

# Detection of Adulterant Seed Oils in Extra Virgin Olive Oils by LC-MS and Principal Components Analysis

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## Overview

Various oils were analyzed using LC-MS/MS and Principal Components Analysis (PCA). These oils included oils advertised as extra virgin olive oils, an authentic extra virgin olive oil, other seed oils, and an authentic extra virgin olive oil manually substituted with varying amounts of other seed oils (manually adulterated extra virgin olive oil) were analyzed using LC-MS. PCA was then used to differentiate extra virgin olive oils from other seed oils, and extra virgin olive oils from adulterated extra virgin olive oil at various levels of adulteration. The presence of adulterants at trace levels was determined, along with the components responsible for the greatest difference between these groups. Hazelnut oil, a common, hard-to-detect adulterant, was also detected at trace levels.

## Introduction

Olive oil is an expensive, popular, edible oil mainly produced and consumed in Mediterranean regions. It is an important oil that is high in nutritional value due to its high content of antioxidants (including Vitamin E), its ability to lower LDL cholesterol, and its recently reported possible anti-inflammatory activity.<sup>1</sup>

Among all olive oils, extra virgin olive oil contains the most health benefits.

It is common practice today to adulterate extra virgin olive oils with refined oils or less expensive seed oils for financial gain; hazelnut oil is a commonly used adulterant.<sup>2-3</sup>

Although adulterated extra virgin olive oils are sold as extra virgin olive oil, they do not offer the full nutritional health benefits, and in some cases, may pose a health risk. Methods capable of exploiting these differences in order to distinguish between authentic and adulterated versions of a product are of great importance for both producers and consumers. In this work, an LC-MS method was used in conjunction with a statistical tool to perform Principal Components Analysis (PCA) to detect the adulteration of extra virgin olive oil.



## Experimental

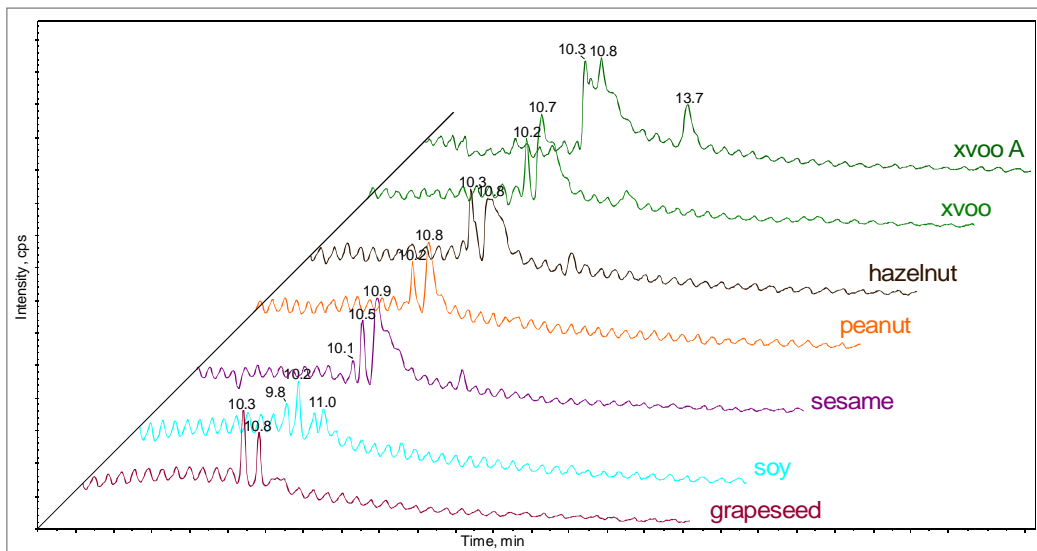
### Samples

Store-bought olive oils (oo), oils advertised as extra virgin olive oils (xvoo), various seed oils (canola, corn, hazelnut, grapeseed, hot chili sesame, peanut, safflower, sesame, soy, and sunflower) and a known, authentic extra virgin olive oil were used. The authentic extra virgin olive oil (xvoo A) was manually adulterated with the seed oils in ratios of 50/50, 75/25, 80/20, 90/10, and 99/1 (xvoo A/seed oil). All samples were diluted by a factor of 100 in hexane/isopropanol (99.7/0.3) for injection into the LC-MS system.

### LC/MS

The major components of seed oils are triacylglycerols, which are typically analyzed by LC-MS with Atmospheric Pressure Chemical Ionization (APCI).<sup>4</sup>

Normal-phase liquid chromatography was used for this analysis. Chromatographic separation was carried out using a Keystone Scientific Spherisorb Silica column (250 x 4.6 mm, 5  $\mu$ m, 80 Å). The mobile phase consisted of hexane (solvent A) and isopropanol (solvent B). A linear gradient starting at 0.3% B for 5 min was ramped to 100% B in 23 min and held at 100% B for 5 min. The flow rate was 1 mL/min and the effluent was directed into the orthogonal APCI source of the mass spectrometer.



**Figure 1.** Total Ion Chromatogram LC/MS With Atmospheric Pressure Chemical Ionization in negative polarity of extra virgin olive oils (xvoo) and different seed oils

without flow splitting. Aliquots of 10  $\mu$ L of diluted oil samples were injected for analysis in triplicate.

Data was collected on an AB SCIEX 3200 QTRAP<sup>®</sup> LC/MS/MS system - a hybrid triple-quadrupole linear ion trap mass spectrometer - in negative and positive polarity over the mass range of 50 to 1700 Da. The 3200 QTRAP<sup>®</sup> system was coupled to an Agilent 1100 HPLC system.

hazelnut oil have similar profiles, while the chromatograms of all other oils are relatively distinct. However, differentiating between these oils based on the comparison of LC-MS chromatographic profiles alone is not sufficient - further data mining is required at the spectral level as well. Processing such data manually is difficult and very time consuming; additionally, such a manual procedure is incomplete and will not allow the user to distinguish

Samples were analyzed in random order to ensure that any observed differences were due to the intrinsic differences of each oil. The data was processed through PCA using MarkerView<sup>™</sup> software.

## Results and Discussion

Representative chromatograms of authentic xvoo A, a supposed xvoo, hazelnut oil, peanut oil, sesame oil, soy oil, and grapeseed oil are displayed in Figure 1. The overlay indicates that each oil has its own representative profile. It can be seen that xvoo A, xvoo, and

hazelnut oil have similar profiles, while the chromatograms of all other oils are relatively distinct. However, differentiating between these oils based on the comparison of LC-MS chromatographic profiles alone is not sufficient - further data mining is required at the spectral level as well. Processing such data manually is difficult and very time consuming; additionally, such a manual procedure is incomplete and will not allow the user to distinguish authentic extra virgin oil from adulterated oils.



**Figure 2.** Principal Components Analysis (PCA) plot of extra virgin olive oils (xvoo), refined olive oils (oo), and different seed oils.

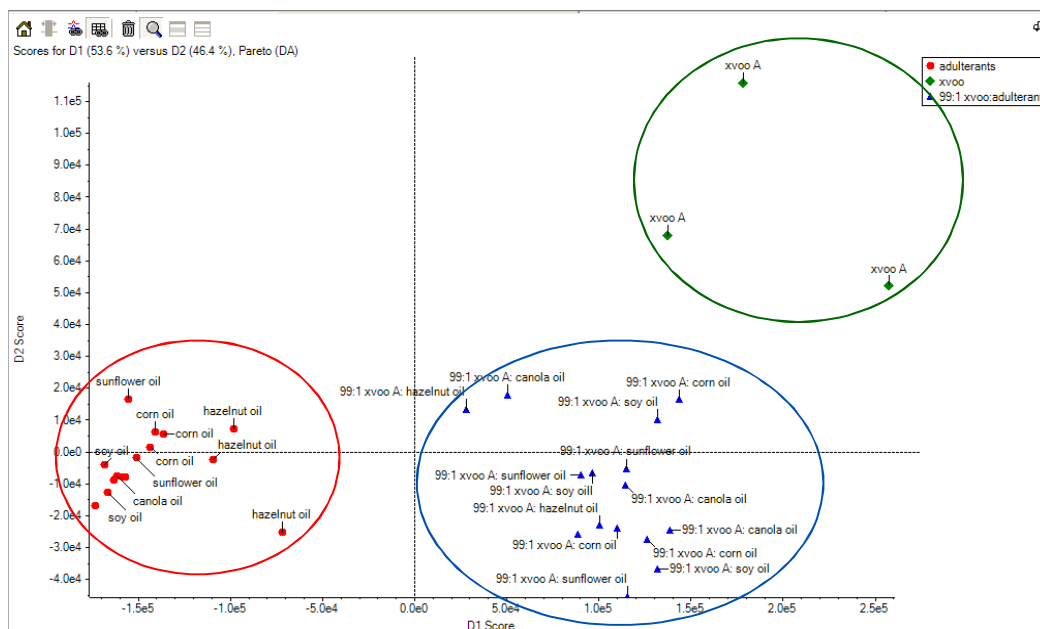
Principal Components Analysis (PCA) was performed on all full-scan data using MarkerView<sup>™</sup> software. PCA finds combinations of variables (LC-MS signals in this case) that explain most of the variance present in the data set. For each principal component (PC), every sample has a score and every variable has a loading that represents its contribution to the combination. It is common practice to plot the scores and loadings for the first two PCs. Discriminant Analysis (DA) was employed, which is a supervised version of PCA wherein prior knowledge of

sample groups is used to determine the variables that maximize the variation between groups and those which minimize the variation within a group.

In the preliminary analysis, the various seed oils, extra virgin olive oils, and refined olive oils were plotted together. From Figure 2, the groupings between the extra virgin olive oil and other seed oils are shown to be clearly demarcated. From this analysis, it was evident that the LC/MS method, developed with data processing based on PCA-DA, was valuable for making distinctions between groups and that the analysis could be extended to include authentic extra virgin olive oil and manually adulterated extra virgin olive oil.

Hazelnut oil is a common adulterant added to xvoo, due to the similarity in fatty acid composition as well its physical properties (including viscosity and color).<sup>2</sup> For this reason, it is also considered the most difficult adulterant to detect and therefore one of the more important for our analysis.

Comparing hazelnut oil, authentic xvoo A, and xvoo A manually adulterated with hazelnut oil (99/1) reveals that a 1% detection level for hazelnut oil in xvoo is possible. As seen in Figure 4, authentic xvoo A is clearly separated from both the adulterant group and the mixture group. The loadings plot, demonstrating the variables that make the most difference in separating of xvoo A from the rest of the group, can be found on the right side of Figure 4. It reveals a group of masses that set hazelnut oil apart from authentic xvoo.



**Figure 3.** Principal Components Analysis (PCA) plot of authentic extra virgin olive oil (xvoo A), seed oils, and manually adulterated extra virgin olive oil with hazelnut oil (99/1)

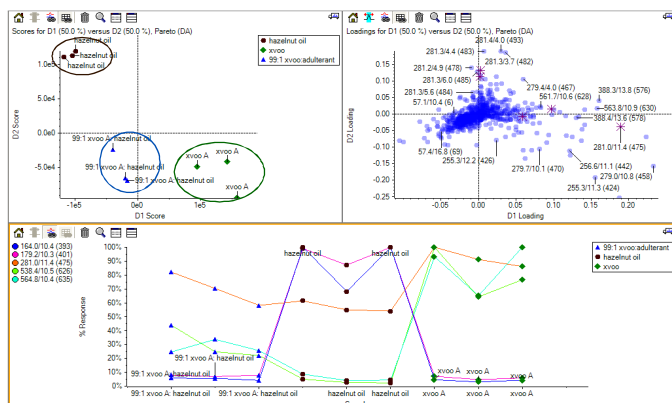
Data from the known authentic extra virgin olive oil was plotted with the manual adulterated mixtures and different seed oils (Figure 3); the authentic xvoo A is clearly separated from both the adulterants group and the seed oils group, even at an adulteration level of 99/1 (xvoo A/seed oil). Adulterated oils and xvoo A at higher adulteration levels were even more separated (data not shown). In the case of adulteration, it is assumed that adulterants would be present in quantities high enough to offer financial benefit but low enough to potentially escape detection. The plot in Figure 3 reveals that exposing adulteration at the 1% level is possible using the MarkerView™ software.

Plotting the profiles for those components that separate hazelnut oil from extra virgin olive oil is easily done through MarkerView™ software. The lower panel of Figure 4 explores the distinguishing masses identified at a retention time of 10.4 min. The masses dominant in xvoo A as compared to hazelnut oil include m/z 538 and 564; note that these masses also appear in hazelnut oil, but to a lesser degree. This is not surprising, as the fatty acid compositions of olive oil and hazelnut oil are quite similar.<sup>2</sup> By comparison, the ions most dominant for hazelnut oil for the same retention time include m/z 164 and 179, among others.

Many masses were found as distinguishing peaks between hazelnut oil and xvoo A. Through MarkerView™ software, it is possible to mark the masses of interest and include them in a table for later review. Table 1 lists the distinguishing masses found during analysis; many of these correspond to free fatty acids, including oleic acid (m/z 281) and linoleic acid (m/z 279) (both significant in extra virgin olive oil), and palmitic acid (m/z 255). In contrast, m/z 164 and 179 are unique to hazelnut oil (Figure 5), to a possible flavoring or sterol.

**Table 1.** Interest list

	m/z	R.T.	Found in
1	891	3.7	xvoo A
2	279	10.1	xvoo A
3	902	10.1	xvoo A
4	874	10.2	xvoo A
5	164	10.3	hazelnut oil
6	179	10.3	hazelnut oil
7	255	10.3	xvoo A
8	902	10.3	xvoo A
9	903	10.3	xvoo A
10	876	10.3	xvoo A
11	900	10.3	xvoo A
12	564	10.4	xvoo A
13	538	10.6	xvoo A
14	561	10.6	xvoo A
15	279	10.8	xvoo A
16	876	10.8	xvoo A
17	559	10.9	xvoo A
18	564	10.9	xvoo A
19	903	10.9	xvoo A
20	900	10.9	xvoo A
21	902	10.9	xvoo A
22	256	11.0	xvoo A
23	253	11.0	xvoo A
24	667	11.1	xvoo A
25	564	11.2	xvoo A
26	277	11.2	xvoo A
27	281	11.4	xvoo A
28	389	13.6	xvoo A
29	386	13.7	xvoo A
30	179	14.0	hazelnut oil



**Figure 4.** Principal Components Analysis (PCA) plot of authentic extra virgin olive oil (xvoo A), hazelnut oil, and manually adulterated extra virgin olive oil with hazelnut oil (99/1)

Since m/z 164 and 179 are unique to hazelnut oil, these masses could potentially serve as an important marker for the adulteration of xvoo with hazelnut oil. Figure 5 demonstrates the utility of monitoring these masses as markers for adulteration.

## Conclusion

The use of LC-MS using the 3200 QTRAP<sup>®</sup> LC/MS/MS system with the MarkerView<sup>™</sup> software to perform Principle Components Analysis was shown to be effective in differentiating between different types of seed oils; authentic extra virgin olive oil could be identified as being distinct from other pure seed oils and manually adulterated extra virgin olive oil. The detection of adulterated oils was also shown to be possible at trace levels. This is extremely useful in the analysis of contaminants or impurities in foods.

Through the use of the MarkerView<sup>™</sup> software, hazelnut oil - a common and hard-to-detect adulterant - was distinguished as a pure seed oil and as an adulterant. This software also demonstrated potential for distinguishing between oils based not only on major fatty acid components, but also on minor components present in oils that make them unique, such as flavorings and antioxidants.

Further studies using the fast and highly sensitive Enhanced Product Ion (EPI) scanning of the 3200 QTRAP<sup>®</sup> system and the high mass accuracy of the TripleTOF<sup>™</sup> 5600 LC/MS/MS system are planned to more accurately identify and confirm the unique chemical components of authentic extra virgin olive oil and common adulterants. The identified and confirmed markers will be used to screen for adulteration using the highly sensitive and selective Multiple Reaction Monitoring (MRM) feature of the 3200 QTRAP<sup>®</sup> system.

## References

- <sup>1</sup> Beauchamp, G.K. et al. (2005) Nature 437:45–46
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- <sup>3</sup> Parcerisa, J. et al. (2000) J Chromatogr A 881:149–158
- <sup>4</sup> L Jakab, A. et al. (2002) J Chromatogr A 976:255–63

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