The Use of Right Angle Fluorescence Spectroscopy to Distinguish the Botanical Origin of Greek Common Honey Varieties

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Abstract: The standardization of the botanical origin of honey reflects the commercial value and quality of honey. Nowadays, most consumers are looking for a unifloral honey. The aim of the present study was to develop a novel method for honey classification using chemometric models based on phenolic compounds analyzed with right angle fluorescence spectroscopy, coupled with stepwise linear discriminant analysis (LDA). The deconstructed spectrum from three-dimensional-emission excitation matrix (3D-EEM) spectra provided a correct classification score of 94.9% calibration and cross-validation at an excitation wavelength (λex) of 330 nm. Subsequently, a score of 81.4% and 79.7%, respectively, at an excitation wavelength (λex) of 360 nm was achieved. Each chemometric model confirmed its power through the external validation with a score of 82.1% for both. Differentiation could be correlated with hydroxycinnamic and hydroxybenzoic acids, which absorb in this region of the spectrum. Fluorescence spectroscopy constitutes a rapid and sensitive technique, which, when combined with the stepwise algorithm and LDA method, can be used as a reliable and predictive authentication tool for honey. This study indicates that the developed methodology is a promising technique for determination of the botanical origin of common Greek honey varieties. Our long-term ambition is to support producers and suppliers to remain in a competitive national and international market.

Keywords: fir honey; pine honey; thyme honey; citrus honey; botanical origin; right angle fluorescence spectroscopy; discrimination

1. Introduction

In the European Union (EU), beekeeping remains an ever-expanding sector, with the EU establishing itself as the second largest global producer of honey after China, producing 280,000 tons every year [1].

Based on their botanical origins, each variant of unifloral honey commands a premium price due to its organoleptic properties; particularly related to the interest of consumers regarding correctly labeling a honey’s origin. The EU safeguards authenticity by enforcing strict legislation establishing physicochemical characteristics [2], which Greece reinforces by enacting stricter physicochemical characteristics and melissopalynological analyses [3]. Irrespective of these enforced regulations to ascertain the exact origin via scientific means, a number of these parameters are somewhat correlated with large-scale dispersion. Therefore, the resulting chemometric models, using LDA, which are based on physicochemical parameters, are unreliable [4]. Furthermore, it should be considered that these types of analyses...
are both expensive and time-consuming, and, in the case of melissopalynological analyses, require specialized staff. Honey from pine, fir, thyme, and citrus are commercially available in Greece and their demand from consumers worldwide is constantly increasing. They represent about 60–65%, 15%, 10%, and 5% of the country’s total annual honey production, respectively. In recent years, numerous spectroscopic methods have been proposed to determine honey types of different botanical origins. The most frequently cited spectroscopic techniques include Fourier-transform infrared spectroscopy (FTIR) [5,6], near-infrared (NIR) [7], Raman spectroscopy [6,8], and nuclear magnetic resonance (NMR) [9].

Fluorescence spectroscopy is simple and 100 to 1000 times more sensitive than other spectroscopical techniques [10]. It has been utilized for the determination of honey adulteration [11–13], botanical discrimination [14–17], geographical differentiation [18], and fluorescence marker detection [19,20]. Various statistical methods have been used in factorial discriminant analysis [15], principal component analysis (PCA) [13,16–18], partial least squares (PLS) [21], partial least squares discriminant analysis (PLS-DA) [22,23], soft independent modeling of class analogy (SIMCA) [16], and hierarchical cluster analysis (HCA) [17] based on fluorescence spectra data.

Fluorescence excitation-emission matrix (EEM) spectra contain information about a variety of intrinsic fluorochrome compounds, including aromatic amino acids, phenolics, flavins, and Maillard reaction products [13,14,24]. EEM includes spectra regions often complicated due to both Raman and Rayleigh scattering. Nevertheless, honey contains phenolic compounds (phenyl carboxylic acids) that do not coincide with scattering, and their concentrations vary depending on their botanical origins [25–28]. The abovementioned dispersion can be overcome by subtracting a blank EEM or by using the interpolation method [29]. Thus, the right-angle fluorescence spectroscopic technique can be used.

The aim of this study was the development of an alternative novel method to distinguish honey, based on fluorophore compounds, mainly hydroxycinnamic and other phenyl carboxylic acids. For this purpose, four confirmed unifloral Greek honey varieties were selected.

2. Materials and Methods

2.1. Honey Samples

The honey samples used in this study were provided directly from beekeepers during the 2018 and 2019 harvest years. The botanical origins were confirmed based on physico-chemical and mellisopalynological analyses, as defined by European and Greek legislation. A total of 87 unifloral honey samples were derived from the four separate botanical sources (32 thyme, 18 pine, 21 fir, and 16 citrus). The samples were stored in dark at 23 ± 1 °C and their fluorescence spectra were recorded within one month.

2.2. Reagents and Solutions

Standards of phenolics, including kaempferol, catechin, chrysin, hesperetin, isorhamentin, naringenin, and phenolic acids (gallic, chlorogenic, ellagic, gentisic, homogentisic, p-coumaric, protocatechuic, sinapic) were purchased from Extrasynthese (Genay, France). Methyl syringate, caffeic, ferulic, syringic, and vanillic acids were purchased from Sigma-Aldrich (Steinheim, Germany). The purities of the standards were 98–99%. The chemical structures are shown in Table S1. All solutions were prepared by dissolving the abovementioned phenolic compounds in MS-grade acetonitrile (0.01 mg/mL) (Sigma-Aldrich Merck KGaA, Darmstadt, Germany).

2.3. Fluorescence Spectroscopy

Three-dimensional-emission excitation matrices (3D-EEMs) were acquired using a FluoroMate FS-2 spectrometer (CE Mark. Scinco Nieuwegein, NLD) equipped with a continuous wave xenon-arc lamp light source with 500 W of output power. The type of electronic transition was $S_1 \rightarrow S_0$ with a timescale of $10^{-9}$ to $10^{-6}$ s. Honey samples were homogenized in a water bath at 50 °C for 10 min and introduced into a quartz cuvette.
EEM spectra were recorded in duplicate using a right-angle sample holder. Following optimization of the spectrum acquisition, the emission wavelength ($\lambda_{\text{em}}$) was set from 270 to 620 nm at 5 nm intervals and the excitation wavelength ($\lambda_{\text{ex}}$) was set from 240 to 500 nm at 5 nm intervals. The fluorescence spectra were obtained on a computer supported by FluoroMasterPlus software (CE Mark. Scinco, Nieuwegein, The Netherlands).

Each 3D-EEM spectrum was saved as a CSV file and pre-treatment was performed using XLSTAT-3DPlot (XLStat ver 2019.2.2, Addinsoft Inc., New York, NY, USA). Then, all data were normalized using software (The Unscrambler X ver.10.4, CAMO Software AS., Oslo, Norway) before statistical analyses.

2.4. Physicochemical and Melissopalynological Analysis

Regarding physicochemical parameters, sugars (fructose, glucose, and sucrose), electrical conductivity, and moisture content were determined according to Association of Official Analytical Chemists (AOAC) [30] and International Honey Commission (IHC) protocols [31]. Specifically, determination of honey sugars was performed using an HPLC Shimadzu CTO-10A, equipped with a Shimadzu RID-20A detector (Shimadzu Corporation, Kyoto, Japan), and electrical conductivity was determined with a Consort C3010 multi-parameter analyzer (Consort bvba, Turnhout, Belgium). Moisture was measured with a refractometer (Bellingham and Stanley Ltd., Kent, UK).

Melissopalynological analyses were performed with a Kruss microscope (A. Kruss Optronic GmbH, Hamburg, Germany) according to Louveaux et al. [32].

2.5. Statistical Analysis

A total of 87 unifloral honey samples were randomly separated into calibration and test sets. The first group was comprised of 59 honey samples (18 thyme, 13 pine, 16 fir, and 12 citrus) and was named as “standards”; the second group was made up of 28 samples (14 thyme, 5 pine, 5 fir, and 4 citrus) and was named as “unknown”. This was subsequently followed by the development of two chemometric models, based on $\lambda_{\text{ex}}$ = 330 and 360 nm, using the stepwise-LDA statistical technique. Botanical classification was based on EEM spectra of fluorophore phenolic compounds. Before the development of the discriminant analysis, the homogeneity of the covariance matrices was ensured since the ratio of the largest group (thyme, n = 18) divided by the smallest group (citrus, n = 12) was equal or less than 1.5. [33]. Each chemometric model was examined using cross-validation and external validation. The statistical analyses were performed using SPSS v.25 (IBM, SPSS Inc., Statistics, New York, NY, USA) software.

3. Results and Discussion

3.1. Physicochemical and Melissopalynological Analysis

The physicochemical parameters of all samples were in line with legislation. The amount of fructose and glucose was found to be 60.1 and 66.2 (%w/w) for thyme and citrus honey and 46.4 and 46.3 (%w/w) for pine and fir honey, respectively. Sucrose content was no more than 5 (%w/w) for any of the selected honey varieties. Values of electrical conductivity were $\leq$600 $\mu$S cm$^{-1}$ and $\leq$324 $\mu$S cm$^{-1}$ for thyme and citrus honey and $\geq$911 $\mu$S cm$^{-1}$ and $\geq$1041 $\mu$S cm$^{-1}$ for pine and fir honey, respectively. Finally, regarding moisture content, legislation demands were met, as the moisture content was less than 20 (%w/w) for thyme, citrus, and pine honey, whilst for fir honey, it was less than 18.5 (%w/w). Table 1 shows a summary of the results of the physicochemical analyses.

The results of the melissopalynological analyses agree with the botanical origin of the honey samples (Table S2).
Table 1. Results of physicochemical analysis.

| Botanical Source | Aggregate Functions | Fructose + Glucose (% w/w) | Sucrose (% w/w) | Electrical Conductivity (µS cm⁻¹) | Moisture (% w/w) |
|------------------|---------------------|-----------------------------|-----------------|-----------------------------------|-----------------|
| Thyme honey      | Min                 | 60.1                        | 0.0             | 251                               | 14.3            |
|                  | Max                 | 86.4                        | 3.7             | 600                               | 17.9            |
|                  | Average             | 68.2                        | 0.7             | 435                               | 15.8            |
| Pine honey       | Min                 | 46.4                        | 0.0             | 911                               | 14.6            |
|                  | Max                 | 77.2                        | 1.0             | 1431                              | 17.7            |
|                  | Average             | 58.9                        | 0.1             | 1122                              | 16.1            |
| Fir honey        | Min                 | 46.3                        | 0.0             | 1041                              | 13.7            |
|                  | Max                 | 64.5                        | 2.4             | 2000                              | 18.4            |
|                  | Average             | 55.0                        | 0.2             | 1526                              | 15.5            |
| Citrus honey     | Min                 | 66.2                        | 0.0             | 181                               | 15.3            |
|                  | Max                 | 76.2                        | 4.8             | 324                               | 18.7            |
|                  | Average             | 71.9                        | 1.0             | 275                               | 17.0            |

3.2. 3D-EEM Spectra of Standards Phenolic Compounds

Standard phenolic compounds exhibited excitation with λex ranging between 260 and 360 nm and emission with λem ranging from 315 to 420 nm. Moreover, some flavonoids presented a low fluorescence intensity [24]. Detailed results are shown in Table 2 and the spectra of the standard phenolic compounds are presented in Figure S1.

Table 2. The wavelengths of λex and λem of standards phenolic compounds.

| Phenolic Compound        | λex (nm) | λem (nm) |
|--------------------------|----------|----------|
| Caffeic acid             | 310–360  | 410      |
| Chlorogenic acid         | 300–360  | 416      |
| p-Coumaric acid          | 320–340  | 380      |
| Ferulic acid             | 310–360  | 400      |
| Sinapic acid             | 300–360  | 415      |
| Ellagic acid             | 280–380  | 400      |
| Homogentisic acid        | 280–320  | 335      |
| Gallic acid              | 265–315  | 345      |
| Protocatechuic acid      | 265–315  | 335      |
| Syringic acid            | 250–315  | 335      |
| Vanillic acid            | 260–315  | 330      |
| Methyl syringate         | 250–320  | 340      |
| Gentisic acid            | 290–360  | 400 and 475 |
| Kaempferol               | 280–320  | 430 and 500 |
| Catechin                 | 250–310  | 315      |
| Chrysin                  |          | Low intensity |
| Hesperetin               |          | Low intensity |
| Isorhamentin             |          | Low intensity |
| Naringenin               |          | Low intensity |
3.3. 3D-EEM Spectra of Honey Samples

Characteristic 3D-EEM spectra for each honey type are presented in Figures 1–4. Honey spectra showed intensities at different λex values as follows (Figure 5): aromatic amino acids, including phenylalanine, tryptophan and tyrosine residues (λex = 240–280 nm) [17,23,34], phenolic compounds (λex = 280–330 nm and λex = 310–380 nm) [13,17,18,24], Maillard reaction compounds such as furosine and hydroxymethylofurfural (HMF) (λex = 380–440 nm) [13,22], and flavins (λex = 440–500 nm) [11,23,24].

Figure 1. Characteristic 3D-EEM spectra of thyme honey.

Figure 2. Characteristic 3D-EEM spectra of pine honey.
Figure 3. Characteristic 3D-EEM spectra of fir honey.

Figure 4. Characteristic 3D-EEM spectra of citrus honey.
Figure 5. The 3D-EEM spectra intensities at $\lambda_{ex} = 240–400$ and $\lambda_{em} = 250–640$.

Fluorescence spectra of thyme, pine, fir, and citrus honey highlight similar 3D-EEM patterns. Samples were complex due to the presence of several fluorophore compounds with overlapping regions. Therefore, raw fluorescence EEMs spectra cannot lead to the determination of phenolic compounds. Some researchers overcame this difficulty by simultaneously scanning excitation and emission wavelengths ($\Delta \lambda$) with synchronous fluorescence spectroscopy [35]. In this study, from 3D-EEM, only the 2D spectra that correspond to phenolic compounds were chosen.

Figures 6 and 7 show the emission spectra at $\lambda_{ex} = 330$ and 360 nm, respectively. These regions were attributed to phenolic compounds and apparent differences were observed among the different honey varieties. Each emission spectrum from a honey sample can be considered as a fingerprint of phenolic substances. These spectra consisted of concomitant high or low concentrations of heterogeneous phenolic compounds accordingly to the honey’s nature. Fluorescence spectra of the phenolic standard compounds provided more details. More specifically, $\lambda_{ex}$ between 330 to 360 nm was attributed mainly to hydroxycinnamic acids while ($\lambda_{ex}$) 330 nm had a significant contribution of other phenyl carboxylic acids.

Generally, specific phenolic compounds, particularly hydroxycinnamic and phenyl carboxylic acid derivatives, have been detected in several types of blossom honey, as in the case of thyme honey from Italy [36], Greece [37], and citrus honey from China [38], Italy [39,40], Iran [41] and Greece [37]. Similar results were obtained for honeydew honey from Germany [42], pine honey from Poland [43] and Greece [37,44], and fir honey from Greece [37,44].
Generally, specific phenolic compounds, particularly hydroxycinnamic and phenyl carboxylic acid derivatives, have been detected in several types of blossom honey, as in the case of thyme honey from Italy [36], Greece [37], and citrus honey from China [38], Italy [39,40], Iran [41] and Greece [37]. Similar results were obtained for honeydew honey from Germany [42], pine honey from Poland [43] and Greece [37,44], and fir honey from Greece [37,44].

Several studies have suggested possible correlations between the botanical origin and certain hydroxycinnamic and other phenyl carboxylic acids. Kıvrak et al. [45] reported a notable variation in the content of phenolic compounds of 19 types of honey from Turkey, with ferulic, homogentisic, gentisic, and protocatechuic acids being the most abundant compared to other phenolics. Specifically, the highest levels of homogentisic acid were obtained from thyme, citrus, and protocatechuic acid from pine honey. In addition, pine honey had a high content of syringic acid. Furthermore, all samples contained a significant amount of gentisic, syringic, 3,4-dihydroxybenzoic, caffeic, and ferulic acids. Tsiapara et al. [44] found differences in phenolic acid fractions among Greek honey extracts. Fir
and pine honey were richer in protocatechuic acid, whereas the vanillin acid content was found to be higher in thyme honey. Spilioti et al. [37] observed that protocatechuic, p-hydroxybenzoic, vanillic, caffeic, and p-coumaric acid were the major phenolic acids in 12 honey variants (thyme, pine, fir, citrus) from Greece. Thyme and citrus honey had a lower content of protocatechuic and caffeic acid than pine and fir, and p-hydroxybenzoic acid was the dominant compound in thyme honey.

From the above, it can be inferred that the qualitative and quantitative profiles of phenolics, especially phenolic acids, in unifloral honeys, undoubtedly provide key information about their botanical origins.

3.4. Stepwise-LDA of Fluorescence Spectra

The chemometric analysis of fluorescence spectra ($\lambda_{ex} = 330$ and $360$ nm) for the classification of thyme, pine, fir, and citrus honey was performed using the stepwise-LDA algorithm. After random separation of the samples, the ratio of the largest group (thyme honey) divided by the smallest group (citrus honey) was calculated at 1.5, confirming the homogeneity of the covariance matrices. Subsequently, the calibration set ($n = 59$) was subjected to a stepwise algorithm under the Mahalanobis distance method. Following the development of LDA models, their performance was evaluated using the cross-validation method. Furthermore, a test set ($n = 28$) was used for external validation to examine the robustness of the models.

The chemometric model, based on $\lambda_{ex} = 330$ nm, demonstrated that nine stepwise steps ($p < 0.05$) were formed. The score values for both calibration and cross-validation were 94.9%. The group centroid values are also plotted in Figure 8.

![Figure 8. Discrimination results based on $\lambda_{ex} = 330$ nm.](image_url)

The results of the Wilks’s lambda ($\Lambda$) of the canonical functions (first: 0.120, $p < 0.05$; second: 0.240, $p < 0.05$; third: 0.653, $p < 0.05$) indicated a significant difference between the mean vectors of the four honey botanical origins. Additionally, the eigenvalues and canonical correlation of the discriminant functions (first: 18.213, 97.4%; second: 1.724, 79.6%; third: 0.531, 58.9%) confirmed the calibration model. After, external validation of “unknown” samples evaluated the ability of the discrimination. A total of 82.1% were correctly classified while 17.9% were misclassified. Hence, a low variation between the
cross-validation and external validation indicates good performance of the chemometric model to predict new data (Table 3).

Table 3. Discrimination results based on fluorescence at $\lambda_{ex} = 330$ nm.

| Predicted Group Membership | Labels          | Thyme Honey | Pine Honey | Fir Honey | Citrus Honey | Total |
|----------------------------|-----------------|-------------|------------|-----------|--------------|-------|
| **Original** a             | Thyme honey     | 18          | 0          | 0         | 0            | 18    |
| Count                      | Pine honey      | 1           | 11         | 1         | 0            | 13    |
|                           | Fir honey       | 0           | 1          | 15        | 0            | 16    |
|                           | Citrus honey    | 0           | 0          | 0         | 12           | 12    |
|                           | Thyme honey     | 100.0       | 0.0        | 0.0       | 0.0          | 100.0 |
|                           | Pine honey      | 7.7         | 84.6       | 7.7       | 0.0          | 100.0 |
|                           | Fir honey       | 0.0         | 6.3        | 93.8      | 0.0          | 100.0 |
|                           | Citrus honey    | 0.0         | 0.0        | 0.0       | 100.0        | 100.0 |
| **Cross-validated** b,c    | Thyme honey     | 18          | 0          | 0         | 0            | 18    |
| Count                      | Pine honey      | 1           | 11         | 1         | 0            | 13    |
|                           | Fir honey       | 0           | 1          | 15        | 0            | 16    |
|                           | Citrus honey    | 0           | 0          | 0         | 12           | 12    |
|                           | Thyme honey     | 100.0       | 0.0        | 0.0       | 0.0          | 100.0 |
|                           | Pine honey      | 7.7         | 84.6       | 7.7       | 0.0          | 100.0 |
|                           | Fir honey       | 0.0         | 6.3        | 93.8      | 0.0          | 100.0 |
|                           | Citrus honey    | 0.0         | 0.0        | 0.0       | 100.0        | 100.0 |
| **External Validation** d  | Thyme honey     | 11          | 0          | 3         | 0            | 14    |
| Count                      | Pine honey      | 1           | 4          | 0         | 0            | 5     |
|                           | Fir honey       | 0           | 1          | 4         | 0            | 5     |
|                           | Citrus honey    | 0           | 0          | 0         | 4            | 4     |
|                           | Thyme honey     | 78.6        | 0.0        | 21.4      | 0            | 100.0 |
|                           | Pine honey      | 20.0        | 80.0       | 0.0       | 0            | 100.0 |
|                           | Fir honey       | 0.0         | 20.0       | 80.0      | 0            | 100.0 |
|                           | Citrus honey    | 0.0         | 0.0        | 0.0       | 100.0        | 100.0 |

a 94.9% of original grouped cases correctly classified; b 94.9% of cross-validated grouped cases correctly classified; c cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case; d 82.1% of external validated test set correctly classified.

When applying the stepwise algorithm on $\lambda_{ex} = 360$ nm spectra three steps were formed ($p < 0.05$). The separation of the four honey botanical origins are shown in Figure 9.
Group centroids of pine and fir honey failed to display the same clear separation as was evident between the thyme and citrus honey. Specifically, the rate of samples that were classified correctly was 81.4%, while cross-validation was 79.7%. The statistical test of Λ was 0.081, 0.707m and 0.992 ($p < 0.05$) for the first, second, and third discriminant functions, respectively. Furthermore, the eigenvalues of these functions were estimated at 7.685 with canonical correlation at 94.1% for the first, 0.403 with 53.6% for the second, and 0.008 with 0.9% for the third. Finally, the correct classification of external validation (82.1%) further confirmed the reliability of the model. More detailed results are presented in Table 4.

Table 4. Discrimination results based on fluorescence at \(\lambda_{\text{ex}} = 360\) nm.

| Predicted Group Membership | Thyme Honey | Pine Honey | Fir Honey | Citrus Honey | Total |
|----------------------------|-------------|------------|-----------|--------------|-------|
| **Labels**                 |             |            |           |              |       |
| Thyme honey                | 17          | 1          | 0         | 0            | 18    |
| Pine honey                 | 0           | 10         | 3         | 0            | 13    |
| Fir honey                  | 0           | 6          | 10        | 0            | 16    |
| Citrus honey               | 1           | 0          | 0         | 11           | 12    |
| **%**                      |             |            |           |              |       |
| Thyme honey                | 94.4        | 5.6        | 0.0       | 0.0          | 100.0 |
| Pine honey                 | 0.0         | 76.9       | 23.1      | 0.0          | 100.0 |
| Fir honey                  | 0.0         | 37.5       | 62.5      | 0.0          | 100.0 |
| Citrus honey               | 8.3         | 0.0        | 0.0       | 91.7         | 100.0 |
Classification results and case-wise statistics from both chemometric models were similar, though the first chemometric model based at $\lambda_{ex} = 330$ provided a slightly higher discriminant score (94.9%) compared to the second model (81.4%) based at $\lambda_{ex} = 360$. Additionally, group centroids for both chemometric models can be explained. Specifically, as observed from the discriminant scatter plot, pine and fir honey are located near each other and are somewhat distinct from thyme and even more so from citrus honey. These findings are confirmed by the literature, as pine and fir honey is honeydew honey and, therefore, share several similarities [46]. Standardized canonical discriminant function coefficients responded to phenolic compounds and specifically to hydroxycinnamic and other phenyl carboxylic acids. Despite the presence of several fluorophore phenolics in this spectra region, the development of robust models remained unaffected by overlaps. No research on Greek honey using fluorescence spectroscopy has been previously conducted. Although the stepwise-LDA has not been applied for the classification of honey botanical origins, fluorescence spectroscopy studies from other researchers confirm the successful distinguishing of honey samples using the spectral region of phenolic compounds. Ruoff et al. [14] differentiated honey with an average score from 70% to 100% using PCA-LDA, while noting that spectra region $\lambda_{ex} 290–440$ nm was the most useful region. Furthermore, in another study, Karoui et al. (2007) suggested a PCA-FDA method to discriminate seven botanical origins. Recent studies also utilized the spectral region of phenolic compounds coupled with SIMCA [16] and HCA [17] for the botanical authentication of honey. The results of the present study confirmed that the fluorophore phenolic profile is related to the botanical origin of monofloral honeys, so it can be used as a robust tool for honey authentication. Consequently, the novel methodology developed in this study is robust and can be successfully applied for the authentication of honey botanical sources.
4. Conclusions

In this study, two chemometric models based on fluorescence spectra (λ\text{ex} = 330; 360 nm) and the LDA statistical method were developed to distinguish the botanical origins of four well-known and commercial honey varieties (thyme, pine, fir, and citrus). Chemometric models are considered successful. The first (λ\text{ex} = 330) was found to be more effective, providing a reliable score of 94.9% against the 81.4% of the second model (360 nm). Cross and external validations reinforced these results, verifying the high robustness of the chemometric models. Furthermore, the proposed chemometric models are non-time-consuming, economical, and do not alter the environmental fingerprint. The novel methodology based on right-angle fluorescence spectroscopy and the stepwise-LDA algorithm can be used for routine analyses in the industry for the differentiation of honey botanical origins, thereby enhancing the competitiveness of producers and suppliers in national and international markets.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/app11094047/s1, Table S1: The structure of the standard phenolic compounds. Table S2: Results of melissopalynological analysis. Figure S1: Emission spectra of caffeic acid; chlorogenic acid; p-coumaric acid; ferulic acid; sinapic acid and ellagic acid at λ\text{ex} 330. Emission spectra of homogentisic acid; gallic acid; protocatechuic acidid syringic acid; vanillic acid; methyl syringate; gentisic acid; keamferol and catechin at λ\text{ex} 300.

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