Development of a Novel Angular Fluorescence Spectroscopic Method for Characterizing Olive Oils from Different Geographic Origins

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ABSTRACT— The geographic origin and optical characteristics of olive oils are important for quality assessment, authenticity, and traceability. In this study, a novel angular fluorescence method was developed to characterize olive oils from eight regions in Iran using Laser-Induced Fluorescence (LIF) spectroscopy. Although conventional fluorescence spectra did not reveal clear differences among samples, angular fluorescence measurements provided a useful means of examining their optical responses. Differential fluorescence intensities at 675 nm were recorded across multiple detection angles, and distinct angular patterns were observed for samples from each region. Regression parameters derived from these patterns were plotted in phase space, where the olive oil samples formed separate, non-overlapping clusters. Separation in the slope–intercept feature space demonstrates the potential of angular fluorescence measurements for characterizing the investigated olive oils according to their reported geographic origins. The regression models yielded R^2 values of 0.95–0.99, indicating good fits to the measured angular responses rather than predictive classification accuracy. Because independent validation using previously unseen samples was not performed, these findings should be considered a proof of concept. Further validation is required to determine whether the method can potentially support reliable geographic-origin assessment of unknown olive oils.

KEYWORDS: Fluorescence spectroscopy, Laser-induced fluorescence, Olive oils, Phase space

I. INTRODUCTION

The olive oil industry is a cornerstone of both the Mediterranean economy and global agriculture, encompassing olive groves, oil mills, and refineries. Beyond its culinary significance, olive oil is celebrated for its nutritional and medicinal benefits, making it a key ingredient in various industries. From table olives to high-value products in the pharmaceutical, cosmetic, and dietary sectors, its versatility is unmatched. This multifaceted industry continues to thrive, blending tradition with innovation to meet diverse consumer needs [1]-[3].

Authentication plays a crucial role in ensuring the production of high-quality olive oils [4]. In this context, determining the geographic origin has become a key strategy for identifying Protected Designations of Origin (PDOs) and Protected Geographical Indications (PGIs) in various countries [5], [6]. Growing consumer demand for authentic olive oils has driven research efforts to investigate and verify their geographic origins [7]. Consequently, robust systems for quality assurance, purity verification, and safety control are essential to meet these expectations.

In addition to the importance of determining the geographical origin, another issue with olive oils available in the market is the presence of adulteration [8]. Various methods are used to detect adulteration in oils, including spectroscopic techniques such as Fourier

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transform infrared (FTIR) [9], [10], near-infrared (NIR) [11], [12], and Raman spectroscopy [13]. One of the simplest methods for detecting adulteration and identifying oils is the use of the laser-induced fluorescence (LIF) technique [14]-[16].

LIF has gained attention as a rapid, cost-effective, and portable analytical technique with minimal sample preparation. Utilizing a laser light source, LIF offers high sensitivity and specificity, effectively detecting minor adulterations in olive oil by capturing detailed molecular spectral data. Compared to traditional fluorescence methods, LIF enhances fluorescence efficiency and detection sensitivity while being non-destructive and environmentally friendly. Its real-time analysis capabilities and suitability for routine quality control make it an ideal tool for the food industry [17]-[20].

While extensive research has been conducted on detecting adulteration, differentiating geographical origins of olive oil, and analyzing its fluorescence spectra, a critical gap remains in the development of advanced statistical methods capable of accurately capturing and distinguishing the subtle spectral variations between olive oils. Current approaches often fall short in fully leveraging these nuanced differences. Thus, the development of a novel, highly precise statistical framework is essential to overcome this limitation and significantly improve the reliability and accuracy of such analyses.

II. Materials and Method

A. *Materials*

Three olive oil samples were purchased from each of eight provinces in Iran: Gilan, Qazvin, Isfahan, Semnan, Golestan, Lorestan, Sistan, and Zanjan.

B. *LIF Setup*

The LIF spectroscopy setup utilized in this study was designed based on the methodology outlined in [21]. Olive oil samples were placed in cylindrical cuvettes with a 20 mm inner diameter and a 1 mm wall thickness. A continuous-wave (CW) diode laser operating at a wavelength of 405 nm served as the excitation source, illuminating the samples in a symmetrical configuration. The emitted fluorescence was collected using a collimator and transmitted to an OPCT-V550 spectrometer via a fiber optic probe, providing a spectral resolution of 0.6 nm. To investigate the angular dependence of the fluorescence spectra, the fiber optic probe was mounted on a rotatable arm, allowing precise positioning near the surface of the cuvette.

C. *Optical Method*

In LIF spectroscopy of bulk media, a narrow laser beam excites fluorophores within a confined region known as the fluorescence observation field (FOF), and a detection probe situated outside the medium collects the emitted fluorescence photons from this region [22]. As the laser propagates through the medium, its intensity diminishes due to absorption events. Furthermore, the emitted fluorescence encounters reabsorption by non-excited (ground-state) fluorophores outside the FOF and scattering by molecules and small particles throughout the medium.

The attenuation of LIF intensity is influenced by factors such as dye concentration, the density of scattering centers within the medium, and the excitation and emission wavelengths. In cases of self-absorption, where reabsorption events are exclusively performed by ground-state fluorophores, the attenuation also depends on the angle of detection, i.e., the position of the probe relative to the FOF [22], [23]. These attenuations are collectively referred to as fluorescence inner filter effects (IFEs). There are two types of IFEs: the attenuation of the laser beam, termed primary-IFE, and the fluorescence reabsorption, known as secondary-IFE [22].

The heterogeneous distribution of non-excited fluorophores contributes to the secondary-IFE by reabsorbing fluorescence photons emitted from the FOF region. Consequently, secondary-IFE of the LIF emission occurs with varying strengths in different detection geometries. Figure 1 illustrates the heterogeneous

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distribution of the population of excited molecules in a cylindrical cuvette due to the IFEs. Detected LIF emissions at different detection angles are subjected to different reabsorption rates as a result of the secondary-IFE.

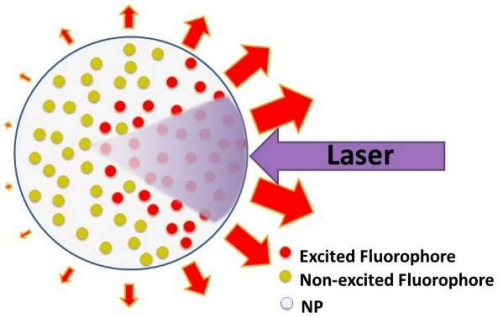


Fig. 1. Detected LIF emissions in different angles of detection are subjected to different reabsorption rate as secondary-IFE.

In the diffusion model of light propagation within an optical medium, the reabsorption rate is determined by the Beer-Lambert Law (Eq. (1)). This law relates the reduction of the original fluorescence intensity $I_{0,fluor.}(\lambda)$, after traveling an optical path length l, to the density of the non-excited fluorophores and the reabsorption cross-section $\sigma_{reabs.}(\lambda)$. The reabsorption cross-section is highly dependent on the fluorescence Stokes shift, which determines the crossover area between the absorption and fluorescence spectra [24]. The equation is as follows:

$$I_{fluor.}(\lambda,l) = I_{0,fluor.} \exp\left[\int_0^l \sigma_{reabs.}(\lambda) N_{Fl.}^{G}(l')dl'\right] \quad (1)$$

Accordingly, an increase in the traveling length l, the density of non-excited molecules, or the reabsorption cross-section $\sigma_{reabs.}(\lambda)$ results in more reabsorption events (secondary-IFE) and a subsequent reduction in the detected fluorescence intensity [24], [25].

When there is a non-uniform overlap between the absorption and emission spectra, the likelihood of reabsorption varies for different wavelengths within the fluorescence spectrum. This uneven probability leads to a wavelength-dependent reduction of emission intensity in the crossover region. As a result, there is not only attenuation of the fluorescence intensity but also a potential shift in the wavelength at the peak of the detected fluorescence [24]. Such non-intrinsic spectral shifts in fluorescence emission can serve as optical parameters in the design of molecular sensors for chemical and biomedical applications [24].

III. Results and Discussion

A. *Fluorescence and Novel Method*

In this study, a novel method was developed to distinguish olive oils originating from geographically distinct regions. Specifically, three olive oil samples were purchased from each of eight provinces in Iran: Gilan, Qazvin, Isfahan, Semnan, Golestan, Lorestan, Sistan, and Zanjan. The primary objective was to investigate whether olive oils from these regions could be differentiated based on their fluorescence characteristics. Initially, the LIF spectra of all olive oil samples were obtained, as shown in Fig. 2. Upon examining the spectra, it was observed that the fluorescence profiles of the oils did not exhibit significant differences. This indicated that conventional fluorescence spectra alone might not be sufficient for distinguishing olive oils from different regions. To address this challenge, a more refined approach was implemented.

The fluorescence spectrum of each olive oil sample was recorded at six different angles: 20°, 30°, 40°, 50°, 60°, and 70°. These spectra were then normalized to ensure comparability. Subsequently, the fluorescence intensity at each wavelength for a given angle was subtracted from the corresponding intensity at 20°. This differential intensity calculation revealed a critical observation: for all olive oil samples, the largest intensity differences occurred around a wavelength of 675 nm. This finding provided a key insight into the potential for distinguishing olive oils based on their angular fluorescence response. The differential intensities at 675 nm were plotted as a function of angle, as depicted in Fig. 3. Remarkably, the resulting curves for the olive oils from different regions were entirely distinct and non-overlapping. The angular response patterns showed separation among the measured

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samples from the eight geographic groups. The fitted regression models yielded R^2 values ranging from 0.95 to 0.99, indicating that the linear models described the measured angular responses well. However, these goodness-of-fit values do not quantify geographic classification accuracy or establish predictive performance for previously unseen samples.

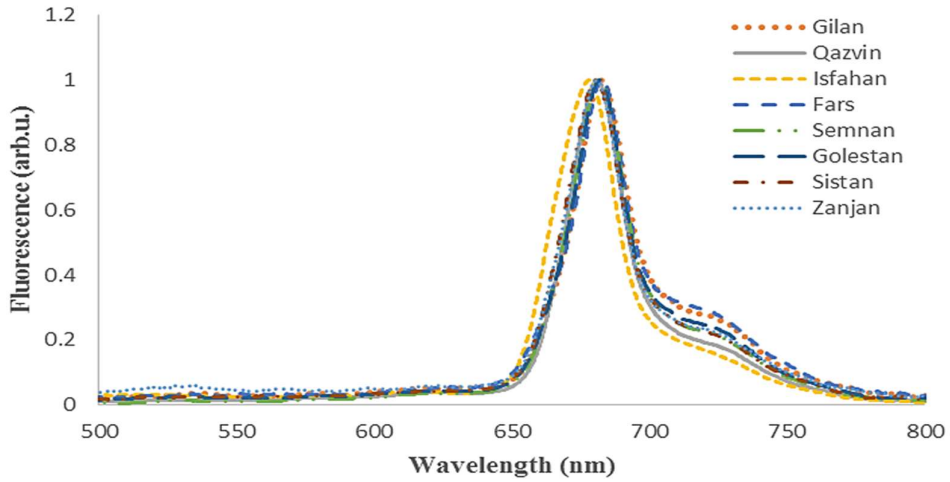


Fig. 2. Typical LIF spectrum of olive oil samples excited by a laser at 405 nm and recorded at an angle of θ=20°.

The observed differences in the angular fluorescence response may be associated with compositional variations among the olive oil samples from different regions. Previous studies have reported that geographic region and environmental conditions can influence olive oil composition, including fatty acid profiles [26]. However, the concentrations of chlorophylls, carotenoids, and fatty acids were not independently measured in the present study. Therefore, their specific contributions to the observed angular fluorescence response and secondary inner filter effect cannot be directly established from the present results. It is hypothesized that variations in the concentrations of absorbing and fluorescent constituents may alter the overlap between absorption and emission spectra and, consequently, affect wavelength-dependent fluorescence reabsorption. Nevertheless, this proposed mechanism remains tentative and requires further investigation through independent chemical characterization and optical measurements. The present findings demonstrate distinct angular fluorescence response patterns among the measured samples, suggesting the potential of this approach for further investigation of olive oil differentiation.

B. *Formulation of a New Method*

1) *Recording Fluorescence Spectra at Different Angles*

For each olive oil sample, the fluorescence intensity $I_\theta(\lambda)$ was measured at angles, $\theta = 20°, 30°, 40°, 50°, 60°, 70°$.

2) *Normalizing the Spectra*

To ensure accurate comparisons between the spectra, all fluorescence intensities were normalized:

$$I_{norm,\theta}(\lambda) = I_\theta(\lambda) / I_{\theta,\max} \quad (2)$$

where $I_{\theta,\max}$ is the maximum intensity at angle θ.

3) *Calculating Differences in Fluorescence Intensities*

The difference in fluorescence intensity for each angle relative to the reference angle 20° was calculated:

$$\Delta I_\theta(\lambda) = I_{norm,\theta}(\lambda) - I_{norm,20°}(\lambda) \quad (3)$$

4) *Focusing on Wavelength (λ_{LID})*

In this study it was observed that the Largest Intensity Differences (LID) occurred at the wavelength 675 nm. Therefore, ΔI_θ (675 nm) was selected for further analysis.

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5) *Plotting Intensity Differences as a Function of Angle*
The values of ΔI_θ (LID) were plotted against the angle θ.

6) *Data Analysis and Modeling*
A regression line was fitted to the data for each sample:

$$\Delta I_\theta (LID) = a\theta + b \quad (4)$$

where a and b are the regression coefficients.

The regression used detection angle as the independent variable and normalized differential fluorescence intensity at 675 nm as the dependent variable; the reported R^2 values describe goodness of fit, not geographic classification accuracy, which requires separate validation using metrics such as accuracy, sensitivity, precision, and F1-score.

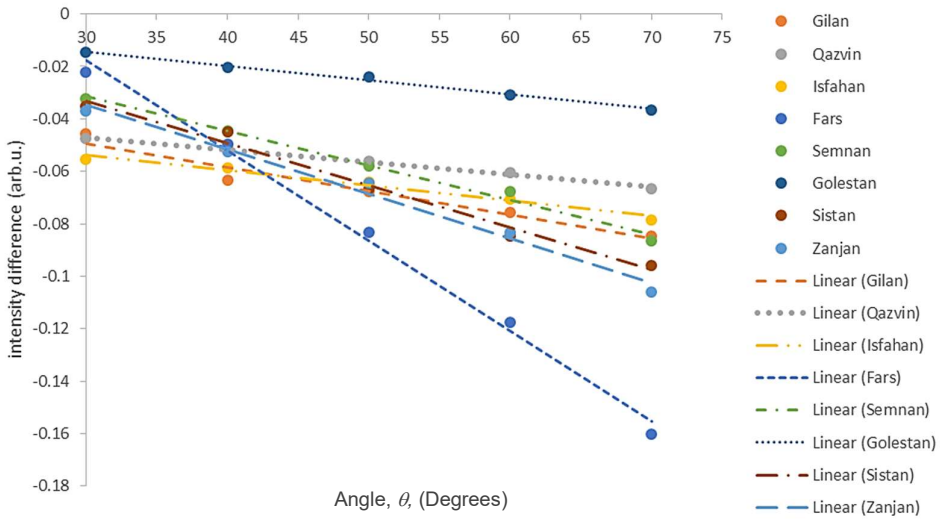


Fig. 3. Regression lines of the fluorescence intensity differences of olive oils from different regions at a wavelength of 675 nm at various angles, with the reference angle set to 20°.

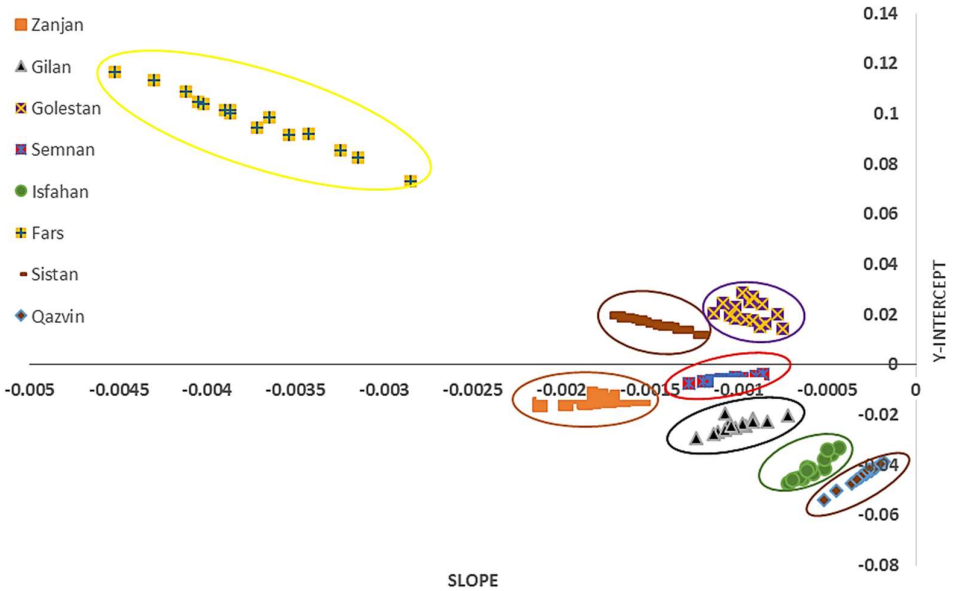


Fig. 4. Phase space representation of olive oil samples, showing distinct clustering of samples based on the slope (x-axis) and intercept (y-axis).

C. *Phase Space*

The analysis of the fluorescence spectra of olive oil samples from different regions revealed a clear distinction among the samples when their angular fluorescence responses were examined. By focusing on the differential fluorescence

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intensities at a wavelength of 675 nm, the data demonstrated that the olive oils from various geographic origins exhibited unique and non-overlapping patterns. This differentiation was further validated by plotting the regression parameters of the fluorescence intensity differences in a phase space, where the horizontal axis represented the slope and the vertical axis represented the intercept of the regression lines.

As shown in Fig. 4, the phase space analysis provided a robust visualization of the separation among the olive oil samples. Each group of 15 measurements corresponding to olive oils from a specific province clustered tightly within a distinct region of the phase space. These clusters were reasonably well differentiated, with no substantial overlap observed between the groups. These observations suggest that the proposed approach may be useful for distinguishing olive oils based on their geographic origin. Furthermore, the relatively compact distribution of data points within each cluster may indicate acceptable reproducibility of the method, as the measurements corresponding to each group were predominantly located within a specific region of the phase space.

The phase space representation revealed distinct patterns in the angular fluorescence responses of the olive oil samples investigated in this study. The separation observed in the slope–intercept feature space indicates differences in the measured angular fluorescence responses among the samples. These differences may be associated with variations in sample composition; however, the concentrations of chlorophylls, carotenoids, and fatty acids were not independently measured in the present study. Therefore, their specific contributions to the observed fluorescence responses and the secondary inner filter effect remain unverified. The proposed relationship between compositional variations and the observed separation should consequently be considered a possible interpretation rather than a confirmed mechanism.

Overall, the results demonstrate that angular fluorescence measurements combined with regression-derived slope and intercept parameters can reveal distinct patterns among the olive oil samples examined. Although the phase space representation provides a useful means of visualizing these differences, the observed separation does not, by itself, establish classification accuracy or confirm the ability to identify the geographic origin of previously unseen samples. Further studies involving independent chemical characterization, a larger number of samples, and external validation are required to investigate the underlying mechanisms and determine the applicability of this approach to geographic authentication of olive oils.

IV. CONCLUSION

This study evaluated angular laser-induced fluorescence spectroscopy as a proof-of-concept method for optically characterizing olive oil samples collected from eight reported geographic groups in Iran. Differential fluorescence intensities at 675 nm and regression-derived slope and intercept parameters produced distinct angular-response patterns and separated the investigated samples in a two-dimensional feature space. The R^2 values of 0.95–0.99 indicate good fits to the measured angular-response data; however, they should not be interpreted as measures of classification accuracy or geographic-authentication performance. Because information regarding olive cultivar, harvesting date, production and processing conditions, storage history, and other relevant variables was not fully available or controlled, the observed differences cannot be attributed solely to geographic factors. Moreover, independent validation using previously unseen oils and external datasets was not performed. Therefore, the proposed method should be regarded as a promising preliminary approach for the optical characterization of olive oils, with potential to support geographic-origin assessment, rather than as a validated authentication tool. Future studies should employ controlled sampling designs, appropriate reference analyses, explicit classification rules, independent

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sample-level validation, and external datasets covering broader cultivar, production, temporal, and storage variability.

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