Determination of Olive Oil Adulteration by Principal Component Analysis with HPLC–Charged Aerosol Detector Data

Marc Plante, Bruce Bailey and Ian N. Acworth
Thermo Fisher Scientific, Chelmsford, MA, USA

Thermo SCIENTIFIC

Overview

Purpose: To examine the use of targeted principal component analysis (PCA) with HPLC-charged aerosol detector data as a means of determining adulteration of olive oils.

Method: Oil samples were analyzed as both untreated and hydrolyzed, using a C30 HPLC method and charged aerosol detection. PCA results were determined using peak area percent values of the acylglycerides and free fatty acids.

Results: It was found that the high pressure liquid chromatography (HPLC) and charged aerosol detector method can be used for the PCA analysis of extra virgin olive oils (EVOO) for purity without base-catalyzed hydrolysis.

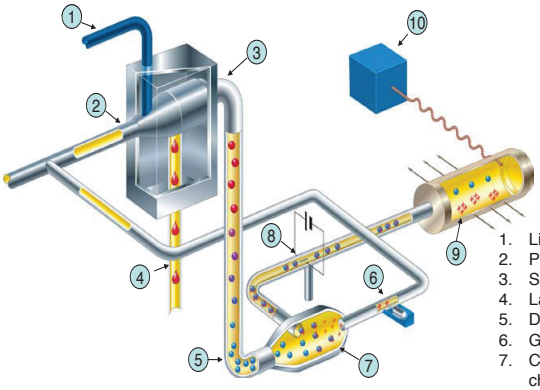
Introduction

Adulteration is a common problem typically found with high-value products. Less costly materials are often added to high-cost materials for sale. Adulteration of food has occurred for hundreds of years and analytical techniques are always improving reliability in detecting such adulteration. Some recent examples include the adulteration of orange juice with other juices,[1] the use of marjoram and thyme being added into oregano,[2] and other vegetable oils (lampante grade,[3] canola oil,[4] as well as avocado, palm, and sunflower oils[5]), for olive oil.[6,7]

With the anticipated future shortages of olive oil, combined with the associated increases in value, it is likely that adulteration will become an escalating issue for olive oil in the market. Reliable and accurate determinations of olive oil quality will be required to maintain the integrity of olive oil products. A comparison of HPLC and charged aerosol detection data from triglyceride analysis of whole oils or from free fatty acid analysis from hydrolyzed oil samples using principal component analysis was made to evaluate the best method for determination of adulteration of different olive oil samples.

The charged aerosol detector is a sensitive, mass-based detector, especially well-suited for the determination of non-volatile and many semi-volatile analytes. As shown in Figure 1, the detector uses nebulization to create aerosol droplets. The mobile phase evaporates in the drying tube, leaving analyte particles, which become charged in the mixing chamber. The charge is then measured by a femtoampere-level electrometer, providing reproducible, nanogram-level detection limits. This technology has greater sensitivity and precision than evaporative light scattering (ELS), and is simpler and less expensive to operate than a mass spectrometer (MS). Typical characteristics of chromatography with charged aerosol detection include: over four orders of magnitude of dynamic range, high precision results, typically less than two percent of peak area RSD, and analyte response largely independent of chemical structure. The detector does not require analytes to ionize, unlike MS, and the detector does not require a chromophore, which lipids typically do not possess. This factor, coupled with sensitivity and precision, make the Corona charged aerosol detectors essential to the successful analysis of these oils.

FIGURE 1. Schematic and functioning of charged aerosol detection.



1. Liquid eluent enters from HPLC system
2. Pneumatic nebulization occurs
3. Small droplets enter drying tube
4. Large droplets exit to drain
5. Dried particles enter mixing chamber
6. Gas stream passes over corona needle
7. Charged gas collides with particles and charge is transferred
8. High mobility species are removed
9. Charge is measured by a highly sensitive electrometer
10. Signal transferred to chromatographic software

Methods

Sample Preparations
For samples that were hydrolyzed under base-catalyzed conditions, aliquots of 50 µL of a sample were added to 4 mL of 2-propanol/water (3:2). To this, 1 mL of 5 M potassium hydroxide was added. Samples were heated in a water bath at 80 °C for one hour. From the top layer (wet 2-propanol), a 500 µL aliquot was transferred to an HPLC vial and 25 µL of formic acid was added. Vials were capped and shaken.

Unhydrolyzed oil samples were diluted 100 µL into 900 µL of methanol/chloroform (1:1).

Liquid Chromatography System

HPLC System:	Thermo Scientific™ Dionex™ UltiMate™ 3000 system consisting of a DGP-3600RS pump, WPS-3000RS autosampler, and TCC-3000RS column oven

Liquid Chromatography–Omega Free Fatty Acids from Hydrolyzed Oils

HPLC Column:	Thermo Scientific™ Accucore™ C30, 2.6 µm, 2.1 × 250 mm at 50 °C
Mobile Phase A:	Methanol/MP B/acetic acid (900:100:3.6)
Mobile Phase B:	Acetone/acetonitrile/tetrahydrofuran/acetic acid (675:225:100:4)
Detector:	Thermo Scientific™ Dionex™ Corona™ ultra RS™
Detector Filter:	4
Power Function:	1.00
Data Acquisition Rate:	10 Hz
Nebulizer Temperature:	12 °C
Flow Rate:	0.4 mL/min
Injection Volumes:	2 µL

Flow Gradient:

Time	%A	%B
-5	100	0
0	100	0
1	40	60
13	30	70
22	5	95
24	5	95
24	100	0
27	100	0

Liquid Chromatography–Untreated Oils

HPLC Column:	Accucore C18, 2.6 µm, 3.0 × 100 mm at 50 °C
Mobile Phase A:	Acetonitrile
Mobile Phase B:	2-Propanol
Detector:	Corona ultra RS
Detector Filter:	4
Power Function:	1.20
Data Acquisition Rate:	10 Hz
Nebulizer Temperature:	Ambient
Injection Volumes:	5 µL

Flow Gradient:

Time	Flow Rate (mL/min)	%A	%B
-6.0	1.0	100	0
-0.2	1.2	100	0
-0.1	0.8	100	0
0.0	0.8	100	0
0.2	1.2	100	0
2.0	1.2	90	10
25.0	1.2	60	40
30.0	1.0	40	60
30.0	1.0	100	0

Data Analysis
All HPLC chromatograms were obtained and compiled using Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, 7.1 SR 1. PCA results were obtained using Pirouette® version 4.5 software.

Results

Principal Component Analysis

PCA is a technique that yields results based on differences and similarities between various samples. PCA has been used to differentiate adulterants in orange juices using HPLC and electrochemical array detectors,[1] and it can provide a measure of how much of an adulterant is present. For a full principal component analysis with HPLC data, raw chromatograms can be used to compare samples. This kind of analysis, however, requires careful alignment of analyte retention times and a consistent baseline, which is difficult to control even under the best of conditions. In a targeted analysis of HPLC data, integrated peak areas can be used. This allows for greater variance, which occurs typically and especially over large analytical sequences. For these PCA results, percent peak areas for components of the different samples were used as the basis of the PCA calculation. The power function of the Corona ultra RS detector was also used to eliminate the bias of nonlinear changes with concentration to apparent composition. With a non-linear correlation, some peak areas will change more than others with different amounts of sample injected. PCA would reveal this as a different oil composition, in this case.

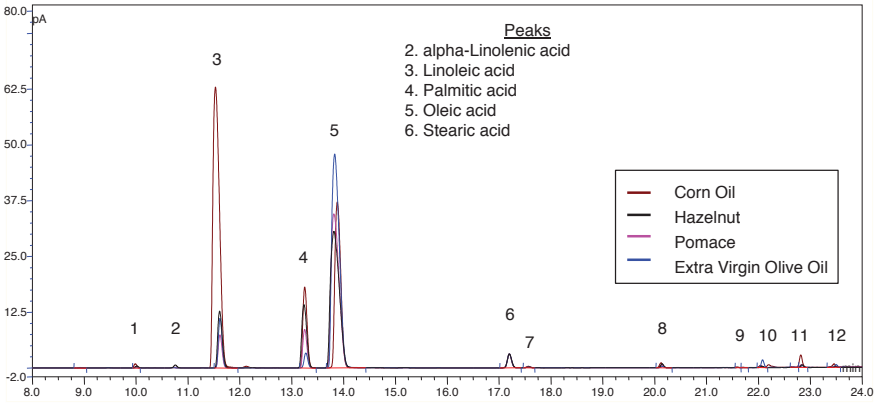
Omega Free Fatty Acids Analysis

Mixed EVOO samples were prepared containing 1, 5, 10, 25, 50, and 75 mass-percent of adulterants hazelnut oil and pomace oil (added to EVOO-2) as well as corn oil (added to EVOO-1). Aliquots were hydrolyzed as previously described and analyzed using the omega fatty acids method. All analytes were integrated and subjected to targeted PCA analysis using percent peak area values.

After an oil is hydrolyzed, the acylglycerols are converted into free fatty acids, which can then be analyzed using an HPLC method specifically designed for the different unsaturated fatty acids found in natural oils. The use of a C30 column proved to be extremely useful in separating these fatty acids, some of which only differ by a double bond.

Two common adulterants are cold-pressed hazelnut oil and olive pomace oil, due to their purported similarity in fatty acid content making it difficult to differentiate when blended into EVOO. Chromatogram overlays of EVOO with cold-pressed hazelnut, pomace, and corn oils are shown in Figure 2. Clear differences were found between the oils, relative to stearic acid (Peak 6), for peaks 3, 4, and 5.

FIGURE 2. HPLC-Charged aerosol chromatogram overlay (normalized on peak 6) of hydrolyzed EVOO, hazelnut, olive pomace, and corn oils.

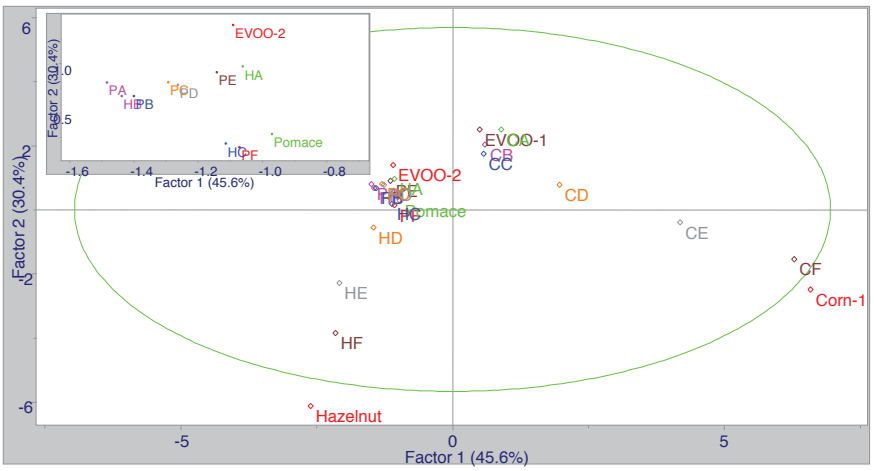


Aliquots were hydrolyzed as described and analyzed by HPLC using the free fatty analysis method. The resulting free fatty acid peaks were normalized to the stearic acid peak, integrated for percent peak area, and then processed by targeted PCA using three factors. The calculated results are shown in Figure 3, and both the hazelnut and corn oil adulterants were clearly identified. Adulteration with pomace oil was not as evident.

Pomace oil is produced using heat and solvents to remove the last oils from the remains of olives from previous pressings and extractions. As a result, this oil should have similar profiles to olive oil and EVOO, and this is evident in the chromatograms, as well as the PCA results. No significant correlations were found between the EVOO-2 oil and the pomace oil: the mixtures (PA – PF) do not form a line between the two pure-oil points, as shown in the Figure 3 inset.

The results from the untreated trigylcerides analysis proved to be of greater clarity, yielding a good correlation between the three adulterants, including that for the pomace oil adulterant.

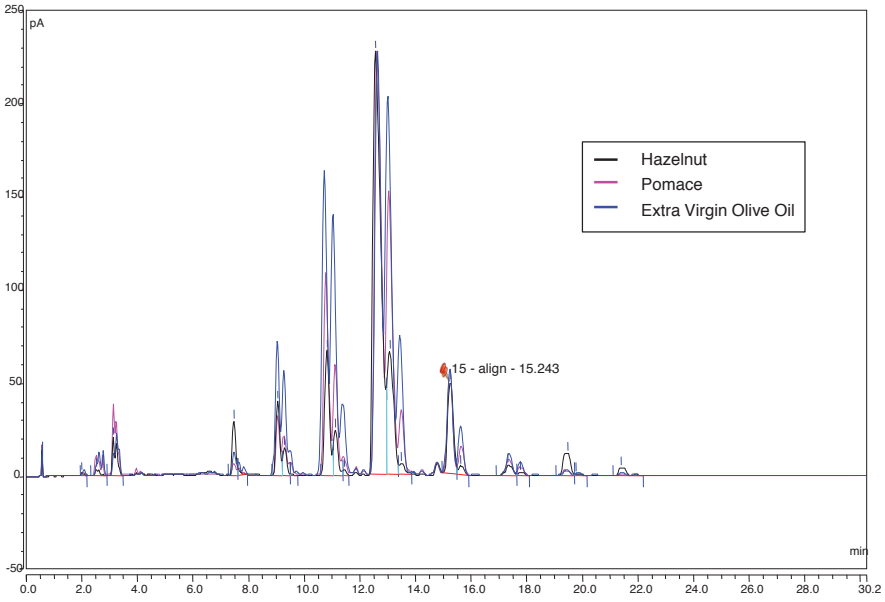
FIGURE 3. Calculated PCA results of hydrolyzed EVOO with hazelnut (HA-HF), corn (CA–CF), and pomace oil (PA–PF) adulteration at six levels (1, 5, 10, 25, 50, and 75 mass-%, respectively) using hydrolyzed oil data. Inset results for pomace oil analysis.



Untreated Oils Analysis

The same mixed oil and blend samples were diluted and analyzed directly using a solid-core C18 column. HPLC chromatograms overlaid in Figure 4 show the differences that are found between EVOO-2, hazelnut, and pomace oils. As can be seen when comparing the chromatograms in Figure 4 to those in Figure 2, untreated oils have many more peaks and more significant differences than hydrolyzed samples.

FIGURE 4. HPLC-Charged aerosol chromatogram overlay (normalized on peak 15) of non-hydrolyzed EVOO, with hazelnut oil, and olive pomace oil.



The triglyceride (and some diglyceride) peaks in the resulting chromatograms were integrated and processed by targeted PCA. The results appeared to be better correlated between the original EVOO sample and the additions of the corn oil and the hazelnut oil, as shown in Figure 5. In the inset, the correlation of adulteration of pomace oil to EVOO-2 was improved over the results using the hydrolyzed oil.

A possible measure of quality was seen with distance from the pomace oil point, as shown in Figure 6. Two known, high-quality EVOOs (EVOO-1 and EVOO-2) were found to have the greatest difference to pomace oil. An olive oil (OO), which is extracted from the remains of olives after EVOO is obtained, was found to lie between EVOO and pomace oil. Two other EVOO samples, of lesser quality, were also found closer to either olive oil or pomace oil.

FIGURE 5. Calculated PCA results of EVOO with hazelnut (HA-HF), corn (CA–CF), and pomace oil (PA–PF) adulteration at six levels (1, 5, 10, 25, 50, and 75 mass-%, respectively) using unhydrolyzed oil data. Inset results for pomace oil analysis.

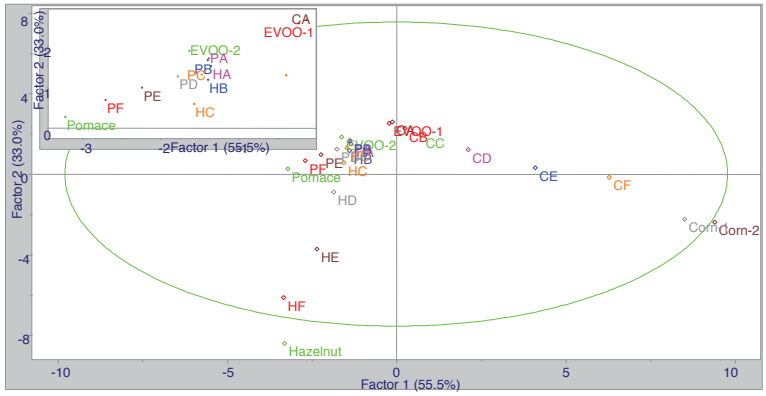
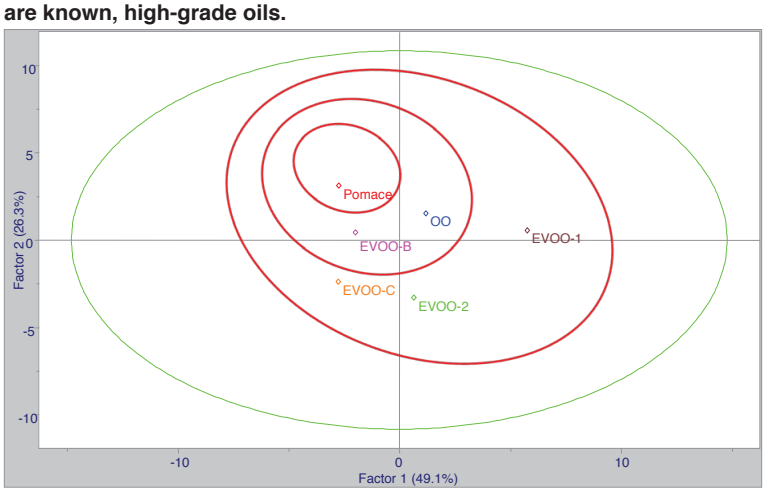


FIGURE 6. PCA plot of unhydrolyzed oils, of different EVOOs, including olive oil (OO) relative to pomace oil. EVOOs with a number are known, high-grade oils.



Conclusions

- HPLC and charged aerosol detection, using only sample dilution and analysis, could readily determine EVOO adulteration with most of the commonly used oil adulterants.
- PCA data from unhydrolyzed oils more readily revealed adulteration than data from the fatty acid analysis of hydrolyzed samples.
- A measure of EVOO quality, relative to that of pomace oil, is shown.

References

1. Gamache, P. et al. Coulometric array detection for HPLC in the analysis of juice products. In: *Methods to Detect Adulteration of Fruit Juice Beverages*. Nagy, S., Wade, R. L., Eds.; Agscience, Inc.: Auburndale, FL, 1995; pp 120-144.
2. Spice Adulteration white paper. American Spice Trade Association [online], http://www.astaspice.org/files/public/SpiceAdulteration.pdf (accessed 19 April 2013)
3. Higgins, C. Suspected Fraud Hits Major Israeli Supermarkets. Olive Oil Times, 02 Oct 2012 http://www.oliveoiltimes.com/olive-oil-business/africa-middle-east/olive-oil-fraud-supermarkets-israel/29281 (accessed 19 April 2013).
4. Cord, C. New Study Finds Some Foodservice Olive Oil 'Not Fit for Consumption'. Olive Oil Times, 18 Sep 2012 http://www.oliveoiltimes.com/olive-oil-basics/some-foodservice-olive-oil-not-fit-for-consumption/28788 (accessed 19 April 2013).
5. Butler, J. Spanish Police Say Palm, Avocado, Sunflower Was Passed Off as Olive Oil, Olive Oil Times, 14 Feb 2012 http://www.oliveoiltimes.com/olive-oil-business/europe/operation-lucerna-olive-oil-fraud/24815 (accessed 19 April 2013).
6. Marini, D. HPLC of Lipids in Nollet LML. *Food Analysis by HPLC*, Marcel Dekker: New York, 2000; pp 231-232.
7. Aparicio, R., Aparicio-Ruiz, R. Authentication of vegetable oils by chromatographic techniques. *J. Chromatogr. A.* **2000**, 881(1-2), 93-104.

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