Authentication and Geographical Origin Analysis of Plant-Derived Edible Oil Using the SCIEX X500R QTOF System

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Plant-derived cooking oil is an important source of nutrition for human consumers; this source of fatty acids plays an important role in the body as one of the three primary macronutrients[1,2,3]. Fatty acids such as those from edible vegetable oils provide constituents for human cells and tissues as well as a variety of important physiologically active substances and functions. Triacylglycerols (TAGs) are the main components of plant oils more than 95%[4]. The physicochemical and nutritional properties of plant oils are determined by TAG molecule structure (Figure 1). The nutritional value of vegetable oils varies with the different fatty acids of TAG structure. Therefore, for studying the authenticity and traceability of vegetable oils, identification of TAGs structure and fat acids composition can provide more accurate, direct, and reliable evidence.

There are many challenges in identifying the authenticity and geographical origin of edible vegetable oils, and in developing robust methods for lipids analysis or even nontargeted screening analysis. Selecting appropriate representative samples to study is key. The complexity of the lipids in samples is high, especially considering the glycerides in oil, with many isomers and their higher molecular weight. Chromatography of lipid samples is not straightforward, especially if required to match previously published studies of lipids. Finally data analysis typically requires high expertise.

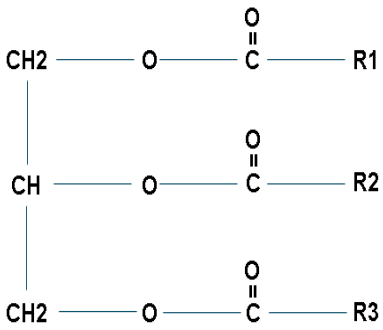


Figure 1. Triglyceride Backbone Structure Diagram. R1,R2,R3: Potential sites for fatty acid chains: Mono-Acyl-Glycerols contain one fatty acid chain (MAG); Di-Acyl-Glycerols contain two fatty acid chains (DAG);Tri-Acyl-Glycerols contain three fatty acid chains (TAG).

In this study, high performance liquid chromatography tandem mass spectrometry on the SCIEX X500R QTOF system was used to perform global profiling to establish a workflow for determining the authenticity and geographical origin of some plants important to edible oil production. Vegetable oil quality analysis such as this could provide an important tool for assessing fraudulent or adulterated products in the global food supply.

Advantages of X500R for Authenticity and Origin Analysis

- X500R QTOF system scanning speed (100Hz) ensures that all relevant spectral information can be collected without compromising the integrity of the data. The unique Dynamic Background Subtraction (DBS) feature maintains high quality and relevance of the spectral data acquired.
- Excellent demonstrated reproducibility over 290 injections is indicative of the robustness and stability of the system even with complex matrices
- SCIEX OS Q provides intuitive and powerful tools for LC-MS quantitation as well as the ability to confirm identity of target analytes using their accurate mass MS/MS spectra.
- LipidView™ Software in conjunction with its lipid-centric database and tools, offers generation of comprehensive customized methods for data processing and lipid confirmation.
- MarkerView™ software enhances the data analysis with statistical tools like PCA and t-test, to quickly find differences between samples and identify distinguishing features.

Experimental Methods

Sample Collection: For this experiment, oils from seven ordinary oil producing plants were obtained: namely, olive oil, peanut oil, corn oil, sunflower oil, rapeseed oil, sesame oil, soybean (Figure 2).

Figure 2. Seven Kinds of Common Edible Oil Plants. When selecting oils for the study, common edible oils were selected as samples.

Pretreatment: A quick and simple sample preparation was employed, using methanol / isopropanol (1 / 1,5mM ammonium acetate) and directly diluting the sample for analysis.

Chromatography Conditions: The SCIEX ExionLC™ AC system was employed for analytical separation using a Phenomenex C18 column (2.6 µm, 2.1 × 100mm). The mobile phases were as follows: Buffer A - Water / methanol / acetonitrile (3/1/1, with ammonium acetate at 5mM), Buffer B: Isopropanol (containing 5mM ammonium acetate). The flow rate was 0.35 mL/min with a column temperature of 60 °C. The injection volume was 2 µL.

MS Conditions: The X500R system (SCIEX) was operated using an IDA method, with a TOF MS survey scan and multiple MS/MS spectra acquired per cycle. Method details are in Table 1.

Data Processing: First, target lipid lists for TAGs, DAGs, and FFA were prepared in LipidView Software 1.2 using the LV Method Exporter tool. These lists of compounds and masses were imported into SCIEX OS-Q for extraction of the TOF MS peak areas for each of the species. Confirmation of identities using MS/MS was performed manually in combination with the Catalogue and Calculator in LipidView software. Then, areas for the identified lipids were exported for analysis in MarkerView™ Software 1.3.1 for statistical analysis to find differences between sample types.

Table 1. Ion Source and MS Parameters.

Parameter	Setting
TOF MS (Da)	*100 - 1000*
TOF MS/MS (Da)	*50 - 1000*
Dynamic Background Substraction	*On*
# of Candidate MS/MS per cycle	*12*

Repeatability and Stability of Method

The experiment consisted of a total of 290 samples (145 injections in positive ion mode, 145 injections in negative ion mode). Aliquots of each sample were mixed to prepare a QC sample. In the injection process, each QC sample injection was separated by 6 samples. While the samples were injected, it was also included in the design that six QC check samples were collected, spaced evenly throughout the injection sequence every 24 samples. Three different ions with different retention times were selected for evaluation for repeatability and stability. The sampling period of different molecular weights present in the ion difference reproducibility difference. Shown in Figure 3, although the instrument sample collection spanned almost eight days without interruption, the three different *m/z* values with differing retention times were demonstrated to be reproducible throughout the run. The instrument is thus shown to be able to cope with complex matrix introduction, as well as excellent stability, ensuring the reliability of all the sequence data.

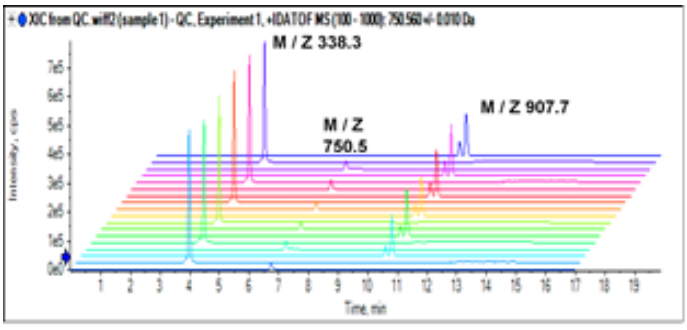


Figure 3. Quality Control Samples Across Study. The QC samples were analyzed across the full study and analyzed for workflow reproducibility.

MS Scan Speed

Oil is a complex sample matrix and co-elution of many constituents in the sample means the mass spectrometer must have very high acquisition rates to achieve the collection of all relevant spectral information for species identification and quantification.

The X500R system can acquire MS and MS/MS spectra at very high acquisition rates (up to 100 Hz), allowing for high resolution spectra to be acquired on large numbers of lipids in one injection. With the LC strategy used here, the LC peaks have a chromatographic width of only 0.2 min, but high acquisition rates ensured that 15 points were collected each peak, strongly benefiting the accuracy and reproducibility of integrations during quantification (Figure 4). After 12 hours of continuous operation with back to back injections, instrument maintains a 1 ppm mass accuracy, well within the required tolerance (Figure 5).

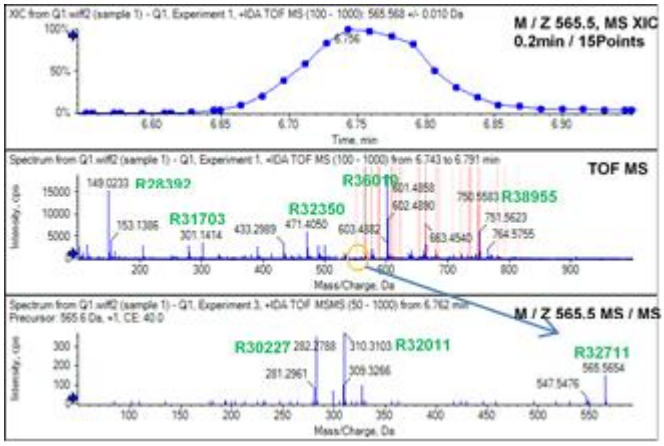


Figure 4. Ultra-Fast Scan Speed is Beneficial for Mass Resolution, High Sensitivity, and High Accuracy. (Top) The collection of a sufficient number of data points across the LC peak profile accurate samples peak shape and improves quantitation accuracy. In the second pane, the red lines indicate where MS/MS spectra have been triggered by the MS signal. Fast acquisition rates ensure that a very high number of MS/MS is acquired across the chromatogram for high sample coverage.

Dynamic Background Subtraction (DBS)

The DBS function performs 'on the fly' analysis of all the peaks eluting off the column, across the chromatographic run, to selectively distinguish the background ions from the sample ions, to ensure most MS/MS spectra is acquired on analytes from the sample of interest and not background ions. So only ions with a chromatographic peak profile will be sent for MS/MS acquisition and steady background ions will not. This also helps ensure MS/MS spectra is acquired on low level analytes, even in the presence of more intense background ions.

Index	Sample Name	Component Name	Formula	Precursor Mass	Found At Mass	Expected RT	Mass Error...	Mass Error (ppm...
9	QC	TAG 52:1	C55H104O6	878.817	878.8179	10.40	✓	0.9
10	QC	TAG 52:2	C55H102O6	876.801	876.8008	10.39	✓	-0.8
11	QC	TAG 52:3	C55H100O6	874.786	874.7851	10.18	✓	-0.9
12	QC	TAG 52:4	C55H98O6	872.770	872.7698	9.95	✓	-0.4
13	QC	TAG 52:5	C55H96O6	870.755	870.7542	9.71	✓	-0.4
14	QC	TAG 52:6	C55H94O6	868.739	868.7394	9.48	✓	0.7

Index	Sample Name	Component Name	Formula	Precursor Mass	Found At Mass	Expected RT	Mass Error...	Mass Error (ppm...
1	QC	TAG 52:1	C55H104O6	878.817	878.8165	10.40	✓	-0.7
2	QC	TAG 52:2	C55H102O6	876.801	876.8014	10.39	✓	-0.1
3	QC	TAG 52:3	C55H100O6	874.786	874.7851	10.18	✓	-0.9
4	QC	TAG 52:4	C55H98O6	872.770	872.7694	9.95	✓	-0.9
5	QC	TAG 52:5	C55H96O6	870.755	870.7541	9.71	✓	-0.5
6	QC	TAG 52:6	C55H94O6	868.739	868.7393	9.48	✓	0.5

Figure 5. Stable Instrument Mass Accuracy. After 12 hours of continuous injection, the instrument mass error stays within 1PPM, demonstrating an excellence in mass calibration stability during operation.

Identification of the Lipid Samples

It has been reported in the literature[1-3] that more than 95% of the oil component in plants are triglycerides, with a small amount of free fatty acids, phospholipids, vitamins, hormones, phytosterols and other components. This study uses a list of lipid species (TAGs, DAG, FFAs) exported from LipidView software to perform targeted analysis of the IDA data to detect, confirm and quantify the lipid species.

The lipid identification process was based on the precursor mass, the MS/MS fragments as well as the known retention time behaviors of sets of lipids. Shown in Figure 6 is an example of a TAG; the results showed that the TAG with fewer carbon double bonds had stronger chromatographic retention than the TAGs with more carbon double bonds. The retention interval of TAG is the same for every difference of one carbon double bond. The TAGs were detected as NH4+ ions.

The main MS/MS fragments of TAGs were formed after the precursor ions lose different fatty acid chains. The example in Figure 7, TAG 52:3 fragments into three different fatty acids FA16:0_FA18:1_FA18:2.

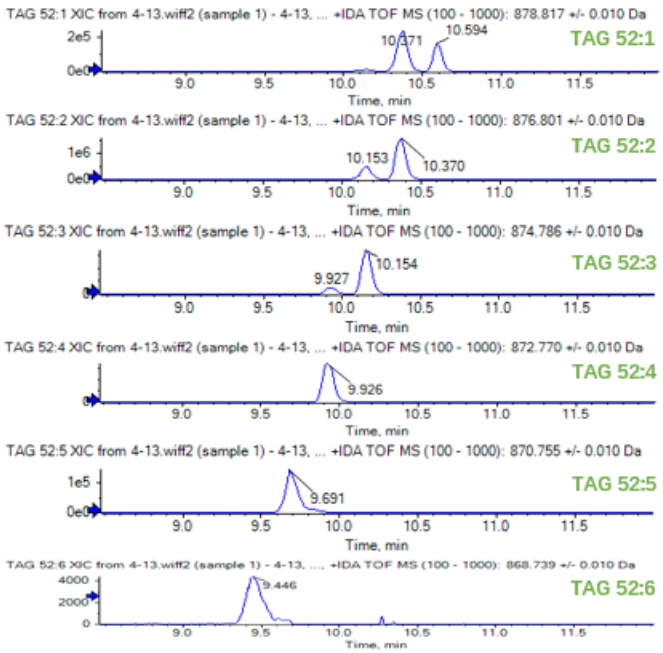


Figure 6. Relative Retention Times of Triglycerides. TAGs with increasing numbers of double bonds have elute earlier off the LC column, due to reduced hydrophobicity.

Because free fatty acids do not have unique MS/MS fragments, qualitative analysis of free fatty acids was evaluated by the rules of retention times. Figure 8 showed that the retention interval was 0.2 min for FFA 18:0, FFA 18:1, FFA 18:2, FFA 18:3. In addition, the fewer carbon double bonds of FFA had stronger chromatographic retention than the greater carbon double bonds of FFA.

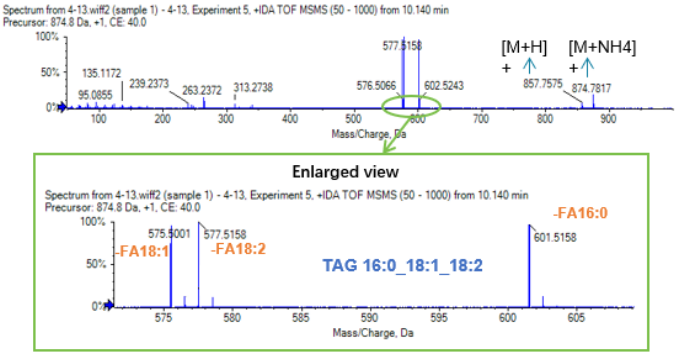


Figure 7. Triglycerides Identified by Precursor Mass and Fragment Ions. Fragmentation of the TAG 52:3 at 874.4 m/z produces a relative simple MS/MS spectrum, mainly at the fatty acid chains. Observed fatty acids are FA16:0_FA18:1_FA18:2.

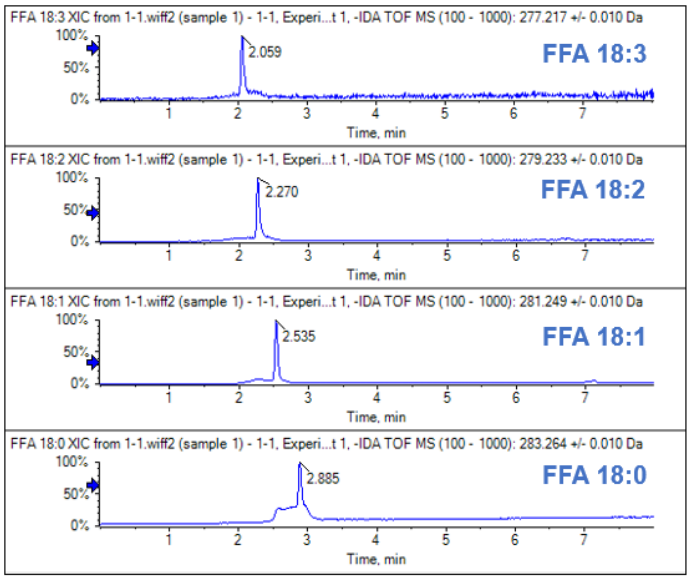


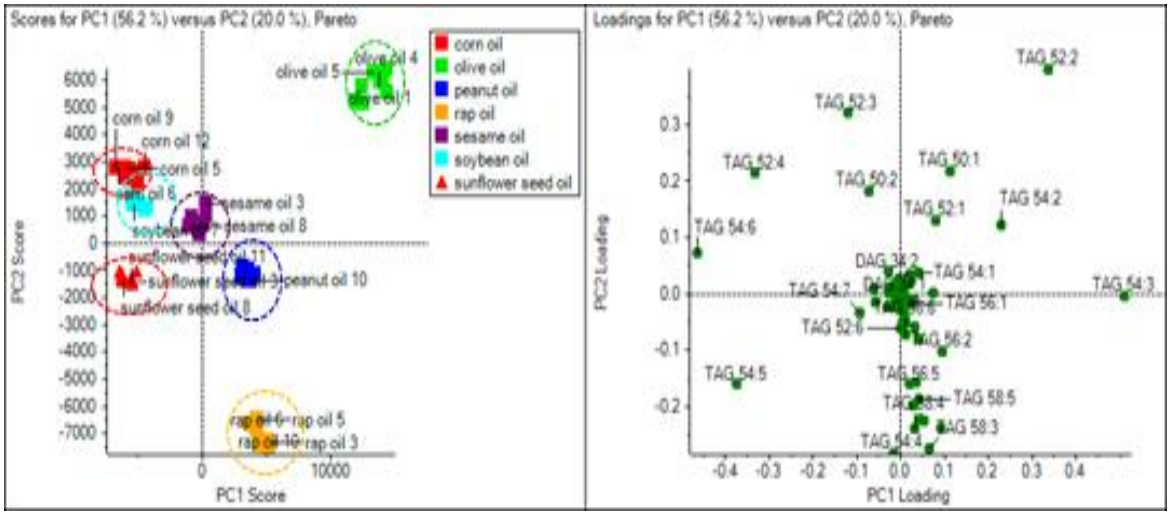
Figure 8. Relative Retention Times of Free Fatty Acids. Similar to the TAG example, as the number of double bonds increases, the FFA elutes earlier from the LC column.

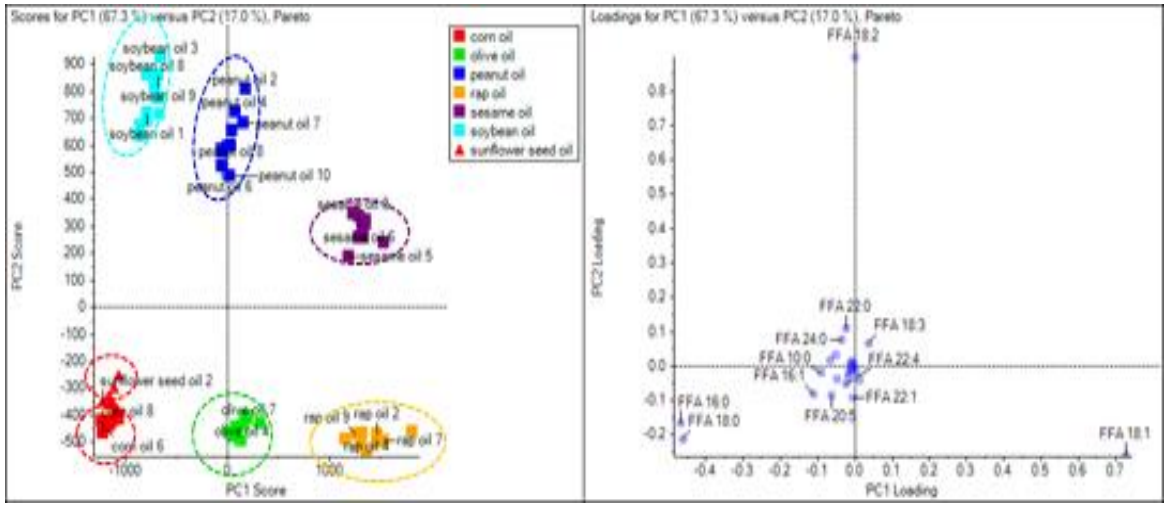
Authenticity Analysis of Seven Edible Oils

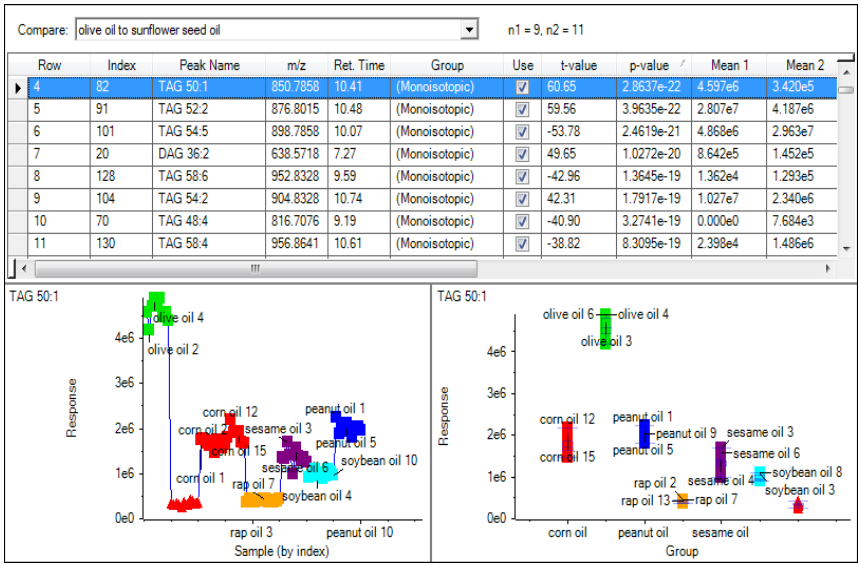
Data processing for authenticity of the oils was done using a multi-step process. Lipid profiling was performed by using a list of masses determined in LipidView software then imported into SCIEX OS-Q for targeted extraction. The profiling results, primarily from vegetable oil, showed the main components as TAG, DAG, and FFA. There were 283 triglycerides and diglycerides and 27 FFAs identified. The peak areas of the identified components are then imported into to MarkerView™ Software for statistical data processing (PCA, T-test). Omics approaches use MarkerView data processing software as it is a simple and intuitive way to quickly find the differences between the seven kinds of oil.

Good grouping of replicates of oil types was observed using the TAG data, showing clear oil type differentiation (Figure 9, top). Using the FFAs, good clustering of sample replicates was also observed with the exception of corn and sunflower oils which were not significantly separated in the Scores plot (Figure 9, middle).

T-test results indicated significant differences between sample sets for 39 kinds of glyceride compounds and nine kinds of free fatty acids (Figure 9, bottom). These potential markers were applied to authenticity identification and geographical origin characterization of seven vegetable oils.







Row	Index	Peak Name	m/z	Ret. Time	Group	Use	t-value	p-value	Mean 1	Mean 2
4	82	TAG 50:1	850.7858	10.41	(Monoisotopic)	☑	60.65	2.8637e-22	4.597e6	3.420e5
5	91	TAG 52:2	876.8015	10.48	(Monoisotopic)	☑	59.56	3.9635e-22	2.807e7	4.187e6
6	101	TAG 54:5	898.7858	10.07	(Monoisotopic)	☑	-53.78	2.4619e-21	4.868e6	2.963e7
7	20	DAG 36:2	638.5718	7.27	(Monoisotopic)	☑	49.65	1.0272e-20	8.642e5	1.452e5
8	128	TAG 58:6	952.8328	9.59	(Monoisotopic)	☑	-42.96	1.3645e-19	1.362e4	1.293e5
9	104	TAG 54:2	904.8328	10.74	(Monoisotopic)	☑	42.31	1.7917e-19	1.027e7	2.340e6
10	70	TAG 48:4	816.7076	9.19	(Monoisotopic)	☑	-40.90	3.2741e-19	0.000e0	7.684e3
11	130	TAG 58:4	956.8641	10.61	(Monoisotopic)	☑	-38.82	8.3095e-19	2.398e4	1.486e6

Figure 9. Statistical Analysis of the Seven Edible Oils Using MarkerView Software. Principal Component Analysis (PCA) was performed using the quantitative results from the identified TAG and DAG species (top) and the FFA species (middle). The scores plot on the left shows that in both cases the samples from the different types of oils clearly separate, with replicates clustering tightly together. Using the Loadings plot on the right, the lipid species responsible for the separation can be determined. Bottom) A t-test can also be performed which provides an additional way to determine specific lipid species that differ significantly between the different oils. Shown is TAG 50:1, significant higher in the Olive oil samples.

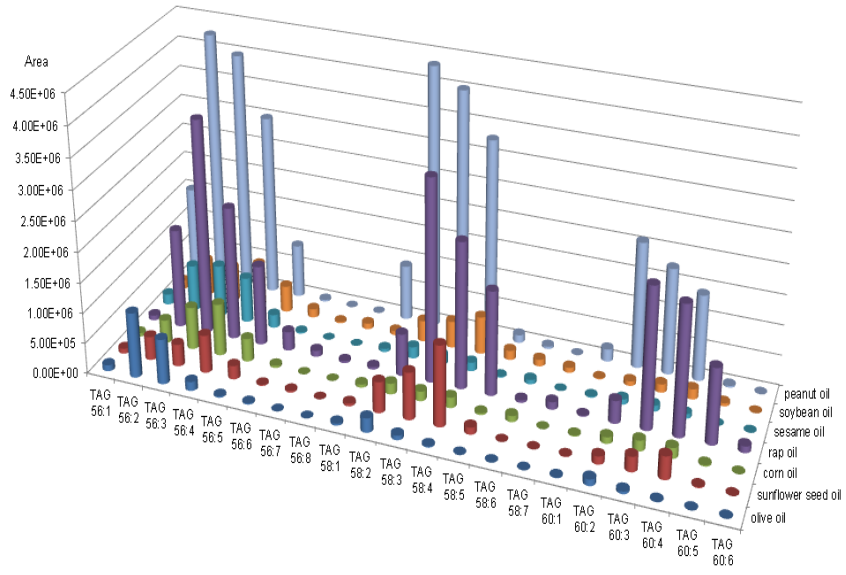


Figure 10. Differing Composition of TAG Species Across the Oils. By plotting the average peak areas (n=10) for each of the TAG species across the different oils, interesting patterns emerge and potential lipid markers of oil types can be visualized.

Some olive oil producers will add a cheaper oil to more expensive oil products to extend product and increase profits. To further verify this experimental approach and find markers which can be applied to identify the authenticity of edible oil in a routine quantitative analysis, evaluation experiments were performed using blind sample testing. Vegetable cooking oil from a blind sample brand manufacturer was shown to be mainly comprised of different proportions of sunflower oil and olive oil, according to experiment results. The ratio of the sum of the peak area of the markers in olive oil and sunflower seed oil to the peak area of the markers in blend oil was close to 1 (Figure 11), indicating that the blend oil can be identified by using the found TAG markers. And results suggest this approach could be used to test oil authenticity.

After identifying the distinguishing compounds in the edible oils, the peak areas were used to further assess of the differences in concentration levels of the marker compounds in the seven kinds of vegetable oil (Figure 10). Peanut oil and rapeseed oil have many obvious differences compared with other five kinds vegetable oil, mainly in TAG 56:3, TAG 56:4, TAG 56:5, TAG 58:3, TAG 58:4, TAG 58:5, TAG 60:3, TAG 60:4, TAG 60:5; indicating that these glycerides may be used as the basis for identification of these two types of oil.

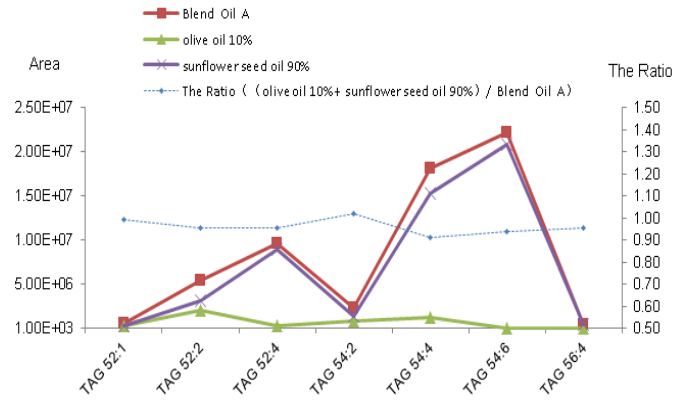


Figure 11. Testing Cooking Oil. The cooking oil is 90% sunflower oil and 10% olive oil composition. The red line is the peak area of markers in blend oil A. The green line is the peak area of markers in olive oil 10%. The purple is the peak area of markers in sunflower seed oil 90%. The dash line is the ratio of the sum of the peak area of the markers in olive oil 10% and sunflower seed oil 90% to the peak area of the markers in blend oil.

Geographical Origin Analysis of Olive Oil and Soybean Oil

Olive oil and soybean oil were collected from different countries and regions. Olive oil is the main oil produced by Italy, Greece, Spain; soybean oil is the main oil produced in China, Brazil, and the United States. The geographical origin analysis experiments found a total of 34 distinguishing markers.

Due to geographical and climatic differences, components of leading olive oils from three different countries can have differences in glycerides and free fatty acids content. In Figure 12, according to the results of PCA analysis using the FFAs, there is clear differentiation between the olive oils from the different countries (Italy, Greece, Spain). And the groupings of oils from within each country were quite tight.

For soybean oil, grouping using the FFAs for the oils from China, Brazil, and the United States did not product clear differentiation, however the triglycerides groups are obvious (Figure 13).

These results suggest that profiling of the TAGs and FFAs in oil will enable differentiation of oils by type and country.

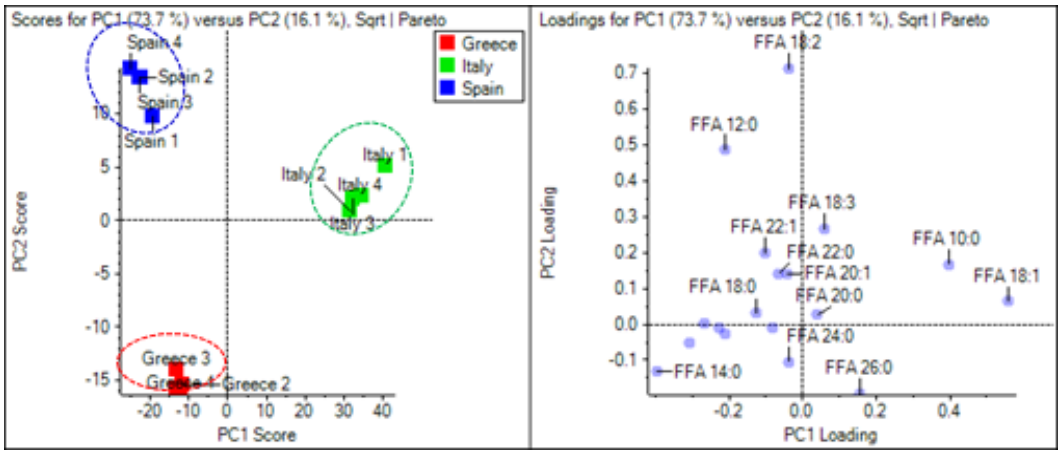


Figure 12. Olive Oil Free Fatty Acids, Showing PCA Grouping of Samples from Three Countries. This indicates that geographical origin can be distinguished by the content of free fatty acids for these three countries tested.

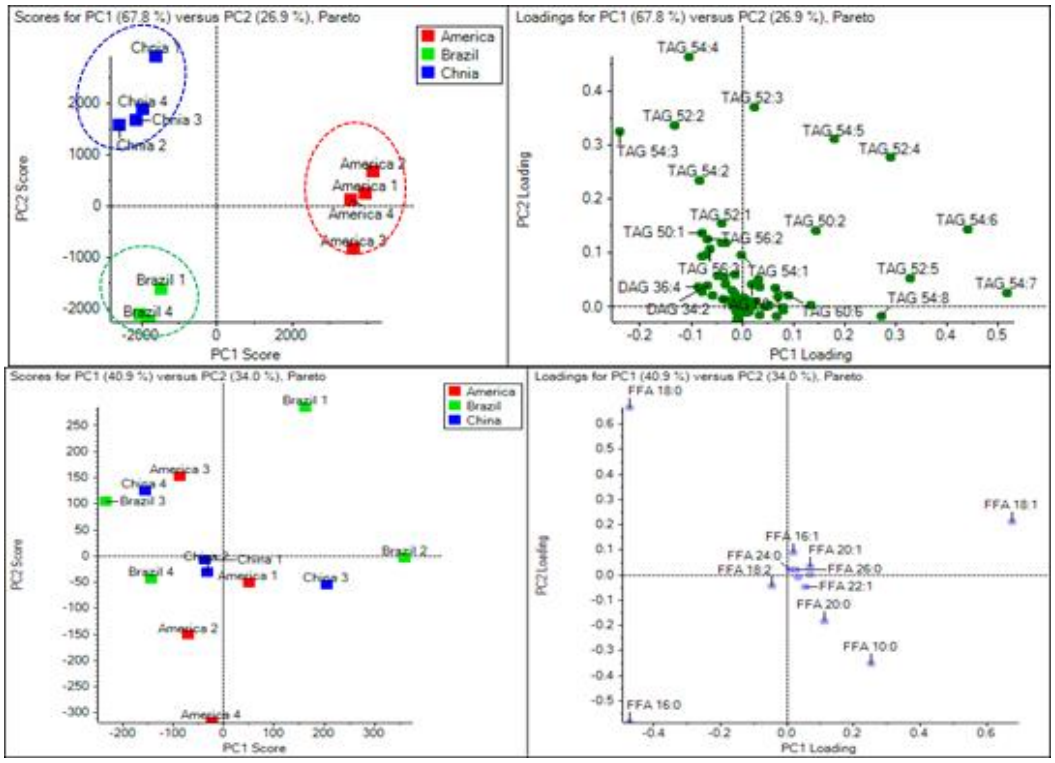


Figure 13. Soybean Glycerides and Free Fatty Acids, Showing PCA Grouping of Samples from Three Countries. Top panels indicate that these geographical origin can be distinguished by the content of the TAGs, but the bottom panels show that there is no distinction in FFA content between geographical origins in this example, America, Brazil and China.

Summary

- The SCIEX X500R QTOF System was used successfully to develop a workflow to identify the authenticity and geographical origin of edible oils.
- Despite the complex matrix of edible vegetable oil, the performance of the X500R system (such as DBS, mass resolution, high sensitivity, high accuracy, and fast scanning speed) ensures that good quality information is collected, and the data are of high quality.
- QC data show that, despite continuous collection days, the reproducibility was good across the full experiment.
- Using a targeted list of lipid species of interest from LipidView software, lipid identifications and quantitative peak areas can be extracted from SCIEX OS-Q software.
- MarkerView software was employed for principal component analysis (PCA) and t-test, which were critical statistical analysis that allowed for the rapid detection of potential marker compounds.
- There were 39 species of lipid markers and 9 free fatty acids found in this experiment which were successfully monitored in oil samples to assess the authenticity and geographical origin.

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Document number: RUO-MKT-02-10076-A

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