Characterization of Adulterated Olive Oils in Cases of Food Fraud by Comprehensive Two-Dimensional Gas Chromatography with Time-of-Flight Mass Spectrometry (GC×GC-TOFMS)

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Introduction

Food fraud has become an increasing problem in the global marketplace and is loosely defined as the deliberate misrepresentation of a product to a consumer for the purpose of monetary gain. Olive oil adulteration ranks near the top of all reported food fraud cases with common adulterations including the substitution of olive derived oils with other less expensive edible oils (for example; soybean, corn, vegetable, canola, sunflower, peanut, etc.), or the mislabeling of regular olive oils as extra virgin olive oil. Detecting food fraud is complicated by the inherent variations of natural products and by the wide range of methods of adulteration. Non-targeted analytical methods that characterize complex food products and isolate individual analytes within the food matrix are useful for recognizing these adulterations. The analysis of adulterated olive oils was explored using comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-TOFMS). This analytical approach isolates individual analytes both chromatographically and with mathematical deconvolution, identifies the individual analyte components through library matching of the mass spectral data, and yields distinct visual two-dimensional chromatograms. The complex samples benefit from the two-dimensional separations, and the data processing features within the ChromaTOF® software facilitate comparisons. These methods allowed for comparing edible oil varieties by their chromatographic fingerprints, and with the identification of individual analyte differences between the samples with the potential to identify food fraud.

Methods

Sample Preparation

Edible oil samples were acquired from the grocery store. Three extra virgin olive oil, and one each of light flavored olive oil, peanut oil, vegetable oil, and grape seed oil were analyzed. Each oil was diluted 1:10 in cyclohexane. Mixtures were prepared to simulate extra virgin olive oil adulteration with other oil varieties. Each oil variety was mixed with extra virgin olive oil at 50% and 10% levels.

Instrumental Conditions

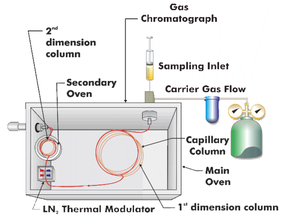
GC×GC analyses were performed with LECO's Pegasus® 4D consisting of an Agilent 7890 GC equipped with a GERSTEL MPS2 Auto Sampler and LECO's dual stage quad jet thermal modulator, secondary oven, and Pegasus 4D TOFMS.

Table 1. Instrument Parameters

GC×GC-TOFMS (Pegasus 4D) Conditions	
Injection	1.0 µL splitless with inlet @ 250°C
Carrier Gas	He @ 1.0 ml/min, corrected with pressure ramps for constant flow
Column One	Rxi-5Sil MS, 30 m x 0.25 mm x 0.25 µm (Restek, Bellefonte, PA)
Column Two	Rxi-17Sil MS, 1.25 m x 0.18 mm x 0.18 µm (Restek, Bellefonte, PA)
Temperature Program	1 min at 40°C, ramped 8°C/min to 300°C, held 5 min; Secondary oven maintained +10°C relative to primary
Modulation	3 s with temperature maintained +15°C relative to 2nd oven
Transfer Line	Temperature set to 250°C
Solvent Delay	240 s
Mass Range	35-500 m/z
Acquisition Rate	200 spectra/s
Source Temp	250°C
Data Processing	ChromaTOF 4.50

Comprehensive GC×GC-TOFMS

GCxGC can be beneficial in the analysis of complex samples by offering both improved peak capacity and lower-level detection to better isolate and detect individual analytes. These benefits are attributed to having two dimensions of complementary separation and to the cryogenic focusing effects of thermal modulation.



Extra Virgin Olive Oil Characterization

Three extra virgin olive oil samples were analyzed with GC×GC-TOFMS. Representative chromatograms are shown below. The complex oils benefit from the additional separation dimension. The signals for lower level analytes are enhanced with thermal focusing at the modulator which helps in detecting more analytes.

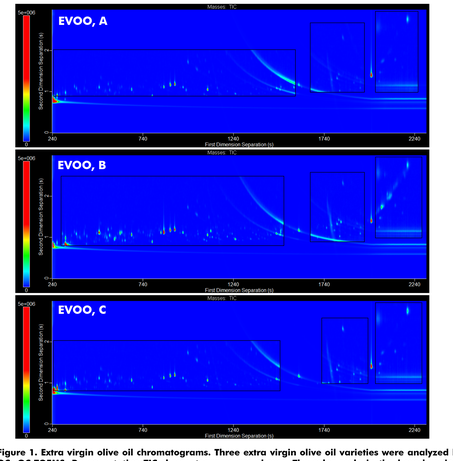


Figure 1. Extra virgin olive oil chromatograms. Three extra virgin olive oil varieties were analyzed by GC×GC-TOFMS. Representative TIC chromatograms are shown. The color scale in the boxed regions has 10x lower intensity to better visualize the lower level analytes. There are some differences and many similarities in these samples.

Edible Oil Characterization

A combined list of the 10 analytes with the largest S/N in each extra virgin olive oil sample is provided in Table 2. Due to the similarities in the varieties, this list consists of 15 analytes. This sampling procedure detects hundreds of analytes, but does not capture some of the main components of edible oils. Other sampling methods will be considered in future work. Many of the analytes that have the largest S/N in extra virgin olive oil are also present in the other edible oil varieties, shown in Figure 2 and Table 2.

Table 2. Largest S/N Analytes (Average S/N reported for each edible oil variety)

Name	Similarity	R.T. (s)	EVOO, A	EVOO, B	EVOO, C	OO	Peanut	Grape	Vegetable	Mass
Norbornane	953	264 , 0.870	10803	9327	9024	8271	8710	10481	7630	68
Octane (CAS)	943	309 , 0.820	5015	25062	4123	1472	2038	2164	2070	71
Nonanal (CAS)	917	675 , 1.070	1480	2673	1953	489	301	288	376	57
1-Undecene (CAS)	879	789 , 0.900	1486	1305	868	21.9	15.2	23.1	29.8	57
2-Decenal, (E)-	838	837 , 1.120	404	3481	494	218	257	214	210	70
2-Decenal, (Z)-	931	852 , 1.130	2039	17256	2454	999	1079	892	966	70
2,4 DECADIENAL	925	888 , 1.175	6901	10089	6598	2738	616	1823	4768	81
2,4-Decadienal, (E,E)-	939	912 , 1.195	7775	11553	7297	3221	880	2868	8034	81
α-Farnesene	874	1095 , 1.065	3120	2558	2426	33.8				93
Hexadecanoic acid (CAS)	909	1473 , 1.100	6493	7639	6450	4481	6279	7056	8484	60
Oleic Acid	937	1599 , 1.165	17791	18489	16677	17138	17197	14846	9051	83
Eicosane (CAS)	900	1701 , 0.995	336	467	2678	416				57
2-Butanone, 3-amino-4-phenyl-	706	1842 , 2.315	2558	2864	5175	577	57.9	58.9	69.6	120
trans-Geranylgeraniol	885	1998 , 1.395	134849	124803	124435	121070	7866	16329	8873	69
Unknown		2199 , 2.760	4319	3615	5939	3117	3621	13369	1560	165

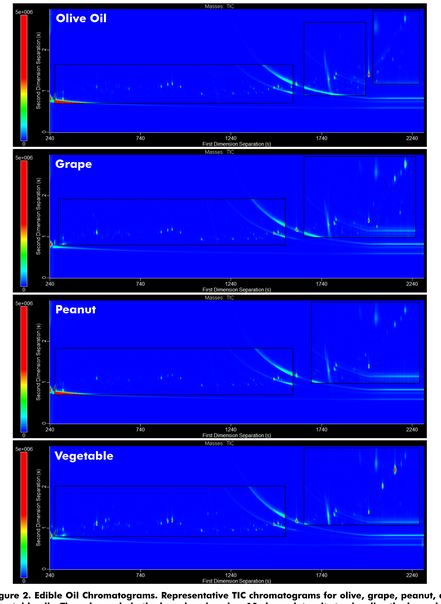


Figure 2. Edible Oil Chromatograms. Representative TIC chromatograms for olive, grape, peanut, and vegetable oils. The color scale in the boxed regions has 10x lower intensity to visualize the lower level analytes. There are some distinct differences between these and extra virgin olive oil (Figure 1), but there are also many similarities.

Feature Selection and Data Analysis

To determine analytes that differ between the oil varieties, the data were prepared for further analyses with the Statistical Compare data analysis tools in LECO's ChromaTOF software. Analyte information across multiple sample replicates was aligned based on retention time and mass spectral similarities. The resulting peak table compiled these data in a format amenable to further data processing, including feature selection and PCA. Feature selection, which is intended to determine analytes that can distinguish samples from each other, can be an important part of data analysis. This is especially helpful for these similar edible oil samples where the largest S/N analytes are common to most of the oil varieties. The ability to determine analytes that distinguish edible oil varieties could lead to the ability to detect food fraud. The Fisher Ratio is a ratio of the between-class variance relative to the within-class variance, as shown below, and is one approach to feature selection.

Fisher Ratio	σ^2**class / σ^2error**
Between-Class Variance	$\sigma^2\text{class} = (\Sigma\,(x^i - x)^2 n_i\,) / (k - 1)$
Within-Class Variance	$\sigma^2\text{error} = (\Sigma(\Sigma\,(x^{ii} - x)^2) - (\Sigma(x^i - x)^2 n_i)) / (N - k)$

x^i is the mean of the i^{th} class, x is the overall mean, n^i is the number of samples in the i^{th} class, k is the number of classes, x^{ii} is the i^{th} sample of the i^{th} class, N is the total number of samples.

Fisher Ratios were calculated pair-wise with each edible oil type compared to extra virgin olive oil. A combined list of the analytes with the largest Fisher Ratios in each pair was compiled. PCA was used to compare the analyte information across all samples, with and without feature selection. The corresponding scores plots are shown in Figure 3.

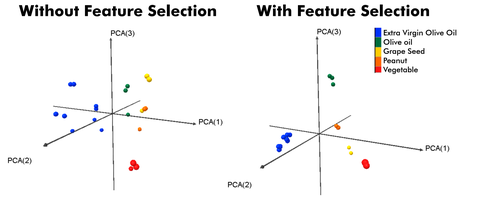


Figure 3. PCA scores plots. Without feature selection, there are not clear clusters associated with each edible oil type. Through Fisher Ratio feature selection, a subset of analytes were selected that help to distinguish the samples from each other. PCA with the selected analytes shows each edible oil type tightly clustered and apart from the others.

A collection of representative analytes that were determined with Fisher Ratio feature selection are shown in Figure 4. Three replicates for each oil variety are shown. Additionally, peak areas for a 50% and 10% mixture of each oil variety into extra virgin olive oil are shown. These mixtures simulate adulteration of blending other edible oil varieties into extra virgin olive oil. There are clear differences in these analytes between oil varieties.

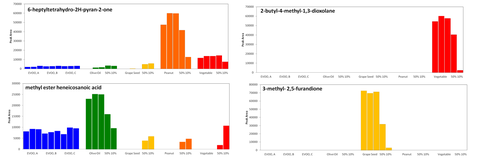


Figure 4. Peak areas for representative analytes. These analytes, determined by Fisher Ratio feature selection, have peak areas that differ between the oil varieties and the various mixtures (to simulate adulteration). The analytes shown are: 6-heptyltetrahydro-2H-pyran-2-one (similarity, 830); 2-butyl-4-methyl-1,3-dioxolane (similarity, 904); methyl ester heneicosanoic acid (similarity, 864); and 3-methyl-2,5-furandione (similarity, 862)

Representative Analytes

In addition to differing between oil varieties, the peak areas for the 50% and 10% mixtures are between the edible oil variety and that of extra virgin olive oil. These analytes show potential for detecting food fraud as they changed with adulteration.

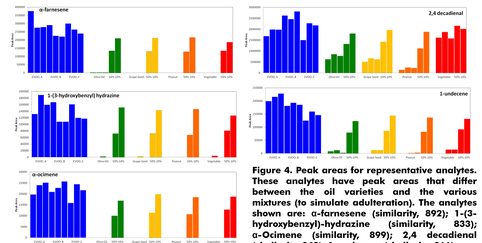


Figure 4. Peak areas for representative analytes. These analytes have peak areas that differ between the oil varieties and the various mixtures (to simulate adulteration). The analytes shown are: α-farnesene (similarity, 892); 1-(3-hydroxybenzyl)-hydrazine (similarity, 833); α-Ocimene (similarity, 899); 2,4 decadienal (similarity, 943); 1-undecene (similarity, 866).

PCA was repeated with the analytes selected with Fisher Ratios and the 50% mixture samples, as shown in Figure 5. These mixtures simulate adulteration of blending other edible oil varieties into extra virgin olive oil, and have scores that fall between the pure edible oil varieties. This suggests that adulteration with these oil types could be detected with these approaches. Detecting adulteration with other edible oil varieties would likely also be possible, but may require additional feature selection to select appropriate analytes for monitoring.

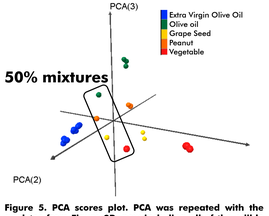


Figure 5. PCA scores plot. PCA was repeated with the analytes from Figure 3B, now including all of the edible oil varieties and the 50% mixtures.

Conclusions

This poster demonstrates that edible oil varieties can be characterized with GC×GC-TOFMS. Further sample preparation optimization will be performed in future works. The data analysis approaches utilized here, including Fisher Ratio feature selection and PCA, show promise for distinguishing edible oil varieties and mixtures of each oil variety with extra virgin olive oil. This suggests that adulteration of olive oil by the substitution of other less expensive edible oils, or the mislabeling of regular olive oils as extra virgin olive oil could be detected with these techniques, given sufficient data to model oil varieties and optimized sampling procedures. These methods allowed for comparing edible oils by chromatographic fingerprints, and by the identification of individual analyte differences between the samples with the potential to identify food fraud.

Pegasus 4D GCxGC-TOFMS