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Usefulness of Flavonoids and Phenolic Acids in Differentiating Honeys Based on Geographical Origin: The Case of Dominican Republic and Spanish Honeys

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Featured Application

To this point, there have been no targeted studies examining antioxidant compounds in Dominican honeys, making the present findings both novel and important for expanding scientific knowledge of honey of this origin. Moreover, putting the spotlight on the antioxidant value of Dominican honey could strengthen its appeal in the global marketplace and contribute to improving the socioeconomic well-being of rural areas, where apiculture is a sustainable activity with high growth potential.

Abstract

As a novel approach, polyfloral honey originating from the three regions of the Caribbean Island of the Dominican Republic (D.R.) was analyzed. Using the HPLC-DAD technique, 10 specific flavonoids (FLV) together with 9 phenolic acids (PHA) were identified and compared with Spanish polyflorals (commercial brands, artisanal beekeepers, and experimental apiaries). On average, the total content of FLV and PHA was much higher in Spanish (14.2 and 20.1 mg/kg) than in D.R. (10.8 and 4.5 mg/kg) honeys. Unlike in Dominican honeys, chrysin (in FLV) and vanillic acid (in PHA) had the greatest impact on Spanish honey, with the latter alone accounting for more than 50% of the quantified PHAs. Unsupervised Principal Component Analysis (PCA) showed that the information provided by both FLV and PHA allowed us to differentiate honeys according to their geographical origin, particularly at the country level. Furthermore, a stepwise discriminant-analysis identified the PHA ferulic acid followed by the FLVs apigenin-7-glucoside, chrysin, and naringenin as the most influential compounds for distinguishing among groups of honeys. The resulting model correctly classified 80.3% of the original and 71.2% of the cross-validated cases, indicating acceptable efficiency and robustness. These findings highlight the potential of the analyzed compounds for the geographical authentication of honey, providing the beekeeping sector with valuable tools for ensuring honey provenance.

Keywords: honey differentiation; country origin; antioxidant compounds

Academic Editors: Otilia Bobis, Daniel Severus Dezmirean and Claudia Pașca

Received: 21 September 2025
Revised: 10 October 2025
Accepted: 15 October 2025
Published: 18 October 2025

Citation: Ogando-Rivas, P.; Juan-Borrás, M.; Caja, G.; Escriche, I. Usefulness of Flavonoids and Phenolic Acids in Differentiating Honeys Based on Geographical Origin: The Case of Dominican Republic and Spanish Honeys. *Appl. Sci.* **2025**, *15*, 11181. https://doi.org/10.3390/app152011181

1. Introduction

Honey is primarily composed of sugars, such as fructose and glucose, and water. It also contains minor components, including organic acids, enzymes, antioxidants, amino acids, aromatic compounds, vitamins, and minerals [1,2]. Its composition may vary depending on the botanical origin of the nectar and plant secretions collected by bees, as well as by environmental conditions, harvesting season, and geographical location [3,4]. Pollen content has been widely used to classify honey according to its botanical and geographical origin [5,6]. However, this analysis remains a challenge for the beekeeping sector, as the traditional method—based on melissopalynological analysis via optical microscopy—is time-consuming, requires trained personnel, and is affected by subjectivity and interference, making it complex and costly [7]. Therefore, researchers continue to seek objective analytical techniques to identify honey origin [6,8,9]. The qualitative and quantitative information provided by certain minor plant secondary components—such as antioxidant compound profiles—has been proposed as a promising alternative for authenticating the botanical and geographical origin of honey, as reported by different authors [10,11].

Currently, antioxidants are highly valued in food due to their nutritional and therapeutic properties, particularly for their ability to delay or prevent oxidative processes and counteract the harmful effects of free radicals [12]. These substances play an essential role in health, as well as in the prevention and treatment of certain diseases [13,14]. High antioxidant intake is associated with a reduced risk of developing specific pathologies such as cancer, cardiovascular diseases, immune system disorders, cataracts, and various inflammatory conditions [15–17]. For this reason, its presence in food is often highlighted in marketing strategies. A good example of this is Manuka honey, which, due to its high antioxidant content, is one of the most expensive on the market [18,19].

Among the most important groups of compounds with antioxidant activity (enzymes, vitamins, carotenoids, and polyphenols), honey is particularly rich in flavonoids (FLVs) and phenolic acids (PHAs). Numerous studies have identified these compounds as the main contributors to the honey antioxidant capacity [8,12,20–26]. Considering FLVs present in honey, four main families with similar structures stand out: flavanols (e.g., epicatechin); flavanones (e.g., naringenin, hesperetin); flavones (e.g., apigenin-7-glucoside, chrysin); and flavonols (e.g., galangin, kaempferol, myricetin, quercetin, quercitrin, quercetin-3-glucoside, rutin) [13,24]. PHAs are molecules containing one or more hydroxyl group attached to an aromatic ring. Their antioxidant activity largely depends on the number and position of these hydroxyl groups [26]. Two PHA families are recognized: hydroxybenzoic acids (e.g., ellagic acid, gallic acid, 4-hydroxybenzoic acid, vanillic acid) and hydroxycinnamic acids (e.g., caffeic acid, ferulic acid, *p*-coumaric acid, sinapic acid, cinnamic acid, chlorogenic acid). Specifically, hydroxycinnamic acids are considered more effective in terms of antioxidant activity due to the presence of more than one carboxyl group and the spatial separation between the carbonyl group and the aromatic ring [27].

Many research efforts on honeys from various sources have focused on finding markers that can clearly indicate their geographical or botanical origin. In Greek honeys, for instance, chlorogenic acid has been recognized as a marker for chestnut honey, while homogentisic acid and 2-cis,4-trans-abscisic acid are specifically associated with arbutus (strawberry tree) honey [28]. Additionally, the combined presence of gallic acid and protocatechuic acid has been proposed as a distinctive marker for oak honeydew honey from this country. In *Triadica cochinchinensis* honey from China, gallocatechin gallate was identified as a reliable flavonoid marker for this type of honey [29]. In tropical Colombian honeys, *p*-coumaric, caffeic, and cinnamic acids, along with the flavones luteolin, quercetin, and isorhamnetin, have been identified as relevant chemical markers. These compounds were consistently detected across samples, regardless of their geographical origin or botanical source within the country [30].

The procedures used to measure antioxidant activity in food have undergone changes in recent years [14,31]. The spectrophotometric methods (ABTS, DPPH, FRAP, ORAC, etc.) have been widely used in honey for this purpose [32]. Although these are cost-effective (reducing the need for individual analytical standards) and do not require expensive equipment or highly trained personnel, they lack specificity. These spectrophotometric methods provide only a rough estimate of total phenolic content, without quantitatively measuring the individual compounds responsible for the antioxidant activity in the food [32]. Therefore, the latest guidelines from IUPAC (International Union of Pure and Applied Chemistry) and AOAC (Association of Official Agricultural Chemists) recommend avoiding total antioxidant activity measurements based on spectrophotometry [33]. Only targeted analysis of specific compounds enables the differentiation of beehive products according to type and geographical origin [33–37]. Chromatographic techniques, especially high-performance liquid chromatography (HPLC-UV), are the most widely used for the separation and quantification of the specific compounds of the phenolic profile found in hive products. Nevertheless, the use of this technique as a routine procedure is generally limited due to high operational costs [34].

Honey production has been developed worldwide as both an economic asset and a nutritional resource. Yet, in countries such as the Dominican Republic, commercial exploitation of this resource remains minimal, even though it holds significant potential for fostering sustainable livelihoods in rural areas. The country (located in the Antilles arc, between Cuba and Puerto Rico, and sharing the island of Hispaniola with Haiti) has favorable conditions for beekeeping, including a suitable climate, nectar-producing plants, and large forested areas. Its wild flora (near 70% forest) is characterized by conifer, broad-leaved, dry, and swamp forests, as well as high-elevation savannahs, wetlands, and arid zones. It is the second-largest country in the Greater Antilles and is considered one of the Caribbean islands with high biodiversity and endemism, due to the island's complex geological history and terrain [38]. Average annual rainfall is greater than 1300 mm, ranging from 2500 mm in the mountainous NE (windward side) to 500 mm in the SW valleys (rain shadow side).

Despite all this, in the D.R., the beekeeping sector remains in a relatively rudimentary state, characterized by low levels of technological development, with most apiaries relying on artisanal practices and tools. In recent years, annual honey production has decreased from 804 t in 2022 to 356 t in 2024 [39,40]. The average honey production per hive/year is approximately 20 kg/year.

Several studies have focused on the characterization of the antioxidant properties of European honeys [2,23,41,42]; however, scientific data on honeys from tropical regions remain scarce. Recent research has begun to shed light on the bioactive profiles and antioxidant potential of some tropical honeys from Brazil [43–46], Colombia [24,30], and Cuba [47,48], among others.

To our knowledge, honey from the D.R. remains unstudied in terms of the identity and content of antioxidant compounds. Advancing the understanding of this little-known type of honey could significantly enhance its potential value. Furthermore, such information could help to improve livelihoods by creating economic opportunities in rural areas, where beekeeping may serve as a sustainable source of income. Along these lines, this study considers the Dominican Republic and Spanish types of honey to evaluate the influence of their geographical origin on the profile of specific flavonoids and phenolic acids.

2. Materials and Methods

2.1. Honey Samples

For this study, a total of 131 polyfloral honey samples from different origins (collected throughout 2023 and 2024 and stored at room temperature in dark conditions), were used.

A group of 72 samples came from artisanal beekeepers from the three different political regions of the D.R. (and different provinces in each region): North region, n = 24 (Monseñor Nouel, Santiago, Monte Cristi, Dajabón, Santiago Rodríguez, and Puerto Plata); South region, n = 26 (Peravia, San Cristóbal, Elías Piña, Azua, and Bahoruco); and East region, n = 22 (Monte Plata, San Pedro de Macorís, Santo Domingo, and Hato Mayor). The location of the different provinces in each region and the sample collection points is shown in Figure 1a. This country has a rich diversity of vegetation and agriculture in these three regions, characterized by dominant crops and native wild species. The North region is characterized by the most fertile soils in the country and supports humid and tropical forest formations dominated by palms, ferns, mahogany, and guano palms, as well as plantations of coffee, maize, cacao, and plantain. The South region presents an arid-to-semi-arid climate, with lower rainfall and higher temperatures, where thorny shrubs, cactus, bayahonda trees, and crops such as plantain, mango, grape, and coffee predominate. The East region, with its warm and humid climate, is distinguished by palms, coconut trees, natural savannas, and extensive sugarcane cultivation (historically the country's largest sugar-producing area), along with coconut and cacao crops.

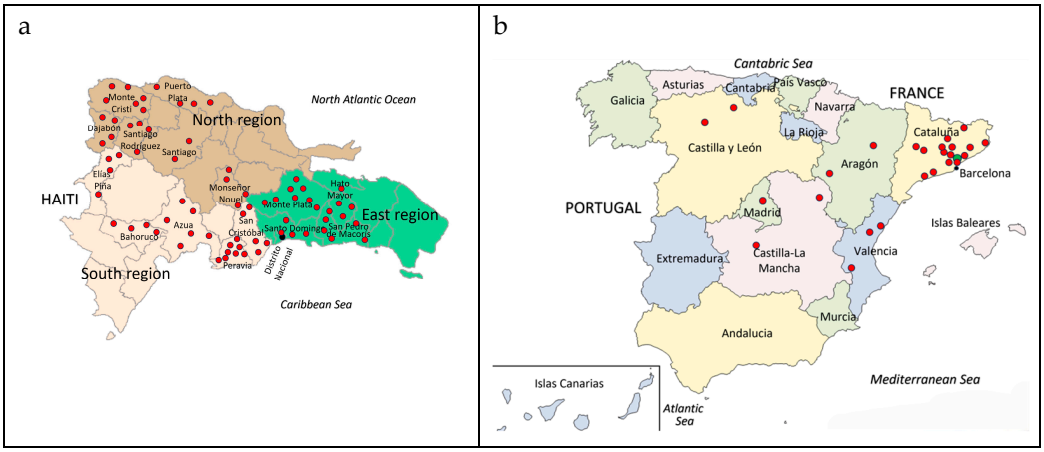

Figure 1. Origin of honey samples. (**a**) Map of the Dominican Republic showing the regions and provinces where artisanal honey samples were collected (red dots). Source: Adapted from www.geografiabasicarepublicadominicana.edu.do (accessed on 5 September 2025). (**b**) Map of Spain showing the provinces where the artisanal (red dots) and experimental samples (green dot) were collected. Source: the image obtained is free from copyright. In the figures, the colors represent the different regions in each country.

Another group of 59 samples came from different origins from Spain (commercial brands, n = 27; artisanal beekeepers, n = 25; isolated experimental apiary, n = 7). Honey samples from Spain were also polyfloral and came from different origins. The group of commercial brands consisted of samples randomly bought from different retailers throughout Spain (local origin not specified) and labelled as Spanish honey since they contained more than 50% from Spain in line with the EU legislation. The artisanal beekeepers' honey samples were obtained in Catalonia from beekeepers registered with the ASAB (Associació d'Apicultors/Beekeepers Association, Barcelona, Spain) and having their apiaries mainly located in the Barcelona province of the Catalonia region, in the northeast of Spain. This area is characterized by a Mediterranean climate (600 mm annual rainfall) and supports dry land cultures and maquis and xeric forest flora (e.g., thyme, rosemary, heather, arbutus, pines, and oaks). Some of the artisanal beekeepers (n = 10) make transhumance during summer or collected their honey samples from their original locations outside Catalonia (Aragón, Castilla-La Mancha, Castilla y León, Madrid, and Valencia regions). Finally, the remaining seven samples were collected from the experimental apiary (UABee) of the

Universitat Autònoma de Barcelona (UAB), located at the university campus in Bellaterra (Cerdanyola, Vallès Occidental County, Barcelona Province) under fully controlled conditions. The campus has Mediterranean forests and maquis floral conditions, cultivated dry forages, and irrigated gardens with large areas of aromatic plants. The UABee hives are stationary and are managed as fixed under semi-intensive conditions and harvested in mid-spring and -summer, receiving supplementary feeding during winter [49,50]. The origin of the Spanish artisanal and experimental honey samples is shown in Figure 1b.

All samples were sent and analyzed at the Universitat Politècnica de València (Polytechnic University of Valencia) in the Laboratory of Quality Control of Honey (LABMIEL, Valencia, Spain), where they were also stored at room temperature and in the dark until analysis.

2.2. Analysis of Phenolic Compounds by Liquid Chromatography (HPLC)

2.2.1. Standards and Reagents

A total of 19 analytical standards with a purity above 99% (Sigma-Aldrich, St. Louis, MO, USA) were used: apigenin-7-glucoside, caffeic acid, chrysin, epicatechin, ellagic acid, ferulic acid, galangin, gallic acid, 4-hydroxybenzoic acid, kaempferol, naringenin, *p*-coumaric acid, quercetin, quercitrin, quercetin-3-glucoside, sinapic acid, cinnamic acid, vanillic acid, and rutin. Table 1 shows the list of the 19 compounds ordered by retention time, along with their corresponding wavelengths (λ) of maximum absorbance. These wavelengths were used for the subsequent quantification of each compound. To quantify the concentration of each compound, the corresponding calibration curves were constructed. The R^2 values of the linear fits are also presented in this Table 1, together with the limit of quantification (LOQ) for each compound. Good linearity was observed in the method, with R^2 values ranging from 0.991 to 0.999.

Table 1. Retention time (RT), wavelengths of maximum absorbance (λ), linear fits R^2, and limit of quantification (LOQ) for each phenolic compound detected.

Phenolic Compounds	RT (min)	λ (nm)	R^2	LOQ (mg/kg)
Flavonoids (FLV)				
Epicatechin	13.036	280	0.992	0.015
Rutin	15.400	260	0.999	0.015
Quercitin-3-glucoside	16.416	260	0.992	0.015
Quercitrin	18.032	260	0.992	0.015
Apigenin-7-glucoside	18.192	320	0.994	0.015
Quercetin	23.429	380	0.996	0.015
Naringenin	26.147	290	0.992	0.015
Kaempferol	27.204	380	0.995	0.015
Chrysin	33.953	250	0.992	0.030
Galangin	34.605	250	0.997	0.015
Phenolic acids (PHA)				
Gallic acid	06.751	280	0.996	0.015
4-Hydroxybenzoic acid	12.849	260	0.997	0.015
Caffeic acid	13.606	320	0.992	0.015
Vanillic acid	13.663	250	0.992	0.015
p-Coumaric acid	16.803	320	0.991	0.015
Ellagic acid	16.804	250	0.993	0.015
Sinapic acid	17.419	320	0.994	0.015
Ferulic acid	17.625	320	0.992	0.015
Cinnamic acid	24.679	280	0.991	0.015

For each of these compounds, working standard solutions were prepared by diluting an appropriate amount of the stock solution (1000 mg/L in MeOH) to obtain seven calibration levels with the following final concentrations: 0.5, 1, 3, 6, 10, 20, and 40 mg/L (ppm). All dilutions, including stock and working solutions, were stored at −20 °C until they were used. The solvents (HPLC grade) used for the analysis were as follows: acetonitrile (ACN), methanol (MeOH), and formic acid, purchased from VWR (Gliwice, Poland). Throughout the extraction process, bidistilled water from a Milli-Q system was used.

2.2.2. Extraction

Phenolic compounds were extracted from honey samples using the method described by Bertoncelj et al. [51]. Briefly, in each analysis, 10 g of honey and 15 mL of water (pH 2 acidified with concentrated HCl) were mixed until complete dissolution. The resulting solution was filtered through a 24 mm diameter glass fiber filter (No. 693, VWR, France) to remove any insoluble solids present in the sample. To eliminate interfering compounds prior to chromatographic analysis, this solution was further passed through solid-phase extraction (SPE) using Strata-X 33 µm Polymeric Reversed Phase cartridges (200 mg/3 mL, Phenomenex, Torrance, CA, USA). Cartridges were preconditioned with 3 mL of MeOH followed by 3 mL of Milli-Q water. After loading the sample, cartridges were washed with 5 mL of pH 2 water and 15 mL of Milli-Q water, then dried under vacuum for 2 min. Phenolic compounds were eluted using 3 mL of MeOH/ACN (2:1) mixture. Prior to chromatographic injection, 1 mL of the eluate was filtered using a 0.45 µm nylon membrane filter. To verify the stability of the method, a standard solution was injected at the start of each analytical session. All analyses were conducted in triplicate, using a new sample for each replicate. Results were reported as mg/kg of honey.

2.2.3. Chromatographic Conditions

The analysis of the extracts was carried out using an Agilent Technologies 1200 Series liquid chromatograph, equipped with a degasser, a binary pump, an autosampler, and a thermostatted column compartment, coupled to a diode array detector (DAD). Compound separation was achieved using a Kinetex C18 column (250 × 4.6 mm, 5 µm particle size, 100 Å; Phenomenex, Torrance, CA, USA). The column temperature was set at 30 °C, the flow rate was 0.5 mL/min, and the injection volume 10 µL.

The mobile phase consisted of solvent A (water with 1% formic acid) and solvent B (ACN, 50% B); 33 min (30% A, 70% B); 40 min (10% A, 90% B); 43 min (90% A, 10% B); 45 min (90% A, 10% B).

Data acquisition and processing were performed using MassHunter Workstation Software version B.09.00. Chromatograms were recorded at different wavelengths between 200 and 400 nm. Flavonoids and phenolic acids were identified by comparing retention times and UV spectral characteristics with those of authentic standards and those published in the literature [52].

2.3. Statistical Analysis

A one-way analysis of variance (ANOVA) was performed using Statgraphics Centurion XIX for Windows to assess the statistical significance of the differences found in the concentrations of FLV and PHA, considering the following factors: country (the D.R. and Spain), regions of the D.R. (North, South, and East), and Spain origins (commercial, artisanal, and experimental) where the polyfloral honey samples were collected. The least significant difference (LSD) test was applied for mean comparison, with a significance level of $\alpha = 0.05$. Additionally, a Principal Component Analysis (PCA) was conducted using XLSTAT 2020 3.1 [53] to explore the relationships between the quantified compounds and origin. To identify the most discriminant variables, a stepwise linear discriminant analysis

(SLDA) was performed using the forward method in PASW Statistics 18. Classification functions for each group were obtained, and the predictive ability of the discriminant model was evaluated using leave-one-out cross-validation.

3. Results and Discussion

3.1. Identification and Quantification of Phenolic Compounds

The quantitative results of the compounds identified in the samples, expressed as average values (mg of compound per kg of honey) with the corresponding standard deviation, are shown in Table 2. It also includes the ANOVA results for each factor (with homogeneous groups, F-ratio, and significant level). The 10 FLV and 9 PHA compounds were grouped into four families: FLV [flavanols (epicatechin), flavanones (naringenin), flavones (apigenin-7-glucoside and chrysin), and flavonols (galangin, kaempferol, quercetin, quercitrin, quercetin-3-glucoside, and rutin)] and PHA [hydroxybenzoic acids (ellagic acid, gallic acid, 4-hydroxybenzoic acid, and vanillic acid) and hydroxycinnamic acids (caffeic acid, ferulic acid, *p*-coumaric acid, sinapic acid, and cinnamic acid)]. Mean values were higher in Spain than in the D.R. for both FLV and PHA. However, three compounds in each group did not show significant differences between countries: FLV (epicatechin, galangin, and quercetin-3 glucoside) and PHA (4-hydroxybenzoic acid, ferulic acid, and cinnamic acid).

Despite not differing between countries, epicatechin was the most abundant FLV in both the D.R. and in Spain, and its concentration significantly varied among types of samples inside each country. That is, epicatechin mean values ranged from 1.6 mg/kg to 5.3 mg/kg from the East to the South of the D.R., and from 0.63 to 4.2 mg/kg from the experimental to the commercial origins in Spain. This may be a consequence of the floral origin of the honeys, as many flowers of tropical plants are recognized for their high epicatechin content, such as tea (*Camellia sinensis*), cocoa (*Theobroma cacao*), coffee (*Coffea* spp.), or the yellow flamboyant tree (*Peltophorum pterocarpum*). In these plants, epicatechin contributes to defensive properties against pests, with its concentration being influenced by plant stress and flowering stages [54]. The labelling of Spanish commercial honeys only warranted that 50% of the honey come from Spain, the rest being from foreign countries which may explain the difference.

On the contrary, the FLV chrysin (usually detected in propolis) was very high in the Spanish honeys, with values ranging from 1.4 to 4.8 mg/kg in commercial to experimental samples, respectively, and with significant differences according to their origin. Plants from Geranium spp. and Pelargonium spp. genders are rich in chrysin and are common in Mediterranean gardens, especially those located around the UABee experimental apiary at the Universitat Autònoma de Barcelona. Very high values of chrysin (12.2 mg/kg), greater than those found in our study, were reported in Spanish honeys from the Andalucian region (Granada province) [55] where the previously described plants are very popular. Additionally, chrysin has also been found in different concentrations in Italian (3.9 mg/kg), Portuguese (1.1 mg/kg), and Spanish (0.824 mg/kg) honeys [20]. In Greek honeys, the content of chrysin differed according to their botanical origin [42]: blossom honey (3.6 mg/kg), oak honey (0.72 mg/kg), pine honeydew (0.65 mg/kg), fir honeydew (0.35 mg/kg), and thyme honey (0.061 mg/kg). In contrast, this compound was not detected in the D.R. samples, suggesting that the botanical specimen of origin was not common in the Caribbean flora and that it can be used for differentiation of origin.

Table 2. Mean (and standard deviation) of flavonoids (FLV) and phenolic acids (PHA) quantified (mg/kg of honey) in polyfloral honey samples from different regions of the Dominican Republic (North, South, and East) and different origins from Spain (commercial, artisanal, and experimental). The ANOVA F-ratio is given for the "country" and "origin" factors for every country.

	Country			Dominican Republic (D.R.) Regions				Spain Origins			
	D. R. Mean (SD)	**Spain Mean (SD)**	**Anova F-Ratio**	**North**	**South**	**East**	**Anova F-Ratio**	**Commercial**	**Artisanal**	**Experimental**	**Anova F-Ratio**
FLV											
Flavanols											
Epicatechin	3.2 (3.7)	3.1 (6.1)	ns	2.3 (2.7) [a]	5.3 (4.9) [b]	1.6 (0.4) [a]	8 ***	4.2 (7.7) [b]	2.6 (4.6) [b]	0.63 (0.16) [a]	1 **
Flavanones											
Naringenin	0.17 (0.05) [b]	0.10(0.13) [a]	20 ***	0.20 (0.07) [b]	0.16 (0.08) [a]	0.16 (0.10) [a]	6 **	0.07 (0.16)	0.11 (0.11)	0.14 (0.03)	ns
Flavones											
Apigenin-7-glucoside	0.4 (0.8) [b]	0.011 (0.034) [a]	16 **	0.6 (0.9)	0.15 (0.10)	0.6 (0.9)	ns	0.002 (0.005)	0.002 (0.04)	<LOQ	2 *
Chrysin	<LOQ	2.3 (1.6)	139 ***	<LOQ	<LOQ	<LOQ	ns	1.4 (1.0) [a]	2.8 (1.6) [b]	4.8 (0.33) [c]	20 ***
Flavonols											
Galangin	<LOQ	1.01 (0.51)	ns	<LOQ	<LOQ	<LOQ	ns	0.8 (0.4) [a]	1.1 (0.5) [b]	1.8 (0.19) [c]	17 ***
Kaempferol	0.17 (0.02) [a]	0.4 (0.3) [b]	48 ***	0.17 (0.03)	0.16 (0.01)	0.17 (0.01)	ns	0.3 (0.2) [a]	0.5 (0.4) [b]	0.338 (0.012) [a]	3 *
Quercetin	0.3 (0.11) [a]	0.7 (0.19) [b]	216 ***	0.31 (0.15) [b]	0.34 (0.01) [b]	0.21 (0.04) [a]	10 ***	0.70 (0.19) [b]	0.7 (0.2) [b]	0.53(0.06) [a]	3 *
Quercitrin	0.8 (0.8) [b]	0.5 (0.5) [a]	5 *	0.8 (0.7)	0.8 (0.6)	0.9 (1.6)	ns	0.3 (0.5)	0.6 (0.6)	0.65 (0.13)	ns
Quercitin-3-glucoside	1.1 (4.3)	1.0 (2.8)	ns	2.4 (7.3)	0.3 (0.3)	0.5 (0.4)	ns	0.3 (0.2) [b]	2 (4) [a]	0.10 (0.03) [b]	3 *
Rutin	1.1 (1.0)	<LOQ	63 ***	1.04 (1.14) [b]	1.9 (0.9) [c]	0.35 (0.67) [a]	16 ***	<LOQ	<LOQ	<LOQ	
Σ average FLV	**10.8**	**14.2**		**7.8**	**8.8**	**4.5**		**8.1**	**10.4**	**9.0**	
PHA											
Hydroxybenzoic acids											
Ellagic acid	<LOQ	4.0 (4.1)	69 ***	<LOQ	<LOQ	<LOQ	ns	3.3 (1.6)	4.6. (6.0)	5.1 (1.3)	ns
Gallic acid	0.3 (0.3)	0.04 (0.12)	27 ***	0.4 (0.6) [b]	0.24(0.15) [a,b]	0.18 (0.06) [a]	3 *	0.05 (0.15)	0.03 (0.12)	<LOQ	ns
4-Hydroxybenzoic acid	2.0 (1.2)	2.4 (1.7)	ns	1.9 (1.1)	2.2 (1.4)	1.7 (1.2)	ns	2.5 (2.6)	2.3 (0.7)	1.9 (0.4)	ns
Vanillic acid	0.6 (0.5)	12.0 (11.0)	74 ***	0.6 (0.3) [a,b]	0.8 (0.3) [b]	0.4 (0.2) [a]	4 *	6.2 (4.4) [a]	14.7 (13.3) [b]	27.0 (2.3) [c]	15 ***
Hydroxycinnamic acids											
Caffeic acid	0.4 (0.4) [a]	1.8 (1.8) [b]	39 ***	0.4 (0.4)	0.5(0.5)	0.4 (0.4)	ns	0.8 (0.7) [a]	2.1 (1.9) [b]	4.8 (0.5) [c]	24 ***
Ferulic acid	0.4 (0.6)	0.5 (0.3)	ns	0.5 (0.7)	0.22 (0.07)	0.5 (0.7)	ns	0.39 (0.24)	0.5 (0.4)	0.60 (0.04)	ns
p-Coumaric acid	0.3 (0.2)	2.6 (0.45)	18 ***	0.30 (0.24)	0.27 (0.11)	0.27 (0.02)	ns	1.5 (1.1)	3.7 (6.6)	2.5 (0.3)	ns
Sinapic acid	0.20 (0.11)	0.031 (0.13)	64 ***	0.15 (0.01) [a]	0.26 (0.13) [b]	0.19 (0.11) [a]	8 ***	0.006 (0.021)	0.07 (0.21)	<LOQ	ns
Cinnamic acid	0.3 (1.1)	0.3 (0.7)	ns	0.156 (0.014)	0.5 (1.8)	0.16 (0.02)	ns	0.25 (0.75)	0.4 (0.9)	0.11 (0.04)	ns
Σ average PHA	**4.5**	**20.1**		**4.4**	**4.7**	**3.8**		**15.0**	**28.3**	**42.0**	

Different letters in the same row indicate significant differences at the 95% confidence level as obtained by the LSD test. ns: Not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. LOQ: Limit of quantification (0.001 mg/kg).

In the case of the flavonol rutin, the opposite trend was observed. It was detected in all samples from the D.R., ranging from 0.35 to 1.9 mg/kg, but was not present in any of the Spanish samples. Under tropical conditions comparable to those in the D.R., rutin has also been quantified in honeys from other countries, specifically, in Brazilian orange blossom honeys (3.89 mg/kg) [43] and in Colombian honeys produced under both conventional (0.19 mg/kg) and organic (0.17 mg/kg) management [30].

In contrast, the flavonol galangin was absent in the D.R. samples, but was detected in Spanish honeys, with concentrations ranging from 0.8 to 1.8 mg/kg in commercial and experimental samples, respectively. These values were lower than those reported in the honey from Granada [43] where the average value of this compound was 5.41 mg/kg.

Among other analyzed FLVs, it is worth highlighting that the flavone apigenin-7-glucoside was found in samples from both countries; however, significant differences were observed, with average concentrations of 0.40 mg/kg in D.R. honeys and lower levels of 0.011 mg/kg in Spanish honeys.

Considering all the antioxidant compounds analyzed, PHAs were the most abundant in Spanish samples. Notably, vanillic acid showed the highest concentration in this country, with an average of 12 mg/kg. This compound alone represents over 50% of the total PHAs present in the samples from Spain, showing the highest content in the experimental honey samples (27 mg/kg), followed by the artisanal (14.7 mg/kg) and commercial (6.2 mg/kg) groups. This compound was also identified by Cheung et al. [56] in commercial honey samples purchased in Chinese supermarkets, originating from various countries, including Spain. In that analysis, vanillic acid was detected exclusively in Spanish thyme honeys (with a predominance of *Thymus vulgaris* pollen), with a concentration of 5.41 mg/kg. The authors suggested that this compound could be considered a relevant chemical marker for the authentication of Spanish thyme honey. Moreover, vanillic acid is also present in extracts of other plants from the *Lamiaceae* family (e.g., *Origanum vulgare*, *Salvia rosmarinus*, *Salvia officinalis*) also common in Spain. These findings suggest that it is possible that the Spanish honeys in the present study, particularly the experimental ones, contained a high percentage of *Lamiaceae*'s nectar, even if not enough for a monofloral classification (required >10% of thyme pollen) [6]. We confirmed the abundance of *Lamiaceae* spp. in the flora around the UABee apiary which accounted for 1 to 10% of the pollen identified in the UABee's honeys (G. Caja, unpublished data).

Hydroxybenzoic acid was the predominant PHA in the D.R. honeys (average of 2.0 mg/kg) and one of the most abundant in the Spanish honeys (2.4 mg/kg). No significant differences were observed between countries or among regions or origins within the same country. Among the hydroxycinnamic acids, *p*-coumaric (average, 2.6 mg/kg) and caffeic acids (1.8 mg/kg) stood out in the Spanish honeys, but not in those from the D.R. (with averages values of 0.3 and 0.4 mg/kg, respectively).

According to the ANOVA results (Table 2), the highest F-ratios for the country factor were observed for quercetin, chrysin, and ellagic acid, suggesting that these compounds may be key discriminators between honeys from the two countries.

After analyzing the individual effect of the factors on each PHA and FLV, a Principal Component Analysis (PCA) was conducted to evaluate the overall relationship between all of the compounds and the origin of the honeys from Spain and the D.R. This approach reduced the complexity of the dataset by creating new dimensions (principal components). In addition, it helped to identify the variables that best explain the general variability within the dataset. The first four principal components explain over 65% of the data variability. Figure 2 shows the biplot resulting from this analysis, representing the first two principal components, which account for 30.01% and 12.94% of the total variability, respectively.

The plot of Figure 2 reflects both the origin of the samples from the two countries and the associated compounds. A clear separation of the samples by country is evident, with all Spanish samples positioned in the right quadrant, and those from the D.R. in the left. The greater contribution of the variables to this distribution were the FLVs galangin and chrysin and the PHAs vanillic and caffeic acid in the positive first component, mainly associated with the Spanish samples, and the FLV rutin in the negative component, most strongly associated with the D.R. samples.

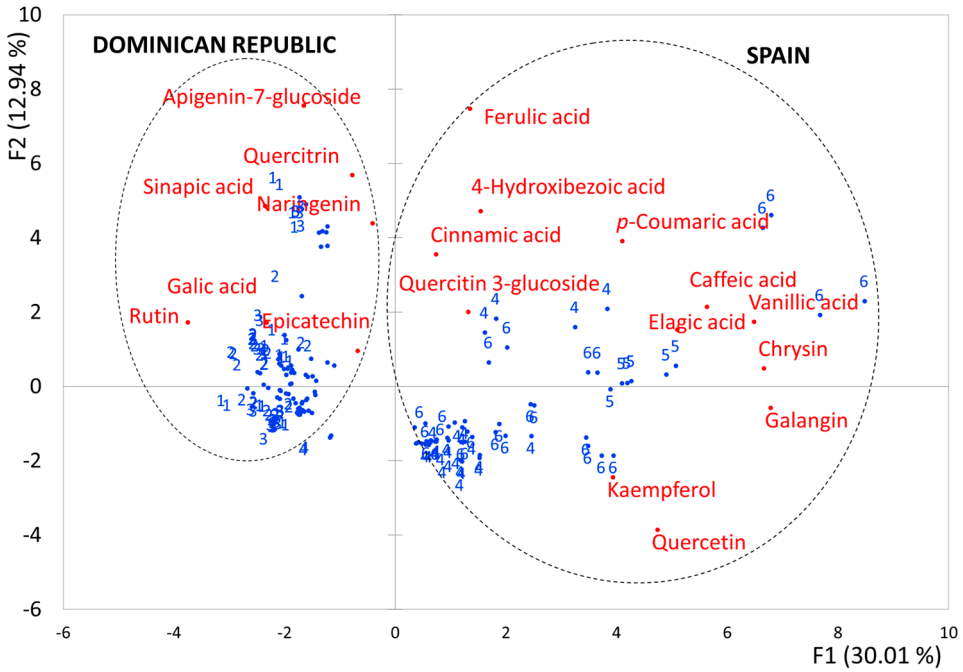

Figure 2. PCA biplot of score [samples of polyfloral honey from different regions of Dominican Republic North (1, n = 24), South (2, n = 26), and East (3, n = 22) and Spain: commercial brand (4, n = 27), experimental apiary (5, n = 7), and artisanal beekeeper (6, n = 25)] and loading (specific flavonoids and phenolic compounds).

3.2. Identification of the Variables with the Highest Discriminant Power

The results obtained from both ANOVA and PCA analyses indicate that certain variables play a more significant role in differentiating honey samples. Therefore, a linear discriminant analysis (LDA) was performed to classify honey samples by origin and to examine their distribution based on their PHA and FLV compounds. The analysis also sought to identify which compounds contribute most significantly to group differentiation. The independent variables were the phenolic compounds, while the grouping factor consisted of the six honey origins: three from the Dominican Republic and three from Spain. The first three discriminant functions were statistically significant, with Wilks' Lambda (λ) values below 0.3: F1 (λ = 0.005, *p*-value = 0.0000); F2 (λ = 0.099, *p*-value = 0.0000); F3 (λ = 0.290, *p*-value = 0.0000). These functions explain 83.8%, 8.8%, and 4.1% of the total variability, respectively, accounting for 96.7% overall. Figure 3 presents the distribution of sample origins in the space defined by the first two discriminant functions. F1 (with 83.8% of the variability of the data) clearly differentiates Spanish groups (SP: placed in the left quadrant) from those that belong to DR (placed in the right quadrant), whereas Function 2 (8.8% of the variability of the data) separates the group of SP experimental samples from the rest.

Table 3 presents the standardized canonical discriminant function coefficients derived from the model. Variables with higher absolute coefficients have greater discriminative influence. The first canonical function provided the greatest discrimination between honey

groups, as it accounted for the largest portion of the total variability. The variables contributing most significantly to the functions were ferulic acid, apigenin-7-glucoside, chrysin, and naringenin, associated with Function 1, whereas caffeic acid and quercitin 3-glucoside were the main contributors to Function 2.

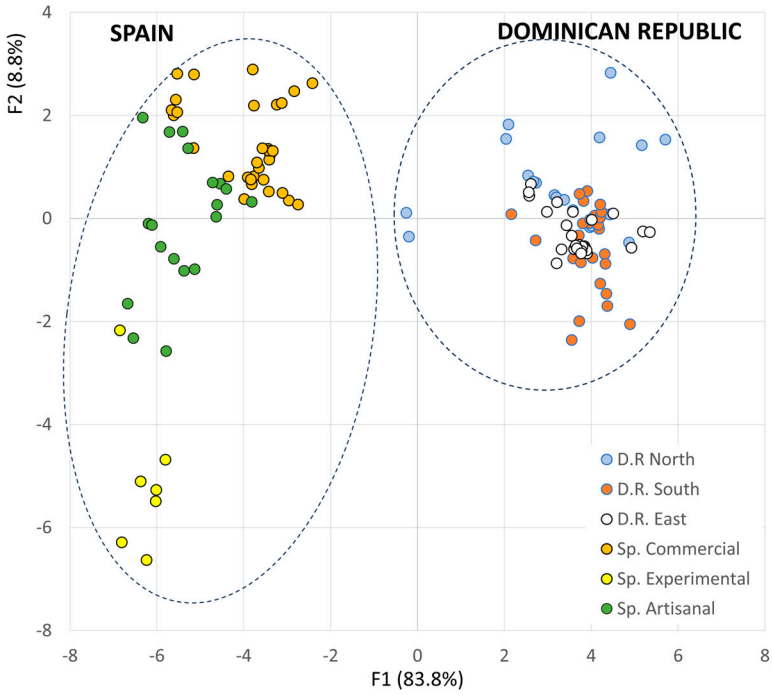

Figure 3. Discriminant function score plot from the LDA model constructed to classify the six honey groups by origin, showing Function 1 versus Function 2.

Table 3. Standardized canonical discriminant function coefficients.

	Function 1 83.8%	**Function 2 8.8%**
Ferulic acid	−1.623	0.494
Apigenin-7-glucoside	1.428	−0.027
Chrysin	−1.124	0.919
Naringenin	1.121	0.451
Quercetin	−0.697	0.725
Caffeic acid	0.664	−1.892
Quercitin 3-glucoside	0.649	1.047
Sinapic acid	0.509	−0.254
Galangin	−0.469	−0.570
Galic acid	−0.467	−0.669
Rutin	0.163	0.054
4-Hydroxibezoic acid	0.052	0.205

Table 4 shows the classification results of the discriminant analysis carried out with the original samples (using all variables to estimate the classification model) and by a cross-validated procedure (leave-one-out) to estimate its robustness. The number of samples correctly classified was 80.3% of the original grouped cases and 71.2% through cross-validation. These satisfactory percentages demonstrate the effectiveness of the model in discriminating among the types of honey considered in the present study. Spanish—Experimental apiary honey samples were the best classified with 100% of the cases, showing

complete differentiation from the other samples, which demonstrates high specificity in FLV and PHA of the honeys of this type of origin.

Table 4. Classification results of the discriminant analysis carried out by original and cross-validated procedure. Percentage of samples well classified by the model.

		Predicted Group Membership						
Country Group	Subgroup	Dominican Republic (D.R.)			Spain			Total
		North	South	East	Commercial	Artisanal	Experimental	
		Original %						
D.R.	North	**54.2**	8.3	37.5	0.0	0.0	0.0	100.0
	South	15.4	**84.6**	0.0	0.0	0.0	0.0	100.0
	East	0.0	9.1	**90.9**	0.0	0.0	0.0	100.0
Spain	Commercial brands	7.1	0.0	0.0	**92.9**	0.0	0.0	100.0
	Artisanal beekeepers	0.0	0.0	0.0	23.1	**73.1**	3.8	100.0
	Experimental apiary	0.0	0.0	0.0	0.0	0.0	**100.0**	100.0
		Cross-Validation %						
D.R.	North	**37.5**	16.7	45.8	0.0	0.0	0.0	100.0
	South	15.4	**84.6**	0.0	0.0	0.0	0.0	100.0
	East	18.2	9.1	**72.7**	0.0	0.0	0.0	100.0
Spain	Commercial brands	7.1	0.0	0.0	**89.3**	0.0	3.6	100.0
	Artisanal beekeepers	0.0	0.0	0.0	26.9	**61.5**	11.5	100.0
	Experimental apiary	0.0	0.0	0.0	0.0	0.0	**100.0**	100.0

In all of the groups, misclassification was limited to samples from the same country, which is logical given their botanical similarity. The exception was the Spanish–Commercial brand group, in which 7.1% of the samples were misclassified as Dominican honeys. This result is unsurprising, as commercial polyfloral honeys often contain mixtures of honey from different countries which can result in antioxidant properties differing from those of honeys collected within a single country [25,56]. Specifically, the presence of tropical South American honeys is highly plausible, since between 2023 and 2024 (the period during which the samples in this study were collected) Spain annually imported almost 6000 t of honey from South American countries. Furthermore, some of the honey may have been acquired from other EU countries (e.g., Portugal), which could themselves have sourced it from a tropical region [57].

For the remaining groups, as mentioned before, the misclassifications occurred between groups from the same country. For instance, Spanish—Artisanal honeys (61.5% correctly classified) were misclassified as Spanish—Commercial in 26.9% of cases and as Spanish—Experimental in 11.5%. Regarding the honeys from the Dominican Republic, only 37.5% of the honeys from the North were correctly classified, while errors occurred with those from the South (16.7%) and the East (45.8). This is to be expected, as the division between the Northern and Eastern regions is political rather than geographical and biological. These adjacent regions share semi-humid climatic conditions and important melliferous resources for local beekeeping, particularly citrus trees, orange, lemon, and grapefruit (*Citrus* spp.), as well as coffee (*Coffea arabica*) [38,58].

Similarly, 84.6% of honeys from the South were correctly classified, while 15.4% were misclassified as being from the North. This could also be explained by the fact that some of the honey production in both regions originates from predominantly arid areas. These areas are dominated by subtropical dry forests, with the presence of trees such as cambrón

(*Prosopis juliflora*) and guayacán (*Guaiacum officinale*), as well as cultivated species like coffee (*Coffea arabica*) [38,58].

Honeys from the Eastern region of the D.R. were correctly classified in 72.7% of cases, while 18.2% were misclassified as originating from the North and 9.1% from the South. The possible confusion between honeys from the South and the East lies in the fact that both regions share adjacent areas with the same humid-dry climate and, therefore, similar vegetation. Common melliferous species in these areas include coconut (*Cocos nucifera*), sugarcane (*Saccharum officinarum*), and palm (*Roystonea hispaniolana*), among others [38,58].

4. Conclusions

This study has demonstrated that the analysis of specific flavonoids and phenolic acids is a valuable tool for differentiating the geographical origin of honeys. Particular attention should be given to the flavonoid rutin, identified as a characteristic compound of Dominican honeys, and to chrysin, predominantly found in Spanish honeys. These compounds are associated with the distinctive flora of Caribbean and Mediterranean ecosystems, respectively. To date, no specific studies have been conducted on the antioxidant compounds of Dominican honeys, making these findings a novel and significant contribution to the scientific understanding of this type of honey. Moreover, emphasizing the antioxidant value of Dominican honey could enhance its competitiveness in the international market and support the socioeconomic development of rural communities, where beekeeping is a sustainable activity with considerable growth potential. To date, the findings have been very promising; nevertheless, a broader sampling from different countries would be required to conclusively state that the method proposed here is applicable to differentiate other geographical origins.

Author Contributions: Conceptualization, I.E.; methodology, M.J.-B.; formal analysis, P.O.-R. and M.J.-B.; investigation and data curation, P.O.-R. and M.J.-B.; writing—original draft preparation, P.O.-R. and M.J.-B.; writing—review and editing, I.E. and G.C.; supervision, I.E. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Next Generation European Union and the Plan de Recuperación, Transformación y Resiliencia of the Spanish Government, with the support of Generalitat Valenciana (project AGROALNEXT/2022/043). The authors also wish to thank the Spanish MAPA (Ministerio de Agricultura Pesca y Alimentación) for the research contract titled "Estudio De Caracterización De Las Principales Mieles Monoflorales Españolas" entered into in collaboration with the Laboratory of Honey Quality Control (LABMIEL) of the Universitat Politecnica de Valencia (UPV). Honey sample sharing with the UABee Interdisciplinary Group (E. Rojas and A. El Hadi) is specially recognized. Paola T. Ogando Rivas acknowledges the Ministerio de Educación Superior, Ciencia y Tecnología (MESCYT) of the Dominican Republic for the scholarship awarded.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed at the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

D.R.	Dominican Republic
FLV	Flavonoids
PHA	Phenolic Acids

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