APPLICATION NOTE

Liquid Chromatography/
Mass Spectrometry

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Differentiation of Olive Varieties by the Triacylglyceride Levels of Oils –Statistical Analysis of LC/MS Results

Introduction

Olives of different cultivars are used to produce oils with different particular flavors and stability. Oils of known cultivars from designated areas may be certified for authenticity, and are marketed internationally, commanding a price premium.

This study reports on the measurement of the triacylglyceride composition of a large number of olive oils from known origins using a simple LC/MS method, together with statistical analysis of the results to find indicators for different cultivars. Such marker compounds could be used to test the authenticity of an unknown sample.

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Method

The oil samples

Extra virgin olive oils (EVOO) from California and Spain and other lower grade olive oils were analyzed. A set of 35 Spanish EVOO brands were donated by suppliers and consisted of monocultivar oils cold pressed from olives of a single cultivar or blends. The cultivars were Manzanilla Cacereña, Arbequina, Picual, Arbequina, Empeltre and Cornicabra olives and various blends of Hojiblanco, Picudo and Picual cultivars. The Californian EVOOs were purchased locally, and included Arbequina oil and an Arbequina-rich blend. Commercial virgin olive oil, olive pomace oil and hazelnut oil, a common olive oil adulterant, were also analyzed for comparison with the EVOO samples.

Analysis of triacylglycerides by LC/MS

The levels of a number of triglycerides (TAGs) in the oils were detected with a positive mode LC/MS analysis method as described in a previous applications note (see list of references).

Statistical analysis method

An algorithm to extract the significant features from each LC/MS dataset and combine the features into a data table has been described previously (see list of references). TIBCO Spotfire® software was used for statistical analysis with the S-Plus Principal Component Analysis (PCA) function. Results are displayed in TIBCO Spotfire® 2D or 3D scatter plots, with color coding of the samples by oil type.

Results

TAG levels for cultivars

The PCA of the TAG levels for all samples gave component scores and loadings which were displayed in scatter plots. A Scores Plot of PC 1 v. 2 (Figure 1 left panel) has the triplicate analyses for each sample color-coded by cultivar. The plot shows that Cornicabra, Picual and Manzanilla Cacereña cultivar samples are clustered into groups in the lower right quadrant and are distinct from those of Arbequina and Empeltre cultivar groups, clustered in the upper left quadrant.

Samples from several blended Californian oils (colored green) are close to those of the Arbequina cultivar in red; at least two of these blends are known to include oil from Arbequina olives. A hazelnut oil sample is distinctly separated from all of the olive oil samples.

The corresponding Loadings Plot of PC 1 v. 2 (Figure 1 right panel) shows the features which most cause the differentiation of the cultivar groups.

Assignment of these features to known TAGs was based on the accurate mass of the features. The rounded m/z and retention times of the features in the table were correlated to accurate masses of a component in a spectrum at that retention time in the original dataset.

The mass and identity of the detected TAGs are summarized below (Table 1). The TAGs OOL, OOO and OOP having the most influence in this Loadings Plot.

Arbequina and blends have lower levels of OOO. Picual, Cornicabra and Manzanilla Cacereña cultivars have higher levels of OOP and lower levels of OOL. Empeltre oils had the highest levels of the TAGs OOL and LLO.

Table 1. TAGs in the oils which are most significant to the sample grouping by cultivar.

m/z	time/min	TAG
904.8328	10.17	SOO
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	POPo

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

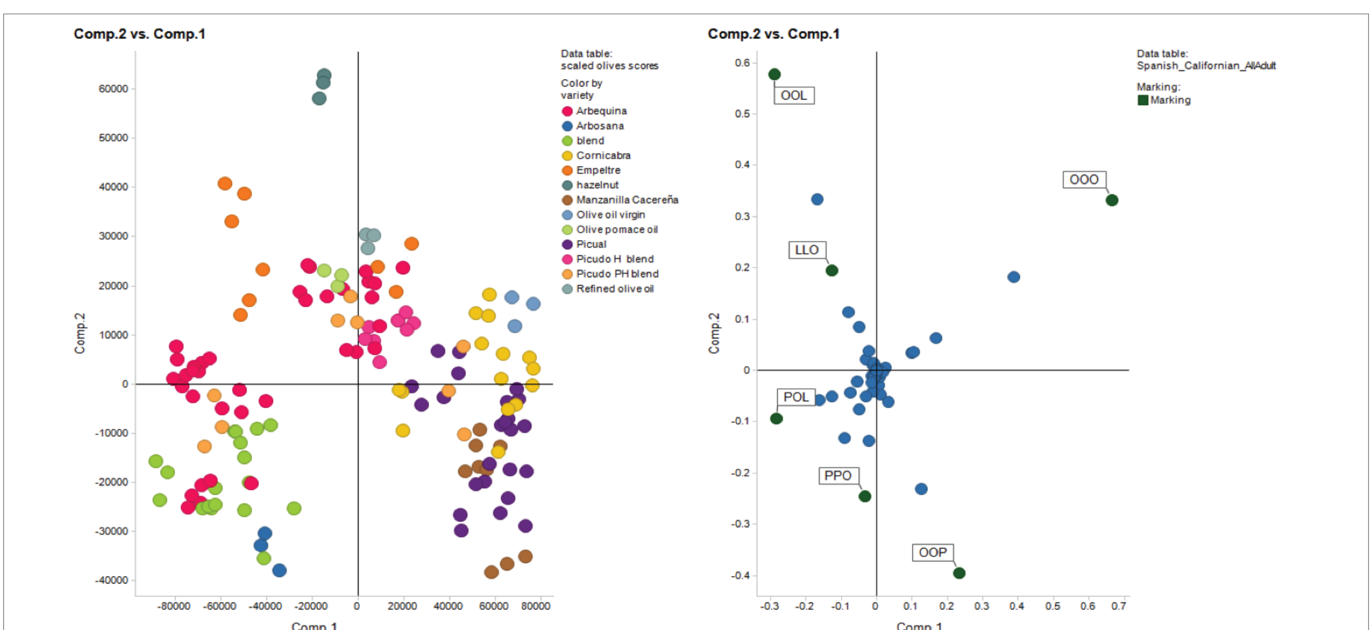

Figure 1. PCA Scores and Loadings Plots of PC 1 v. 2 from the TAG levels of olive oils. Clustering of color-coded cultivar samples is observed. Picual, Manzanilla Cacereña, and Cornicabra oils group in the lower right quadrant and Arbequina and Empeltre oils group in the upper left quadrant. The TAGs OOL, OOO and OOP have most influence on this grouping.

We found from these PCA results that the levels of OOO and OOL were the strongest indicators of cultivar for these Spanish olive oils. The ratios of the intensities for the TAGs OOO and OOL for each sample, colored by cultivar, are summarized in a bar chart in Figure 2. The ratio is highest for Cornicabra, Picual and Manzanilla Cacereña cultivar samples.

The TAG levels of Cornicabra oils have been reported[1]. In that previous study, SOO levels were higher than oils from many other cultivars including Manzanilla Cacereña, but similar to those in Picual.0Levels of POL and PPO were similar to those of Manzanilla Cacereña and Picual cultivars.

In our work, higher levels of SOO were also observed for Cornicabra and Picual varietals than for all other cultivars. The bar chart in Figure 3 shows the levels of SOO for replicates of each sample, colored by cultivar.

The levels of the three cultivar-indicative TAGs OOO, OOL and OOP are compared in a three-dimensional scatter plot in Figure 4. Samples for Picual, Cornicabra and Manzanilla Cacereña cultivars are clearly separated from those of Arbequina and Empeltre varieties.

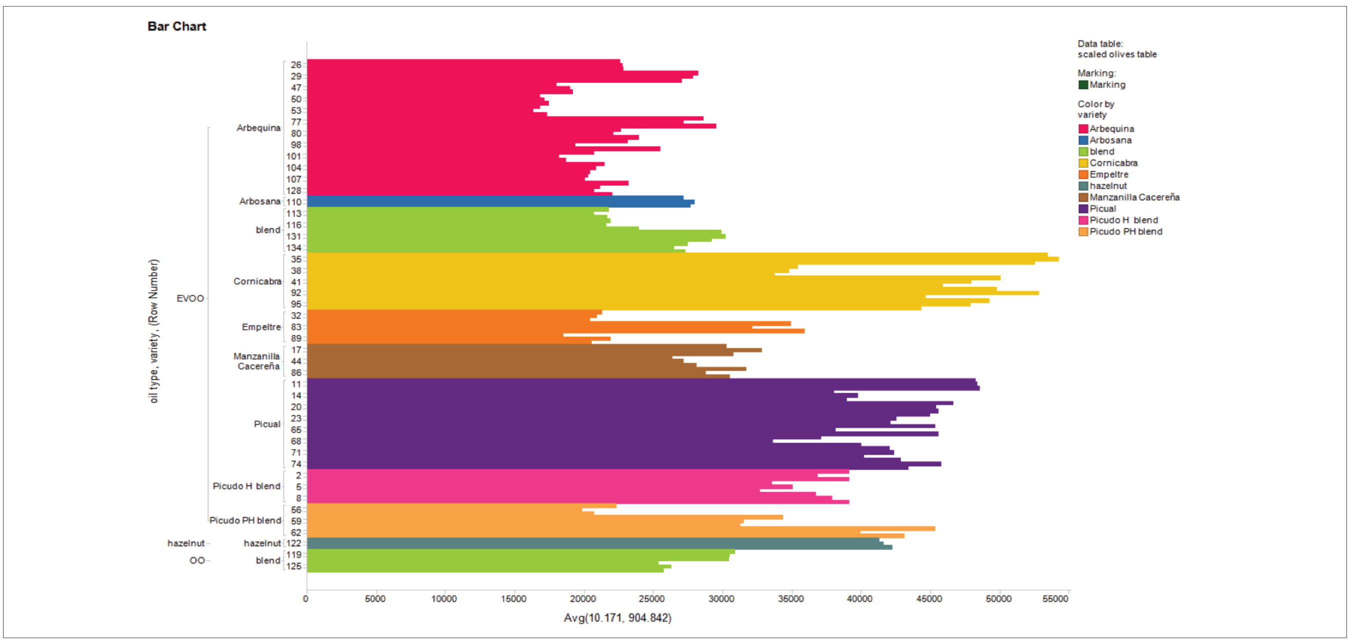

Figure 2. Chart showing the ratio of the intensities of the TAGs OOO and OOL for all replicates of each sample, colored by variety. The ratios are higher for Cornicabra in yellow, Picual in purple and Manzanilla Cacereña in brown.

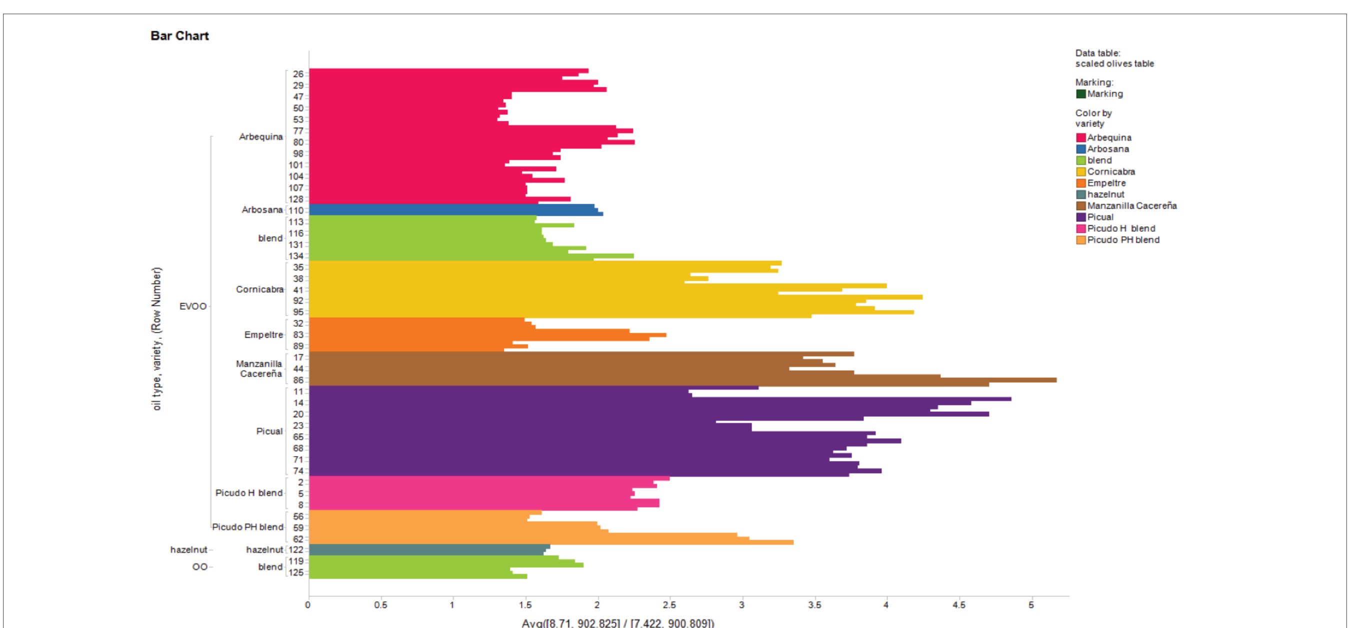

Figure 3. Chart showing the intensities of the TAG SOO for replicates of each sample, color coded by cultivar. Highest levels are for the Cornicabra and Picual varietals in yellow and purple.

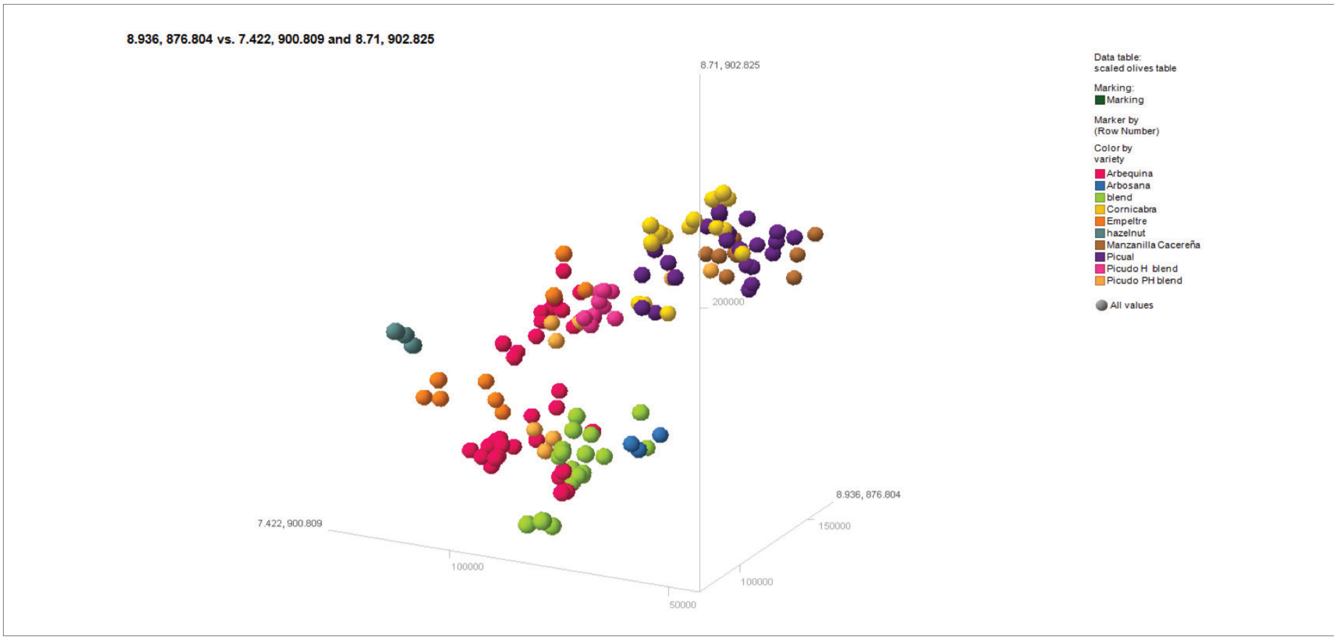

Figure 4. 3D scatter plot showing a separation of samples into cultivar groups, with the axes being the intensities of the three TAGs OOL v. OOO v. OOP.

TAG levels by region

One cultivar in this study included samples from olives grown in different regions. Arbequina cultivar oils originated from either the Cataluña or Navarro regions of Spain or from California. Oils from each region are shown with different shapes in the Scores Plot of PC 2 v. 1 (Figure 5), while other oil cultivars are greyed out for clarity. Clustering of sample groups by geographical origin was observed. Differences between the groups are primarily due to varying levels of the TAGs OOO, OOPo and OOL.

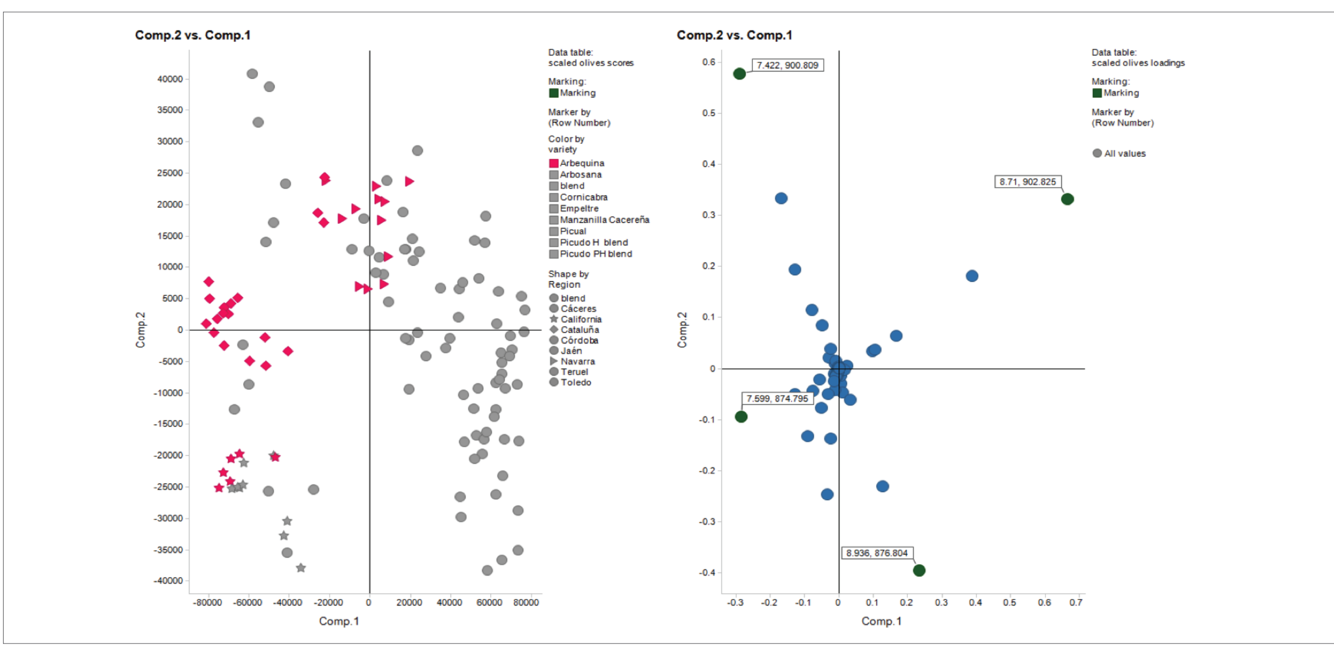

Figure 5. Scores and Loading Plots of PC 2 v. 1 showing separation of Arbequina oils in red into groups related to the region of origin, shown by shape. Samples from the Spanish regions Navarra are triangles, Cataluña are diamonds and the Californian samples are stars. Other cultivars are shown in grey.

Conclusion

Statistical analysis was used to distinguish EVOOs produced from different olive oil cultivars in Spain and California by their pattern of characteristic TAGs. Levels of the TAGs OOO, OOL and OOP are the most significant indicators for the cultivars. Levels of OOO and OOL also showed differences between oils of a single olive cultivar grown in different locations.

References

1. Differentiation of Cultivars of Spanish Olive Oils Using Multiple LC/MS Analysis Methods, Application Note, Robert Seward & Catherine Stacey, PerkinElmer, Inc.
2. Markers for Spanish Olive Oil Cultivars – Statistical Analysis of Polar Compounds from LC/MS Results, Application Note, Robert Seward and Catherine Stacey, PerkinElmer, Inc.
3. Differentiation of Olive, Vegetable and Seed Oils by LC/MS Analysis of Triacylglycerides, Application Note, Robert Seward and Catherine Stacey, PerkinElmer, Inc.
4. F. Aranda, S. Gomez-Aonso, R.M. Rivera del alamo, M.D. Salvaror, G. Fregapane. Triglyceride, total and 2-position fatty acid composition of Cornicarbra virgin olive oil: Comparison with other Spanish cultivars. *Food Chemistry*, 86 (2004), 485-492.

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