Diffuse-Light Absorption Spectroscopy and Chemometrics for Discrimination and Quantification of Extra Virgin Olive Oil Adulterants

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***Abstract* — A selection of top-quality extra virgin olive oils with excellent nutraceutic properties was artificially adulterated by means of lower-grade olive oils commonly used in commercial counterfeits. The oil sample set was analyzed by means of diffuse-light absorption spectroscopy performed in the wide 400-1700 nm spectral range. A setup based on optical fiber technology was experimented, which made use of an optical fiber *supercontinuum* source. The spectroscopic library was processed by means of a *Partial Least Squares* regression for quantifying the adulterant concentration, followed by a *Principal Component Analysis* and *Linear Discriminant Analysis* for identifying the type of adulterant.**

I. Protecting Extra Virgin Olive Oil

Extra virgin olive oil (EVOO) is the only vegetable oil that is consumed as it is – freshly extracted from the fruit. Thanks to its balanced taste and flavored aroma, EVOO is capable of enhancing the most popular gastronomic recipes and is thus considered the chef's gold. EVOO also offers highly beneficial health effects, thanks to both its high content of monounsaturated fatty acids, vitamins, and polyphenols – the antioxidant substances.

EVOO, besides containing the highest levels of antioxidants, has the highest amount of monounsaturated fatty acids. It is a "healing fat", since it controls the *"bad"* LDL cholesterol levels while raising the *"good"* HDL ones [1,2]. Studies have shown that people who consumed 25 ml (about 2 tablespoons) of EVOO daily for 1 week presented less oxidation of LDL cholesterol and higher levels of antioxidant compounds in the blood [3], and, more generally, that EVOO is a panacea of the entire cardiovascular system [4].

Because of the time-consuming agronomical practices used for EVOO production, and the low production efficiency – frequently lower than 20% as oil yield – the price of EVOO is high, especially compared with that of other vegetable oils. Consequently, EVOO is prone to adulteration with cheaper oils in order to increase profits. In addition to an economic burden, EVOO adulteration is detrimental if consumers react by buying other cooking fats or dressings, thinking that EVOO cannot be trusted. The negative implications on consumer confidence are even worse than the economic ones. Lastly, EVOO protection measures also imply the product area conservation, as far as landscape, tourism, and job preservation are concerned.

While the European Commission regulations are indicating the characteristics of olive oil types, and are suggesting the methods of analysis [5], a lot of research is currently carried out, in order to experiment innovative techniques for authenticating extra virgin olive oils and predicting potential frauds.

Numerous methodologies exist for EVOO authentication, both for adulteration detection and quantification.

Chromatographic, thermal, nuclear magnetic resonance techniques are frequently used, as well as dielectric spectroscopy [6-9]. They are mostly suitable for laboratory use, since the instrumentation is cumbersome and some treatments of the analyzed sample are required. Optical spectroscopy is also frequently used and sometimes preferred because it allows a rapid and non-destructive analysis and requires minimum or no sample preparation. Infrared, mid-infrared, and fluorescence spectroscopic techniques have been proposed, as well as absorption spectroscopy in the ultraviolet, visible or near-infrared spectral ranges [10-13]. These optical techniques are usually combined with chemometric methods for spectroscopic data processing, thus providing an excellent EVOO authentication [14]. However, none of the absorption spectroscopy techniques experimented so far takes into account the intrinsic turbidity of the olive oil, which can considerably impair absorption measurements because of the unavoidable scattering effects produced by suspended particles.

This paper shows an absorption spectroscopy experiment carried out in the wide 400-1700 nm spectral range by means of optical fiber technology to predict the adulteration of authentic EVOOs produced in the Italian region of Tuscany, caused by lower-grade olive oils. Olive-pomace oil (OPO), refined olive-pomace oil (ROPO), refined olive oil (ROO), and deodorized olive oil (DOO) were considered as adulterants. While the detection of EVOO adulteration caused by OPO, ROPO, and ROO has been previously achieved by means of absorption spectroscopy [15], we innovatively tested the adulteration caused by DOO, an emerging adulterant, the detection of which is hard to achieve by means of conventional techniques.

II. Diffuse-Light Absorption Spectroscopy

Diffuse-light absorption spectroscopy makes use of an integrating sphere that contains the sample under test. The source and the detector are butt-coupled to the sphere. Almost all the light impinging on the sphere surface is diffusely reflected, and the detector can be placed anywhere in the sphere in order to gather the average flux [16,17]. By inserting an absorbing medium in the cavity, a reduction of the radiance in the sphere occurs. The reduction is related to the absorption of the medium and to its volume, and is independent of non-absorbing objects within it, such as suspended scattering particles.

Efficient diffuse-light measurements need bright sources. A conventional deuterium/halogen lamp is enough, provided that it is butt-coupled to the integrating sphere. However, when optical fibers are needed for a better geometrical versatility of the measuring system, conventional lamps provide poor and insufficient light intensity.

Recently, the revolutionary advent of compact, high brightness *supercontinuum* fiber optic sources has changed the perspectives of optical spectroscopy [18,19]. This innovative source is made of a holey optical fiber, typically a photonic crystal fiber, which is pumped by a high-power nanosecond or femtosecond laser. The bright light generated by the holey fiber over a wide spectral range can easily be coupled to a conventional optical fiber and guided to the input port of an integrating sphere. Another port of the sphere can accommodate an optical fiber coupled to a spectrometric detector, so as to achieve an efficient setup for diffuse-light absorption spectroscopy, as shown in Figure 1.

Commercially-available components were used for the practical implementation of the experimental setup [20]. The Fianium-SC400 fiber optic *supercontinuum* source was used for illumination: it emits 4 Watts throughout the entire 415-1800 nm spectral range. The Instrument System-Spectro 320 fiber optic spectrometer was used as detector, which scanned the wide 400-1700 nm spectral range with a resolution of 1.37 nm. The Labsphere-LMS100 cavity was used as a diffusing sphere, the ports of which were equipped by means of fiber optic connectors for coupling to both the source and the detector. The olive oil sample under test was contained in a glass vial having a volume of 32 cm^3. This setup was previously used for lubricant oil analysis – it allowed for a successful spectral fingerprinting of the lubricant oil and for predicting functional parameters and wear indicators [21].

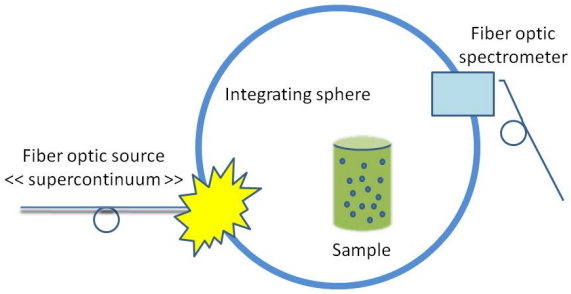

Figure 1. Setup for diffuse-light absorption spectroscopy by means of optical fiber technology.

III. The Collection of Authentic Extra Virgin Olive Oils and Adulterants

Authentic EVOOs were four different types of oils collected in Tuscany, which were produced according to local traditions around the area of Grosseto. The lower-grade olive oils – OPO, ROO, ROPO, and DOO – were provided by the Università degli Studi di Udine. Table I summarizes the codes used for identifying the various oil types.

Four series of EVOO-adulterant mixtures were prepared by spiking each authentic EVOO with 5%, 25%, 50%, 75%, and 95% w/w of adulterant. They were used for calibration procedures. Also, replica mixtures of EVOOs with 25%, 50%, 75% w/w of adulterants were prepared for validation purposes. The entire collection of measured oils consisted of 136 samples, 88 for calibration and 48 for validation, respectively.

Figure 2 and 3 show the measured diffuse-light absorption spectra of all authentic EVOOs and adulterants, respectively. Their mixtures show intermediate spectra: as an example,

Figure 4 and 5 show the O1 EVOO adulterated by means of two different adulterants, OPO and DOO, respectively, providing very similar (O1F1 mix) or highly different (O1F4 mix) spectral signatures.

A multivariate processing of the spectroscopic data allowed for predicting the fraction and the type of adulterant. All data processing were carried out in Matlab® code, by means of customized programs.

TABLE I. CODES OF EXPERIMENTED AUTHENTIC EXTRA VIRGIN OLIVE OILS AND ADULTERANTS.

Code	Type of oil
O1	EVOO from Tuscany
O2	EVOO from Tuscany
O3	EVOO from Tuscany
O4	EVOO from Tuscany
F1	OPO
F2	ROO
F4	DOO
F5	ROPO

IV. PREDICTING THE ADULTERANT FRACTION

As a first processing, the spectra were smoothed by means of Savitsky-Golay algorithm, employing a 2nd degree polynomial and a smoothing window of 15 points (30 nm). Then, the prediction of the adulterant fraction in the mixtures was achieved by using a multivariate analysis method called *Partial Least Squares* regression (PLS) [22]. PLS looks for a limited number of PLS "factors" (PF) which are linear combinations of the original predictors. These new variables are mutually orthogonal (thus uncorrelated) and have the maximum possible covariance with the target variable, among all possible combinations of the original predictors.

The optimal number of factors was assessed by testing each PLS model on the validation set and by choosing that minimizing the RMSEP (Root Mean Square Error of Prediction). Two other parameters were evaluated in order to assess the goodness of the fit: the RMSEC (Root Mean Square Error of Calibration) and the determination coefficient, R^2. RMSEC is, like RMSEP, an estimation of the "expected" prediction error, but is evaluated on the calibration set. R^2 is the squared correlation coefficients between predicted and reference values, for the calibration set; thus the fit is as better as this value is closer to 1.

Table II summarizes the values of these parameters for each EVOO-adulterant mixture, together with the chosen number of PF (# PF). Note that all mixtures involving F4 needed 2 PFs for achieving the best fit. Indeed, the values of R^2 are very good. The best prediction is obtained for O3 EVOO adulterated by means of ROO (F2), showing R^2=0.997 and RMSEP=0.02. The worse prediction, which is still very good, is obtained for O2 EVOO adulterated again by means of ROO (F2), showing R^2=0.933 and RMSEP=0.06.

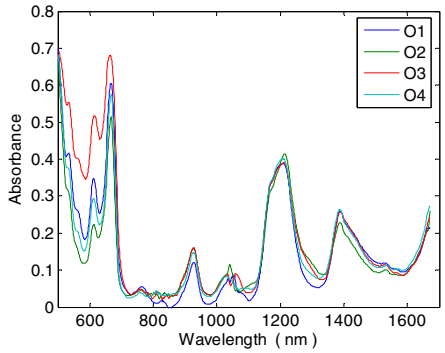

Figure 2. Diffuse-light absorption spectra of authentic EVOOs.

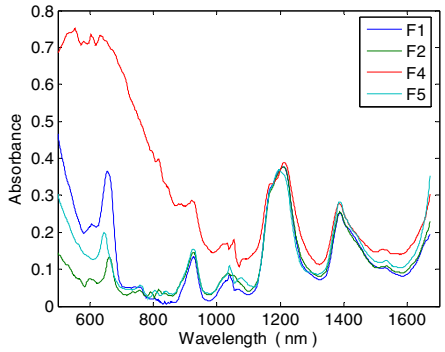

Figure 3. Diffuse-light absorption spectra of adulterants.

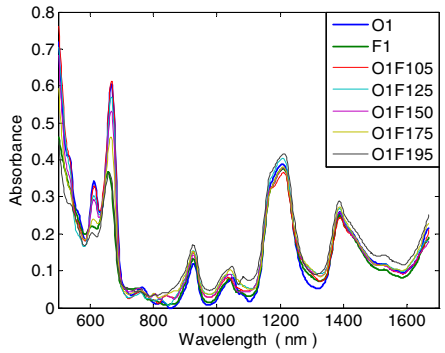

Figure 4. Diffuse-light absorption spectra of O1F1 mix – example of very similar spectral signatures.

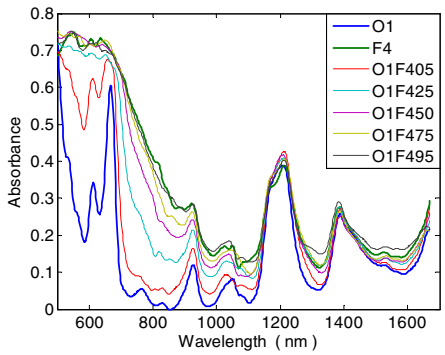

Figure 5. Diffuse-light absorption spectra of O1F4 mix – example of highly different spectral signatures.

V. Discriminating the Adulterant Type

The previous section showed how to predict the fraction of adulterant when the adulterant type is known *a priori*. However, in practice, the type of adulterant is usually unknown. Therefore we investigated how to discriminate among the different types of adulterants by means of multivariate calibration and classification methods [23].

The *Principal Component Analysis* (PCA) was firstly applied. For each EVOO, the spectra of pure adulterants and of calibration mixtures were considered, thus taking into account 24 samples.

Figure 6 shows the results of PCA processing obtained for O1 EVOO; similar results were obtained for the other EVOOs. This score plot highlights that DOO (the F4 adulterant) can be easily distinguished along the PC1 axis. In fact, PC1 is linked to the average absorbance in the 500÷900 nm range, where DOO absorbance is higher and broader than any other adulterant.

Then, for a better discrimination of the other three adulterants, the *Linear Discriminant Analysis* (LDA) was applied. Since overfitting is likely with LDA direct processing of large variable sets, like spectra, a two-step PCA + LDA model was considered. For each EVOO, calibration and validation sets were created. The calibration set was made by the spectra of pure OPO, ROO and ROPO adulterants, and of the relative calibration mixtures (total 18 samples), while the validation set was made by the validation mixtures of the same adulterants (total 9 samples). PCA was applied to the calibration set, showing that two PCs only were sufficient to obtain explained variances of 96% or higher in any case. LDA was then performed on the PCA score matrix, obtaining two Discrimination Functions (DF). Finally, the decision boundaries separating the three classes of adulterants were calculated.

Figure 7 and 8 show the results of PCA + LDA processing for discriminating OPO, ROO and ROPO (F1, F2 and F5) in O1 EVOO; similar figures were obtained for the other EVOOs. Empty and filled dots represent the calibration and validation samples, respectively. Figure 7 shows the discriminating map, where labels indicating the adulterant percentage in the mixture are added, while adulterants are simply identified by their code. Figure 8 shows the discriminating map also including the discriminating boundaries.

As expected, the best discrimination among the adulterants is achieved with high adulterant concentrations, and the dots converge towards a point where the pure EVOO should be. Note that the spectrum of authentic EVOO was not considered in the LDA processing, for not introducing a fourth class populated by a single element, which contrasts with LDA principles.

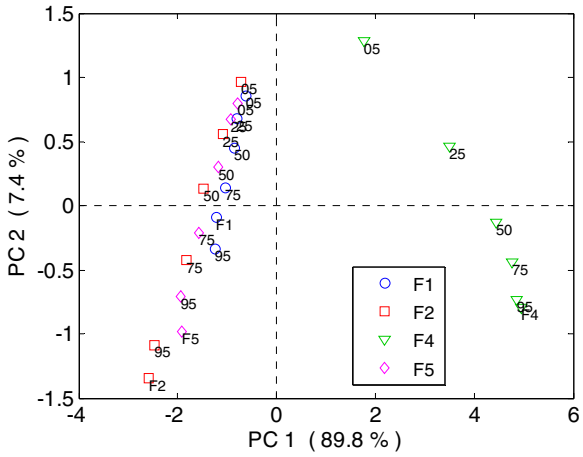

Figure 6. Results of PCA processing obtained for O1 EVOO.

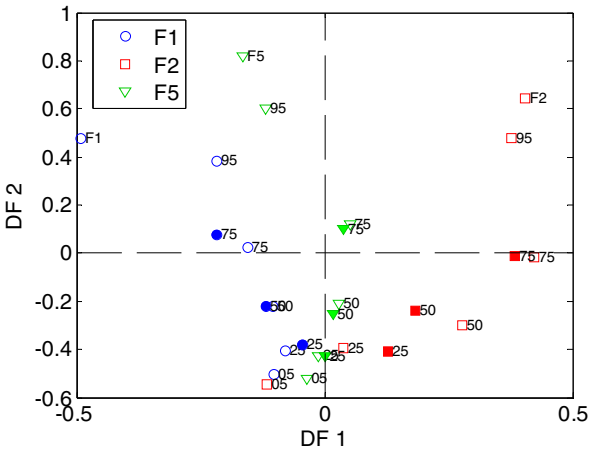

Figure 7. Results of PCA + LDA processing for discriminating OPO, ROO and ROPO (F1, F2 and F5) in O1 EVOO: discriminating map with labels indicating the adulterant percentage in the mixture.

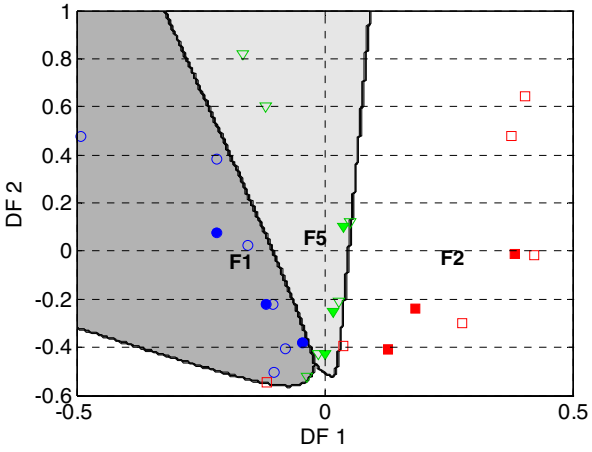

Figure 8. Results of PCA + LDA processing for discriminating OPO, ROO and ROPO (F1, F2 and F5) in O1 EVOO: discriminating map with boundaries.

TABLE II. SUMMARY OF PARAMETERS FOR PREDICTING THE FRACTION OF ADULTERANT.

EVOO-adulterant Mix	# PF	RMSEC	RMSEP	R^2
O1F1	1	0.09	0.05	0.947
O1F2	1	0.06	0.07	0.975
O1F4	2	0.07	0.05	0.971
O1F5	1	0.07	0.13	0.964
O2F1	2	0.03	0.06	0.996
O2F2	1	0.01	0.06	0.933
O2F4	2	0.07	0.06	0.969
O2F5	2	0.02	0.08	0.997
O3F1	2	0.02	0.05	0.996
O3F2	2	0.02	0.02	0.997
O3F4	2	0.05	0.04	0.985
O3F5	1	0.10	0.10	0.932
O4F1	1	0.10	0.03	0.926
O4F2	1	0.07	0.07	0.966
O4F4	2	0.07	0.06	0.968
O4F5	2	0.04	0.08	0.990

VI. PERSPECTIVES

Diffuse-light absorption spectroscopy performed in the 400-1700 nm spectral range, combined with a multivariate processing of spectroscopic data, have demonstrated the capability of predicting the adulteration of concentration of diverse lower-grade olive oils which are frequently used as adulterants of authentic EVOOs produced in Tuscany, a centrally located Italian region. Being scattering-independent, this technique can be used for EVOO analysis during the entire shelf-life of the product.

To the best of our knowledge we have demonstrated for the first time that optical spectroscopy can be successfully used to identify and quantify the fraction of DOO in authentic EVOO.

Verifying the authenticity of EVOOs is just one of the many other potential applications that diffuse-light absorption spectroscopy has, especially in combination with a suitable processing of the spectroscopic data. Other types of expensive foodstuffs can be authenticated, such as bio-juices, honeys, alcoholic beverages, as well as many other liquids, the most promising of which can be dietary supplements based on herbs and naturals cosmetics.

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