Liquid Chromatography/ Mass Spectrometry

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Differentiation of Cultivars of Spanish Olive Oils Using Multiple LC/MS Analysis Methods

Introduction

For high value olive oil, the provenance may have a dramatic effect on the price. Falsifying the origin of oils is financially an attractive proposition to fraudsters. The patterns of organic components in olive oils relate to the geographic origin, cultivar and processing methods for the olives. In this study a statistical analysis of the chemical components of authentic monovariatal virgin olive oils from Spain was used to determine markers for the cultivars. These markers could be used to confirm the provenance and origins of olive oil samples.

Different LC/MS methods were used to measure the levels of a large number of polar and non-polar organic components in the oils. Results from the separate analyses were combined with data fusion. A statistical comparison with the combined results improved the discrimination of cultivar groups, when compared to a single LC/MS analytical method.

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Method

The Spanish Olive Oil Samples

Olive oils produced from a number of olive cultivars grown in Spain were analyzed. Each brand of extra virgin olive oil (EVOO) was pressed from locally grown olives comprised by a single cultivar or blend. In most cases, each cultivar was limited to one distinct geographical region. Olive brands from one cultivar came from two distinct regions.

Oils were produced from either a single olive cultivar of Manzanilla Cacereña, Arbequina, Picual, Arbequina, Empeltre and Cornicabra olives or various blends of Hojiblanco, Picudo and Picual cultivars.

Analytical Methods

The concept of Principal Component Analysis and the implementation of this function within TIBCO Spotfire® software has been described previously (see list of references). A method to extract intensities for each significant feature from LC/MS datasets, resulting in a table with each feature labeled with the mass and elution time of the peaks in the datasets has also been described. The intensities in the table are used as variables for PCA within TIBCO Spotfire®.

Three different sample preparations and LC/MS methods for the analysis of various categories of compounds from olive oil were described in previous application notes relating to olive oil analysis (see list of references). Compounds detected in the oils included triacylglycerides (TAGs), secoiridoids, lignans and fatty acids.

Considerable variation was observed in the levels of these compounds between different brands from the same cultivar, related to different growing conditions, production methods and storable conditions for the oils.

Due to these variations, PCA was unable to separate all the oils from each cultivar into distinct groups using results from any one of the analytical methods described above.

Combining Separate Results Tables with Data Fusion

A data fusion method was explored as a way to improve the discrimination between these chemically very similar oil sample groups. Three separate data tables of LC/MS-derived compound intensities were combined into one fused table for all the detected compounds for each sample. The new fused table enabled a single PCA with all these intensities.

Since the mass spectrometer response varies by compound type, LC conditions, electrospray source parameters and ionization mode; intensities from each separate analysis method have different ranges. Intensities in each table were normalized to unity before the tables were combined.

Grouping Spanish Olive Oils by Cultivar with Data Fusion

The Scores plot (Figure 1, left panel) from PCA of the fused data table produced a clearer grouping of the samples into cultivar groups than was obtained from any single analytical method (see list of references).

For visual clarity, samples from the blended oils were excluded from display in the Scores Plot, although these samples were included in the PCA. The blends clustered in the center of the Scores Plot.
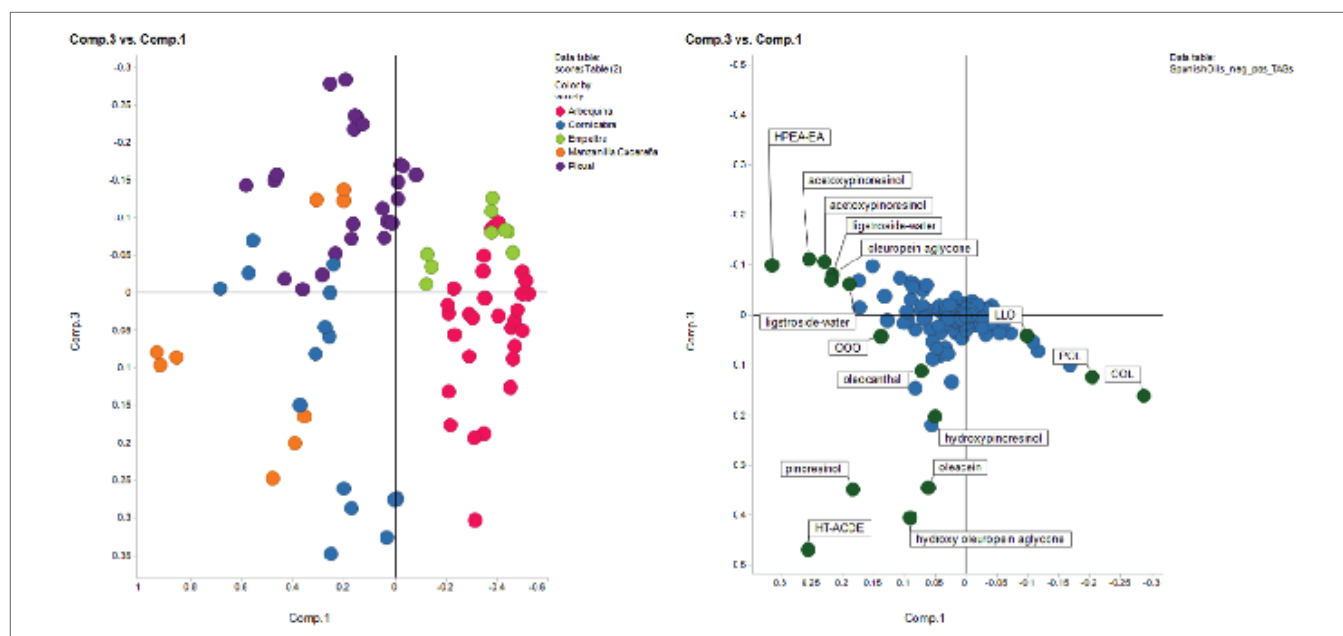

Figure 1. The PC 1 v. 3 Scores and Loadings plots from the PCA of fused data from TAGs and polar compounds detected in negative and positive modes. In the Scores Plot samples are color-coded by cultivar. The plot shows a discrimination of Arbequina and Empeltre cultivars in green and red from those of other olive cultivars.

A distinct separation of oils from Arbequina and Empeltre cultivars from those of the Picual, Cornicabra and Manzanilla Cacereña cultivars was observed, except for one brand of Manzanilla Cacereña oil which did not group with others of that cultivar.

Groups were most clearly differentiated by the variables indicated in the Loadings Plot. Arbequina and Empeltre cultivars had higher levels of the TAGs OOL and OLP and lower levels of many polar compounds, when compared with other cultivars. Picual cultivars had the highest levels of oleuropein aglycone, acetoxypinoresinol and HPEA-EA, or ligstroside aglycone. Cornicabra and Manzanilla

Cacereña cultivars had high levels of a number of compounds including pinoresinol, oleacein and HT-ACDE, a hydrotyrosol enolate ester.

Geographic Origins

Brands of Arbequina cultivar oil from two different regions of Spain could be partly separated in a PCA Scores plot due to difference in the levels of a number of TAGs (Figure 2). The level of polar components was not significantly different between oils from the different regions.
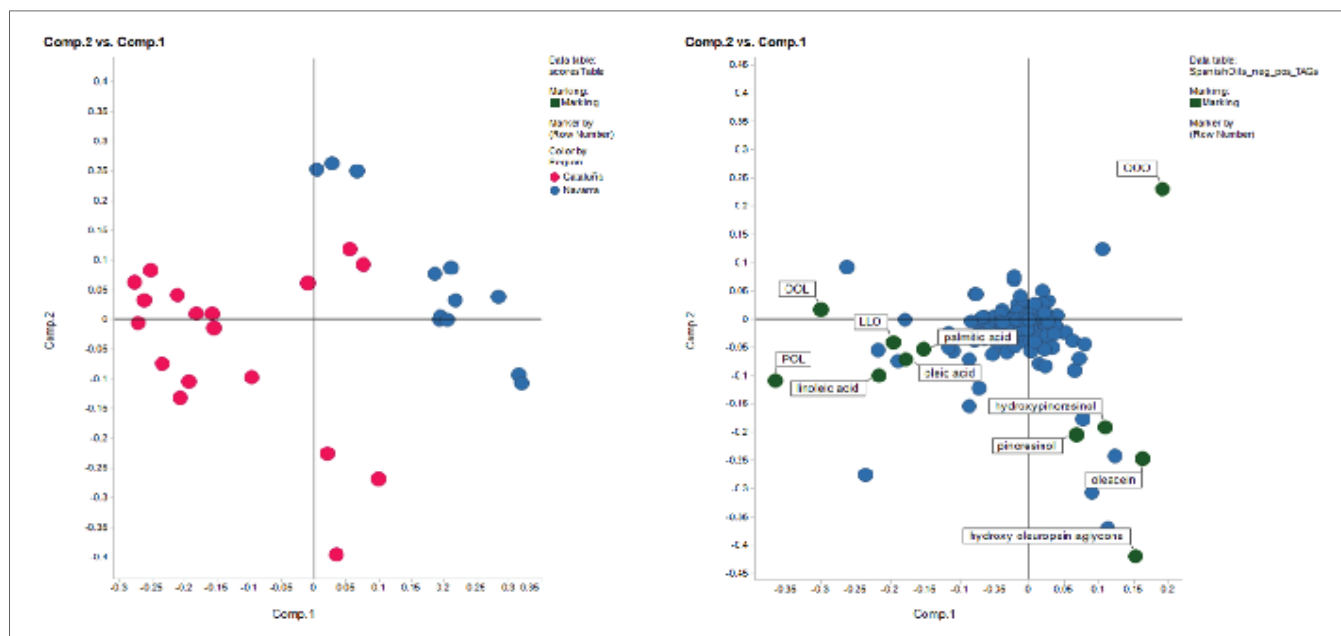

Figure 2. PCA Scores and Loadings plots calculated with the fused information for the Arbequina cultivar samples from two different regions. Partial separation into groups is primarily due to differences in the levels of a number of TAGs.

Conclusion

Data fusion of results from different sample preparation and LC/MS analytical methods allowed for distinct cultivar groups in a PCA Scores Plot. An improved discrimination was produced when compared with PCA using any single LC/MS method.

This concept of fusing together results from separate analytical methods could be extended to include other analytical techniques such as UV and infrared spectral measurements and intensities of compounds from GC/MS analysis and of elements from ICP-MS analysis.

References

1. Differentiation of Olive Varieties by the Triacylglyceride Levels of Oils – Statistical Analysis of LC/MS Results, Application Note, Robert Seward and 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.