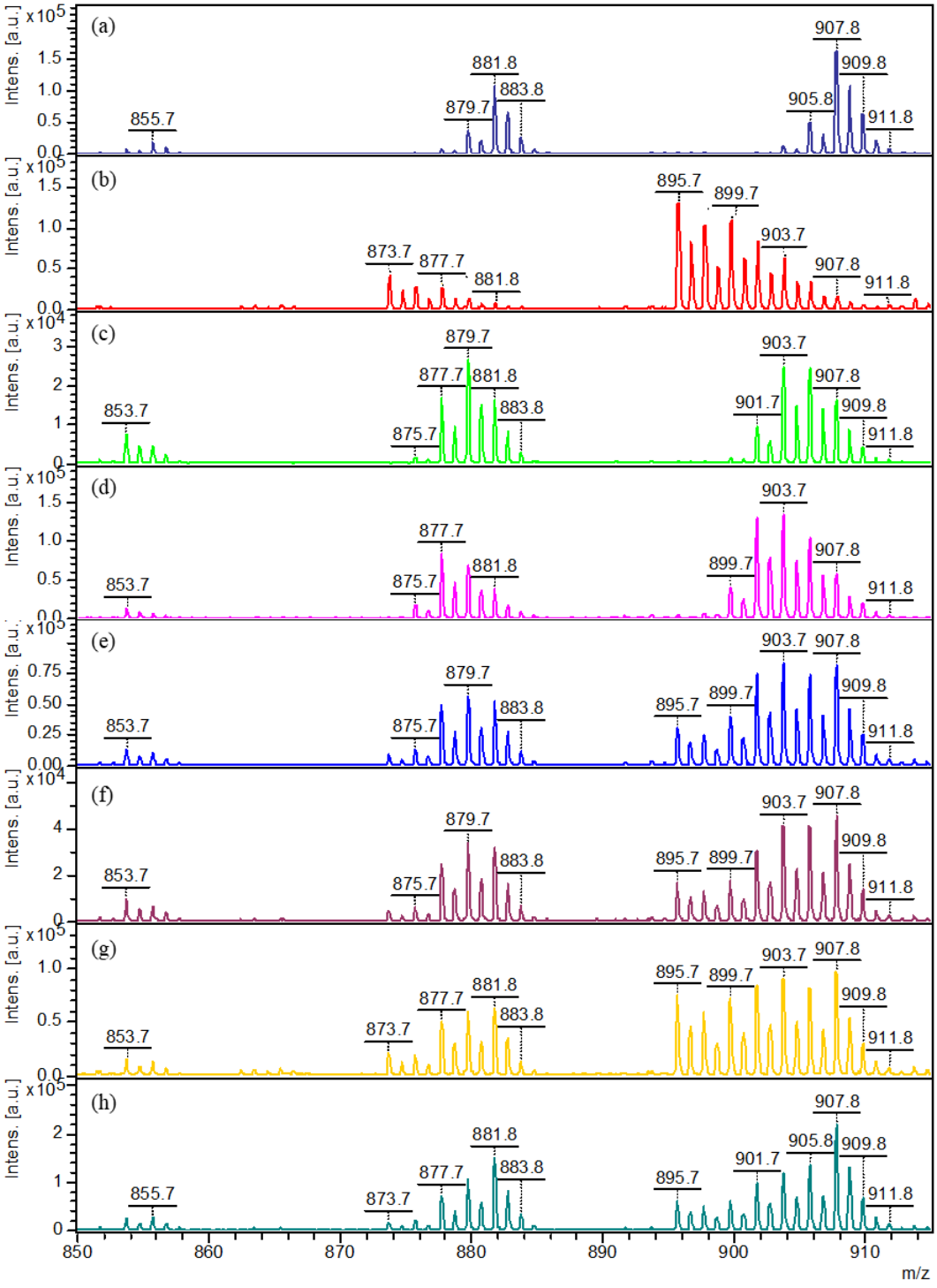

Figure 2-16. The TAG region of the MALDI-MS spectra for (a) 100% olive oil, (b) 100% flaxseed oil, (c) 100% rice bran oil, (d) 100% soybean oil, (e) 20% olive oil – 20% flaxseed oil – 20% rice bran – 40% soybean oil blend, (f) 20% olive oil – 20% flaxseed oil – 40% rice bran – 20% soybean oil blend, (g) 20% olive oil – 40% flaxseed oil – 20% rice bran – 20% soybean oil blend and (h) 40% olive oil – 20% flaxseed oil – 20% rice bran – 20% soybean oil blend.

As shown in Equation (2-21), the intensity ratio of marker ions in ternary blended oils can also be expressed by the weight ratios of oil compositions, so the quantitative analysis of ternary blended oils can be realized as

$$\begin{cases} f(r_1)' = \dfrac{A_1' R_{21} + a_1'}{R_{31} + b_1'} \\ f(r_2)' = \dfrac{A_2' R_{21} + a_2'}{R_{31} + b_2'} \end{cases} \tag{2-33}$$

where $f(r)' = \left(\frac{r}{Q} - C_1'\right)^{-1} - C_2'$, $f(r_1)'$ and $f(r_2)'$ are 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 in the blends, i.e., $R_{21} = \frac{p_2}{p_1}$ and $R_{31} = \frac{p_3}{p_1}$.

For the quantitative analysis of olive oil – flaxseed oil – rice bran oil – soybean oil blends, if olive oil is selected as the standard composition for the group of olive oil, rice bran oil and soybean, so the quantitative results of this group would be the ratio of olive oil to olive oil (i.e., unity), the ratio of rice bran to olive oil ($R_{(\text{rice bran/olive})}$) and the ratio of soybean oil to olive oil ($R_{(\text{soybean/olive})}$). Similarly, if flaxseed oil is selected as the standard composition for the group of flaxseed oil, rice bran oil and soybean, the quantitative results should be the ratio of flaxseed oil to flaxseed oil (i.e., unity), the ratio of rice bran to flaxseed oil ($R_{(\text{rice bran/flaxseed})}$) and the ratio of soybean oil to flaxseed oil ($R_{(\text{soybean/flaxseed})}$). Since rice bran oil and soybean oil are included in both groups, the ratio of olive oil to flaxseed oil ($R_{(\text{olive/flaxseed})}$) could be obtained as $\frac{R_{(\text{rice bran/flaxseed})}}{R_{(\text{rice bran/olive})}}$ or $\frac{R_{(\text{soybean/flaxseed})}}{R_{(soybean/\text{olive})}}$, and the actual concentrations of the four oil

compositions could be solved from $R_{(\text{olive/flaxseed})}$, $R_{(\text{flaxseed/flaxseed})}$ (unity), $R_{(\text{rice bran/flaxseed})}$ and $R_{(\text{soybean/flaxseed})}$.

Compared with binary and ternary blended oils, quaternary blended oils showed more complex TAG profiles in the MALDI-MS spectra (Figure 2-16). To improve the reproducibility of the spectral data, each quaternary blended oil sample was analyzed in twenty-four replicates and the average data of eight replicates were used for further analysis. Based on the spectral data of olive oil – flaxseed oil – rice bran oil – soybean oil blends, non-linear relationships referring to Equation (2-21) were developed for the group of olive oil, rice bran oil and soybean oil as well as the group of flaxseed oil, rice bran oil and soybean oil (Table 2-30), and then applied to establish quantitative models as Equation (2-30) showed. All the quantitative models were established by the 1st NLS regression and the details of the models with the best quantitative performance were summarized in Table 2-31.

Table 2-30. Parameters of nonlinear relationships obtained from theoretical calculation and 1st NLS regression for olive oil – rice bran oil – soybean oil group and flaxseed oil – rice bran oil – soybean oil group.

Marker ions (*r*)	**Model**	A'	a'	b'	C_1'	C_2'	**PCC***
Olive oil – rice bran oil – soybean oil group (R_{21}: rice bran oil/olive oil; R_{31}: soybean oil/olive oil)							
$I_{883.8}/I_{879.7}$	Theoretical	42.47	–346.7	15.58	0.150	23.43	0.9854
	1st NLS	35.70	–297.9	13.47	/	/	0.9870
$I_{883.8}/I_{855.7}$	Theoretical	6.51	–2.146	4.518	0.408	2.165	0.9873
	1st NLS	7.43	–2.393	5.916	/	/	0.9879
Olive oil – rice bran oil – soybean oil group (R_{21}: soybean oil/rice bran oil; R_{31}: olive oil/rice bran oil)							
$I_{883.8}/I_{879.7}$	Theoretical	0.238	0.455	–0.123	1.284	0.186	0.9794
	1st NLS	0.298	0.474	–0.135	/	/	0.9799
$I_{909.8}/I_{853.7}$	Theoretical	0.094	0.305	–0.530	3.005	0.077	0.9940
	1st NLS	0.107	0.318	–0.484	/	/	0.9945
Flaxseed oil – rice bran oil – soybean oil group (R_{21}: rice bran oil/flaxseed oil; R_{31}: soybean oil/flaxseed oil)							
$I_{895.7}/I_{899.7}$	Theoretical	–3.724	–1161	–52.75	0.160	–20.82	0.9810
	1st NLS	–1.451	–1153	–52.53	/	/	0.9909
$I_{895.7}/I_{901.7}$	Theoretical	50.96	–15488	104.7	0.020	148.9	0.9940
	1st NLS	38.81	–16141	109.1	/	/	0.9982

Table 2-31. Quantitative results of olive oil – rice bran oil – soybean oil group and flaxseed oil – rice bran oil – soybean oil group.

Marker ions (r_1&r_2)	**Model**	**Ratio of oil compositions**			
		PCC	RMSE	PCC	RMSE
Olive oil – rice bran oil – soybean oil group					
Standard: olive oil		$R_{(\text{rice bran/olive})}$		$R_{(\text{soybean/olive})}$	
$I_{883.8}/I_{879.7}$& $I_{883.8}/I_{855.7}$	Theoretical	0.9855	0.4529	0.9705	1.0733
	1st NLS	0.9853	0.4956	0.9659	0.7366
Standard: rice bran oil		$R_{(\text{soybean/rice bran})}$		$R_{(\text{olive/rice bran})}$	
$I_{883.8}/I_{879.7}$& $I_{909.8}/I_{853.7}$	Theoretical	0.8988	2.4219	0.9835	0.4159
	1st NLS	0.9130	0.9532	0.9913	0.3747
Flaxseed oil – rice bran oil – soybean oil group					
Standard: flaxseed oil		$R_{(\text{rice bran/flaxseed})}$		$R_{(\text{soybean/flaxseed})}$	
$I_{895.7}/I_{899.7}$& $I_{895.7}/I_{901.7}$	Theoretical	0.9434	0.8101	0.9934	0.5935
	1st NLS	0.9857	0.3562	0.9975	0.3865

The developed NLS models were applied to measure the oil compositions in validation samples. For the group of flaxseed oil, rice bran oil and soybean oil, the NLS model based on $I_{895.7}/I_{899.7}$ and $I_{895.7}/I_{901.7}$ showed strong quantitative ability to $R_{(\text{rice bran/flaxseed})}$ and $R_{(\text{soybean/flaxseed})}$ with RMSE as 0.3562 and 0.3865 (Table 2-31). For the group of olive oil, rice bran oil and soybean oil, the NLS 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 the NLS model based on $I_{883.8}/I_{879.7}$ and $I_{883.8}/I_{855.7}$ provided good quantitative results to $R_{(\text{rice bran/olive})}$ (RMSE = 0.4956) (Table 2-31). Combining the results obtained from the NLS models of different groups, the quantitation of oil compositions in validation samples were achieved and results were shown in Table 2-32. Compared with the model

of olive oil – rice bran oil – soybean oil group with olive oil as the standard composition, the model regarding rice bran oil as the standard composition for the olive oil – rice bran oil – soybean oil group provided better quantitative results with RMSE as 0.0437, 0.0289, 0.0251 and 0.0515 for olive oil, flaxseed oil, rice bran oil and soybean oil, respectively, which were 0.0595, 0.0274, 0.0256 and 0.0539 for the results provided by the model using olive oil as the standard composition. For most of validation samples, the measured results were close the actual concentrations with accuracy within ±20%, and the poor accuracy was observed for low-abundance compositions (~10%). Meanwhile, large RSD (>20%) were obtained from the measured results of compositions lower than 20% and for composition at high-levels, the quantitative results showed good precision. Overall, it was feasible to quantify the compositions of quaternary blended oils using the intensity ratio-based method, and the obtained results showed good accuracy and acceptable precision.

Table 2-32. Quantitative results of olive oil (OO) – flaxseed oil (FS) – rice bran oil (RB) – soybean oil (SO) blends.

No.	Oil species	Actual con. (%)	RB–based for OO–RB–SO group			OO–based for OO–RB–SO group		
			Measured con. (%)	Accuracy (%)	RSD (%)	Measured con. (%)	Accuracy (%)	RSD (%)
Q1	OO	10.0	9.8±3.0	–1.6	30.3	9.2±1.6	–7.7	17.8
	FS	10.0	9.6±0.9	–4.2	9.3	9.6±0.8	–3.8	8.8
	RB	9.9	9.4±0.1	–5.4	0.5	9.5±0.2	–4.7	2.0
	SO	70.0	77.6±10.4	10.8	13.4	77.9±10	11.2	12.9
Q2	OO	10.0	9.9±1.1	–0.5	11.1	9.2±1.6	–8.0	17.4
	FS	10.0	9.8±0.4	–2.2	4.4	9.9±0.5	–1.3	4.8
	RB	70.2	70.7±0.3	0.7	0.4	71.3±0.3	1.5	0.4
	SO	9.8	9.6±1.1	–2.2	11.8	9.7±1.2	–1.3	12.3
Q3	OO	10.1	13.2±5.3	30.0	40.2	10.2±2.8	0.5	27.6
	FS	69.8	68.2±6.8	–2.3	9.9	71.4±4.6	2.3	6.5
	RB	9.9	10.4±2.9	4.9	28.2	10.6±3.3	7.7	30.7
	SO	10.2	8.2±1.4	–19.0	17.4	10.2±3.3	0.7	32.1
Q4	OO	69.9	71.9±6.2	3.0	8.6	82.7±1.1	18.4	1.3
	FS	10.0	8.9±3.1	–11.1	34.6	5.3±0.3	–46.5	5.2
	RB	10.1	12.2±0.9	20.1	7.3	7.9±2.4	–22.4	30.8
	SO	10.0	7.0±4.0	–29.8	56.6	4.1±1.1	–59.4	26.3
Q5	OO	20.0	19.9±4.6	–0.7	23.0	21.8±6	9.2	27.4
	FS	20.5	19.7±2.8	–3.7	14.1	19.2±3.1	–6.0	15.9
	RB	19.4	21.9±4.2	12.7	19.4	21.3±3.8	9.6	17.7
	SO	40.1	38.6±6.0	–3.9	15.6	37.7±6.7	–6.2	17.7
Q6	OO	20.1	18.7±0.5	–7.0	2.8	19.1±1.8	–5.1	9.2
	FS	19.9	20.1±0.4	0.7	2.2	20.0±0.3	0.2	1.3
	RB	40.0	39.8±2.6	–0.7	6.5	39.6±3.1	–1.1	7.9
	SO	19.9	21.5±2.5	7.7	11.6	21.3±2.3	7.1	10.8

(To be continued)

Table 2-32-*continued*. Quantitative results of olive oil (OO) – flaxseed oil (FS) – rice bran oil (RB) – soybean oil (SO) blends.

No.	Oil species	Actual con. (%)	RB–based for OO–RB–SO group			OO–based for OO–RB–SO group		
			Measured con. (%)	Accuracy (%)	RSD (%)	Measured con. (%)	Accuracy (%)	RSD (%)
Q7	OO	20.2	22.3±5.1	10.2	23.0	22.4±4.7	10.6	20.9
	FS	40.0	39.0±5.7	–2.6	14.6	40.8±1.3	1.9	3.2
	RB	19.8	20.7±3.6	4.2	17.3	18.4±3.2	–7.3	17.3
	SO	19.9	18.1±2.3	–9.3	12.5	18.5±0.2	–7.3	1.0
Q8	OO	40.1	44.5±8.8	11.0	19.7	44.5±7.7	11.0	17.2
	FS	19.9	18.3±4.6	–7.9	25.0	18.3±3.8	–8.2	20.9
	RB	20.1	20.0±2.5	–0.4	12.4	20.2±3.4	0.3	17.0
	SO	19.9	17.1±5.7	–13.9	33.5	17.1±5.0	–14.2	29.3
Q9	OO	25.1	25.4±1.5	1.5	5.7	29.3±1.7	17.0	5.8
	FS	24.9	24.4±1.7	–2.1	6.8	23.1±1.7	–7.3	7.3
	RB	25.1	26.3±3.2	4.8	12.3	24.9±3.0	–0.8	12.0
	SO	24.9	23.9±3.0	–4.1	12.5	22.6±2.9	–9.1	13.0

2.3.4 Quantitative analysis of commercial products

For binary and ternary blended oils, the general procedures for quantitative analysis using the intensity ratio-based method have been summarized. First, MALDI-MS spectra of pure oils are compared to select potential marker ions. Then, different equations are applied to describe the relationship between the intensity ratio of marker ions and the proportions of oil compositions. For binary blended oils, calibration curves are developed between $\frac{1}{r-r_0}$ and $\frac{1}{p_1}$ for different potential marker ions based on the spectra of calibration samples, and then applied to quantify the oil compositions in

validation samples. Comparing the results measured by different calibration curves, the corresponding ions of the curve providing the best quantitative results are chosen as the optimized marker ions and following utilized to establish the calibration curves based on both oil compositions in the binary blends to achieve more accurate quantitative measurement. For ternary blended oils, at least two groups of potential marker ions are needed to construct the quantitative model as shown in Equation (2-32). Using the spectral data of pure oils, quantitative models based on theoretical values are developed for different potential marker ions and then applied for quantitative analysis of validation samples. Afterwards, the optimization of potential marker ions is performed by evaluating the quantitative performance of different theoretical models, and the optimized marker ions are utilized to establish the 1st NLS model based on the spectra of calibration samples, which will eventually be used for quantitative analysis.

The intensity ratio-based method was applied in the quantitative analysis of commercial blended oil products collected from the market and the obtained results were summarized in Table 2-33. For each binary blended oil product, one oil composition was selected to be quantified. 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 relative errors over 30%. The concentrations of all the compositions in each ternary blended oil product were quantified simultaneously by the 1st NLS 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 of the two compositions.

Table 2-33. Quantitative results of commercial blended oil products.

No.	Blending type	Marker ions (*r*)	Measured oil	Labeled con. (%)	Measured con. (%)	Relative error (%)	RSD (%)
1	Sunflower seed oil – soybean oil	$I_{899.7}/I_{903.7}$	Sunflower	6	9.4±2.5	57.1	26.5
2	Corn oil – soybean oil	$I_{899.7}/I_{903.7}$	Corn	10	10.5±4.4	5.3	41.3
3	Olive oil – peanut oil	$I_{903.7}/I_{907.8}$	Olive	10	21.4±1.7	113.9	8.1
4	Corn oil – canola oil	$I_{901.7}/I_{907.8}$	Corn	56	75.5±1.3	34.8	1.8
5	Peanut oil – corn oil – canola oil	$I_{881.8}/I_{905.8}$ & $I_{907.8}/I_{901.7}$	Peanut	NA*	32.6±5.8	/	17.8
			Corn	NA*	37.1±1.7	/	4.5
			Canola	NA*	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	–37.4	11.1
			Corn	45	37.3±5.8	–17.0	15.4
			Sunflower	45	56.4±5.9	25.4	10.4

* NA: Not available on the label.

2.4 Conclusion

The application of MALDI-MS for rapid quantitation of oil compositions in blended oils has been investigated in this study. Using the developed MALDI-MS approach to profile the TAG contents of blended oils, no sample pretreatment and chromatographic separation are required, and spectra with high quality and high reproducibility were obtained due to the direct analysis of oil samples in their oily states and much reduced variations of sample spots. Compared with the previous protocol, the analysis of oil samples using MALDI-MS has been upgraded to automatic data acquisition, further shortening the analysis time and making the MALDI-MS-based method have higher potential for high-throughput analysis.

The MALDI-MS spectra of blended oils contained quantitative information of the comprised oil compositions, and by investigating the relationship between the intensity ratio of peaks obtained from the spectra and the proportion of oil compositions in the blends, an intensity ratio-based method was developed for the quantitative analysis of blended oils. In addition to binary blended oils, the developed method was applicable to blended oils of multiple compositions, i.e., ternary and quaternary blended oils, and allowed simultaneous quantitation of multiple compositions with good quantitative performance. Based on the analysis of various types of blended oils, general guidelines were determined for the selection of marker ions and the determination of quantitative models, which significantly simplified the quantitative analysis of blended oils,

especially those with multiple compositions. The developed method was convenient, straightforward and user-friendly, and could achieve the semi-quantitative analysis of blended oils using the spectral data of pure oils, making this method advantageous in rapid screening of blended oils to promote quality control and safety assurance. Moreover, the intensity ratio-based method could be extended to studies of other complex mixtures, such as glycans in honey and active compounds in Chinese herbal medicines, and has further application potential for the quantitative analysis based on mass spectrometry.

Chapter 3: Quantitative analysis of blended oils by MALDI-MS and chemometric approach

3.1 Introduction

In the previous chapter, a MALDI-MS-based method has been demonstrated for rapid quantitative analysis of blended oils using the intensity ratio of marker ions. The results showed the potential of MALDI-MS technique for quantitation of oil compositions in blended oils, However, to obtain the best quantitative results, the selection of marker ions needed to be optimized which strongly depends on the species of pure oils contained in the blended oils, requiring exploration of quantitative analysis of different blended oils and adjustment of the quantitative method. The MALDI-MS spectra of blended oils with multiple oil compositions became more complex with increased number of oil compositions, causing larger variations in the measured intensity ratio, and then leading to the deteriorated precision of the quantitative results. Moreover, for blended oils with different numbers of oil compositions, different methods and algorithms were applied for data processing and analysis, making the quantitative analysis complicated and changeable. To overcome such drawbacks, in this chapter, partial least squares regression (PLS-R) has been applied for improvements of the MALDI-MS-based method for quantitative analysis of blended oils.

Partial least squares regression (PLS-R) is a chemometric method that is commonly used to deal with complex and multicollinear data, and it could achieve multivariate

regression[95-96]. Using PLS-R to process multivariate data, the most useful information of the original data is extracted as new variables (called PLS component) which will be used to establish new models. The number of new variables is usually smaller than that of the original variables, leading to dimension reduction of variables and graphical visualization of complex data[97-99].

To perform multivariate regression using PLS-R, the original data is gathered in two matrices, i.e., **X** and **Y**, which represent variables used for quantitation and predictors obtained from the quantitation, respectively. In **X**, a row corresponds to an "observation" which can be a set of physicochemical data or a spectrum of a sample, and a column refers to a "variable" which is one physicochemical data of all the samples that are included in the analysis. The PLS-R model generates coefficients **W** (also called weights) to explain the maximum covariance between **X** and **Y**, and a set of new variables $\boldsymbol{t_a}$ (a = 1, 2, …, A), which are called X-scores, are generated from **X** as Equation (3-1).

$$\mathbf{T} = \mathbf{X}\mathbf{W}^{\mathrm{T}} \tag{3-1}$$

where $\mathbf{W}^{\mathrm{T}}$ is the transform matrix of **W** and **T** is the matrix of $\boldsymbol{t_a}$.

$\boldsymbol{t_a}$ are orthogonal and should be good summaries of **X** through multiplied by loadings (**P**). Therefore, **X** could be expressed as follow,

$$\mathbf{X} = \mathbf{T}\mathbf{P}^{\mathrm{T}} + \mathbf{E} \tag{3-2}$$

where $\mathbf{P}^{\mathrm{T}}$ is the transform matrix of **P**, and **E** is the matrix of residuals which should be small.

In PLS-R, **Y** could be approximated by **T** as

$$\mathbf{Y} = \mathbf{T}\mathbf{C}^{\mathrm{T}} + \mathbf{F} \tag{3-3}$$

where $\mathbf{C}^{\mathbf{T}}$ is the transform matrix of **C** (the weights of **Y**) and **F** is the matrix of residuals.

When **C** is determined, the corresponding Y-scores ($\boldsymbol{u}_a$, a = 1, 2, …, A) could be generated as

$$\mathbf{Y} = \mathbf{U}\mathbf{C}^{\mathrm{T}} + \mathbf{G} \tag{3-4}$$

where **G** is the matrix of new residuals.

Substituting Equation (3-1) into Equation (3-3), it could be obtained

$$\mathbf{Y} = \mathbf{X}\mathbf{W}^{\mathrm{T}}\mathbf{C}^{\mathrm{T}} + \mathbf{F}$$

or

$$\mathbf{Y} = \mathbf{X}\mathbf{B} + \mathbf{F} \tag{3-5}$$

where $\mathbf{B} = \mathbf{W}^{\mathbf{T}}\ \mathbf{C}^{\mathbf{T}}$. Therefore, the linear relationship between **X** and **Y** is established and **B** is the matrix of regression coefficients.

PLS-R is designed to confront the situation of numerous variables that are possibly correlated, while the number of samples is limited, thus PLS-R is powerful in dealing

with multicollinear data and is commonly used for analyzing spectral data[97]. PLS-R has been applied for analysis of various food samples, including quality control of red meats[100-102], assessment of wines[103-105], authentication of milk[106] and milk powders[107], and determination of herbals[108-109]. Quantitation of olive oil in blends with other vegetable oils, e.g., sunflower seed oil, corn oil, sesame oil and soybean oil, has been demonstrated using PLS-R to process the FA contents and TAG profiles obtained from GC-FID and high-temperature GC-MS, respectively[17, 19]. Combining HPLC with PLS-R, the concentrations of the individual oils in blended oils were determined[22, 110]. Direct analysis techniques, such as those based on fluorescence[30, 111], UV[26, 112], Raman [29, 113] and Fourier transform infrared spectroscopy (FTIR)[31, 114], and electrospray ionization mass spectrometry[32] have been used for quantitative analysis of blended oils with the help of PLS-R, illustrating the possibility of using PLS-R to decode the quantitative information contained in the MALDI-MS spectra of blended oils.

In this study, we aimed to improve the developed MALDI-MS-based method for quantitative analysis of blended oils. To enhance the universality of the MALDI-MS-based method for different types of blended oils, all the TAG peaks and their isotopic peaks shown in the MALDI-MS spectra of blended oils were used to quantify the oil compositions. PLS-R was applied to process the numerous and collinear MALDI-MS spectral data and provide simultaneously quantitation of multiple oil compositions. By investigating various types of blended oils, including binary blended oils, ternary

blended oils and quaternary blended oils as well as commercial blended oil products, we demonstrated that for the first time, quantitative analysis of oil compositions in blended oils could be achieved using PLS-R to interpret the MALDI-MS spectra of blended oils, and the PLS-R-based method was simple, high-throughput and allowed simultaneous quantitation of multiple oil compositions with high sensitivity.

3.2 Materials and methods

3.2.1 Chemicals

α-Cyano-4-hydroxycinnanic acid (CHCA) and 2, 5-dihydroxybenzoic acid (DHB) were purchased from Aldrich (St. Louis, USA). HPLC grade acetonitrile and acetone were purchased from Anaqua Chemical Supply (Houston, USA) and Acros Organic (Waltham, USA), respectively. Polyethylene glycol (PEG) standards and sodium iodide (NaI) were purchased from Fluka (St. Louis, USA) and Panreac Química (Barcelona, Spain), respectively. The standard mixture of 37 fatty acid methyl esters was purchased from Supelco (St. Louis, USA), and trimethylsulfonium hydroxide (TMSH) and tert-butyl methyl ether (TBME) were collected from Acros Organic.

3.2.2 Oil samples

Both blended oil samples prepared in laboratory as well as commercial blended oil products collected from the market were analyzed in this study. Details of the collected commercial pure oil products and commercial blended oil are shown in Table 3-1. For

blended oils samples that were prepared by manually mixing pure oils in different proportions (w/w), the prepared samples were divided into two set, i.e., training set and testing set, for establishment and validation of PLS-R models, respectively. For each type of binary blended oils, there were 6 oil samples and 5 oil samples in the training set and the testing set, respectively (Table 3-2); for each type of ternary blended oils, there were 66 oil samples and 12 oil samples in the training set and the testing set, respectively (Table 3-3); for each type of quaternary blended oils, there were 286 oil samples and 13 oil samples in the training set and the testing set, respectively (Table 3-4). All blended oil samples were freshly prepared, sealed and stored in a dark and dry place, and analyzed within a few days.

Table 3-1. Oil products collected from mainland China.

No.	Oil product	Brand
1	Olive oil	A
2	Sunflower seed oil	A
3	Canola oil	A
4	Olive oil - sunflower seed oil blend	A
5	Olive oil - canola oil blend	A
6	Olive oil	B
7	Sunflower seed oil	B
8	Corn oil	B
9	Olive oil - sunflower seed oil blend	B
10	Olive oil - sunflower seed oil blend	B
11	Olive oil - corn oil blend	B
12	Olive oil	C
13	Corn oil	C
14	Olive oil - corn oil blend	C
15	Olive oil	D
16	High oleic acid peanut oil	D
17	Olive oil - high oleic acid peanut oil blend	D
18	Olive oil	E
19	Perilla oil	E
20	Olive oil - perilla oil blend	E
21	Olive oil	F
22	Soybean oil	F
23	Sunflower seed oil	F
24	Olive oil - soybean oil - sunflower seed oil blend	F
25	Olive oil	G
26	Flaxseed oil	G
27	Sunflower seed oil	G
28	Olive oil - flaxseed oil - sunflower seed oil blend	G
29	Olive oil	H
30	Camellia oil	H
31	Flaxseed oil	H
32	Corn oil	H
33	Olive oil – camellia oil - flaxseed oil – corn oil blend	H

Table 3-2. Blended oil samples prepared for PLS-R models of binary blended oils.

Type	Training set (6)		Testing set (5)
Pure (2)	0%:100%	100%:0%	8%:92% 30%:70%
Binary blends (4)	20%:80% 40%:60%	60%:40% 80%:20%	50%:50% 70%:30% 92%:8%

Table 3-3. Blended oil samples prepared for PLS-R models of ternary blended oils.

Type	Training set (66)			Testing set (12)
Pure (3)	0%:0%:100%	0%:100%:0%	100%:0%:0%	
Binary blends (9×3)	0%:10%:90% 0%:20%:80% 0%:30%:70% 0%:40%:60% 0%:50%:50% 0%:60%:40% 0%:70%:30% 0%:80%:20% 0%:90%:10%	10%:90%:0% 20%:80%:0% 30%:70%:0% 40%:60%:0% 50%:50%:0% 60%:40%:0% 70%:30%:0% 80%:20%:0% 90%:10%:0%	10%:0%:90% 20%:0%:80% 30%:0%:70% 40%:0%:60% 50%:0%:50% 60%:0%:40% 70%:0%:30% 80%:0%:20% 90%:0%:10%	50%:50%:0% 50%:0%:50% 0%:50%:50% 5%:5%:90% 5%:90%:5% 90%:5%:5% 20%:20%:60% 20%:60%:20% 60%:20%:20% 0%:0%:100% 0%:100%:0% 100%:0%:0%
Ternary blends (36)	10%:10%:80% 10%:20%:70% 10%:30%:60% 10%:40%:50% 10%:50%:40% 10%:60%:30% 10%:70%:20% 10%:80%:10% 20%:10%:70% 20%:20%:60% 20%:30%:50% 20%:40%:40%	20%:50%:30% 20%:60%:20% 20%:70%:10% 30%:10%:60% 30%:20%:50% 30%:30%:40% 30%:40%:30% 30%:50%:20% 30%:60%:10% 40%:10%:50% 40%:20%:40% 40%:30%:30%	40%:40%:20% 40%:50%:10% 50%:10%:40% 50%:20%:30% 50%:30%:20% 50%:40%:10% 60%:10%:30% 60%:20%:20% 60%:30%:10% 70%:10%:20% 70%:20%:10% 80%:10%:10%	

Table 3-4. Blended oil samples prepared for PLS-R models of quaternary blended oils.

Type	**Training set (286)**				**Testing set (13)**
Pure (4)	0%:0%:0%:100%	0%:0%:100%:0%	0%:100%:0%:0%	100%:0%:0%:0%	
Binary blends (9×6)	10%:0%:0%:90% 20%:0%:0%:80% … 90%:0%:0%:10% 0%:10%:90%:0% 0%:20%:80%:0% … 0%:90%:10%:0%	10%:0%:90%:0% 20%:0%:80%:0% … 90%:0%:10%:0% 0%:10%:0%:90% 0%:20%:0%:80% … 0%:90%:0%:10%	10%:90%:0%:0% 20%:80%:0%:0% … 90%:10%:0%:0% 0%:0%:10%:90% 0%:0%:20%:80% … 0%:0%:90%:10%		10%:10%:10%:70% 10%:10%:70%:10% 10%:70%:10%:10% 70%:10%:10%:10% 20%:20%:20%:40% 20%:20%:40%:20% 20%:40%:20%:20% 40%:20%:20%:20% 25%:25%:25%:25% 0%:0%:0%:100% 0%:0%:100%:0% 0%:100%:0%:0% 100%:0%:0%:0%
Ternary blends (36×4)	10%:10%:80%:0% 10%:20%:70%:0% … 80%:10%:10%:0%	10%:0%:10%:80% 10%:0%:20%:70% … 80%:0%:10%:10%	10%:10%:0%:80% 10%:20%:0%:70% … 80%:10%:0%:10%	0%:10%:10%:80% 0%:10%:20%:70% … 0%:80%:10%:10%	
Quaternary blends (84)	10%:10%:10%:70% 10%:10%:20%:60% … 10%:40%:30%:20%	10%:40%:40%:10% 10%:50%:10%:30% … 20%:30%:30%:20%	20%:30%:40%:10% 20%:40%:10%:30% … 30%:40%:20%:10%	30%:50%:10%:10% 40%:10%:10%:40% … 70%:10%:10%:10%	

3.2.3 MALDI-MS analysis

MALDI-MS analysis of oil samples was performed using a previously reported protocol (see Section 2.2.3 for the details) with an UltrafleXtreme MALDI-TOF/TOF mass spectrometer (Bruker, Billerica, USA) in positive and reflectron mode. The ion source voltage 1, ion source voltage 2, lens voltage, reflector voltage 1 and reflector voltage 2 of the mass spectrometer were set to 19.00 kV, 16.40 kV, 8.00 kV, 21.00 kV and 9.60 kV, respectively. For each oil sample, MALDI-MS mass spectra with a *m/z* range of 500–2000 Da were acquired automatically as described in Section 2.2.3. For some oil samples, their MALDI-MS spectra at *m/z* 500–2000 Da were also acquired manually by irradiating different positions on the sample spot randomly. 8 single spectra showed signals in the region of *m/z* 850–920 Da with the absolute intensity of the highest peak higher than 2000 were accumulated into a final spectrum which was saved for further analysis. Each oil sample was measured in eight replicates.

3.2.4 GC-FID analysis

To perform GC-FID analysis, oil samples were converted into FAME samples according to ISO 12966-3[115]. A 6890N gas chromatograph coupled with a flame ionization detector (Agilent, Santa Clara, USA) and a DB-23 column (Agilent, 60 m, 0.25 mm, 0.25 μm) was utilized to analyze the FAME samples. The temperature of the injector and the detector were set to 250 ºC and 280 ºC, respectively, and the gas flow

rates of hydrogen and nitrogen were set to 40 mL min^{-1} and 4 mL min^{-1}, respectively. To separate the FAME samples, the oven temperature was set to 50 ºC at the beginning. After held at 50 ºC for 1 min, the oven temperature was quickly increased from 50 ºC to 175 ºC at 25 ºC min^{-1}, then slowly increased from 175 ºC to 230 ºC at 3 ºC min^{-1}, and finally held at 230 ºC for 10 mins. To determine the FA contents in oil samples, the obtained chromatograms of oil samples were compared with the chromatograms of the mixture standard of FAMEs. Each FAME sample was analyzed in triplicate.

3.2.5 Statistical analysis

Before statistical analysis, the normalization of each MALDI-MS spectrum was performed by dividing the intensity of individual TAG peak by the total intensity of all the TAG peaks in the spectrum, and the normalization of GC-FID chromatograms was performed in the same way. The normalized spectral or chromatographic data of oil samples as well as the corresponding concentrations of oil compositions were input into a statistics software (Umetrics Simca 14.0, Andover, USA) as X-variables and Y-variables, respectively, to establish the PLS-R models.

3.2.5.1 *Protocol for PLS-R analysis*

The protocol for establishing the PLS-R models was shown in Figure 3-1, with five major steps involved. In the 1st step, PLS components were extracted from original data

to develop a regression relationship between X-variables and Y-variables. Generally, increasing the number of PLS components would result in a better fitting of the PLS-R model to the original data but might decrease its predictive ability due to noise information, which is called over-fitting. Therefore, the number of PLS components (A) is a critical factor for the performance of PLS-R model, and the optimal number of PLS components (optimal A) could be determined using cross-validation[97].

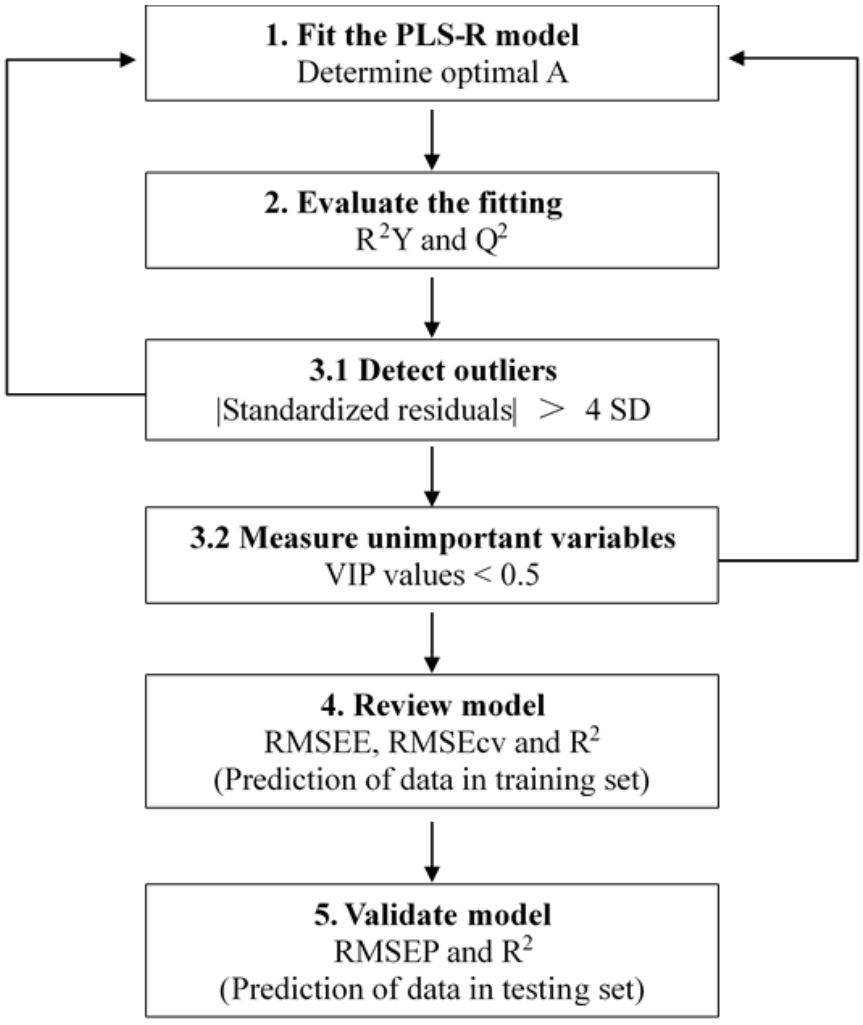

Figure 3-1. Protocol for establishing and optimizing the PLS-R models.

Cross-validation is a standard way to test the predictive power of PLS-R models. To perform cross-validation, the data in training set is equally divided into several parts. Deleting any part of the training data, PLS-R model is developed based on the reduced data, and the Y-values of the deleted data are predicted by the established model with the difference between actual and predicted Y-values obtained. This procedure is repeated with deleting each part of the training data, and the sum of differences between the actual and predicted Y-values is collected from all the parallel models and described as root mean square error of cross-validation (RMSEcv) as shown below,

$$\text{RMSEcv} = \sqrt{\frac{\sum_{i=1}^{N}\left(y_{i,actual} - y_{i,predicted}\right)^2}{N}} \tag{3-6}$$

where N is the number of samples in the training set, and $y_{i,actual}$ and $y_{i,predicted}$ are the actual Y-value and the predicted Y-value of the deleted training sample i, respectively.

In this study, Wold's R criterion[116] and RMSE criterion[34] based on the results of cross-validation were applied to determine the optimal A and prevent over-fitting. Wold's R criterion calculates the ratio of the predicted error sum of squares (PRESS) for (m + *1*) PLS components and m PLS components (called R), which is equal to the square of RMSEcv ratio for (m + *1*) PLS components and m PLS components as shown in Equation (3-8). When R exceeds threshold, an optimal A is obtained as m. For RMSE

criterion, the root mean square error (RMSE) is calculated as Equation (3-9) and the optimal A is obtained with minimal RMSE. 0.90 and 0.95 were selected as thresholds of the Wold's R criterion, and PLS-R models with optimal A determined by the Wold's R criteria (R(0.90) and R(0.95)) and the RMSE criterion were further optimized and then compared to choose the most suitable criterion.

$$\mathrm{PRESS} = \sum_{i=1}^{N} \left(y_{i,actual} - y_{i,predicted}\right)^2 \tag{3-7}$$

$$\mathrm{R} = \frac{\mathrm{PRESS}(m+1)}{\mathrm{PRESS}(m)} = \left(\frac{\mathrm{RMSEcv}(m+1)}{\mathrm{RMSEcv}(m)}\right)^2 \tag{3-8}$$

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{N}\left(y_{i.actual} - y_{i.predicted}\right)^2}{N-A-1}} = \mathrm{RMSEcv} \times \sqrt{\frac{N}{N-A-1}} \tag{3-9}$$

N is the number of samples in the training set, and $y_{i,actual}$ and $y_{i,predicted}$ are the actual Y-value and predicted Y-value of the deleted training sample i, respectively.

In the 2nd step, the PLS-R model was evaluated by two parameters, i.e., R^2Y and Q^2. R^2Y is the percent of variation of all the Y-variables explained by the model. After the establishment of a PLS-R model, the compositions of all the samples in the training set would be predicted by the model, and the obtained fractions of residual sum of squares (RSS) and total sum of squares (TSS) were used to generated R^2Y.

$$R^2Y = 1 - \frac{\mathrm{RSS}}{\mathrm{TSS}} = 1 - \frac{\sum_{i=1}^{N}\left(y_{i,actual} - y_{i,predicted}\right)^2}{\sum_{i=1}^{N}\left(y_{i,actual} - \bar{y}_{actual}\right)^2} \tag{3-10}$$

where N is the number of samples in the training set, and $y_{i,actual}$ and $y_{i,predicted}$ are the actual Y-value and the predicted Y-value of the i^{th} training sample, respectively. $\bar{y}_{actual}$ is the mean of actual Y-values of all the training samples

Q^2 (also called cross-validated R^2Y) is the percent of variation of all the Y-variables predicted by the model, and its calculation is based on the results of cross-validation as Equation (3-11) shows. R^2Y and Q^2 describes the fitting ability and the predictive ability of a PLS-R model, respectively. Normally, a larger R^2Y (close to 1) means better fitting and a larger Q^2 (at least higher than 0.5) represents better predictability[117-118].

$$Q^2 = 1 - \frac{\text{PRESS}}{\text{TSS}} = 1 - \frac{\sum_{i=1}^{N}\left(y_{i,actual} - y_{i,predicted}\right)^2}{\sum_{i=1}^{N}\left(y_{i,actual} - \bar{y}_{actual}\right)^2} \quad (3\text{-}11)$$

where N is the number of samples in the training set, and $y_{i,actual}$ and $y_{i,predicted}$ are the actual Y-value and the predicted Y-value of the deleted training sample i, respectively. $\bar{y}_{actual}$ is the mean of actual Y-values of all the training samples.

In the 3rd step, training samples with standardized residuals exceeding ± 4 standard deviations were defined as outliers and X-variables with variable importance for the projection (VIP) values lower than 0.5 were considered as unimportant variables. To improve the fitting ability of the PLS-R model, these outliers and unimportant variables were excluded from the original data set and the above procedures (step 1-3) were

repeated to carry out new fittings based on the reduced data set. When the obtained PLS-R model showed R^2Y and Q^2 higher than 0.8 but had no outliers and unimportant variables, the model fitting was completed.

In the 4th step, the compositions of samples in the training set were quantified by the established PLS-R model to review the predictive ability of the model. The root mean square error of estimation (RMSEE) was applied to summarize the difference between the actual concentrations and the predicted concentrations for each oil composition of the training samples, and the coefficient of determination (R^2) between the actual ratios and the predicted ratios was also calculated as shown in Equation (3-10).

$$\text{RMSEE} = \sqrt{\frac{\sum_{i=1}^{N}\left(y_{i,actual} - y_{i,predicted}\right)^2}{N}} \quad (3\text{-}12)$$

where $y_{i,actual}$ denotes the actual concentration of the selected composition of sample i in the training set, $y_{i,predicted}$ indicates the predicted concentration of the selected composition of sample i and N indicates the number of training samples used for model building.

In the 5th step, the established PLS-R model was further validated by quantifying the compositions of samples in the testing set. The testing samples were "new" samples for the established model since they were not involved in the development of the PLS-R model. The difference between the actual concentrations and the predicted

concentrations of each oil composition were described by the root mean square error of prediction (RMSEP) and R^2, and Grubbs test with detection level $\alpha = 0.05$ was carried out to detect outliers of the predicted results of testing samples. For a good PLS-R model, the RMSEE, RMSEcv and RMSEP should be small (close to 0) with R^2 close to 1.

$$\text{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n}\left(y_{i,actual} - y_{i,predicted}\right)^2}{n}} \tag{3-13}$$

where $y_{i,actual}$ and $y_{i,predicted}$ are the actual concentration and the predicted concentration, respectively, of the selected composition of sample *i* in the testing set, and *n* is the number of testing samples.

3.2.5.2 *Determination of limit of detection*

The limit of detection (LOD) of the PLS-R model could be calculated using Equation (3-14)[119],

$$\text{LOD}_{cal} = \Delta(\alpha,\beta)\frac{\sigma}{b}\sqrt{\frac{1}{K}+\frac{1}{N}+\frac{(\bar{y}_{actual})^2}{\sum_{i=1}^{N}\left(y_{i,actual}-\bar{y}_{actual}\right)^2}} \tag{3-14}$$

$$\sigma = \sqrt{\frac{\sum_{i=1}^{N}\left(y_{i,measured} - y_{i,actual}\right)^2}{N-2}} \tag{3-15}$$

where $\Delta(\alpha, \beta)$ is the non-centrality parameter of non-central Student's t-distribution with (n-2) degrees of freedom, *N* is the number of training samples, σ is the standard residual deviation of regression curve by curveting the measured concentrations

($y_{i,predicted}$) of samples in the training set against their actual concentrations ($y_{i,actual}$), b is the slope of the regression curve, K is the number of determination performed on each sample, and $\bar{y}_{actual}$ is the mean of actual concentrations of all the samples in the training set.

3.2.5.3 *Similarity evaluation*

Similarity between two MALDI-MS spectra was determined by cosine correlation [120], as defined below,

$$\cos\theta = \frac{\sum i_A i_B}{\sqrt{\sum i_A^2 \sum i_B^2}} \quad (3\text{-}16)$$

where θ is the spectral contrast angle between the MALDI-MS spectra of selected oil sample A and sample B, i_A is the relative intensities of peaks from spectrum A and i_B is the relative intensities of peak from spectrum B. For the first sum, only when a peak at a particular *m/z* is observed in both spectrum A and spectrum B would the relative intensities of this peak in both spectra be multiplied together, otherwise the product of i_A multiplied by i_B should be zero. The calculation of similarity of the GC-FID chromatograms between selected oil sample A and sample B was the same as above.

3.3 Results and discussion

3.3.1 MALDI-MS spectra acquired manually and automatically

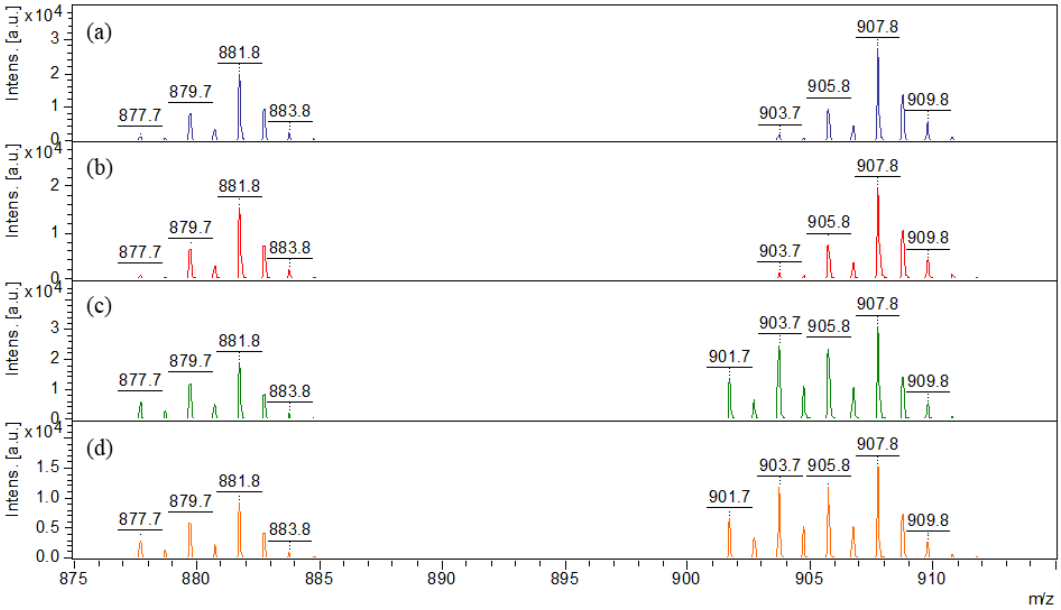

Figure 3-2. The MALDI-MS spectra of 100% olive oil obtained by (a) manual data acquisition and (b) automatic data acquisition, and the MALDI-MS spectra of 50% olive oil – 50% sunflower seed oil blend obtained by (c) manual data acquisition and (d) automatic data acquisition.

After the adjustment of parameters for MALDI-MS analysis, automatic data acquisition was achieved and spectra with high quality were obtained for oil samples. Figure 3-2 showed that the spectra acquired manually and automatically were identical for the same oil sample with slightly variation in the relative intensities (R.I.) of TAG related peaks (Table 3-5). The automatic data acquisition also presented high reproducibility. For the 50% olive oil – 50% sunflower seed oil blend, the precision of the R.I of TAG related peaks was within 1.2-8.0% excluding an extreme RSD (12.8%) belonging to the low-abundance peak (R.I. as 0.17%) at *m/z* 884.8 (Table 3-5). The precision of R.I. obtained by the automatic acquisition was comparable to that obtained by the manual

acquisition, illustrating that the manual acquisition of MALDI-MS spectra could be replaced by automatic acquisition, and the latter one would further shorten the analysis time of oil samples, making the MALDI-MS-based method more advantageous for quantitative analysis of large batches of blended oil samples.

Table 3-5. The precision of R.I. of TAG related peaks for MALDI-MS spectra of 50% olive oil – 50% sunflower seed oil blend obtained by manual data acquisition and automatic data acquisition.

m/z	**Manual acquisition**		**Automatic acquisition**	
	R.I. (%)	RSD (%)	R.I. (%)	RSD (%)
853.7	0.48±0.04	8.3	0.52±0.03	6.3
855.7	0.77±0.08	9.9	0.82±0.05	5.9
877.7	3.10±0.11	3.7	3.05±0.1.0	3.4
879.7	6.13±0.13	2.2	6.07±0.08	1.4
880.7	2.52±0.11	4.5	2.53±0.06	2.5
881.8	9.59±0.09	0.9	9.60±0.12	1.2
882.8	4.17±0.11	2.6	4.31±0.12	2.8
883.8	0.99±0.06	6.3	1.03±0.07	6.4
884.8	0.14±0.01	6.6	0.17±0.02	12.8
901.7	7.12±0.11	1.5	6.98±0.12	1.8
902.7	3.42±0.09	2.7	3.47±0.06	1.8
903.7	12.37±0.18	1.4	12.13±0.17	1.4
904.7	5.61±0.08	1.5	5.58±0.08	1.5
905.8	12.02±0.22	1.9	11.97±0.2	1.7
906.8	5.23±0.09	1.7	5.29±0.10	1.9
907.8	15.51±0.44	2.8	15.38±0.27	1.7
908.8	7.30±0.10	1.4	7.38±0.15	2.0
909.8	2.81±0.07	2.6	2.92±0.13	4.4
910.8	0.59±0.03	4.6	0.65±0.03	5.3
911.8	0.13±0.01	6.8	0.15±0.01	8.0

3.3.2 Determination of optimal A

The number of PLS component (A) is critical for the performance of PLS-R model, and Wold's R criterion and RMSE are commonly used to determine the optimal A of PLS-R models[34, 97, 116]. Although both Wold's R criterion and RMSE are based on the results of cross-validation, different parameters are considered in each case. The criterion for providing PLS-R models with better performance depends on the objectives of analysis and the properties of data[117-118, 121]. Therefore, Wold's R criteria with thresholds at 0.90 (called R(0.90)) and 0.95 (called R(0.95)) and RMSE criterion were applied to determine the optimal A, and the properties of the obtained models were compared to select the most suitable criterion.

For binary blends of olive oil and sunflower seed oil, factors of PLS-R models developed based on different criteria were summarized in Table 3-6. The optimal A of the R(0.90) model, the R(0.95) model and the RMSE model increased sequentially, while the RMSEE, RMSEcv and RMSEP of these models decreased in turn, indicating the improved fitting ability and predictive ability of the models. For PLS-R models of ternary blended oils, the R(0.90) model and the R(0.95) model had the same optimal A, and the RMSE model showed a larger optimal A. Compared with the R(0.90) model and the R(0.95) model, the RMSE model showed better fitting ability but comparable quantitative ability since the RMSEP values of the RMSE model were similar to that of the R(0.90) model and the R(0.95) model (Table 3-7). When the number of oil

composition in blended oils was increased to four, a significant increase was observed in the optimal A of the R(0.90) model, the R(0.95) model and the RMSE model (Table 3-8) as well as the improved fitting ability of the models. For the R(0.90) model and the R(0.95) model, better quantitative results of all the oil compositions were observed with a larger A, while for the R(0.95) model and the RMSE model, there was little improvement or even slightly deterioration in the measured results of some oil compositions, a result that might be related to over-fitting.

The detailed information about the Wold's R criterion and RMSE could be found in Section 3.2.5. Briefly, to prevent over-fitting, a PLS-R model should only include PLS components that are "significant" to the model. Wold's R criterion compares the ratio of RMSEcv (called R) of two continuous PLS components (*m* and *m + 1*) (see Equation (3-8) for details). If R exceeds the threshold, the *m + 1* PLS component is not significant since it could not provide much improved prediction performance to the model. Compared with R(0.95), R(0.90) has a smaller threshold, so it would ignore the PLS components that slightly improve the prediction performance of models and results in more parsimonious models. RMSE considers three parameters, i.e., RMSEcv, the number of training data and A (see Equation (3-9) for details). In this study, the number of training data was much larger than A, so the minimal RMSE was usually obtained with the minimal RMSEcv, making the RMSE criterion tended to choose all the PLS components that could improve the prediction performance of models. Therefore, for

the three criteria investigated in this study, R(0.90) tended to generate the PLS-R model with the smallest A while RMSE usually determined the largest A for the PLS-R model. To achieve a balance between the fitting ability and predictive ability of PLS-R models, R(0.95) was more suitable for the determination of the optimal number of PLS components.

Table 3-6. PLS-R models of olive oil – sunflower seed oil blends determined by different criteria.

Criterion	**A**	**R^2Y**	**Q^2**	**Training set**			**Testing set**	
				RMSEE	RMSEcv	R^2	RMSEP	R^2
R(0.90)	1	0.9995	0.9995	0.0075	0.0076	0.9995	0.0064	0.9996
R(0.95)	2	0.9998	0.9997	0.0052	0.0056	0.9998	0.0058	0.9996
RMSE	3	0.9998	0.9997	0.0049	0.0055	0.9998	0.0054	0.9997

Table 3-7. PLS-R models of olive oil (OO) – perilla oil (PR) – sunflower seed oil (SF) blends determined by different criteria.

Criterion	**A**	**R^2Y**	**Q^2**	**Oil species**	**Training set**			**Testing set**	
					RMSEE	RMSEcv	R^2	RMSEP	R^2
R(0.90) & R(0.95)	5	0.9948	0.9946	OO	0.0181	0.0195	0.9956	0.0283	0.9953
				PR	0.0199	0.0211	0.9946	0.0357	0.9917
				SF	0.0206	0.0221	0.9943	0.0296	0.9951
RMSE	8	0.9956	0.9949	OO	0.0177	0.0195	0.9957	0.0282	0.9954
				PR	0.0180	0.0205	0.9956	0.0358	0.9917
				SF	0.0186	0.0212	0.9954	0.0298	0.9953

Table 3-8. PLS-R models of olive oil (OO) – perilla oil (PR) – rice bran oil (RB) – sunflower seed oil (SF) blends determined by different criteria.

Criterion	A	R^2Y	Q^2	Oil species	Training set			Testing set	
					RMSEE	RMSEcv	R^2	RMSEP	R^2
R(0.90)	8	0.9920	0.9918	OO	0.0181	0.0185	0.9938	0.0235	0.9939
				PR	0.0171	0.0173	0.9944	0.0239	0.9947
				RB	0.0262	0.0265	0.9868	0.0372	0.9900
				SF	0.0188	0.0192	0.9932	0.0331	0.9904
R(0.95)	10	0.9929	0.9927	OO	0.0170	0.0173	0.9945	0.0210	0.9956
				PR	0.0170	0.0171	0.9945	0.0221	0.9948
				RB	0.0238	0.0243	0.9891	0.0287	0.9929
				SF	0.0183	0.0187	0.9936	0.0320	0.9924
RMSE	15	0.9939	0.9935	OO	0.0158	0.0166	0.9953	0.0213	0.9961
				PR	0.0160	0.0167	0.9951	0.0224	0.9946
				RB	0.0219	0.0230	0.9908	0.0278	0.9926
				SF	0.0170	0.0176	0.9945	0.0315	0.9936

3.3.3 Quantitative analysis of binary blended oils

3.3.3.1 *Establishment and validation of the PLS-R model*

Olive oil and sunflower seed oil blends were chosen as reference oils to investigate the quantitative analysis of blended oils using MALDI-MS and PLS-R, since olive oil and sunflower seed oil showed distinct TAG profiles in their MALDI-MS spectra and their blends were common in the market of blended oils. Comparing the MALDI-MS spectra of blended oils with different blending ratios, all the peaks assigned as TAGs (Figure 3-3) or isotopic peaks of TAGs according to the literatures[42, 59, 69, 122] were selected as indicators for quantitative analysis. The R.I. of TAGs related peaks and corresponding

concentrations of oil compositions for samples in training set were used to establish PLS-R model.

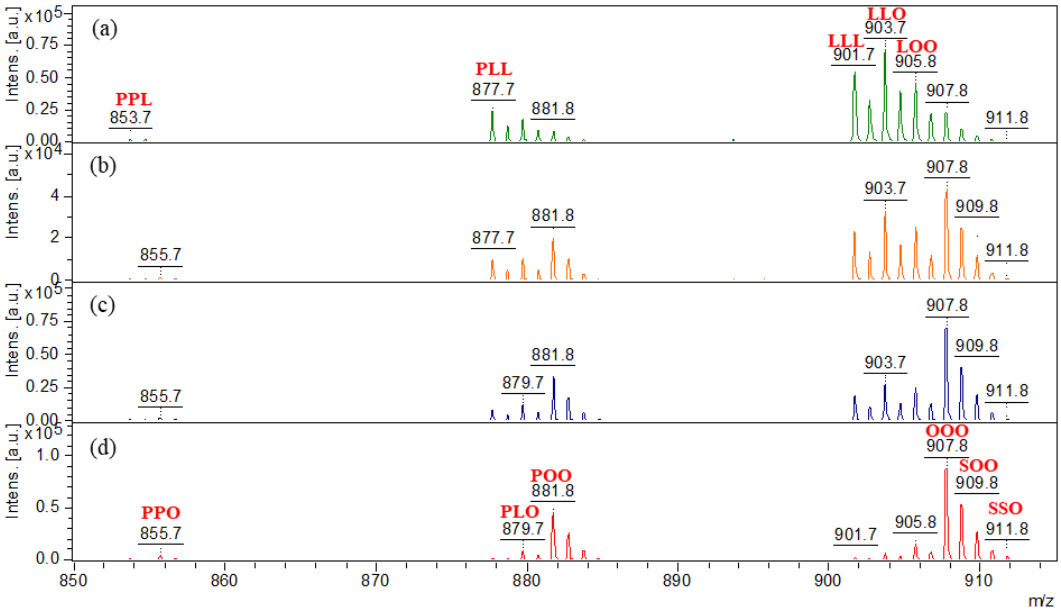

Figure 3-3. The TAG region of the MALDI-MS spectra for (a) 100% sunflower seed oil, (b) 40% olive oil – 60% sunflower seed oil blend, (c) 60% olive oil – 40% sunflower seed oil blend and (d) 100% olive oil, with the identities of the TAGs peaks assigned.

According to Wold's R criterion with 0.95 as threshold, the optimal A of the PLS-R model of olive oil – sunflower seed oil blends was 2 (Table 3-9). After further optimization, the concentrations of olive oil in testing samples were measured by the developed PLS-R model to validate its predictive ability. For all the testing samples, the measured results showed accuracy over 99% and precision within 0.4–4.9% (Table 3-10), illustrating that the PLS-R model had excellent quantitative ability for the olive oil – sunflower seed oil blends. To investigate the reproducibility of the MALDI-MS and PLS-R approach, the same batch of testing samples were analyzed for 8 selected

days in 20 days, i.e., 1st, 2nd, 3rd, 5th, 7th, 10th, 14th and 20th day, and the obtained data were applied to quantify the concentration of olive oil in these samples using the PLS-R model constructed on the 1st day (Table 3-9). As shown in Table 3-10, the accuracy of the measured results was between –16.1% and –0.1% with inter-day precision within 1.6-2.0%, demonstrating the durable applicability of the PLS-R model.

Table 3-9. PLS-R model of olive oil– sunflower seed oil blends for intra-day and inter-day measurements.

Model	A	R^2Y	Q^2	Training set			Testing set	
				RMSEE	RMSEcv	R^2	RMSEP	R^2
Intra-day	2	0.9998	0.9997	0.0052	0.0056	0.9998	0.0058	0.9996
Inter-day	3	0.9986	0.9975	0.0135	0.0173	0.9986	0.0157	0.9973

Table 3-10. Quantitative results of olive oil – sunflower seed oil blends measured on the same day and measured in 8 different days in 20 days.

Actual olive oil con. (%)	Intra-day (n = 8)			Inter-day (n = 8)		
	Measured olive oil con. (%)	Accuracy (%)	RSD (%)	Measured olive oil con. (%)	Accuracy (%)	RSD (%)
7.8	7.7±0.4	–0.4	4.9	6.5±0.8	–16.1	11.8
29.9	29.9±0.5	–0.1	1.8	27.8±0.6	–7.0	2.0
49.8	49.6±0.6	–0.5	1.3	48.3±0.9	–3.2	1.9
69.7	69.6±0.9	–0.2	1.2	69.6±1.4	–0.1	2.0
91.6	91.2±0.4	–0.4	0.4	91.0±1.5	–0.6	1.6

Based on the established PLS-R model, the limit of detection (LOD) of olive oil was calculated. For the PLS-R model of olive oil and sunflower seed oil blends, the degree of freedom was 4 and the $\Delta(\alpha, \beta)$ with confidence level of 95% was 4.07[123]. The slope

and the standard residual deviation of the regression curve between the measured olive oil concentrations and the actual olive oil concentrations of training samples were 1.0 and 0.5%, respectively. Therefore, the LOD of olive oil was calculated as 0.9% using Equation (3-14). Furthermore, a blank sample (i.e., the pure sunflower seed oil) was measured for 20 replicates, and the olive oil concentration measured by the established PLS-R model was –0.4 ± 0.4%. As IUPAC recommended, to make a correct positive detection decision with sufficiently high probability, the probabilities of both false positive and false negative should be considered[124]. Thus, the LOD based on the measured results of the blank sample was calculated also as 0.9% based on Equation (3-17). Two blended oil samples were prepared with 1.0% and 1.5% olive oil, and the PLS-R model measured that the two samples contained 0.7 ± 0.3% and 1.0 ± 0.4% olive oil, respectively. Therefore, for the PLS-R model of olive oil – sunflower seed oil blends, the lowest concentration of olive oil that could be experimentally detected from the oil blends with 95% confidence was 1.5%.

$$\text{LOD} = \text{Average (blank)} + 3.3 \times \text{Standard deviation} \qquad (3\text{-}17)$$

3.3.3.2 *PLS-R models based on manually and automatically acquired spectra*

To further validate the applicability of the developed MALDI-MS method using automatic data acquisition for quantitative analysis of blended oils, the MALDI-MS spectra of the same batch of olive oil and sunflower seed oil blends were acquired manually and automatically, and employed for establishment of PLS-R models. Similar

parameters (e.g., R^2Y, Q^2, RMSEE and RMSEP) were observed from the models based on manual acquisition and automatic acquisition (Table 3-11), indicating these two models had comparable fitting ability and quantitative ability. Both models were applied to quantify the compositions of testing samples based on the MALDI-MS spectra acquired manually and automatically, and the results were summarized in Table 3-12 and Table 3-13. The PLS-R model established based on manual acquisition provided more accurate results for the samples whose spectra were collected manually with RMSEP as 0.0084, which was 0.0095 for the samples whose spectra were collected automatically (Table 3-12). Similar situation was noticed from the results provided by the PLS-R model based on automatic acquisition with smaller RMSEP obtained for the automatically analyzed samples compared with the manually analyzed samples, which were 0.0087 and 0.0094, respectively (Table 3-13). For the same sample, both models based on manual acquisition and automatic acquisition measured higher concentrations for the automatically acquired spectra than the manually acquired spectra, illustrating that there were some differences between the spectra acquired by different methods. However, such differences were very minor and would not affect the quantitative results greatly, because the changes in the quantitative results acquired by different methods were smaller than the variation of quantitative results for replicate measurements. Therefore, the MALDI-MS analysis with automatic data acquisition was available for quantitative analysis of blended oils, and showed comparable quantitative performance as the analysis with manual data acquisition while required less analyzing time.

Table 3-11. PLS-R model of olive oil– sunflower seed oil blends based on manual data acquisition and automatic data acquisition.

Model	A	R^2Y	Q^2	Training set			Testing set	
				RMSEE	RMSEcv	R^2	RMSEP	R^2
Manual acquisition	2	0.9986	0.9981	0.0134	0.0146	0.9986	0.0084	0.9992
Automatic acquisition	2	0.9988	0.9982	0.0124	0.0143	0.9988	0.0087	0.9993

Table 3-12. Quantitative results of olive oil – sunflower seed oil blends measured by the PLS-R model established based on manually acquired spectra.

Actual olive oil con. (%)	Manual acquisition			Automatic acquisition		
	Measured olive oil con. (%)	Accuracy (%)	RSD (%)	Measured olive oil con. (%)	Accuracy (%)	RSD (%)
7.7	8.1±0.7	5.5	8.8	8.6±1.1	11.7	12.3
30.1	29.6±1.2	–1.6	4.2	29.8±1.0	–1.0	3.3
49.7	49.7±1.0	–0.2	1.9	50.2±0.7	1.0	1.5
70.0	70.5±0.4	0.7	0.6	70.7±0.3	1.1	0.5
92.4	92.5±0.3	0.1	0.3	92.6±0.7	0.2	0.7
RMSEP	0.0084			0.0095		

Table 3-13. Quantitative results of olive oil – sunflower seed oil blends measured by the PLS-R model established based on automatically acquired spectra.

Actual olive oil con. (%)	Manual acquisition			Automatic acquisition		
	Measured olive oil con. (%)	Accuracy (%)	RSD (%)	Measured olive oil con. (%)	Accuracy (%)	RSD (%)
7.7	7.1±0.7	–8.1	9.6	7.5±1.0	–1.9	13.4
30.1	29.1±1.2	–3.2	4.1	29.1±1.0	–3.3	3.3
49.7	49.2±0.9	–1.0	1.8	49.7±0.6	–0.2	1.3
70.0	70.2±0.5	0.3	0.7	70.2±0.4	0.2	0.5
92.4	92.1±0.3	0.2	0.3	92.1±0.6	–0.3	0.7
RMSEP	0.0094			0.0087		

3.3.4 Quantitative analysis of blended oils with multiple compositions

3.3.4.1 *Quantitative analysis of ternary and quaternary blended oils*

Blended oils with multiple oil compositions, such as ternary blended oils and quaternary blended oils, are also commonly available in the markets, but such multiple oil compositions make the quantitative analysis of these blended oils become much more complicated. To investigate the applicability of the PLS-R approach to the quantitative analysis of ternary and quaternary blended oils, in this study, olive oil – perilla oil – sunflower seed oil blends and olive oil – perilla oil – rice bran oil – sunflower seed oil blends were prepared as examples because of the distinct TAG patterns of the pure oils (Figure 3-4 and Figure 3-5). The corresponding PLS-R models were established and validated as shown in Figure 3-1.

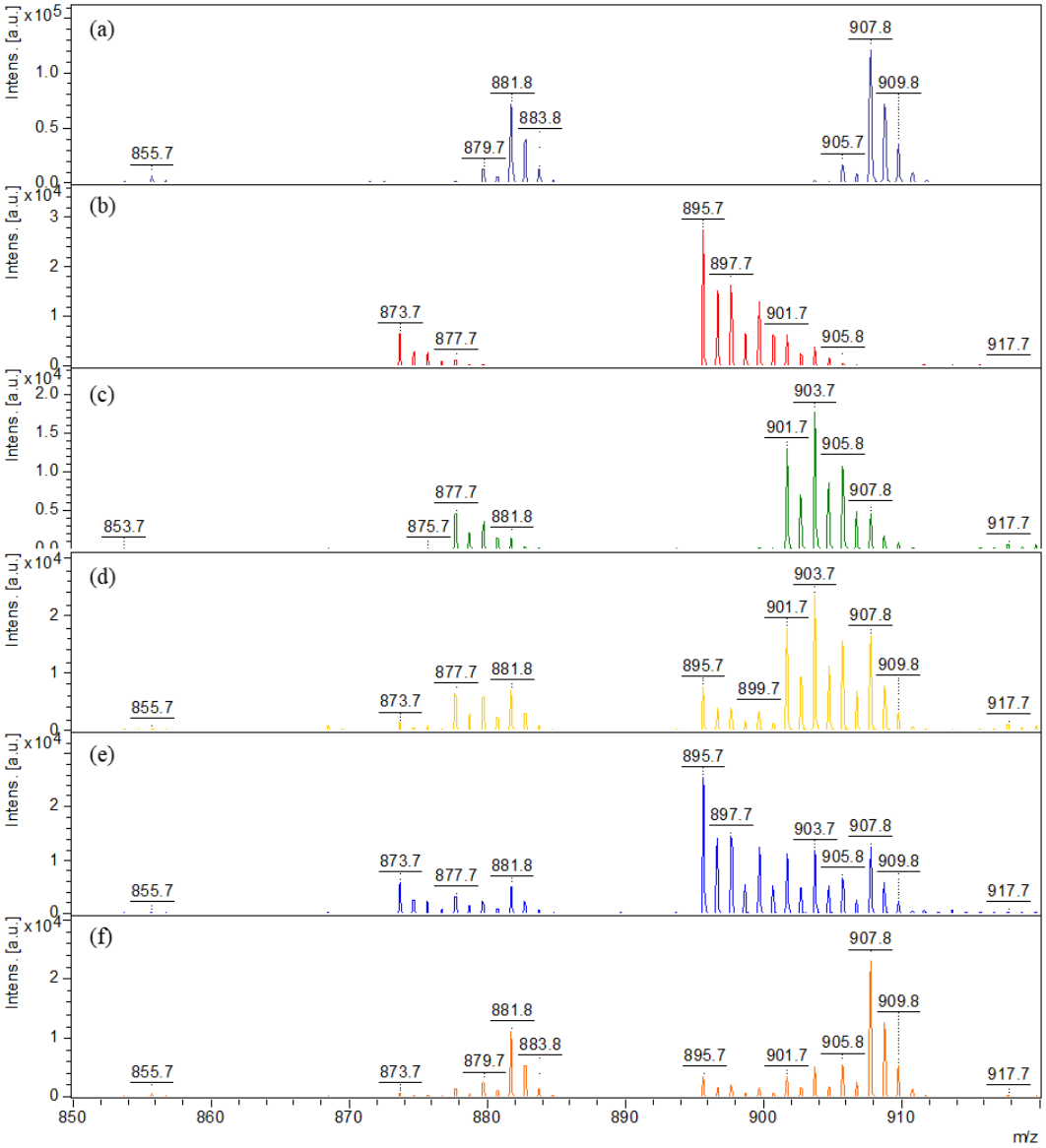

Figure 3-4. The TAG region of the MALDI-MS spectra for (a) 100% olive oil, (b) 100% perilla oil, (c) 100% sunflower seed oil, (d) 20% olive oil – 20% perilla oil– 60% sunflower seed oil blend, (e) 20% olive oil – 60% perilla oil– 20% sunflower seed oil blend and (f) 60% olive oil – 20% perilla oil– 20% sunflower seed oil blend.

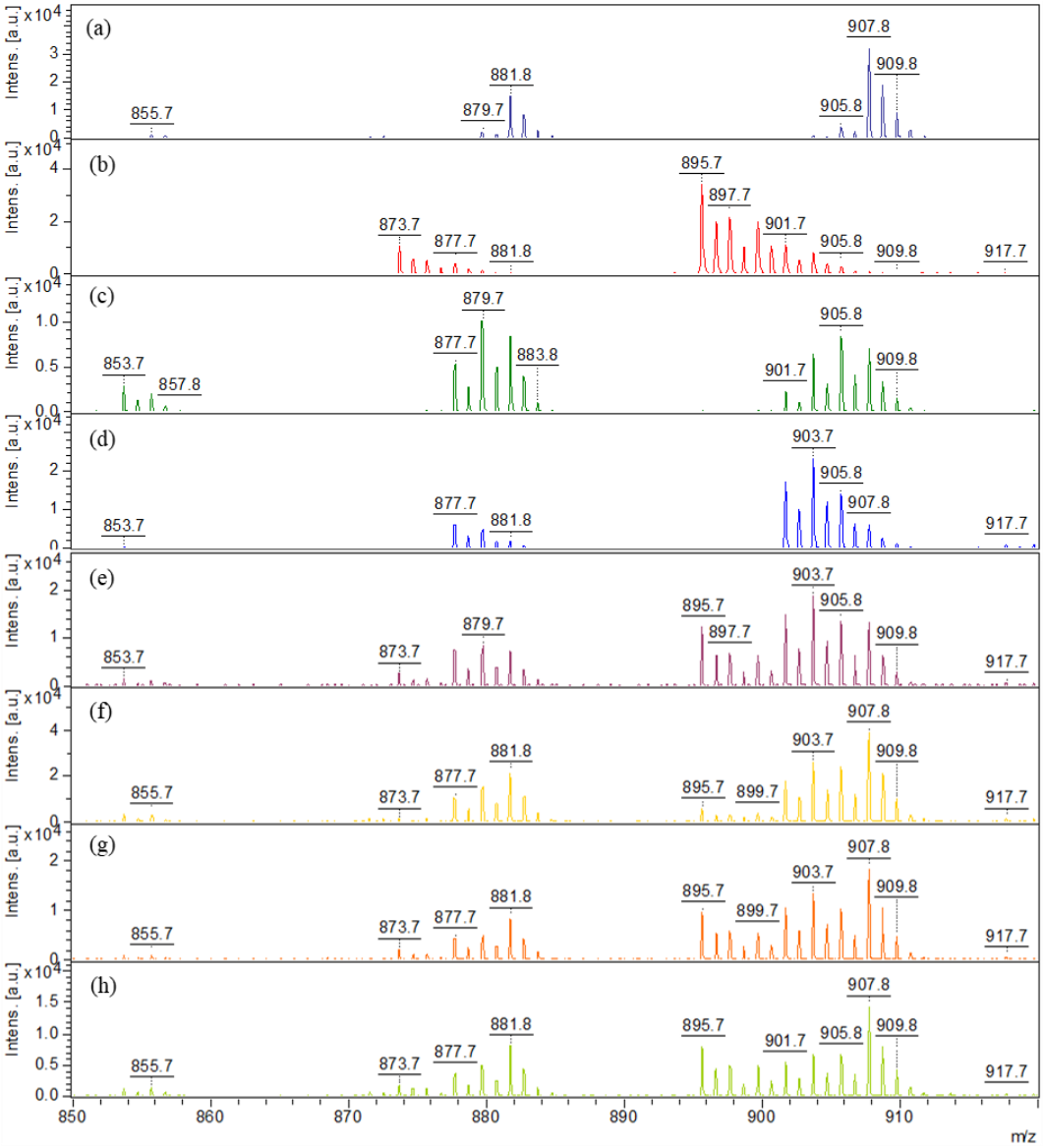

Figure 3-5. The TAG region of the MALDI-MS spectra for (a) 100% olive oil, (b) 100% perilla oil, (c) 100% rice bran oil, (d) 100% sunflower seed oil, (e) 10% olive oil – 30% perilla oil – 30% rice bran oil – 30% sunflower seed oil blend, (f) 30% olive oil – 10% perilla oil – 30% rice bran oil – 30% sunflower seed oil blend, (g) 30% olive oil – 30% perilla oil – 10% rice bran oil – 30% sunflower seed oil blend and (h) 30% olive oil – 30% perilla oil – 30% rice bran oil – 10% sunflower seed oil blend.

For the ternary blended oils and quaternary blended oils, the optimal A of their PLS-R models were determined by R(0.95) as 5 and 10, respectively. The model of olive oil – perilla oil – sunflower seed oil blends showed the best description and quantitation to olive oil with the RMSEE and RMSEcv less than 0.02 and RMSEP less than 0.03. The RMSEP of perilla oil was 0.0357, indicating the quantitative ability of the PLS-R model for perilla oil was slightly worse than that for the other two compositions (Table 3-14). For olive oil – perilla oil – rice bran oil – sunflower seed oil blends, the established PLS-R model showed good fitting ability and quantitative ability to olive oil and perilla oil with RMSEE and RMSEP lower than 0.0170 and 0.0320, respectively, and the largest RMSEcv and RMSEE were observed for rice bran oil and sunflower seed oil, respectively, as 0.0243 and 0.0320, respectively (Table 3-14).

PLS components could present the structural information of original data in a simpler way. Therefore, in the score curve of the PLS-R model of the ternary blended oils, a large triangle was formed by the training samples with the three pure oils located at the three vertices, the binary blends of pure oils formed the three edges, and the ternary blended oils filled the interior of the triangle (Figure 3-6a). Similarly, in the 3D score curve of the PLS-R model of the quaternary blended oils, a tetrahedron was formed by the training samples, and the four faces of the tetrahedron represented the ternary mixtures of pure oils with the inside of the tetrahedron stuffed with the quaternary mixtures (Figure 3-6b).

Table 3-14. PLS-R models of olive oil (OO) – perilla oil (PR) – sunflower seed oil (SF) blends and olive oil (OO) – perilla oil (PR) – rice bran oil (BR) – sunflower seed oil (SF) blends.

A	R^2Y	Q^2	Oil species	Training set			Testing set	
				RMSEE	RMSEcv	R^2	RMSEP	R^2
5	0.9948	0.9946	OO	0.0181	0.0195	0.9956	0.0283	0.9953
			PR	0.0199	0.0211	0.9946	0.0357	0.9917
			SF	0.0206	0.0221	0.9943	0.0296	0.9951
10	0.9929	0.9927	OO	0.0170	0.0173	0.9945	0.0210	0.9956
			PR	0.0170	0.0171	0.9945	0.0221	0.9948
			RB	0.0238	0.0243	0.9891	0.0287	0.9929
			SF	0.0183	0.0187	0.9936	0.0320	0.9924

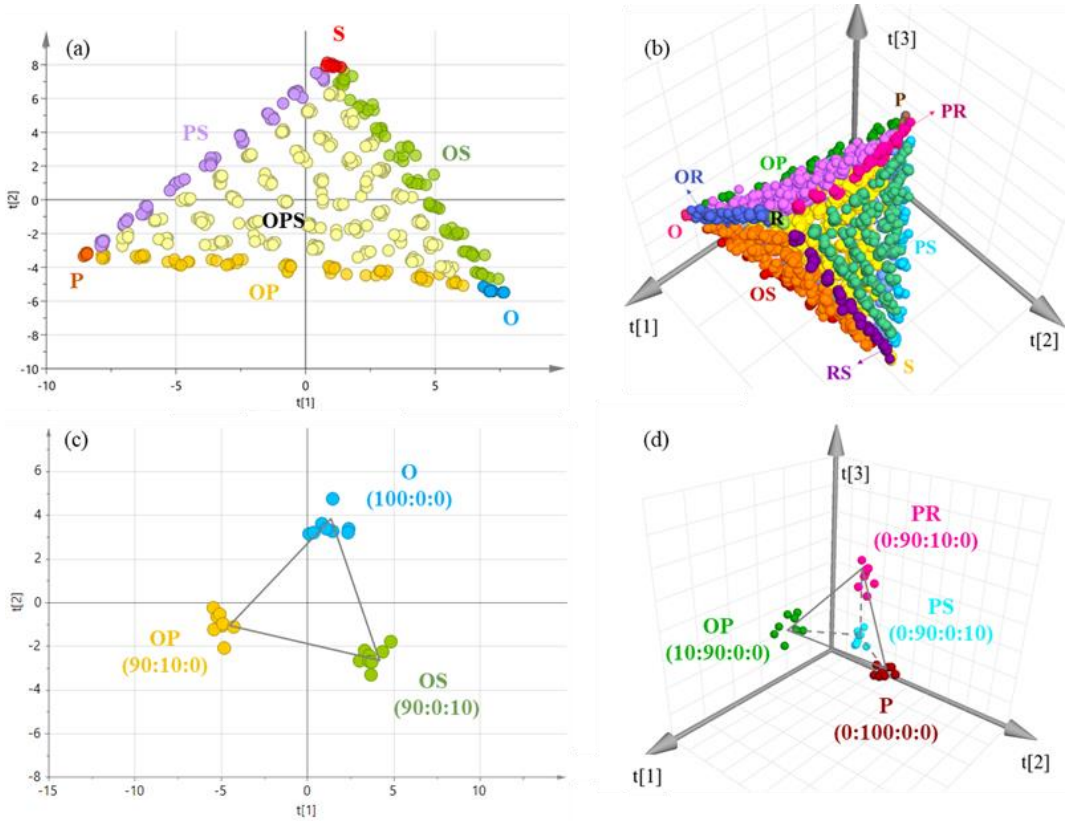

Figure 3-6. The score curves of the (a) full range and (c) zoom-in PLS-R models of olive oil (O), perilla oil (P) and sunflower seed oil (S) blends, and the 3D score curves of the (b) full range and (d) zoom-in PLS-R models of olive oil (O), perilla oil (P), rice bran oil (R) and sunflower seed oil (S) blends.

The predictive ability of the PLS-R approach for blended oils with multiple oil compositions was validated by using the established PLS-R models to measure the abundance of oil compositions in the testing samples, and the results were summarized in Table 3-15 and Table 3-16. For the high-abundance compositions with concentrations higher than 20% in the olive oil – perilla oil – sunflower seed oil blends, the measured results were close to the actual concentrations with the accuracy and precision varied from –16.7% to 12.0% and 0.5% to 4.7%, respectively (Table 3-15). For the high-abundance compositions in the olive oil – perilla oil – rice bran oil – sunflower seed oil blends, satisfactory quantitative results were provided by the PLS-R model with the accuracy and precision in the range of –19.9-10.4% and 0.5-9.2%, respectively (Table 3-16). However, for the oil compositions in the ternary blended oils at low levels, i.e., around 5%, poor quantitative results were measured by the PLS-R model with the accuracy and precision within –45.0-41.9% and 2.6- 38.9%, respectively. Moreover, there was a possibility for the PLS-R models to detect oil compositions that were not present in the oil samples to be at low levels, which was observed for the results of both ternary blended oils and quaternary blended oils, making it possible to wrongly determine pure oils as blended oils.

To establish a PLS-R model for a type of blended oils, the training set should include samples that cover the whole concentration ranges of all the composition. As the number of oil compositions increases, the number of samples that should be included

in the training set significantly increases. For the PLS-R models of olive oil – perilla oil – sunflower seed oil blends and olive oil – perilla oil – rice bran oil – sunflower seed oil blends, the training sets included 528 and 2288 MALDI-MS spectra, respectively, involving pure oils and blended oils. As the PLS-R models were based on the full training sets, it would summarize the maximum differences of all the training samples, which were mainly denoted by the differences between the pure oils. Therefore, some minor differences that were critical to differentiate blended oils with similar oil compositions might not be extracted by the PLS-R models, leading to the decreased predictive ability of the models for oil compositions at low levels. To overcome such drawbacks and prevent false positive determination, a two-step zoom-in strategy was proposed for the first time.

3.3.4.2 *Zoom-in strategy for improved analysis of multi-composition blended oils*

To improve the quantitative results of oil compositions at low levels, zoom-in PLS-R models were established by narrowing the size of training samples. Before processing quantitative analysis using the zoom-in PLS-R models, oil compositions in a blended oil sample were quantified by the full range PLS-R model and based on the obtained results, training samples containing similar compositions to the oil sample were selected to a new PLS-R model (called zoom-in model). The selection of training samples should follow the requirement below. For each composition, a concentration range of 10%, such as 10-20%, 20-30% and 80-90%, was set to include the quantitative

results provided by the full range PLS-R model. For an olive oil – perilla oil – sunflower seed oil blended sample, the concentrations of olive oil, perilla oil and sunflower seed oil were measured by the full range PLS-R model as 95.2%, 0.6% and 4.2%, respectively (Table 3-15, T1), so the corresponding possible concentration ranges of olive oil, perilla oil and sunflower seed oil were 90-100%, 0-10% and 0-10%, respectively. In the whole training set, only three samples, i.e., 100% olive oil – 0% perilla oil – 0% sunflower seed oil blend, 90% olive oil – 10% perilla oil – 0% sunflower seed oil blend and 90% olive oil – 0% perilla oil – 10% sunflower seed oil blend, fulfilled the requirements and these three samples were utilized to develop a zoom-in model for this blended oil sample. As shown in Figure 3-6c, a small triangle was observed in the score curve of the zoom-in model and the blended oil sample was surrounded by the small triangle. The same strategy has been applied to a quaternary blended oil sample (Table 3-16, Q2) to develop the zoom-in PLS-R model and a mini tetrahedron was observed in the score curve of the zoom-in model (Figure 3-6d).

Zoom-in models were established based on the results measured by the full range PLS-R model, thus each blended oil sample had a corresponding zoom-in model. For the olive oil – perilla oil – sunflower seed oil blends, all the testing samples were further quantified by the zoom-in models with the results shown in Table 3-15. For the high-abundance oil compositions in the ternary blended oils, the accuracy of measured results provided by the zoom-in models was in the range of –15.0-14.5% and the

precision was within 0.3-8.0%, which were quite similar to those of the measured results provided by the full range model. On the other hand, for the measured results of the low-abundance oil compositions (~5%) in the ternary blended oils, the ranges of accuracy and precision were narrowed to –22.9-18.3% and 6.6-20.8%, respectively, indicating the quantitative results of oil compositions at low levels were significantly improved. Moreover, for pure oil samples, the abundance of the non-existing oil compositions measured by the zoom-in models was close to 0, and the concentrations of actual oil compositions were measured as higher than 99.5%. According to the results, these samples should be considered as pure oils with very high possibility and the erroneous determination of pure oils as blended oils could be successfully prevented. For the full range PLS-R model, each oil composition had a RMSEP value that described the differences between the actual concentrations and measured concentrations of all the testing samples. Similar calculations were applied on the measured results provided by the zoom-in models, which could be called dummy RMSEP. Significant decreases from RMSEP to dummy RMSEP were observed for all oil compositions, from 0.0283 to 0.0200, 0.0357 to 0.0219, 0.0296 to 0.0130 for olive oil, perilla oil and sunflower seed oil, respectively, indicating that the results measured by the zoom-in models were much closer to their actual concentrations than those measured by the full range model.

The zoom-in strategy was also applied on the quantitative analysis of the olive oil –

perilla oil – rice bran oil – sunflower seed oil blends, and similar situation was observed. Compared with the results provided by the full range model, the measured results provided by the zoom-in models were closer to the actual concentrations, especially for the results of the non-existing oil compositions since the range of the accuracy was significantly narrowed from 90.6-105.0% to 98.7-99.9%. (Table 3-16). Combining the above results, it has been illustrated the full range PLS-R model had excellent quantitative ability to oil compositions not at low levels while the zoom-in PLS-R models could provide better quantitative results to low-abundance oil compositions. Hereby, the general strategy for quantitative analysis of blended oils using PLS-R was to first utilize the full range model for basic screening and then apply the zoom-in model for improved measurements of low-abundance oil compositions.

Table 3-15. Quantitative results of olive oil (OO) – perilla oil (PR) – sunflower seed oil (SF) blends by the full range and zoom-in models.

No.	Oil species	Actual con. (%)	Full range model			Zoom-in model		
			Measured con. (%)	Accuracy (%)	RSD (%)	Measured con. (%)	Accuracy (%)	RSD (%)
T1	OO	100.0	95.2±1.0	–4.8	1.1	99.7±0.3	–0.3	0.3
	PR	0.0	0.6±1.4	/	/	–0.4±0.4	/	/
	SF	0.0	4.2±0.8	/	/	0.7±0.5	/	/
T2	OO	0.0	3.8±0.3	/	/	–0.2±0.4	/	/
	PR	100.0	97.4±1.0	–2.7	1.0	99.8±0.7	–0.2	0.7
	SF	0.0	–1.4±1.2	/	/	0.4±0.6	/	/
T3	OO	0.0	–0.3±0.6	/	/	–1.3±0.6	/	/
	PR	0.0	7.4±1.4	/	/	0.5±0.5	/	/
	SF	100.0	92.6±0.5	–7.4	0.5	99.6±0.7	–0.4	0.7
T4	OO	0.0	1.5±0.5	/	/	0.4±0.4	/	/
	PR	49.8	52.7±1.0	5.9	2.0	52.7±0.8	5.8	1.5
	SF	50.2	45.8±0.7	–8.8	1.6	46.9±0.6	–6.6	1.2
T5	OO	49.8	48.0±0.7	–3.5	1.4	47.3±0.4	–5.0	0.9
	PR	0.0	2.9±0.6	/	/	1.0±0.5	/	/
	SF	50.2	49.4±1.4	–1.5	2.9	51.4±0.5	2.5	1.0
T6	OO	50.2	44.4±0.4	–11.5	1.0	46.3±0.3	–7.7	0.7
	PR	49.8	55.8±1.0	12.0	1.9	53.8±0.5	8.0	1.0
	SF	0.0	–0.2±0.7	/	/	–0.1±0.6	/	/
T7	OO	5.0	4.2±0.7	–15.2	17.6	3.8±0.3	–22.9	8.4
	PR	5.4	7.6±1.5	41.9	19.2	6.4±0.6	18.3	9.0
	SF	89.6	88.2±0.9	–1.7	1.0	90.1±0.8	0.5	0.9
T8	OO	5.0	5.4±0.1	7.7	2.6	4.4±0.3	–12.7	6.6
	PR	89.8	91.7±1.2	2.0	1.3	90.6±0.8	0.8	0.8
	SF	5.1	2.8±1.1	–45.0	38.9	5.0±0.7	–2.3	14.5
T9	OO	89.7	88.7±0.7	–1.1	0.8	89.8±1.1	0.2	1.2
	PR	5.0	4.6±0.8	–8.8	18.5	4.4±0.9	–13.0	20.8
	SF	5.3	6.8±0.8	28.9	12.4	5.8±0.7	9.2	11.4
T10	OO	20.2	18.0±0.7	–10.7	4.1	18.6±1.5	–7.5	8.0
	PR	20.1	21.3±1.0	5.9	4.7	20.6±0.8	2.4	3.9
	SF	59.7	60.7±1.0	1.6	1.6	60.7±0.8	1.7	1.2
T11	OO	20.1	16.7±0.4	–16.7	2.1	17.1±0.3	–15.0	1.6
	PR	59.8	66.1±0.6	10.5	1.0	64.0±0.4	6.9	0.6
	SF	20.1	17.1±0.6	–14.6	3.5	19.0±0.5	–5.5	2.8
T12	OO	59.7	60.2±0.5	0.8	0.9	56.8±0.5	–4.8	0.9
	PR	20.2	19.4±0.2	–3.8	0.9	23.1±0.2	14.5	0.9
	SF	20.1	20.4±0.5	1.3	2.3	20.1±0.4	–0.3	1.9

Table 3-16. Quantitative results of olive oil (OO) – perilla oil (PR) – rice bran oil (RB) – sunflower seed oil (SF) blends by the full range and zoom-in models.

No.	Oil species	Actual con. (%)	Full range model			Zoom-in model		
			Measured con. (%)	Accuracy (%)	RSD (%)	Measured con. (%)	Accuracy (%)	RSD (%)
Q1	OO	100.0	95.2±0.9	–4.8	1.0	99.9±0.8	–0.1	0.8
	PR	0.0	1.5±0.8	/	/	0.0±0.6	/	/
	RB	0.0	0.0±1.4	/	/	–0.1±0.7	/	/
	SF	0.0	3.3±0.8	/	/	0.4±0.2	/	/
Q2	OO	0.0	3.5±1.7	/	/	0.0±0.3	/	/
	PR	100.0	94.6±0.6	–5.4	0.6	99.6±0.8	–0.4	0.8
	RB	0.0	2.7±3.0	/	/	0.9±1.1	/	/
	SF	0.0	–0.4±0.9	/	/	–0.5±0.9	/	/
Q3	OO	0.0	–2.4±1.1	/	/	0.3±0.4	/	/
	PR	0.0	–0.4±0.2	/	/	0.1±0.3	/	/
	RB	100.0	105±1.7	5.0	1.6	99.3±0.8	–0.7	0.8
	SF	0.0	–2.2±1.3	/	/	0.3±0.7	/	/
Q4	OO	0.0	–0.2±0.5	/	/	–0.5±0.4	/	/
	PR	0.0	3.7±0.2	/	/	0.2±0.2	/	/
	RB	0.0	5.8±1.8	/	/	1.6±0.7	/	/
	SF	0.0	90.6±1.5	–9.4	1.6	98.7±0.7	–1.3	0.7
Q5	OO	10.2	9.5±0.3	–7.5	2.9	9.4±0.5	–8.1	5.0
	PR	9.9	9.5±0.1	–4.0	0.8	9.1±0.2	–8.5	2.0
	RB	10.2	11.1±0.8	9.0	7.4	11.0±1.3	7.6	12.1
	SF	69.6	70.0±0.9	0.6	1.3	70.4±1.4	1.2	2.0
Q6	OO	10.1	8.9±0.4	–12.2	4.5	9.9±0.4	–2.0	4.4
	PR	10.6	8.5±0.5	–19.9	5.8	9.9±0.6	–7.2	6.5
	RB	69.4	72.2±1.0	4.1	1.3	69.9±0.9	0.8	1.3
	SF	9.9	10.2±0.7	3.1	6.4	10.3±0.8	4.3	7.5
Q7	OO	10.1	8.7±0.3	–13.9	3.3	9.3±0.4	–7.7	3.9
	PR	69.3	70.9±0.4	2.3	0.5	70.7±0.3	1.9	0.4
	RB	10.3	10.9±0.2	5.2	2.3	10.7±0.6	3.5	5.6
	SF	10.2	9.3±0.4	–9.3	4.2	9.3±0.5	–8.7	5.5
Q8	OO	70.1	70.9±0.5	1.3	0.6	72.7±0.3	3.8	0.5
	PR	10.1	8.9±0.2	–11.8	1.7	5.8±0.3	–42.5	5.4
	RB	9.9	10.1±0.5	2.9	5.3	10.2±0.5	3.7	4.8
	SF	10.0	10.1±0.3	1.0	3.1	11.3±0.3	12.9	2.6

(To be continued)

Table 3-16-*continued*. Quantitative results of olive oil (OO) – perilla oil (PR) – rice bran oil (RB) – sunflower seed oil (SF) blends by the full range and zoom-in models.

No.	Oil species	Actual con. (%)	Full range model			Zoom-in model		
			Measured con. (%)	Accuracy (%)	RSD (%)	Measured con. (%)	Accuracy (%)	RSD (%)
Q9	OO	19.7	19.8±0.7	0.5	3.5	19.3±0.5	–2.0	2.4
	PR	20.4	19.8±0.3	–3.2	1.4	19.9±0.5	–2.5	2.3
	RB	20.2	22.3±1.0	10.4	4.7	25.5±1.2	26.1	4.7
	SF	39.6	38.3±0.8	–3.4	2.0	35.3±1.1	–11.0	3.1
Q10	OO	20.1	20.7±0.6	2.7	3.1	20.9±1.0	3.6	4.6
	PR	19.8	19.0±0.6	–4.1	3.0	19.9±0.6	0.2	3.2
	RB	40.3	39.5±1.0	–2.1	2.4	42.1±0.9	4.4	2.3
	SF	19.8	21.6±0.7	9.1	3.1	17.2±1.3	–12.9	7.4
Q11	OO	19.9	19.2±0.7	–3.7	3.4	17.2±0.3	–13.4	1.9
	PR	40.3	41.8±0.7	3.8	1.7	41.9±0.6	4.0	1.4
	RB	20.0	19.1±1.8	–4.5	9.2	19.7±1.4	–1.6	6.8
	SF	19.8	19.9±0.8	0.6	4.3	21.4±0.9	7.9	4.4
Q12	OO	39.9	40.7±0.6	2.0	1.5	43.6±0.9	9.2	2.1
	PR	19.8	18.6±0.6	–6.0	3.2	18.1±0.4	–8.6	2.2
	RB	20.2	19.1±1.0	–5.5	5.0	15.6±1.1	–22.4	7.1
	SF	20.1	21.6±0.6	7.4	2.7	22.6±0.6	12.6	2.7
Q13	OO	24.6	24.7±0.5	0.7	2.1	24.6±0.7	0.2	2.9
	PR	25.1	24.4±1.0	–2.7	3.9	25.0±0.7	–0.4	2.9
	RB	25.3	23.9±1.5	–5.8	6.4	26.0±1.2	2.6	4.7
	SF	25.0	27.0±0.8	7.9	3.1	24.4±0.5	–2.2	2.0

3.3.5 Quantitation of oil compositions with similar TAG profiles

The chemical compositions of hazelnut oil are very similar to olive oil, including the major composition, i.e., TAGs, and other minor compounds, making it difficult to differentiate the two oils, and thus hazelnut oil was often used in the adulteration of olive oil[125-126]. Camellia oil is known as "eastern olive oil" because of the high abundance of oleic acid and similar FA contents to olive oil[127-128]. As shown in Figure 3-7, the similar FA contents of olive oil, camellia oil and hazelnut oil resulting in very similar MALDI-MS spectra of these three oils, making it difficult to differentiate them. Compared with olive oil and camellia oil, hazelnut oil showed some characteristic peaks at *m/z* 784.6, *m/z* 786.6, *m/z* 808.6 and *m/z* 824.6, which were assigned as phosphatidylcholine (PC) by Calvano et al.[70], and might contribute for the differentiating hazelnut oil from olive oil and camellia oil.

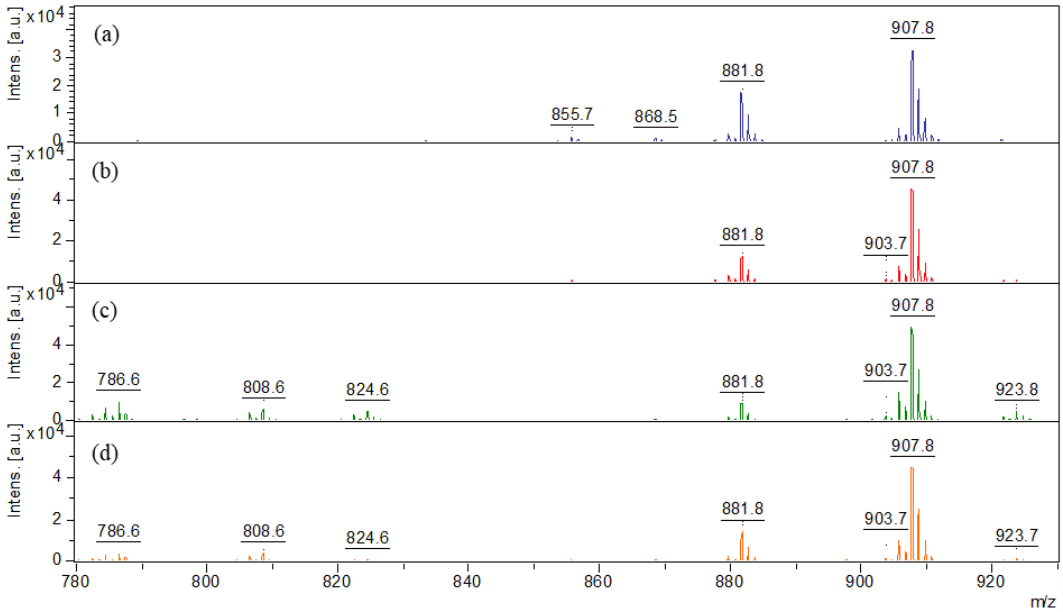

Figure 3-7. The MALDI-MS spectra for (a) 100% olive oil, (b) 100% camellia oil, (c) 100% hazelnut oil and (d) 30% olive oil – 30% camellia oil– 40% hazelnut oil blend.

To investigate the quantitative ability of PLS-R approach for oil compositions with similar MALDI-MS spectra, binary blends of olive oil and camellia oil as well as ternary blends of olive oil, camellia oil and hazelnut oil were prepared for the development of corresponding PLS-R models based on the detected PC and TAG profiles. The PLS-R model of olive oil – camellia oil blends presented powerful fitting ability and predictive ability with RMSEE, RMSEcv and RMSEP smaller than 0.034 (Table 3-17), and provided good quantitative results for testing samples with the accuracy and precision within –4.8-(–1.4)% and 2.7-8.5%, respectively, excluding the extreme accuracy (46.0%) and RSD (13.3%) belonging to the sample with low-abundance olive oil (Table 3-18).

Table 3-17. PLS-R models of olive oil (OO) – camellia oil (CM) binary blends, olive oil– camellia oil – hazelnut oil (HZ) ternary blends and olive oil – camellia oil – flaxseed oil (FS) – corn oil (CO) quaternary blends.

A	**R^2Y**	**Q^2**	**Oil species**	**Training set**			**Testing set**	
				RMSEE	RMSEcv	R^2	RMSEP	R^2
3	0.9939	0.9992	OO	0.0278	0.0306	0.9939	0.0339	0.9925
5	0.9398	0.9301	OO	0.0595	0.0658	0.9514	0.0729	0.9640
			CM	0.0876	0.0939	0.8944	0.0933	0.9510
			HZ	0.0436	0.0443	0.9738	0.0694	0.9762
8	0.9554	0.9537	OO	0.0629	0.0646	0.9244	0.1220	0.8365
			CM	0.0681	0.0698	0.9083	0.1332	0.8568
			FS	0.0203	0.0205	0.9921	0.0208	0.9950
			CO	0.0129	0.0134	0.9968	0.0164	0.9969

Table 3-18. Quantitative results of olive oil– camellia oil blends by the PLS-R model.

Actual olive oil con. (%)	**PLS-R model**		
	Measured olive oil con. (%)	Accuracy (%)	RSD (%)
7.7	11.2±1.5	46.0	13.3
29.9	28.8±2.5	–3.6	8.5
50.1	49.4±1.9	–1.4	3.8
70.2	66.8±2.7	–4.8	4.0
91.8	88.6±2.4	–3.4	2.7

For the olive oil – camellia oil – hazelnut oil blends, the PLS-R model had the best description and prediction for hazelnut oil, and slight worse performance for olive oil and the worst performance for camellia oil among the three compositions (Table 3-17), demonstrating the presence of PC peaks could distinguish hazelnut oil from olive oil and camellia oil. The quantitative results of olive oil – camellia oil – hazelnut oil blends were summarized in Table 3-19. Compared with pure samples and binary blended samples, the measured results of ternary blended samples were much worse, especially for compositions not at high levels (5% and 20%). Zoom-in models were applied for further quantitative analysis, but the improvement in measured results of low-abundance compositions was not significant and even slight deteriorations were observed from the measured results of high-abundance compositions of some samples, indicating the poor quantitative results were caused by the very similar spectral data of the three oils instead of the complex combination of multiple compositions.

Table 3-19. Quantitative results of olive oil (OO) – camellia oil (CM) – hazelnut oil (HZ) blends by the full range and zoom-in PLS-R models.

No.	Oil species	Actual con. (%)	Full range model			Zoom-in model		
			Measured con. (%)	Accuracy (%)	RSD (%)	Measured con. (%)	Accuracy (%)	RSD (%)
T1	OO	100.0	103.4±6.5	3.4	6.3	98.3±1.4	–1.7	1.4
	CM	0.0	–1.4±10.4	/	/	2.7±2.4	/	/
	HZ	0.0	–2.0±4.4	/	/	–1.0±1.3	/	/
T2	OO	0.0	–13.1±5.3	/	/	–4.3±2.5	/	/
	CM	100.0	103.5±7.8	3.5	7.5	101.3±2.7	1.3	2.6
	HZ	0.0	9.7±3.4	/	/	3.0±0.7	/	/
T3	OO	0.0	2.2±7.3	/	/	0.2±1.5	/	/
	CM	0.0	3.2±9.4	/	/	3.3±2.5	/	/
	HZ	100.0	96.3±1.7	–3.7	1.7	96.5±1.9	–3.5	1.9
T4	OO	0.0	–0.2±2.8	/	/	0.9±2.5	/	/
	CM	50.0	43.0±5.4	–13.9	12.5	42.2±2.6	–15.6	6.1
	HZ	50.0	57.2±2.7	14.3	4.8	56.9±0.7	13.8	1.3
T5	OO	51.4	51.9±7.4	1.0	14.3	50.8±3.7	–1.2	7.3
	CM	0.0	–3.7±10.9	/	/	2.0±3.2	/	/
	HZ	48.6	51.7±3.8	6.6	7.4	47.2±1.6	–2.9	3.4
T6	OO	49.8	45.8±6.0	–8.1	13.1	40.0±1.3	–19.6	3.3
	CM	50.2	51.1±8.2	1.7	16.1	57.1±0.7	13.7	1.3
	HZ	0.0	3.2±2.7	/	/	2.9±1.0	/	/
T7	OO	5.2	13.5±3.3	158.3	24.2	4.6±1.6	–10.9	34.5
	CM	4.9	–6.3±6.1	–227.2	–97.2	2.7±3.0	–45.2	109.4
	HZ	89.9	92.8±4.0	3.3	4.3	92.6±1.5	3.1	1.6
T8	OO	5.3	–0.1±2.9	–101.5	–3550.1	7.0±4.8	32.9	68.7
	CM	89.3	84.0±3.6	–5.9	4.3	77.7±4.9	–13.0	6.3
	HZ	5.4	16.1±2.2	197.1	13.7	15.3±1.4	182.2	9.4
T9	OO	89.8	95.3±2.8	6.1	2.9	89.8±1.7	0.0	1.9
	CM	5.0	–3.3±4.2	–166.4	–124.9	1.7±2.2	–66.4	128.1
	HZ	5.2	8.0±1.7	55.2	20.8	8.5±1.3	64.0	15.8
T10	OO	20.4	24.0±3.6	17.4	15.0	26.6±3.9	30.5	14.5
	CM	19.6	10.9±8.4	–44.0	76.3	10.0±2.7	–48.9	26.6
	HZ	60.0	65.1±5.3	8.4	8.1	63.4±2.4	5.6	3.8
T11	OO	20.6	18.3±5.0	–11.4	27.3	14.5±7.1	–29.8	49.1
	CM	59.4	50.4±6.6	–15.2	13.0	56.2±8.7	–5.4	15.4
	HZ	20.0	31.4±2.1	56.8	6.7	29.3±2.1	46.6	7.2
T12	OO	59.9	60.5±5.3	0.9	8.7	64.5±1.1	7.7	1.7
	CM	20.2	16.3±5.6	–19.4	34.1	10.1±1.7	–49.8	16.4
	HZ	19.9	23.3±1.7	16.9	7.5	25.4±1.1	27.5	4.4

A PLS-R model was established for the determination of olive oil and camellia oil from quaternary blends of olive oil, camellia oil, flaxseed oil and corn oil. The latter two oil compositions showed very different TAG patterns with olive oil and camellia oil (Figure 3-8). The established model had larger RMSEP for olive oil and camellia oil, that were 0.1220 and 0.1332, respectively (Table 3-17), indicating the poorer quantitative abilities of the model for these two compositions. The quantitative results of testing samples measured by the PLS-R model presented poor accuracy and precision for olive oil and camellia oil, which varied within –37.9-107.0% and 7.5-72.1%, respectively (Table 3-20), while for flaxseed oil and corn oil, much better quantitative results were obtained with the accuracy and precision in the range of –7.5-9.1% and 1.0-13.1%, respectively, excluding an extreme RSD at 23.6%.

Unlike the PLS-R model of olive oil – camellia oil blends, the PLS-R model of quaternary blends of olive oil, camellia oil, flaxseed oil and corn oil could not differentiate olive oil and camellia oil for accurate quantitation due to their similar TAG patterns, while showed powerful quantitative ability for flaxseed oil and corn oil. If olive oil and camellia oil were regarded as one oil composition, significant improvement was observed in the quantitative results of the olive-camellia combined composition as the ranges of accuracy and precision were narrowed to –6.6-5.0% and 0.6-6.6%, respectively (Table 3-20). Therefore, for the quantitative analysis of olive oil, camellia oil, flaxseed oil and corn oil blends based on PLS-R model, it was

recommended to treat olive oil and camellia oil as one oil composition for more accurate and reliable results.

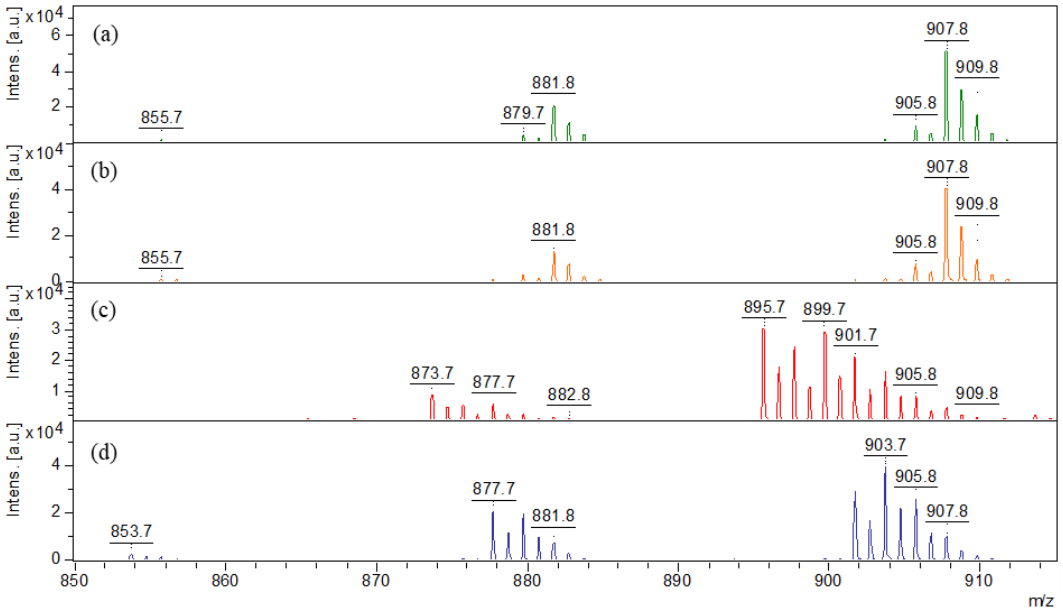

Figure 3-8. The TAG region of the MALDI-MS spectra for (a) 100% olive oil, (b) 100% camellia oil, (c) 100% flaxseed oil and (d) 100% corn oil.

Table 3-20. Quantitative results of olive oil (OO) – camellia oil (CM) – flaxseed oil (FS) – corn oil (CO) blends by the PLS-R model.

No.	Oil species	Actual con. (%)	Single composition			Olive-camellia combined composition		
			Measured con. (%)	Accuracy (%)	RSD (%)	Measured con. (%)	Accuracy (%)	RSD (%)
Q1	OO	100.0	88.5±6.6	–11.5	7.5	98.1±0.6	–1.9	0.6
	CM	0.0	9.6±6.5	/	/			
	FS	0.0	1.9±0.7	/	/	/	/	/
	CO	0.0	0.0±0.6	/	/	/	/	/
Q2	OO	0.0	32.7±13.4	/	/	94.8±0.6	–5.2	0.7
	CM	100.0	62.1±12.9	–37.9	20.7			
	FS	0.0	2.4±1.1	/	/	/	/	/
	CO	0.0	2.8±0.5	/	/	/	/	/
Q3	OO	0.0	6.8±2.2	/	/	0.6±0.5	/	/
	CM	0.0	–6.1±1.8	/	/			
	FS	100.0	103.0±1.0	3.0	1.0	/	/	/
	CO	0.0	–3.4±0.5	/	/	/	/	/
Q4	OO	0.0	–1.3±2.8	/	/	1.3±1.7	/	/
	CM	0.0	2.6±2.5	/	/			
	FS	0.0	0.6±3.1	/	/	/	/	/
	CO	100.0	98.0±1.6	–2.0	1.6	/	/	/
Q5	OO	10.3	12.1±2.8	18.1	23.4	19.5±0.9	–4.2	4.5
	CM	10.1	7.4±3.5	–26.8	47.3			
	FS	10.5	10.4±2.5	–0.6	23.6	/	/	/
	CO	69.1	69.7±1.5	0.9	2.2	/	/	/
Q6	OO	9.9	10.2±2.5	2.3	24.6	19.7±1.3	–2.0	6.6
	CM	10.2	9.6±3.0	–6.2	31.8			
	FS	69.3	69.8±1.3	0.8	1.9	/	/	/
	CO	10.6	10.3±0.4	–2.4	4.4	/	/	/
Q7	OO	10.7	22.2±5.1	107.0	22.9	79.7±1.1	0.1	1.4
	CM	68.9	57.5±5.6	–16.6	9.7			
	FS	9.7	9.0±0.9	–7.5	9.5	/	/	/
	CO	10.6	11.3±0.6	6.5	4.9	/	/	/
Q8	OO	69.9	68.2±8.2	–2.4	12.1	80.5±1.3	0.5	1.6
	CM	10.2	12.3±8.9	20.2	72.1			
	FS	9.9	9.4±1.2	–4.8	13.1	/	/	/
	CO	10.0	10.4±0.6	4.3	6.1	/	/	/

(To be continued)

Table 3-20-*continued*. Quantitative results of olive oil (OO) – camellia oil (CM) – flaxseed oil (FS) – corn oil (CO) blends by the PLS-R model.

No.	Oil species	Actual con. (%)	Single composition			Olive-camellia combined composition		
			Measured con. (%)	Accuracy (%)	RSD (%)	Measured con. (%)	Accuracy (%)	RSD (%)
Q9	OO	18.5	17.5±7.0	–5.4	40.2	38.7±1.1	0.6	2.7
	CM	20.0	21.2±7.5	6.1	35.5			
	FS	19.2	19.5±0.4	1.8	2.0	/	/	/
	CO	42.3	42.0±1.1	–0.8	2.7	/	/	/
Q10	OO	19.9	21.2±3.5	6.6	16.6	41.7±0.7	5.0	1.8
	CM	19.8	20.5±3.6	3.4	17.4			
	FS	40.0	37.3±0.6	–6.8	1.5	/	/	/
	CO	20.2	21.0±0.5	3.7	2.3	/	/	/
Q11	OO	20.3	26.6±4.3	30.9	16.3	60.5±1.2	0.9	2.0
	CM	39.7	34.0±4.0	–14.4	11.8			
	FS	19.9	19.0±1.9	–4.4	9.8	/	/	/
	CO	20.1	20.4±1.1	1.6	5.5	/	/	/
Q12	OO	39.8	37.5±5.6	–5.7	14.9	59.8±1.3	–0.5	2.2
	CM	20.3	22.9±7.3	13.2	31.8			
	FS	20.0	21.1±1.0	5.3	4.8	/	/	/
	CO	19.9	19.2±0.9	–3.4	4.8	/	/	/
Q13	OO	25.0	25.4±3.5	1.6	13.6	46.5±0.4	–6.6	0.9
	CM	24.8	21.4±3.7	–14.0	17.3			
	FS	25.1	27.4±0.7	9.1	2.7	/	/	/
	CO	25.0	25.8±0.8	3.1	3.1	/	/	/

The results discussed above demonstrated that for oil compositions with similar TAG profiles, the PLS-R approach showed good quantitative performance to olive oil and camellia oil in their binary blends, which became poor when one more oil composition with similar TAG profile, i.e., hazelnut oil, was added into the blends. Moreover, when the blends of olive oil and camellia oil were further mixed with other oil compositions that had distinct TAG profiles, the accurate quantitation of olive oil and camellia oil

became challenging, while excellent results were obtained for the oil compositions with different profiles. Therefore, it was speculated that the poor quantitative ability for compositions with similar TAG profiles presented by the PLS-R models of multi-composition blended oils might be improved by extracting the information of similar compositions from that of the whole blends and establishing the corresponding new models, which still needs further investigation.

3.3.6 Comparison of GC-FID- and MALDI-MS-based quantitative methods

3.3.6.1 *Quantitation for low-abundance compositions in blended oils*

The GC-FID method is a conventional method for authentication of edible oils based on the FA contents[16, 115]. To compare the performance of GC-FID and MALDI-MS for quantitation of oil compositions in blended oils, both methods were utilized to analyze the same batch blended oil samples, i.e., olive oil – sunflower seed oil blends, and two PLS-R models were developed based on the obtained chromatograms (Figure 3-9) and mass spectra, respectively, with the detailed information shown in Table 3-21.

The quantitative results of testing samples provided by both models were summarized in Table 3-22. For samples containing olive oil higher than 30%, both GC-FID- and MALDI-MS-based models provided good quantitative results that were very close to the actual concentrations, with the accuracy in the range of –1.0-7.8% and –4.1-0.7%, respectively. For the sample containing 7.4% olive oil, the measured result derived from

the MALDI-MS spectra was more accurate than that derived from the GC-FID chromatograms, with the accuracy as 7.7% and 79.5%, respectively, leading to a smaller RMSEP of the MALDI-MS-based model (Table 3-21). On the other hand, the quantitative results predicted by the GC-FID-based model showed better precision than those provided by the MALDI-MS-based model, although more replicate measurements were performed by the MALDI-MS approach. However, the precision of the results predicted by the MALDI-MS-based model was still pretty good, which was in the range of 0.5-9.2%, suggesting that MALDI-MS could provide quantitative results that were comparable to those of GC-FID.

Table 3-21. PLS-R models of olive oil – sunflower seed oil blends based on GC-FID chromatograms and MALDI-MS spectra.

Method	**A**	**R^2Y**	**Q^2**	**Training set**			**Testing set**	
				RMSEE	RMSEcv	R^2	RMSEP	R^2
GC	4	0.9994	0.9985	0.0095	0.0134	0.9994	0.0297	0.9993
MALDI	3	0.9995	0.9993	0.0078	0.0095	0.9995	0.0093	0.9991

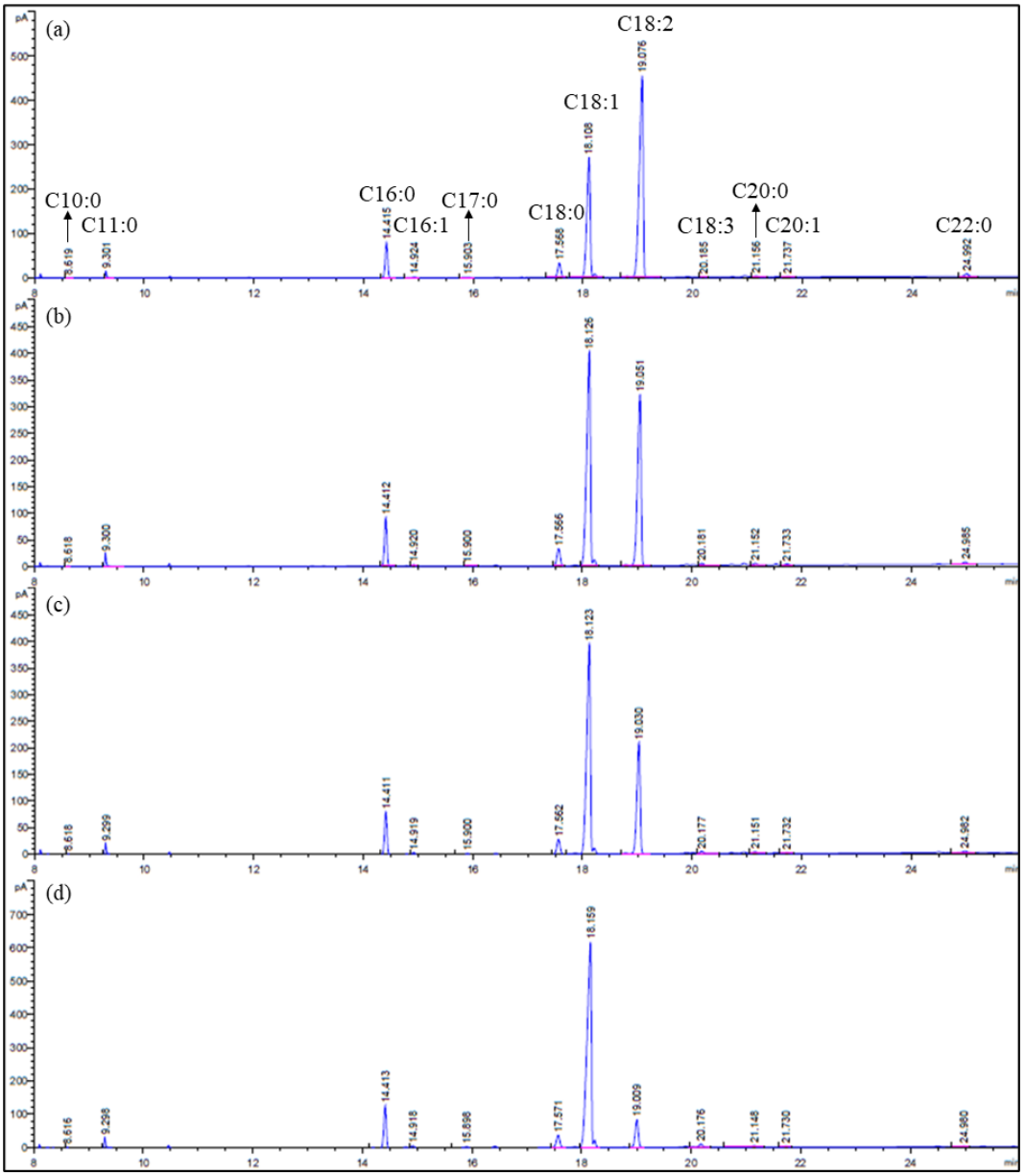

Figure 3-9. GC-FID chromatograms of (a) 100% sunflower seed oil, (b) 40% olive oil – 60% sunflower seed oil blend, (c) 60% olive oil – 40% sunflower seed oil blend, and (d) 100% olive oil.

Table 3-22. Quantitative results of olive oil – sunflower seed oil blends measured by PLS-R models based on the GC-FID chromatograms and MALDI-MS spectra

Actual olive oil con. (%)	**GC-FID**			**MALDI-MS**		
	Measured olive oil con. (%)	Accuracy (%)	RSD (%)	Measured olive oil con. (%)	Accuracy (%)	RSD (%)
1.0	0.1±0.8	–93.5	1173.3	0.8±0.8	–20.0	95.5
3.2	5.0±0.1	56.8	1.7	2.8±0.6	–13.8	20.5
5.2	8.1±0.7	56.4	8.5	5.4±2.1	3.8	38.8
7.4	13.3±0.4	79.5	2.8	8.0±0.7	7.7	9.2
30.1	32.4±0.6	7.8	1.8	28.9±0.8	–4.1	2.8
49.9	51.5±0.5	3.1	1.0	49.7±0.6	–0.5	1.2
69.9	70.4±0.6	0.6	0.8	70.4±0.7	0.7	0.9
92.3	91.3±0.1	–1.0	0.1	92.7±0.5	0.5	0.5

Table 3-23. Cosine similarity scores of GC-FID chromatograms and MALDI-MS spectra between selected olive oil – sunflower seed oil blended samples.

Reference sample	**Method**	**Cosine similarity score**			
		1.0% olive oil	3.2% olive oil	5.2% olive oil	19.9% olive oil
0% olive oil	GC	0.9999	0.9991	0.9979	0.9779
	MALDI	0.9999	0.9990	0.9980	0.9669
19.9% olive oil	GC	0.9807	0.9860	0.9893	/
	MALDI	0.9695	0.9765	0.9805	/

To further compare the performance of GC-FID and MALDI-MS in the quantitative analysis of low-abundance oil compositions, three blended oil samples with only 1.0%, 2.2% and 3.2% olive oil were prepared and analyzed by both methods. For the three samples, the results measured by the MALDI-MS-based model were more accurate than those measured by the GC-FID-based model, which were 0.8%, 2.8% and 5.4%,

and 0.1%, 5.0% and 8.1%, respectively (Table 3-22), illustrating the stronger capability of MALDI-MS for the quantitative analysis of low-abundance oil compositions. This was believed to be due to the fact that MALDI-MS directly analyzed the TAG profiles of oil samples while GC-FID detected the FA contents converted from the TAGs. For pure olive oil and pure sunflower seed oil, the cosine similarity score of their MALDI-MS spectra was 0.3068, while the similarity score of their GC-FID chromatograms was 0.5468, indicating the variation of the chromatograms was smaller than that of the MS spectra, which was caused by the conversion of TAGs to FAs. Regarding the training sample with 0% olive oil as reference sample, the similarity scores between the MALDI-MS spectra of the blended samples with olive oil at extremely low levels and the reference sample were smaller than the similarity scores between the corresponding GC-FID chromatograms (Table 3-23). Regarding the training sample containing 20% olive oil as reference sample, more obvious differences were observed between the similarity scores of the MALDI-MS spectra and the GC-FID chromatograms, with the scores between the samples containing 1.0%, 3.2% and 5.2% olive oil and the reference sample containing 20% olive oil as 0.9695, 0.9765 and 0.9805, and 0.9807, 0.9860 and 0.9893, respectively (Table 3-23), illustrating larger differences between the MALDI-MS spectra. Therefore, compared with the GC-FID method, the MALDI-MS technique is more sensitive to slight changes in oil composition of blended oils and thus is more suitable for quantifying low-abundance oil compositions.

3.3.6.2 *Quantitative analysis of blended oils with similar FA contents*

Optimal PLS-R models of sunflower seed oil – canola oil – grapeseed oil blends were also established based on the GC-FID chromatograms and MALDI-MS spectra. For canola oil, both models showed similar fitting ability and quantitative ability, while for sunflower seed oil and grapeseed oil, the RMSEE, RMSEcv and RMSEP of the MALDI-MS-based model were smaller than those of the GC-FID-based model, revealing the better fitting ability and quantitative ability of the MALDI-MS-based models (Table 3-24). It was noticed that some training samples used to establish PLS-R models had quite similar GC-FID chromatograms although their oil compositions were very different. For the sunflower seed oil – canola oil – grapeseed oil blends with blending ratios at 0%:20%:80%, 20%:20%:60%, 50%:10%:40% and 90%:0%:10%, cosine similarity scores between any two MALDI-MS spectra were lower than those of the corresponding GC-FID chromatograms, which were in the range of 0.9800-0.9980 and 0.9983-0.9997, respectively (Table 3-25). The GC-FID chromatograms of the four samples were more similar to each other than the MALDI-MS spectra, making the differentiation of these samples more challenging by GC-FID compared with MALDI-MS, and resulting in the poorer quantitative ability for the GC-FID-based model.

Table 3-24. PLS-R models of sunflower seed oil (SF) – canola oil (CA) – grapeseed oil (GP) blends based on GC-FID chromatograms and MALDI-MS spectra.

Method	A	R^2Y	Q^2	Oil species	Training set			Testing set	
					RMSEE	RMSEcv	R^2	RMSEP	R^2
GC	3	0.9652	0.9638	SF	0.0622	0.0629	0.9472	0.0888	0.9669
				CA	0.0244	0.0250	0.9916	0.0272	0.9938
				GP	0.0555	0.0555	0.9567	0.0957	0.9572
MALDI	6	0.9832	0.9825	SF	0.0444	0.0450	0.9732	0.0524	0.9832
				CA	0.0212	0.0215	0.9938	0.0228	0.9967
				GP	0.0359	0.0362	0.9825	0.0381	0.9900

Table 3-25. Cosine similarity scores of GC-FID chromatograms and MALDI-MS spectra between selected sunflower seed oil (SF) – canola oil (CA) – grapeseed oil (GP) blended samples.

Method	SF:CA:GP (%)	Cosine similarity score			
		0:20:80	20:20:60	50:10:40	90:0:10
GC-FID chromatograms	0:20:80	1	0.9983	0.9993	0.9996
	20:20:60	/	1	0.9997	0.9986
	50:10:40	/	/	1	0.9996
	90:0:10	/	/	/	1
MALDI-MS spectra	0:20:80	1	0.9980	0.9922	0.9800
	20:20:60	/	1	0.9977	0.9888
	50:10:40	/	/	1	0.9956
	90:0:10	/	/	/	1

Table 3-26 showed the quantitative results of four sunflower seed oil – canola oil – grapeseed oil blended samples measured by the GC-FID- and MALDI-MS-based models. For samples T3 and T4, the two models measured comparable quantitative

results, while for samples T1 and T2, the MALDI-MS-based model provided much better quantitative results than the GC-FID-based model. Sunflower seed oil was not added into sample T1, but the GC-FID-based model measured it contained 16.3% sunflower seed oil. For sample T2, the measured concentration of sunflower seed oil provided by the GC-FID-based model was higher than its actual concentration with RSD up to 38.0%. As shown in Figure 3-10, among the 10 FAMEs detected by GC-FID, only docosanoic acid (C22:0) methyl ester had the VIP value higher than 2 in the PLS-R model, while the VIP values of other FAMEs were lower than 1, revealing the quantitative analysis by the GC-FID-based model was mainly determined by the abundance of docosanoic acid. However, docosanoic acid was very deficient ($\leqslant$0.7%) in the pure sunflower seed oil, pure canola oil and pure grapeseed oil (Table 3-27), making the quantitative results based on GC-FID susceptible to random errors. On the other hand, the MALDI-MS-based model had five variables whose VIP values were higher 1, i.e., *m/z* 904.7, *m/z* 903.7, *m/z* 905.8, *m/z* 853.6 and *m/z* 906.7, and four of them were the major peaks shown in the MALDI-MS spectra (Figure 3-11), which could diminish the influence of random errors and thus provide better differentiation of oil compositions. Compared with GC-FID, the conversion of TAGs to FAs was not required by MALDI-MS, making the quantitative analysis not only simpler but also more accurate due to the maintenance of the delicate TAGs for more sensitive differentiation of complicated oil compositions.

Table 3-26. Quantitative results of sunflower seed oil (SF) – canola oil (CA) – grapeseed oil (GP) ternary blends by PLS-R models based on the GC-FID chromatograms and MALDI-MS spectra.

No.	Oil species	Actual con. (%)	GC-FID			MALDI-MS		
			Measured con. (%)	Accuracy (%)	RSD (%)	Measured con. (%)	Accuracy (%)	RSD (%)
T1	SF	0.0	16.3±9.9	/	/	–0.6±3.2	/	/
	CA	23.0	22.4±1.1	–2.3	5.1	23.4±1.4	1.6	5.8
	GP	77.0	61.2±9.1	–20.5	14.8	77.2±3.3	0.2	4.2
T2	SF	20.0	25.9±9.9	29.6	38.0	18.5±2.5	–7.6	13.5
	CA	18.0	19.4±2.4	8.1	12.5	18.0±0.8	–0.2	4.4
	GP	62.0	54.6±7.5	–11.9	13.8	63.6±2.4	2.5	3.8
T3	SF	50.0	52.4±0.8	4.8	1.6	47.1±2.2	–5.7	4.6
	CA	13.1	14.1±1.4	8.3	9.6	13.5±0.9	3.6	6.8
	GP	37.0	33.5±0.8	–9.4	2.5	39.4±2.5	6.4	6.2
T4	SF	89.8	89.7±1.6	–0.2	1.8	86.8±3.0	–3.4	3.5
	CA	4.1	5.2±1.7	25.8	33.3	5.1±1.5	24.1	29.4
	GP	6.0	5.1±2.6	–15.2	50.8	8.1±2.6	34.0	32.4

Table 3-27. Fatty acid compositions of sunflower seed oil, canola oil and grapeseed oil as determined by GC-FID.

Fatty acid	Sunflower seed oil	Canola oil	Grapeseed oil
C16:0	6.4%	4.4%	7.0%
C16:1	0.1%	0.2%	0.1%
C18:0	3.3%	1.9%	3.9%
C18:1	29.8%	61.9%	20.5%
C18:2	59.3%	20.7%	68.0%
C18:3, n6	0.0%	0.3%	0.0%
C18:3, n3	0.1%	8.3%	0.3%
C20:0	0.2%	0.6%	0.2%
C20:1	0.1%	1.3%	0.2%
C22:0	0.7%	0.3%	0.0%
C22:1	0.0%	0.2%	0.0%

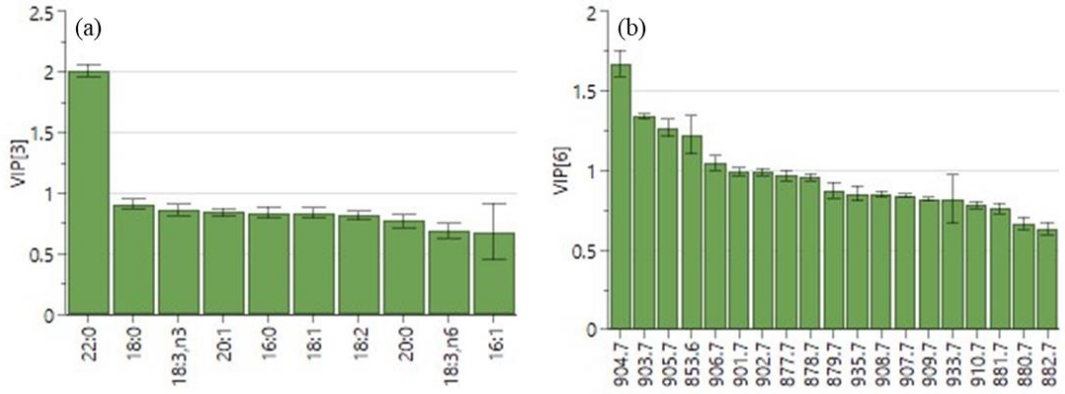

Figure 3-10. VIP values of PLS-R models based on (a) the GC-FID chromatograms and (b) the MALDI-MS spectra of sunflower seed oil – canola oil – grapeseed oil blends.

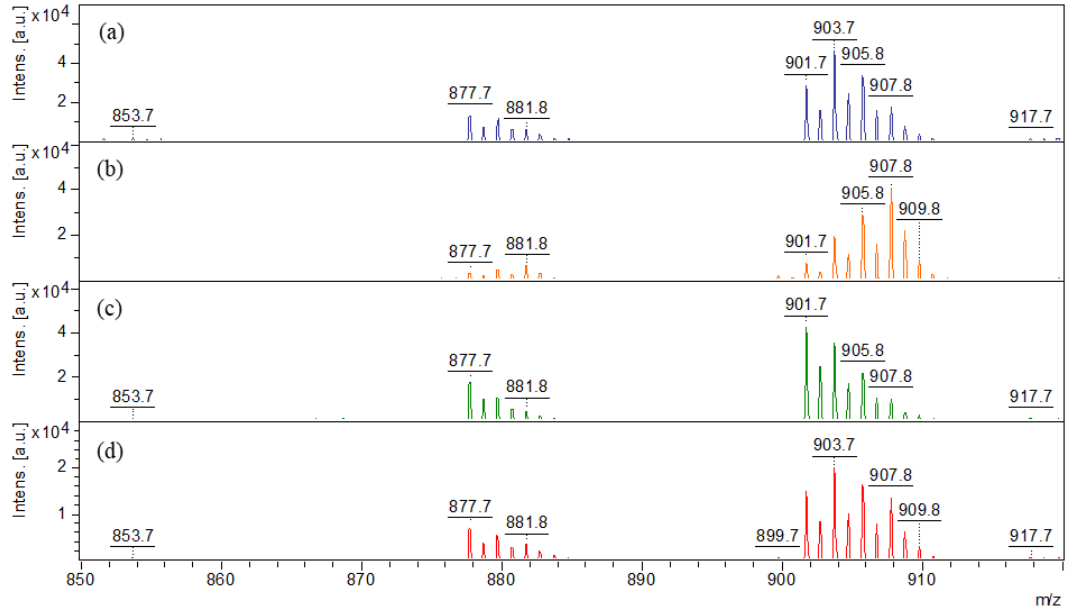

Figure 3-11. The TAG region of the MALDI-MS spectra for (a) 100% sunflower seed oil, (b) 100% canola oil, (c) 100% grapeseed oil and (d) 30% sunflower seed oil – 30% canola oil– 40% grapeseed oil blend.

3.3.7 Quantitative analysis of commercial blended oil products

To apply the MALDI-MS-based PLS-R approach for the quantitative analysis of commercial blended oil products, 11 commercial blended oil products involving 8 different types were collect from the market, i.e., olive oil – sunflower seed oil blends, olive oil – canola oil blend, olive oil – corn oil blend, olive oil – high oleic peanut oil blend, olive oil – perilla oil blend, olive oil – soybean oil – sunflower seed oil blend, olive oil – flaxseed oil – sunflower seed oil blend, and olive oil – camellia oil – flaxseed oil– corn oil blend. Our previous study demonstrated that specific oil products of the same brands showed highly similar TAG profiles[59]. To minimize the variation in oil products from different manufacturers, pure oil products of the same brands as the commercial blended oil products were used to prepare blended oil samples, and these samples were analyzed by MALDI-MS to develop PLS-R models for quantitative analysis (Table 3-28).

For a binary blended oil product, the concentration of olive oil, the composition of major concern, was quantified by the established PLS-R model as the concentration of the other composition could be easily derived. Among the eight binary blended oil products, i.e., products 1-8, the measured results of products 4, 5, 7 and 8 were close to the labeled concentrations with relative errors within ±12%, while for the remaining four products, i.e., products 1, 2, 3 and 6, the relative errors between the measured results and labeled concentrations exceeded ±20%.

Table 3-28. PLS-R models for determination of oil compositions of commercial blended oil products.

Model	Type[a]	A	R^2Y	Q^2	Oil species	Training set			Testing set	
						RMSEE	RMSEcv	R^2	RMSEP	R^2
M1	OO-SF	2	0.9972	0.9966	OO	0.0185	0.0197	0.9972	0.0167	0.9968
M2	OO-SF	2	0.9979	0.9975	OO	0.0161	0.0169	0.9979	0.0139	0.9990
M3	OO-CA	4	0.9985	0.9947	OO	0.0140	0.0261	0.9985	0.0170	0.9969
M4	OO-CO	3	0.9981	0.9971	OO	0.0156	0.0180	0.9981	0.0108	0.9987
M5	OO-CO	3	0.9984	0.9979	OO	0.0144	0.0149	0.9984	0.0107	0.9988
M6	OO-PA	4	0.9967	0.9945	OO	0.0205	0.0249	0.9967	0.0294	0.9899
M7	OO-PR	4	0.9973	0.9953	OO	0.0187	0.0245	0.9973	0.0152	0.9974
M8	OO-FS-SF	5	0.9985	0.9933	OO	0.0170	0.0188	0.9961	0.0202	0.9970
					FS	0.0251	0.0266	0.9914	0.0265	0.9966
					SF	0.0224	0.0243	0.9931	0.0262	0.9955
M9	OO-SO-SF	5	0.9900	0.9896	OO	0.0131	0.0133	0.9977	0.0146	0.9986
					SO	0.0317	0.0320	0.9861	0.0589	0.9844
					SF	0.0315	0.0321	0.9863	0.0578	0.9761
M10	OO-CM-FS-CO	8	0.9554	0.9537	OO	0.0629	0.0646	0.9244	0.1220	0.8365
					CM	0.0681	0.0698	0.9083	0.1332	0.8568
					FS	0.0203	0.0205	0.9921	0.0208	0.9950
					CO	0.0129	0.0134	0.9968	0.0164	0.9969

[a] OO: olive oil, SF: sunflower seed oil, CA: canola oil, CO: corn oil, PA: high oleic acid peanut oil, PR: perilla oil, FS: flaxseed oil, SO: soybean oil, FS: flaxseed oil, CM: camellia oil.

Table 3-29. Quantitative results of commercial blended oil products.

No.	Type[a]	Model	Oil species	Labeled con. (%)	Model range	Measured con. (%)	Relative error (%)	RSD (%)
1	OO-SF	M1	OO	5	Full	6.9±1.2	38.0	16.9
2	OO-SF	M2	OO	10	Full	3.5±0.5	–65.0	15.3
3	OO-SF		OO	20	Full	12.9±0.3	–35.5	2.5
4	OO-CA	M3	OO	10	Full	9.4±1.7	–6.0	18.2
5	OO-CO	M4	OO	6	Full	6.5±0.6	8.3	9.6
6	OO-CO	M5	OO	20	Full	15.6±1.8	–22.0	11.8
7	OO-PA	M6	OO	50	Full	45.0±3.0	–10.0	6.7
8	OO-PR	M7	OO	50	Full	55.6±1.2	11.2	2.1
9	OO-FS-SF	M8	OO	11	Full	4.0±0.5	–63.6	12.3
					Zoom-in	2.8±0.4	–74.5	16.3
			FS	6	Full	10.8±0.4	80.0	4.2
					Zoom-in	6.2±0.6	3.3	9.4
			SF	83	Full	85.3±0.4	2.8	0.4
					Zoom-in	91.1±0.6	9.8	0.7
10	OO-SO-SF	M9	OO	18	Full	4.3±0.4	–76.1	9.2
					Zoom-in	6.8±0.9	–62.2	13.2
			SO	15	Full	85.9±1.0	472.7	1.2
					Zoom-in	84.4±1.9	462.7	2.2
			SF	67	Full	9.8±1.2	–85.4	12.3
					Zoom-in	9.5±0.9	–85.8	9.6
11	OO-CM-FS-CO	M10	OO	6	Full	2.6±3.0	–56.7	114.0
					Zoom-in	–1.5±2.6	/	/
			CM	11	Full	1.6±1.7	–85.5	104.2
					Zoom-in	5.9±2.6	–46.4	44.7
			FS	5	Full	2.9±1.3	–42.0	43.7
					Zoom-in	0.2±0.4	–96.0	229.6
			CO	78	Full	93.8±1.1	20.3	1.2
					Zoom-in	95.4±0.4	22.3	0.4

For ternary and quaternary blended oil products (products 9-11), concentrations of all the oil compositions were measured, and both full range models and zoom-in models were utilized for quantitative analysis since their labels indicated that some oil compositions were dominant in these products. The label of product 9 stated it contained 11% olive oil, 6% flaxseed oil and 83% sunflower seed oil, which were measured as 4.0%, 10.8% and 85.3%, respectively, by the full range model, and 2.8%, 6.2% and 91.1%, respectively, by the zoom-in model. Both full range model and zoom-in model detected sunflower seed oil as the major composition of the product, which was consistent with the label, but the measured results of olive oil were lower than the labeled concentration.

According to the label of product 10, this product was a blend of 18% olive oil, 15% soybean oil and 67% sunflower seed oil, while both the full range model and zoom-in model measured soybean oil as the most abundant composition (>80%) of the product, followed by sunflower seed oil, and then olive oil. Comparing the MALDI-MS spectra of the pure oils, the prepared 10% olive oil – 10% soybean oil – 80% sunflower seed oil blend and the product 10 (Figure 3-12), it was noticed that the spectrum of the product 10 was more similar to the spectrum of the pure soybean oil, rather than the spectrum of the 10% olive oil – 10% soybean oil – 80% sunflower seed oil blend. The spectra of the product 10 and the pure soybean oil showed the characteristic peak of soybean oil at *m/z* 899.7, which was much higher than that of the 10% olive oil – 10%

soybean oil – 80% sunflower seed oil blend. Hence, it could be concluded that product 10 was mislabeled and soybean oil should be the dominant composition of the product instead of the labeled sunflower seed oil.

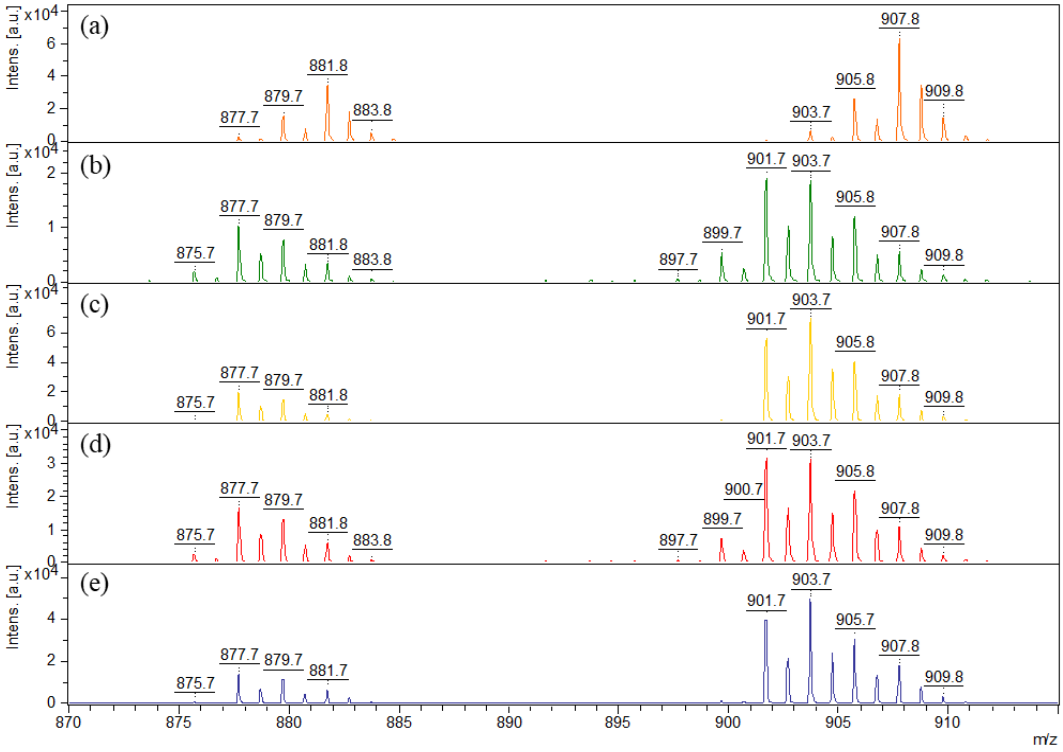

Figure 3-12. The TAG region of the MALDI-MS spectra for (a) 100% olive oil, (b) 100% soybean oil, (c) 100% sunflower seed oil, (d) commercial product 10 and (e) 10% olive oil – 10% soybean oil – 80% sunflower seed oil blend.

As we discussed in Section 3.3.5, for the quantitative analysis of quaternary blends of olive oil, camellia oil, flaxseed oil and corn oil, it was suggested to consider olive oil and camellia oil as one oil composition due to their similar TAG profiles. Product 11 was labeled to contain 78% corn oil, 5% flaxseed oil, and a total of 17% for olive oil and camellia oil, which were measured as 93.8%, 2.9% and 4.2%, respectively, by the

full range model, and 95.4%, 0.2% and 4.4%, respectively, by the zoom-in model (Table 3-29). In the MALDI-MS spectrum of product 11, the characteristic peaks of flaxseed oil at *m/z* 895.7 and *m/z* 899.7 could not be observed, and *m/z* 907.8, the most abundant peak of both olive oil and camellia oil, was significantly lower than the one for the manually prepared 10% olive oil – 10% camellia oil – 10% flaxseed oil – 70% corn oil blend (Figure 3-13). In addition, the characteristic peaks of flaxseed oil at high mass region[59, 129-130], such as *m/z* 1062.6 and *m/z* 1096.6, were very weak in the spectrum of product 11, revealing no or very little flaxseed oil in product 11 (Figure 3-14). According to the quantitative results and comparison of MALDI-MS spectra, it was concluded that product 11 was mislabeled and the concentration of flaxseed oil and the total concentration of olive oil and camellia oil should be lower than the labeled ones.

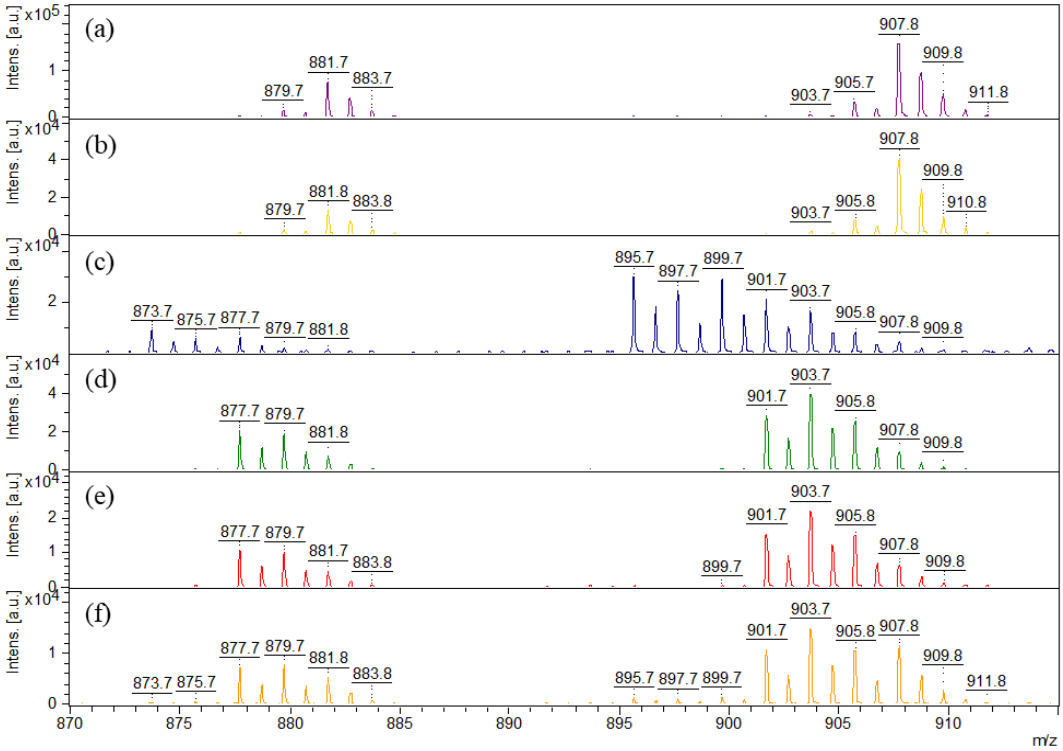

Figure 3-13. The TAG regions of the MALDI-MS spectra for (a) 100% olive oil, (b) 100% camellia oil, (c) 100% flaxseed oil, (d) 100% corn oil, (e) commercial product 11 and (f) 10% olive oil – 10% camellia oil – 10% flaxseed oil – 70% corn oil blend.

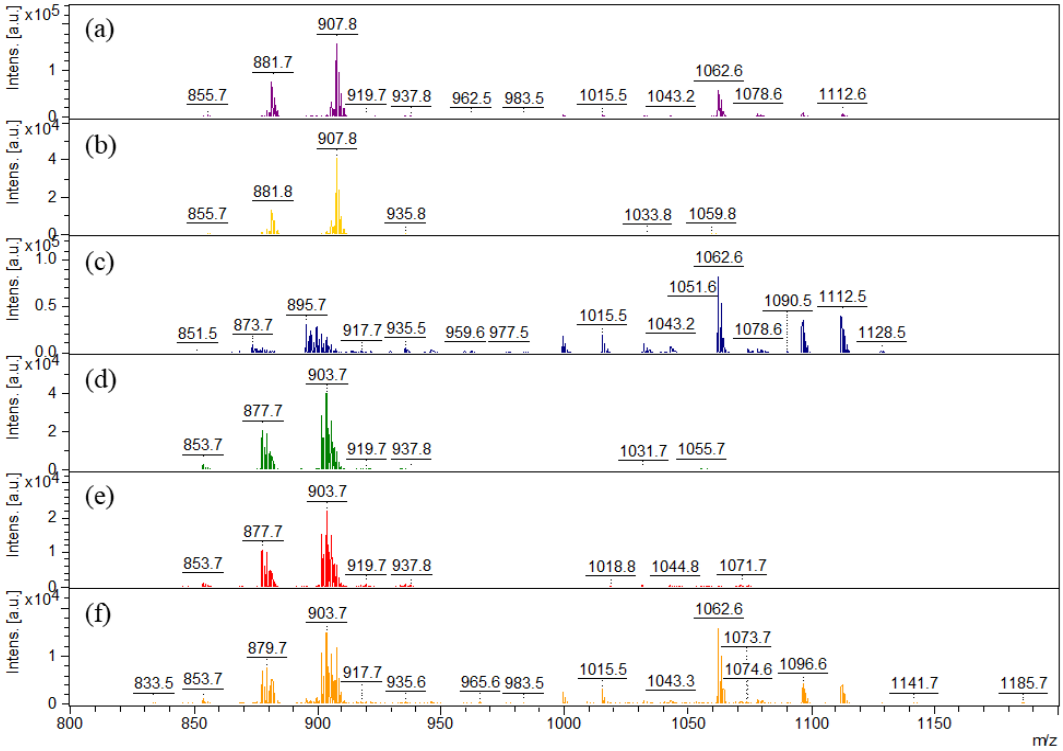

Figure 3-14. The high *m/z* region of the MALDI-MS spectra for (a) 100% olive oil, (b) 100% camellia oil, (c) 100% flaxseed oil, (d) 100% corn oil, (e) commercial product 11 and (f) 10% olive oil – 10% camellia oil – 10% flaxseed oil – 70% corn oil blend.

3.3.8 Comparison of intensity ratio- and PLS-R-based quantitative methods

To compare the quantitative ability of the intensity ratio-based method and PLS-R-based method, the quantitative analysis of three types of ternary blended oils (i.e., peanut oil – corn oil – canola oil blends, sunflower seed oil – camellia oil – canola oil blends and olive oil – corn oil – sunflower seed oil blends) were performed following the protocols of both methods. Details of the calibration models based on the intensity ratio were described in Section 2.3.2.2 (Table 2-26 and Table 2-29), and details of the PLS-R models were shown in Table 3-30. Same batches of blended oil samples were analyzed by corresponding NLS model and PLS model, and the PLS-R models

provided better quantitative results (smaller RMSE) than the NLS models (Table 3-31), indicating the PLS-R-based method is more powerful for accurate quantitative measurement than the intensity ratio-based method.

Table 3-30. PLS-R models of peanut oil (PA) – corn oil (CO) – canola oil (CA) blends, sunflower seed oil (SF) – camellia oil (CM) – canola oil (CA) blends and olive oil (OO) – corn oil (CO) – sunflower seed oil (SF) blends.

Type	**A**	**R^2Y**	**Q^2**	**Oil species**	**Training set**			**Testing set**	
					RMSEE	RMSEcv	R^2	RMSEP	R^2
PA-CO-CA	5	0.9880	0.9870	PA	0.0361	0.0378	0.9824	0.0368	0.9904
				CO	0.0200	0.0214	0.9945	0.0253	0.9949
				CA	0.0298	0.0313	0.9880	0.0313	0.9921
SF-CM-CA	6	0.9920	0.9890	SF	0.0214	0.0262	0.9937	0.0267	0.9948
				CM	0.0221	0.0237	0.9933	0.0299	0.9936
				CA	0.0296	0.0359	0.9880	0.0366	0.9936
OO-CO-SF	6	0.9890	0.9860	OO	0.0146	0.0161	0.9971	0.0149	0.9981
				CO	0.0334	0.0384	0.9848	0.0500	0.9803
				SF	0.0330	0.0373	0.9852	0.0507	0.9800

Table 3-31. Quantitative results of ternary blended oils provided by the NLS models and the PLS-R models.

Type	Oil species	RMSE	
		NLS model	PLS-R model
Peanut oil – corn oil – canola oil blends	Peanut oil	0.0666	0.0368
	Corn oil	0.0322	0.0253
	Canola oil	0.0588	0.0313
Sunflower seed oil – camellia oil – canola oil blends	Sunflower seed oil	0.0172	0.0149
	Camellia oil	0.0946	0.0500
	Canola oil	0.0967	0.0507
Olive oil – corn oil – sunflower seed oil blends	Olive oil	0.0403	0.0267
	Corn oil	0.0629	0.0299
	Sunflower seed oil	0.0391	0.0366

3.4 Conclusion

In this study, a rapid and efficient method for quantitation of oil compositions in blended oils has been developed using MALDI-MS and PLS-R. The developed method showed excellent quantitative ability and could detect the presence of trace-level olive oil (down to 1.5%) in sunflower seed oil. The results obtained for multi-composition blended oils, including the ternary and quaternary blended oils, demonstrated that the developed method could be applied for simultaneous measurements of various oil compositions, and a zoom-in strategy was proposed to improve the quantitative ability of low-abundance oil compositions. The established method was applied for quantitative analysis of various types of commercial blended oil products, and the results indicated that some commercial products were mislabeled. Compared with the

conventional GC-FID approach, the MALDI-MS approach provided comparable quantitative results but required minimal sample pretreatment and was more efficient in the sample analysis. In addition, the MALDI-MS method was advantageous for the quantitation of low-abundance compositions as the variation of TAG profiles was more noticeable than that of FA profiles. Complicated oil compositions in blended oils might bring about similar FA profiles, which were difficult to be quantified by the GC-FID approach, but could also be accurately measured by the MALDI-MS approach. The PLS-R-based method is suitable for quantitative analysis of blended oils, especially for those with multiple compositions, and is expected to generate significant impact on the quality control of the big blend oil market.

Chapter 4: Quantitative analysis of blended oils by MALDI-MS and spectral simulation

4.1 Introduction

It has been demonstrated that the MALDI-MS technique was advantageous in the quantitative analysis of blended oils, especially for the multi-compositions blended oils. No matter using intensity ratio of marker ions or chemometric approach for the quantitative analysis, the establishment of calibration relationship was required, which would be laborious and time-consuming if there were numerous oil compositions (≥4) in blended oils. Moreover, to use the methods developed in the previous chapters for accurate quantitative analysis, the oil compositions contained in the blended oils must be known before the analysis, otherwise the selection of erroneous calibration model would lead to unreliable results. In this chapter, we aimed to propose a new strategy for the quantitative analysis of blended oils, which could determine the compositions of unknow oil samples and provide an approximate quantitation of the determined oil compositions without developing calibration models.

MALDI-MS has been used as a routine technique for identification of bacteria[131-133]. For a typical MALDI-MS based identification, the bacteria should be isolated in pure culture which is difficult and time-consuming for polybacterial samples[134]. To overcome such drawbacks, direct characterization of bacterial samples without pure culture isolation has been investigated based on MALDI-MS. The common

identification of bacteria is based on spectral matching by comparing the spectrum of isolated bacterial sample with the reference spectra in a spectral database. For mixed bacterial samples, the compositions were inferred on the basis of reference spectra of individual bacteria, allowing automatic identification of bacterial mixture[135]. Based on the spectral similarity and biomarkers, the characterization of complex bacterial sample was achieved[136]. A framework has been developed for direct identification of bacterial mixtures based on MALDI-MS spectra, including binary, ternary and quaternary mixtures[137]. Using reference spectra of individual samples and similarity-based ranking algorithms made it available to identify the compositions of mixed samples, because the spectrum of mixed samples could be regarded as linear combination of the spectra of individual samples[138]. Furthermore, a spectral library-based approach for the identification of peptide mixtures allowed the relative quantitation of individual peptides based on the obtained coefficients for simulating the mixture spectra[139], demonstrating that the coefficients used to simulate the spectra of mixed samples were related to the ratios of individual samples in the mixtures.

From the MALDI-MS spectra of pure oils and blended oils discussed in the previous chapters, it has been shown that the spectra of blended oils were the combination of the spectra of pure oils, so the identification of oil compositions in blended oils might be realized by comparing the reference spectra and mixture spectra. In this chapter, the spectral comparison for determination of oil compositions in blended oils was

investigated, and the spectra of blended oils were simulated by the spectra of corresponding pure oils to explore the relationship between the simulated coefficients and the blending ratios. An initial framework was constructed and preliminarily applied on different types of blended oils for verification.

4.2 Materials and methods

4.2.1 Chemicals

2, 5-Dihydroxybenzoic acid (DHB) and α-cyano-4-hydroxycinnanic acid (CHCA) were purchased from Aldrich (St. Louis, MO, USA). HPLC grade acetone and HPLC grade acetonitrile (ACN) were purchased from Acros Organic (Waltham, MA, USA) and Anaqua Chemical Supply (Houston, TX, USA), respectively. Polyethylene glycol (PEG) standards were purchased from Fluka (St. Louis, MO, USA) and sodium iodide (NaI) was purchased from Panreac Química (Barcelona, Spain).

4.2.2 Oil samples and MALDI-MS analysis

Pure oil products were collected from suppliers in Hong Kong and in mainland China and blended oil samples were prepared manually by mixing pure edible oils in different weight ratios. For olive oil – sunflower seed oil blends, olive oil – canola oil blends and olive oil – corn oil blends, the blended samples were prepared with an increment of 5% in concentrations from 5% to 95%. For ternary blended oils, a total of 15 blended samples were prepared with blending ratios at 0%:50%:50% (3), 0%:40%:60% (3),

5%:5%:90% (3), 10%:70%:20% (3) and 20%:30%:50% (3). For quaternary blended oils, a total of 32 blended samples were prepared with blending ratios at 0%:0%:20%:80% (12), 0%:20%:20%:60% (12), 10%:10%:10%:70% (4) and 20%:20%:20%:40% (4). All the oil samples were sealed and stored in a dark and dry place before analysis. The MALDI-MS analysis of oil samples were performed following the protocol described in Section 3.2.3.

4.2.3 Statistical analysis

The MALDI-MS spectra of pure oils and blended oils were normalized by making the total intensities of all the TAG-related peaks in one profile as 100%. The spectral simulation and candidate selection were processed using RStudio (Boston, MA, USA).

Non-negative least squares (NNLS) is a type of linear regression where the estimated coefficients are constrained be non-negative. In this study, it was supposed that the MALDI-MS spectrum of a blended oil samples should be the linear combination of the spectra of corresponding pure oils (Equation (4-1)). Therefore, NNLS is quite suitable to fit the spectra of blended oils using the spectra of pure oils since the contributions of oil compositions to blended oils could only be positive or zero. Another extension of NNLS is penalized non-negative least squares (PNNLS), which allows the further restriction of the estimated non-negative coefficients to have a fixed positive sum. PNNLS is advantageous for fitting the spectra of blended oils since it can restrict the

total of fitted coefficients to be unity, exactly coinciding the total concentrations of all the compositions in blended oils. Both NNLS and PPNLS were applied to fit the spectra of blended oils based on the spectra of selected pure oils by using the R packages, i.e., "nnls" and "lsei", respectively, with the coefficients of pure oils were estimated. The cosine similarity scores between the experimental spectra and simulated spectra of blended oils were calculated using the R package "coop".

$$M = \sum_{i=1}^{m} c_i P_i \tag{4-1}$$

where M represents the spectrum of blended oil, P_i is the spectrum of a pure oil i, c_i is the coefficient of pure oil i contributed to the oil blend and m is the possible number of oil compositions contained in the oil blend.

To determine the compositions of a blended oil sample, the spectrum of the blended sample was compared with the reference spectra, i.e., spectra of pure oils. The Jaccard similarity (Equation (4-2)) between each reference spectrum and the spectrum of blended oil was calculated, and the reference spectra with top six Jaccard scores were selected as candidates. After the candidates were selected based on the Jaccard similarity, the candidates were further examined by Bayesian information criterion (BIC) to filter out some low-potential candidates.

$$J(A,B) = \frac{N_{AB}}{N_A + N_B - N_{AB}} \tag{4-2}$$

where N_A and N_B are the number of peaks shown in the spectrum A and spectrum B,

respectively, and N_{AB} is the number of common peaks shown in both spectrum A and spectrum B.

4.3 Results and discussion

4.3.1 Simulation of spectra of blended oils containing different oil compositions

To investigate the fitting ability of NNLS and PNNLS for simulating the spectra of blended oils based on the spectra of pure oils, pure olive oil, pure sunflower seed oil and their blends with an increment of 5% in blending ratios were prepared. The spectral data of pure olive oil and pure sunflower seed oil were regarded as references and were used to fit the spectral data of different blended samples. The coefficients of both pure olive oil and pure sunflower seed oil were estimated by the NNLS. For both olive oil and sunflower seed oil, the estimated coefficients were close to the concentrations of corresponding compositions in the blended oils, and the R^2 and RMSE between coefficients and concentrations were 0.9988 and 0.9979, and 0.0293 and 0.0196, respectively, for olive oil and sunflower seed oil, respectively (Table 4-1). Compared with blended sample with low-abundance olive oil, larger differences were observed between the estimated coefficients and concentrations for samples contained high-level olive oil. Based on the NNLS coefficients, the spectra of blended oils were simulated as Equation (4-1) shown, and the cosine similarity score between the experimental spectra and the simulated spectra were calculated. For all the blended samples, high similarities were presented between their experimental spectra and the simulated

spectra with the score higher than 0.995 (Table 4-1), illustrating the strong fitting ability of NNLS to the spectra of blended oils.

Table 4-1. Concentrations of olive oil and sunflower seed oil in the blends of olive oil (OO) – sunflower seed oil (SF) measured by NNLS.

No.	Measured by NNLS						
	Olive oil			Sunflower seed oil			CSS[a]
	Con. (%)	Coef. (%)	Accuracy (%)	Con. (%)	Coef. (%)	Accuracy (%)	
1	5.2	5.0	–3.6	94.8	94.5	–0.3	0.9997
2	10.0	10.0	0.0	90.0	89.7	–0.2	0.9995
3	15.6	14.1	–9.2	84.4	85.8	1.7	0.9995
4	20.1	19.9	–1.2	79.9	80.1	0.3	0.9992
5	25.1	23.5	–6.4	74.9	78.5	4.8	0.9991
6	30.1	30.0	–0.4	69.9	73.4	5.1	0.9956
7	35.1	35.7	1.4	64.9	66.2	2.1	0.9991
8	40.4	40.9	1.3	59.6	61.2	2.7	0.9990
9	45.0	47.3	5.2	55.0	55.5	0.9	0.9973
10	49.7	50.5	1.6	50.3	51.3	2.0	0.9987
11	54.9	56.3	2.5	45.1	45.3	0.4	0.9989
12	60.1	62.7	4.3	39.9	40.0	0.2	0.9983
13	65.0	67.3	3.5	35.0	34.1	–2.7	0.9987
14	70.0	72.3	3.3	30.0	28.0	–6.7	0.9993
15	74.6	76.8	2.9	25.4	23.5	–7.4	0.9993
16	79.2	82.6	4.3	20.8	18.4	–11.7	0.9994
17	84.3	90.3	7.1	15.7	12.7	–19.2	0.9987
18	89.5	95.5	6.7	10.5	7.5	–28.2	0.9989
19	94.2	100.9	7.1	5.8	3.1	–46.4	0.9984
R^2		0.9988			0.9979		/
RMSE		0.0293			0.0196		/

[a] CSS: cosine similarity score.

Table 4-2. Concentrations of olive oil in the blends of olive oil (OO) – sunflower seed oil (SF) measured by PNNLS.

No.	Measured by PNNLS			
	Olive oil			CSS[a]
	Con. (%)	Coef. (%)	Accuracy (%)	
1	5.2	5.3	1.0	0.9997
2	10.0	10.2	1.1	0.9995
3	15.6	14.2	–9.1	0.9995
4	20.1	19.9	–1.1	0.9992
5	25.1	22.4	–10.6	0.9991
6	30.1	28.2	–6.4	0.9955
7	35.1	34.7	–1.4	0.9991
8	40.4	39.8	–1.5	0.9990
9	45.0	45.8	1.9	0.9973
10	49.7	49.6	–0.4	0.9987
11	54.9	55.4	1.0	0.9989
12	60.1	61.2	1.9	0.9983
13	65.0	66.6	2.4	0.9987
14	70.0	72.2	3.1	0.9993
15	74.6	76.6	2.7	0.9993
16	79.2	82.1	3.6	0.9994
17	84.3	88.7	5.2	0.9987
18	89.5	93.9	4.9	0.9988
19	94.2	98.8	4.8	0.9982
R^2		0.9987		/
RMSE		0.0223		/

[a] CSS: cosine similarity score.

When PNNLS was applied to fit the spectra of blended oils, the sum of estimated coefficients of olive oil and sunflower seed oil was fixed as 1, which was more similar to the situation of concentrations of the two oil compositions, and the accuracy, R^2 and RMSE of the estimated coefficients for the two compositions were equal. Thus Table 4-2 only showed the results of olive oil for a clear understanding. The coefficients estimated by PNNLS were also quite similar to the actual concentrations with the R^2 and RMSE as 0.9987 and 0.0223, respectively. The coefficients measured by PNNLS seemed to be an average of the results provided by NNLS, which were worse than the NNLS coefficients of olive oil but better than the NNLS coefficients of sunflower seed oil. The simulated spectral data based on NNLS and PNNLS presented close similarities to the corresponding experimental spectral data as the differences between the cosine similarity scores were within ±0.0002, indicating the comparable fitting abilities of the two methods.

The spectral simulation was further investigated for other types of blended oils, including olive oil – corn oil blends, olive oil – canola oil, olive oil – perilla oil – sunflower seed oil blends and olive oil – perilla oil – rice bran oil – sunflower seed oil blends, and the results were summarized in Table 4-3. The simulation of spectra data of binary, ternary and quaternary blended oils were realized using both NNLS and PNNLS, and strong similarities (>0.994) were observed between the simulated spectra and experimental spectra for binary and ternary blended oils with slightly poor similarity

for quaternary blended oils (>0.912). The coefficients of pure oils measured by NNLS and PNNLS were similar to the actual concentrations and showed insignificant differences to each other, so PNNLS was more suitable to fit the spectra for blended oils as it allowed the fixed sum the coefficients. Overall, it has been demonstrated that the quantitation of oil compositions in blended oils could be achieved based on spectral simulation, and the coefficients estimated by PNNLS could be directly used as quantified concentrations without further conversion.

Table 4-3. The quantitative performances of NNLS and PNNLS for different types of blended oil.

Blending type[a]	**Oil species**	**Measured by NNLS**			**Measured by PNNLS**		
		R^2	RMSE	CSS_{min}[b]	R^2	RMSE	CSS_{min}
OO-CO	OO	0.9957	0.0413	0.9982	0.9965	0.0384	0.9982
	CO	0.9975	0.0339		/	/	
OO-CA	OO	0.9951	0.0793	0.9976	0.9956	0.0721	0.9974
	CA	0.9969	0.0347		/	/	
OO-PR-SF	OO	0.9808	0.0654	0.9949	0.9786	0.0579	0.9944
	PR	0.9890	0.0401		0.9879	0.0438	
	SF	0.9722	0.0534		0.9715	0.0538	
OO-PR-RB-SF	OO	0.9906	0.0385	0.9135	0.9910	0.0374	0.9126
	PR	0.9886	0.0379		0.9851	0.0425	
	RB	0.9824	0.0477		0.9859	0.0463	
	SF	0.9840	0.0567		0.9836	0.0561	

[a] OO: olive oil, CO: corn oil, SF: sunflower seed oil, CA: canola oil, PR: perilla oil, RB: rice bran oil.

[b] CSS_{min}: The minimum cosine similarity score.

4.3.2 Determination and quantitation of oil compositions of blended oils

For an unknown blended oil sample, it is necessary to determine its contained oil compositions before the quantitative analysis. A framework about the determination of oil compositions in blended oils has been constructed, which was mainly based on comparing the similarity between reference spectra and mixture spectra. As Figure 4-1 shown, to determine the compositions of blended oils, the spectra of blended oils were compared with the spectra of pure oils (reference spectra) one by one, and the reference spectra with the top six Jaccard similarity scores were selected as potential candidates. Based on the potential candidates, Bayesian information criterion (BIC) was applied to screen out the high-potential candidates by further comparison of the potential reference spectra and the mixture spectra. To validate the performance of the framework for determination of oil compositions, binary blended oil samples and ternary blended oil samples were prepared, and a small library contained the reference spectra of the pure oils involved and not involved in the preparation of blended oil samples was established (Table 4-4).

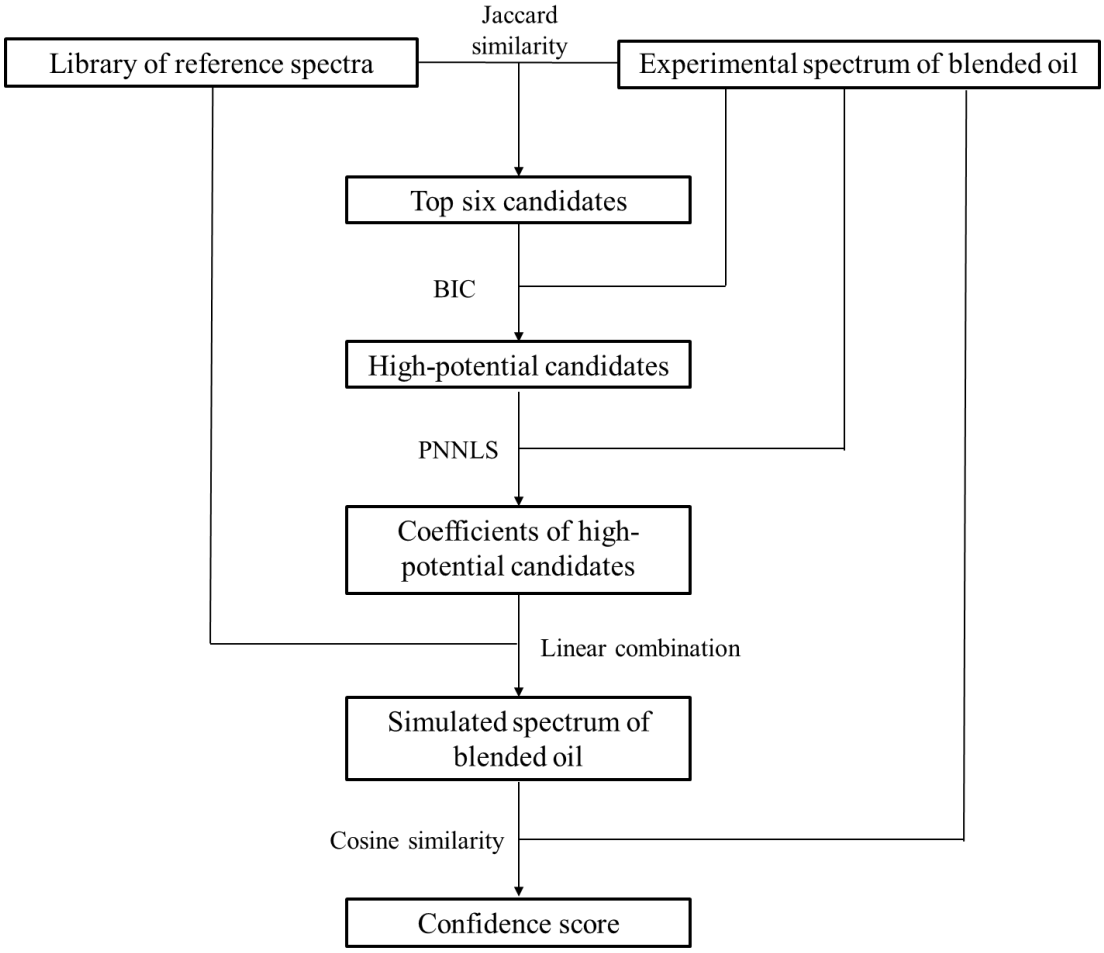

Figure 4-1. Framework for determination and quantitation of oil compositions in blended oils.

Table 4-4. Oil samples prepared for determination of oil compositions in blended oils.

Type	Oil samples	
Reference spectra	Olive oil A Olive oil B Olive oil C Olive oil D Olive oil E Olive oil F	Sunflower seed oil A Sunflower seed oil B Corn oil A Canola oil A Camellia oil A Rice bran oil A Perilla oil A
Testing spectra	Olive oil A – sunflower seed oil A Olive oil B – corn oil A Olive oil C – canola oil A Olive oil D – perilla oil A – sunflower seed oil B Olive oil D Perilla oil A Sunflower seed oil B	

Table 4-5. Sensitivities and error rates in the determination of compositions of oil samples.

Type	Sensitivity	Error rate
Pure oils	100.0% (3/3)	50.0% (3/6)
Binary blends	100.0% (66/66)	17.5% (14/80)
Ternary blends	88.9% (24/27)	20.0% (6/30)

The binary blended oils, ternary blended oils as well as the pure oils contained in the ternary blended oils were used as testing samples, and their oil compositions were determined by the developed framework and reference spectral library. Sensitivities and error rates in the determination of oil compositions of testing samples were summarized in Table 4-5. For pure oils and binary blended oils, all the contained compositions have been successfully determined, and for ternary blended oils, the sensitivity of determination was 88.9% because three low-abundance oil compositions (~5%) were not identified. The false positive determination of non-existing compositions happened to the identified results of all the three types of oil samples with error rates at 17.5% and 20.0% for binary blended oils and ternary blended oils, respectively, and a huge error rate for pure oils (50.0%) due to the small number of samples (Table 4-5). PNNLS was applied to estimate the coefficients of the determined compositions including these non-existing compositions, and the quantitative performance of the framework was evaluated by the differences (i.e., R^2 and RMSE) between the actual concentrations and measured coefficients of each composition that were truly contained in the blended oils.

Table 4-6. Quantitative performance of the framework for different blended oils.

Blending type[a]	Oil species	Measured by PNNLS	
		R^2	RMSE
OO-SF	OO	0.9991	0.0279
	SF	0.9685	0.0584
OO-CA	OO	0.9964	0.0744
	CA	0.9964	0.0744
OO-CO	OO	0.9966	0.0508
	CO	0.9522	0.1525
OO-PR-SF	OO	0.9843	0.0411
	PR	0.9882	0.0431
	SF	0.9843	0.0804

[a] OO: olive oil, SF: sunflower seed oil, CO: corn oil, SF: sunflower seed oil, CA: canola oil, PR: perilla oil, RB: rice bran oil.

Excellent results were obtained from the quantitation of olive oil in olive oil – sunflower seed oil blends with R^2 and RMSE as 0.9991 and 0.0279, respectively (Table 4-6), while the quantitative results for sunflower seed oil in the blends were slightly poor because the incorrect determination and quantitation of non-existing composition, i.e., corn oil, resulted in the lower measured coefficients of sunflower seed oil compared with its actual concentrations (Table 4-7, B1). Such situation was also observed from the quantitative analysis of olive oil – corn oil blends (Table 4-7, B3), where sunflower seed oil was an interference composition for the accurate quantitation of corn oil, leading to the poor quantitative results of corn oil with RMSE as 0.1525 (Table 4-6). For olive oil – canola oil blends, the false positive determination of non-existing compositions did not occur, so the framework presented same quantitative ability for

olive oil and canola oil. Compared with the spectrum of sunflower seed oil, the spectrum of canola oil was more similar to the spectrum of olive oil, making the quantitation of olive oil in olive oil – canola oil blends more challenging than the quantitation of olive oil in olive oil – sunflower seed oil blends, consequently larger RMSE (0.0744) for the quantitative results of olive oil in the olive oil – canola oil blends (Table 4-6). For ternary blends of olive oil, perilla oil and sunflower seed oil, the quantitation of sunflower seed oil might be affected by the false positive determination of canola oil (Table 4-7, T1&T2), thus the quantitative results of sunflower seed oil in the ternary blends were not as good as those of the other two compositions but were still acceptable with R^2 and RMSE as 0.9843 and 0.0804, respectively (Table 4-6).

The obtained quantitative results were used as coefficients to generate simulated spectra of the blended oils, and the similarity between the experimental spectra and the simulated spectra was expressed by the cosine similarity score. Table 4-7 showed some detailed quantitative results as examples. For some samples, the measured results of some compositions were significant different from their actual concentrations, while the simulated data presented high similarity to the experimental data with the cosine similarity scores higher than 0.9960 (Table 4-7), which were even higher than the cosine similarity scores of some simulated spectra based on accurate quantitative results (Table 4-3). Therefore, the cosine similarity score was not available to screen out the poor quantitative results and could not be used as the criterion for evaluating the confidence

of quantitative results.

Table 4-7. Details of some poor quantitative results provided by the developed framework.

No.[a]	Blending type[b]	Oil species	Measured by PNNLS			
			Actual con. (%)	Measured con. (%)	Accuracy (%)	Cosine similarity
B1	OO-SF	OO	20.1	18.5	–7.8	0.9996
		SF	79.9	69.1	–13.5	
		CO	/	12.4	/	
B2	OO-CA	OO	70.7	81.4	15.1	0.9971
		CA	29.3	18.6	–36.6	
B3	OO-CO	OO	45.9	53.3	16.1	0.9995
		CO	54.1	24.4	–54.9	
		SF	/	22.3	/	
T1	OO-PR-SF	OO	50.1	51.6	3.1	0.9960
		PR	30.0	25.7	–14.4	
		SF	19.9	10.0	–49.9	
		CA	/	12.7	/	
T2	OO-PR-SF	OO	60.0	59.5	–0.9	0.9986
		PR	0.0	0.0	/	
		SF	40.0	22.6	–43.4	
		CA	/	17.9	/	

[a] B: binary blended oils, T: ternary blended oils.

[b] OO: olive oil, SF: sunflower seed oil, CO: corn oil, SF: sunflower seed oil, CA: canola oil, PR: perilla oil, RB: rice bran oil.

4.4 Conclusion

A new strategy for rapid quantitative analysis of blended oils has been proposed and preliminarily implemented based on the MALDI-MS analysis. Referring to the proposed strategy, an initial framework was constructed with two main parts, i.e., determination of oil compositions of blended oils and quantitation of the determined oil compositions. The determination of oil compositions was achieved using spectral similarity comparison, and the determined results of different types of blended oils showed sensitivity higher than 88% with all the major compositions in blended oils successfully identified. The false-positive determination of non-existing compositions were observed occasionally due to the similar TAG profiles of some pure oils, such as sunflower seed oil and corn oil, indicating further optimization is needed for the framework for improved identification ability. The quantitation of oil compositions was realized based on spectral simulation, and PNNLS was powerful for fitting the spectra of blended oils by the reference spectra of pure oils. The coefficients of pure oils for the spectral simulation could directly correspond to the concentrations of pure oils contained in blended oils, because the MALDI-MS detected the TAG profiles of edible oils which have similar MS responses due to their similar molecular structures. The initial framework has been applied on different binary and ternary blended oils, and the preliminary results demonstrated that approximate quantitative measurement of unknown blended oils could be achieved using the reference spectra of pure oils.

Chapter 5: General conclusions and prospects

The rapid quantitation of oil compositions of blended oils by MALDI-MS has been developed in this study to meet the strong demand of society for the quality control of blended oils. MALDI-MS is an effective tool for profiling TAGs in edible oils, which have similar molecular structures and similar MALDI-MS responses. In the whole study, the quantitative analysis was based on the normalized abundances (intensity ratio or relative intensity) of TAGs in the MALDI-MS spectra and comparison with reference data acquired under the same conditions, minimizing the ion suppression effects. Due to the direct analysis of oil samples in their oily states and much reduced variations of sample spots, the developed MALDI-MS technique offer mass spectra of edible oils with high quality and high reproducibility. Compared with the conventional GC-FID, MALDI-MS could allow direct analysis of blended oils, analysis of one blended oil sample within minutes, and accurate quantitation of low-abundance oil compositions and blended oils with similar FA contents. The equipment of MALDI-MS is more expensive than that of GC-FID, but considering the labor, time and consumables used, the operation cost for analyzing oil samples using MALDI-MS is significantly lower, due to its minimal sample pretreatment and high analysis efficiency, making the MALDI-MS technique robust and economical for analysis of massive samples.

Different approaches have been applied to analyze the MALDI-MS spectra of blended oils for quantitative analysis. The first attempt was using the intensity ratio of TAG

peaks as indicators for quantitative analysis, and the results demonstrated that the relationships between the intensity ratio of marker ions and the concentration of oil compositions were not linear and would become much more complex for blended oils with multiple compositions. With optimization of marker ions, good quantitative results could be easily obtained for binary blended oils and ternary blended oils using the developed intensity ratio-based method, and the quantitative analysis of quaternary blended oils is feasible using the intensity ratio-based method with complementary strategy. The intensity ratio-based method is not limited for analysis of blended oils, but also available for quantitative measurements of mixed analytes showing overlapped peaks in the MS spectra. The quantitative analysis of binary and ternary mixtures has been comprehensively studied by applying the developed method to various types of blended oils, and a reliable and exhaustive guideline for the selection of marker ions has been proposed which could provide suggestions for other similar studies. However, for mixtures containing multiple compositions ($\geqslant$4), quantitative analysis using the intensity ratio-based method is still challenging, thus further investigation is required to find alternative indicators or algorithms to simplify the calculation.

To make full use of the numerous peaks shown in the MALDI-MS spectra of blended oils, PLS-R was applied to establish multivariate calibration models for quantitative analysis of blended oils. Benefiting from the powerful data processing capability, the PLS-R approach allows simultaneous quantitation of all the compositions in blended

oils, and shows excellent quantitative ability for blended oils, especially for multi-composition blended oils, i.e., ternary and quaternary blended oils. The PLS-R is available for quantitative analysis of various blended oils and would not be limited by the number of oil compositions. However, the quantitative performance of the PLS-R approach might deteriorate as the number of oil compositions increases, demanding complementary strategy to overcome such drawbacks. The number of training samples required for establishment of PLS-R models also significantly increases as the number of oil compositions increases, making it laborious to prepare the massive blended oil samples.

Both approaches using intensity ratio and PLS-R to quantify oil compositions of blended oils require preparation of blended oil samples for establishing calibration models, and the blending types of blended oils need to be provided before quantitative analysis. A preliminary framework has been constructed for determining oil compositions of unknown blended oils and quantifying the determined compositions based on the reference MALDI-MS spectra of pure oils. The framework aims to provide rapid screening and semi-quantitation of blended oils, and the preparation of blended oil samples is not necessary, which would significantly simplify the procedure and shorten the time of quantitative analysis. Although further improvement and optimization of the framework are required, it shows high potential for wide applications and could be coupled with the intensity ratio- and the PLS-R-based

approaches to compensate the shortcomings of each other.

Overall, the MALDI-MS technique for rapid quantitative analysis of blended oils has been developed with different data processing approaches. Both intensity ratio- and PLS-R-based approaches have good quantitative capabilities. Compared with the intensity ratio-based approach, the PLS-R-based approach presents better quantitative performance while requiring more oil samples for the establishment of PLS-R models. The intensity ratio-based approach is easy for understanding and operating but not suitable for blended oils with numerous compositions; the PLS-R-based approach is advantageous for quantitative analysis of multi-compositions blended oils while demanding basic statistic knowledge for operating. Therefore, the intensity ratio-based approach is suitable for rapid and efficient analysis with acceptable errors, and the PLS-R-based approach is recommended for measurements requiring highly accurate results. With further improvement and optimization of the preliminarily developed framework, the framework could be applied as an initial step for the analysis of blended oils, where the compositions of blended oils will be determined and approximately quantified. After that, the intensity ratio- and PLS-R-based approaches could be employed for accurate quantitation depending on the determined results. In addition, our previous study has established a MALDI-MS spectral database for classification of pure oils, which could be further integrated with the framework to realize rapid identification of unknown edible oils, including authentication of pure oils, semi-quantitation of blended

oils and screening of cooked oils. The MALDI-MS-based method is expected to generate positive impacts on the big edible oil market.

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