APPLICATION NOTE

Liquid Chromatography/
Mass Spectrometry

Authors:

Robert Seward

Catherine Stacey

PerkinElmer, Inc.
Waltham, MA

Differentiation of Olive, Vegetable and Seed Oils by LC/MS Analysis of Triacylglycerides

Introduction

Extra virgin olive oil is known for its nutritional value, high antioxidant content and low saturated fat content when compared to other oils.[1] This expensive oil has been adulterated throughout history with less expensive oils such as soybean or hazelnut oil. Detecting adulteration is important for maintaining both the safety and consumer confidence of this product.

This study reports on the measurement of the triacylglyceride composition of a large number of olive oils and other seed and vegetable oils using a simple LC/MS method, together with statistical analysis of the results to find indicators for different oil types.

PerkinElmer®
For the Better

Method

Oil Samples

A number of different seed and vegetable oils, together with samples of virgin and refined olive oils, olive pomace oil, light olive oil and extra virgin olives oils (EVOOs) from California and Spain were analyzed. Samples were purchased from supermarkets or donated by suppliers.

LC-TOF-MS Analysis

The triglycerides (TAG) levels of the oils were detected using a positive mode LC/MS analysis method. Because TAGs are the main constituents of olive oils, only a simple dilution of the oils in acetonitrile was required prior to analysis.

Samples were analyzed with a reversed-phase separation using a 2 mm x 10 cm Brownlee SPP C18 column and an acetonitrile/ethyl acetate gradient at a flow rate of 0.4 mL/min from a Flexar™ FX-10 UHPLC pump. A post-column addition of a 10 mM aqueous ammonium formate solution at 0.2 mL/min through a T-fitting was used to promote the formation of $[M+NH_4]^+$ ammonium adduct ions. TAGs were detected with positive mode electrospray ionization on an AxION® 2 TOF MS fitted with an Ultraspray™ 2 ion source. Each of the oils were analyzed in triplicate.

PCA Analysis

All of the LC/MS datasets were processed in a batch with a proprietary algorithm to extract the intensities for the significant features in each dataset. Each feature is produced by a chemical component with a distinct m/z value and a profile in the time domain that is consistent with the typical LC peak widths for that HPLC column.

The processing results are exported into a table, with rows for each dataset and intensities aligned into columns for each feature; the column headings are a text summary of rounded m/z and retention time values for that feature.

TIBCO Spotfire® was used for statistical analysis of the data table using an S-Plus Principal Component Analysis function and for graphical display of result in scatter plots and bar charts. The intensities in the table are the variables used for the statistical analysis. The column headings are used for labels of features in plots.

Categories such as oil type, country of origin and olive type for each sample were linked to the table. This enabled different grouping and sorting options and color coding of graphs.

PCA transforms or projects the variables for each sample into a lower dimensional space while retaining the maximal amount of information about the variables. Resulting principal components for each sample are a combination of the original variables after the transformation. The largest difference in the combined variables between the samples is described by Principle Component 1 (PC1), the next largest by PC2 and so on.

Scores Plots of principal components are used to investigate the relationship between samples. Samples that group together have similar levels of variables. A Loadings Plot is a means of interpreting the patterns seen in a Scores Plot, with variables furthest from the origin having the most significant contribution to sample grouping.

Results and Statistical Analysis of a Small Set of Californian EVOO, Seed and Vegetable Oils

An initial small scale study of a number of Californian EVOOs and adulterant oils was made to test out the validity of the TAG analysis method.

PCA of the TAG features in the datasets results in a Scores Plot for PC1 v. 2 (Figure 1 left panel) showing a close grouping of all olive oil samples and complete separation of this group from other oil types. The soybean, grape seed and sunflower oils are widely separated from the EVOOs along the PC2 axis, with canola oil less separated on the same axis.

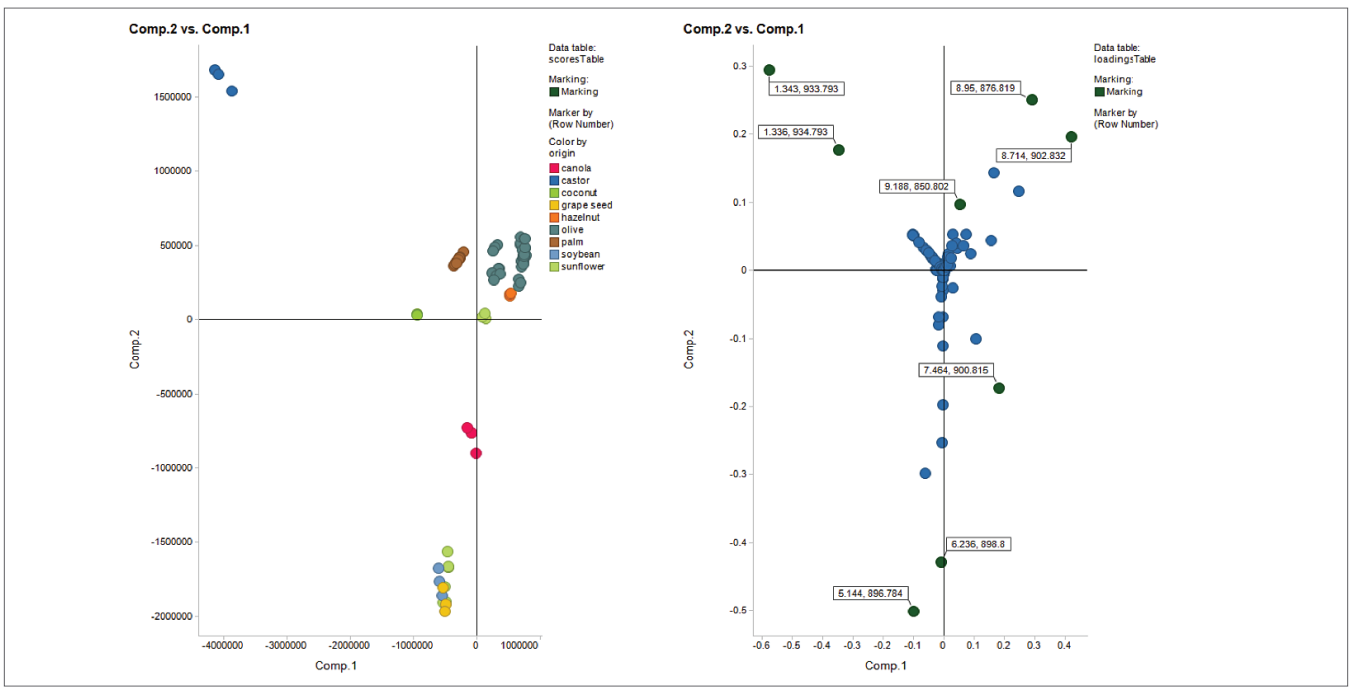

Figure 1. PCA Scores and Loadings Plots of the TAG levels with samples colored by oil type. Only California EVOO oils, shown in blue-green, are included in this initial analysis. Most significant loadings variables are m/z 896, 898, 933, and 876.

The loadings plot (Figure 1 right panel) shows the features on the PC2 axis which most influences this grouping.

Each feature is labeled with a rounded m/z value and retention time created by the extraction algorithm. The identification of all the TAG ions was confirmed from the accurate masses of the molecular ions in peaks in the original datasets. The detected TAGs are listed in Table 1.

In this Loadings Plot, soybean, grape seed and sunflower oils have higher levels of the TAGs at LLL and LLO than other oils, with canola oil also showing elevated levels.

Table 1. The detected TAGs which are most significant to the sample grouping by cultivar.

m/z	time/min	TAG
902.8171	8.71	OOO
900.8015	7.42	OOL
898.7858	6.29	LLO
876.8015	8.93	OOP
874.7858	7.60	OOPo/POL
850.7858	9.19	OPP
848.7702	7.74	OPPo
933.7753	1.34	$[M+H]^+$ for RRR

m/z for $[M+NH_4]+$ ions of the TAGs
Fatty acid residues in the TAGs indicated as S= stearic, O = oleic, P = palmitic, L = linoleic, Ln= linolenic, Po= palmitoleic, R=ricinoleic

Castor oil is widely separated from all other oils in the upper left quadrant of the Scores Plot. The variable in the same direction in the Loadings Plot is a triglyceride of the hydroxylated long chain fatty acid ricinoleic acid. Castor oil is the only oil known to yield this TAG[2].

Palm and coconut oils group closely to the olive oils in the center of the PC 1 v. 2 Scores Plot. In a different projection of the PCA scores in a Score Plot for PC 3 v. 2 (Figure 2) these two oils are separated from EVOOs due to increased levels of OPP and OPPo.

Tree nut oils are known to have a higher proportion of C16 acid (palmitic and palmitoleic acids) containing TAGs, when compared with olive oils.

Hazelnut oil was not well separated from the olive oils in these initial PCA Scores plots, since both oils have similar levels of all the measured TAGs.

The statistically significant separation of most potential adulterant oils from the olive oils by the pattern of TAGs shows that this information could be used to confirm the oil type and detect adulteration of olive oils with these oils.

Results for a Large Scale Study of Oils Including Spanish EVOOs

A larger study was performed with 35 different monovarietal and blended Spanish EVOOs, together with lower grade olive oils. These results were combined with those from the initial analyses of Californian EVOOs and seed and vegetable oils. This larger group of oils was used to confirm and expand on the earlier findings.

Analyzes of samples were conducted in batches over several days, so the initial PCA Scores Plot of PC 1.v 2 shows differences between sample groups caused by small variations in analysis conditions. The PCA was recalculated using scaled variables; the intensities for each variable were scaled by a factor derived from the sum of the intensities for all variables for that sample.

In the scaled PCA Scores Plot of PC 2 v. 1, oils in the expanded sample set are grouped by oil type (Figure 3). EVOOs, refined and regular olive oils are grouped closely together and most adulterant oils are separated from them, in agreement with the earlier study.

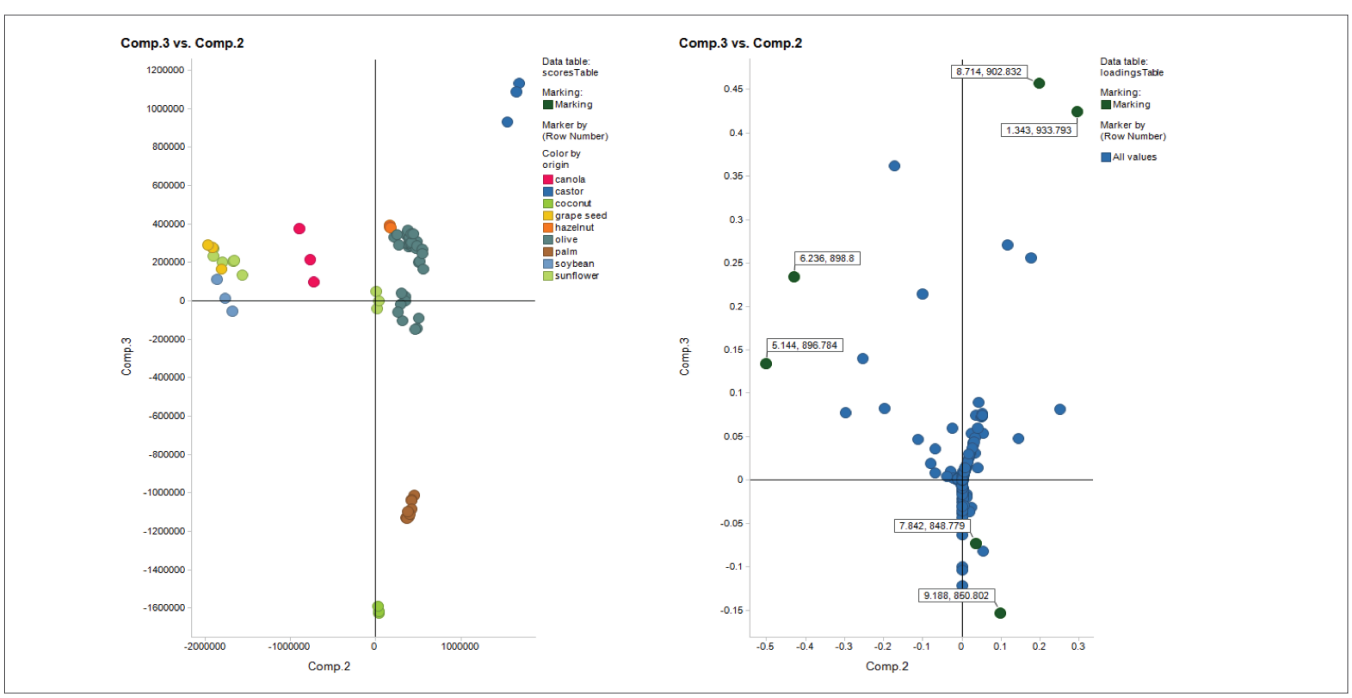

Figure 2. PCA Scores and Loadings Plot of PC 3 v. 2 of Californian olive and other oils. In this projection the palm and coconut oils are separated from the olive oils by higher levels of the TAGs OPP and POPo.

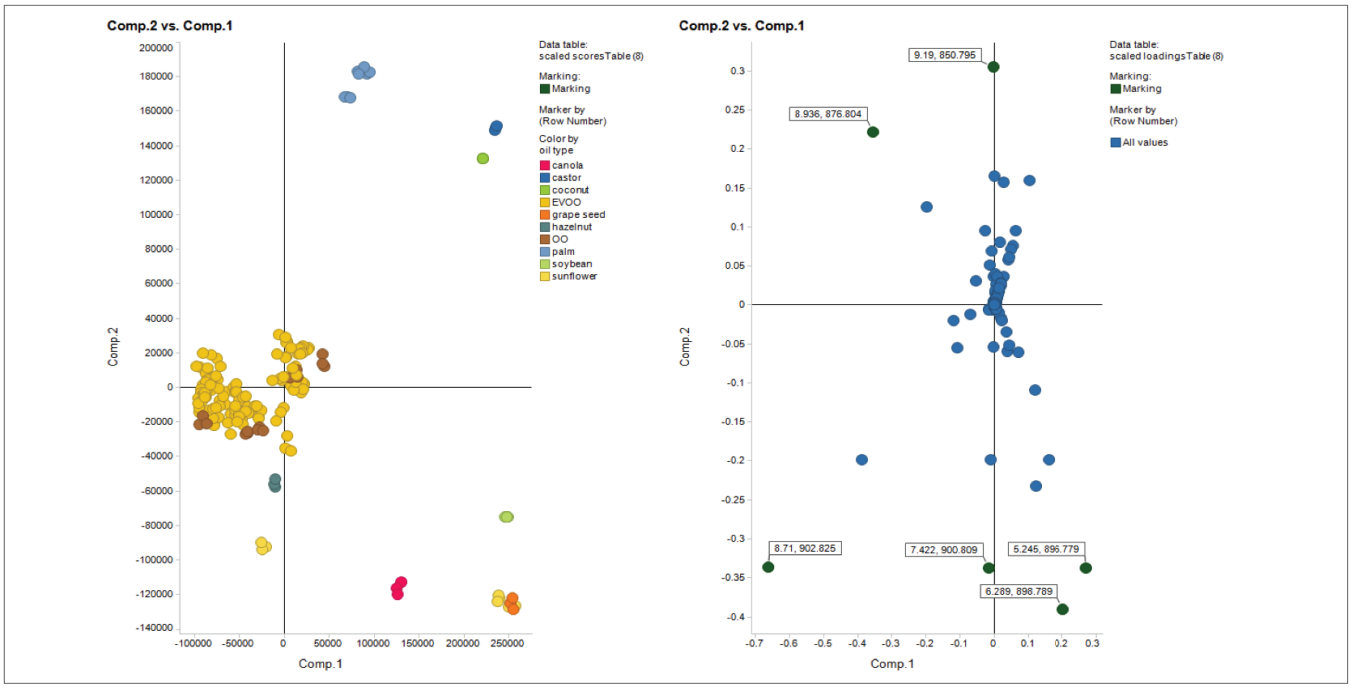

Figure 3. Scores and Loadings Plots of PCA of using scaled variables shows that Spanish and Californian EVOOs in yellow and other olive oils in brown are grouped and separated from other oil types.

Hazelnut oil is separated from all of the olive oil samples in this PCA Scores projection, but has a similar TAG profile to the high oleic acid sunflower oil. The Loadings Plot shows that both of these oils have lower levels of PPO, PLO and POO than the olive oils, but higher levels of OOO.

In a different PCA Scores projection, the Scores Plot of PC 6 v. 2 (Figure 4) shows separation of hazelnut and high oleic acid sunflower oils from olive oils. The differences are due to the levels of the TAGs OPP, OOPo, OOP and OOO in these oils as shown in the Loadings Plot.

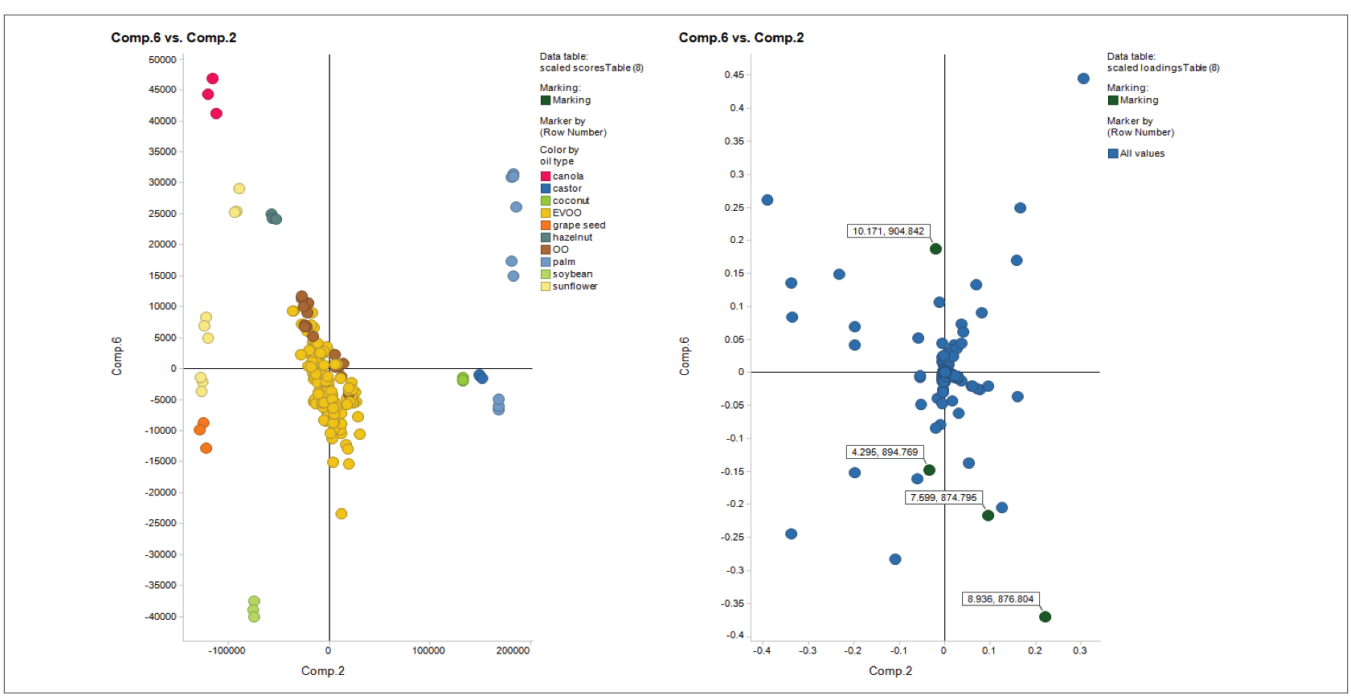

Figure 4. Scores and Loadings Plots of PC 6 v. 2 showing separation of oils. Hazelnut oil is separated from all of the olive oils and also from high oleic acid sunflower oil.

The PCA results suggest that hazelnut oil has a unique profile for these TAGs. This information could be used to detect adulteration of olive oil with hazelnut or sunflower oil, two of the most commonly used adulterants. For example, a bar chart of the ratio of PPO to the most abundant TAG OOO (Figure 5) shows a distinctly different ratio for the adulterant oils. The ratio can be used as a marker for the presence of these adulterant oils in olive oils.

Conclusion

A fast LC/MS method with a minimum of sample preparation was used to detect the TAGs profiles of a large number of seed, vegetable and olive oils.

Statistical analysis of the TAG levels found patterns of TAGs which are characteristic for each oil type. This information could be used to find characteristic markers for different types of oils and to investigate adulteration.

A more detailed analysis of the oils from different Spanish olive cultivars will be covered in a separate applications note.

References

1. Garcia-Gonzalez L. D., Aparicio-Ruiz R., Aparicio R., Virgin olive oil - chemical implications on quality and health. *Eur. J. Lipid Sci. Technol,* 110 (2008), 602-607.
2. Achaya, K.T., Craig, B.M., and Youngs, C.G. The Component Fatty Acids and Glycerides of Castor Oil. *J. Am. Oil Chem. Soc.,* 41 (1964), 783–784.

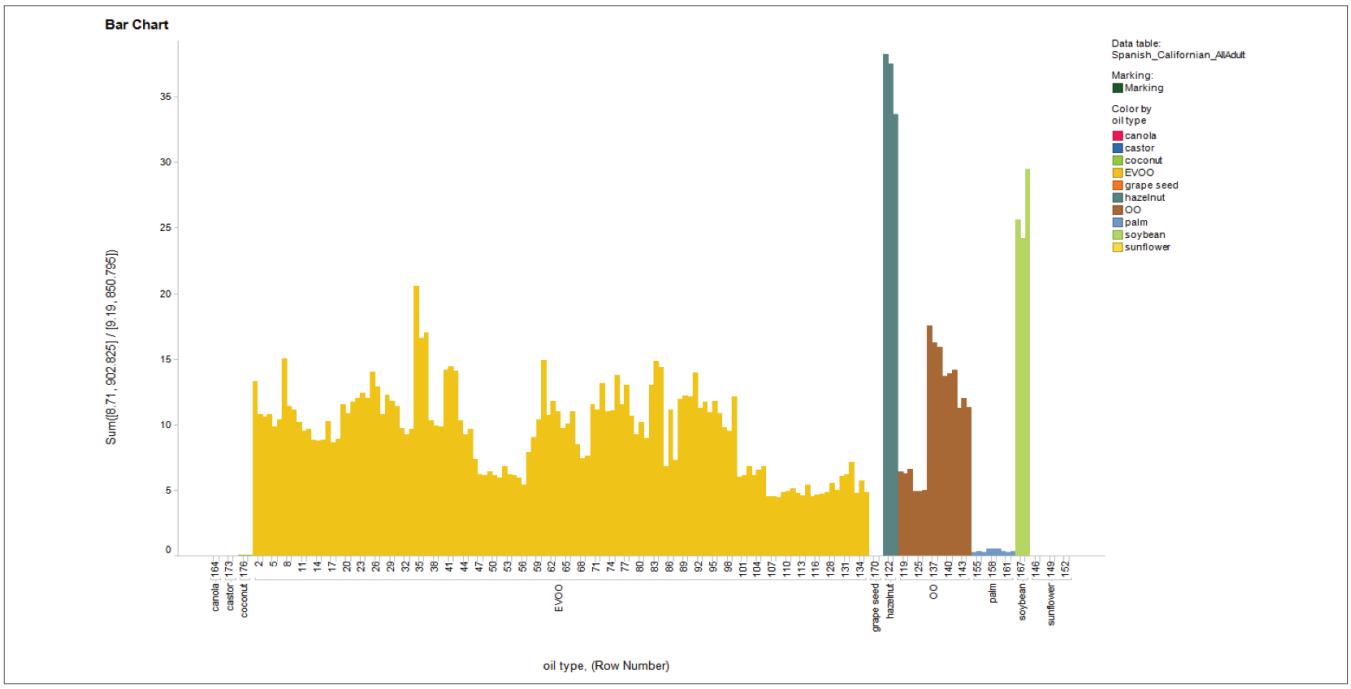

Figure 5. A bar chart of the ratios of OOO to PPO in each oil shows that the hazelnut oil has the highest ratio, with high oleic acid sunflower oil also having a higher ratio than EVOO or refined olive oils.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

011988_01 PKI