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Ultraviolet-Visible Spectroscopy and Chemometrics Analysis of Clover, Citrus and Sugar Feeding Honey.

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Abstract

This work evaluate fast and accurate analytical method model developed for the authentication of honey adulteration by sugar-feeding syrup using UV-VIS (Ultraviolet-visible) spectroscopy in absorbance mode together with chemometric techniques of principal component analysis (PCA) and canonical discrimination analysis, thirty-five honey samples from three types of honey (18 samples of clover honey,14 samples of citrus honey and 3 samples of sugar-feeding honey samples), were measured by a spectrophotometer with recorded wavelength range from 200 to 900 nm, in two different extract solution (water and ethanol), the method of PCA was used to lower the number of inputs (wavelengths), then, the principal components were used with canonical discrimination analysis on the following wavelength ranges individually: UV (200-400nm), VIS (400-900nm) and UV-VIS (200-900nm) for water and ethanolic extract honey samples. The results of the experiment clearly support for the UV-VIS spectral wavelengths with Chemometrics Analysis can be reduced at large spacing interval, which allows easing data analysis as well as developing a simpler and cheaper sensor for honey discrimination in practice. PCA and canonical discrimination were successfully able to present a preliminary clustering pattern to segregate the feeding sugar syrup honey samples from the clover and citrus honey sample in UV and UV-VIS region spectroscopy by water extract.

Keywords: UV-VIS spectroscopy, honey, adulteration, discrimination, PCA.

1. Introduction

Honey as a sweet honeybee product made from the nectar of flowers or from secretions of living parts of plants or excretions of plant sucking insects on the plants, which is collected by bees, transformed by combining with specific substances of their bodies, deposited, dehydrated, stored and left to mature in honeycombs [1,2]. Honey contains water, sugars, amino acids, minerals and specific enzymes produced by bees [3].

Natural honey production is a laborious time-consuming and expensive process. For this reason, oftentimes honey is the subject to falsification by artificially adding sugar and other impurities. In addition, since some types of honey are more expensive than others, in order to prevent fraud in the labeling, a methodology should be elaborated to differentiate between the various honey type [4] adulteration techniques of honey are based on various principles including extension as well as bee feeding with sugar and/or syrups, and mixing with honey originating from different floral or geographical origins [5].

A couple of methods have been devised to determine of the honey floral origin, among which the most popular ones are pollen recognition and sensory analysis. Nevertheless, the method of honey pollen content analysis is time- consuming and suffers certain restrictions. The rest of the methods mainly involve analysis of the honey's sugar profile, aroma compounds, flavonoid pattern, non-flavonoid phenolics, isotopic relations, organic acids, and protein and amino acid compositions, as well as marker presence [6,7]. Melissopalynology, i.e. pollen analysis by light microscopy, is the oldest method used to determine the botanical origin of honey [8]. This method considers the qualitative and quantitative determination of pollen and honeydew elements from honey samples [9,10]. Regardless of the inexpensive instrumentation,

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Melissopalynology has numerous disadvantages and limitations such as the need for highly specialised personnel, great seasonal variation in pollen amount or the fact that pollen may be added fraudulently [6].

The increasing demand for honey in the market has made it absolutely necessary to establish reliable methods of analysis in order to ensure the authenticity of honey and to protect the consumer against any form of falsification. Consequently, a lot of research has been devoted to addressing this authentication issue, in order to develop robust and efficient analytical methods suitable to provide information on the quality and the safety of honey. Techniques based on the analysis of the molecular composition of honey (High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC), as reference methods, are typically time-consuming, require the use of expensive and environmentally polluting reagents and can only be carried out by qualified technicians. therefore, spectroscopic techniques such as visible spectroscopy (VIS), Near-Infrared Spectroscopy (NIRS), Mid Infrared Spectroscopy (MIR), and fluorescence, combined with appropriate chemometrics multivariate methods, have gained importance in food safety control [11]. These methods have been recognized as a rapid and nondestructive alternative to detect and quantify the presence of adulterants since it provides important information on the presence of certain functional groups. It is also considered as a "fingerprinting technique," which means that no two kinds of honey have the same FTIR (Fourier-Transform Infrared Spectroscopy) spectra, whether in the number of peaks or in the intensity of the peaks [12].

The advantages of the technique of spectroscopy (ultraviolet, visible, near and middle infrared, fluorescent) with respect to other methods are the non-invasive approach, the relatively easy and quick data acquisition. Recently, both near infrared and middle infrared spectroscopy have been successfully used for the classification of unfloral and multifloral honeys [13, 14].

Some authors have used UV and VIS spectrometry for the same purpose [15–17]. Besides, the majority of the analytical techniques require certain pre-treatment of the samples. Compared to other analytical methods, the technique of ultraviolet - visible (UV-VIS) and infrared (IR) spectroscopy is a non-invasive approach that further benefits from the relatively easy and quick data acquisition [4].

Principal Components Analysis (PCA) is a well-known method for revealing the hidden structure within large data sets [18]. By using an orthogonal transformation, which could calculate the eigenvectors of the covariance matrix of the original inputs, PCA converts a set of observations of possibly correlated variables (wavelengths) into a set of values of uncorrelated variables called principal components (PCs) [17].

In order to suggest the criteria for reliable characterization and classification of honey, several chemometric techniques such as principal component analysis (PCA), cluster analysis (CA), and linear discriminant analysis (LDA) have been used [8,19].

The purpose of the paper is to research the possibility of honey discrimination on the basis of its botanical origin and adulteration using the technique of UV-VIS spectroscopy in absorbance mode and the obtained spectroscopic data are subject for statistical processing that involves principal components analysis (PCA) for reducing the classifiers' number of inputs and the obtained components were discriminated by the Canonical discriminant function.

2. Materials and method

Thirty-five samples of three different type of honey (18 sample of clover honey, 14 sample of citrus honey and 3 sample of sugar feeding honey), the clover and citrus honey samples were collected by beekeepers between March and July of 2020 from apiaries located in different provinces of Egypt. The floral authenticity of honey samples were verified by pollen analysis [20]. Sugar feeding honey samples were collected after feeding the colonies (with empty combs from honey) by sugar solution 50% (sucrose solution (1: 1 w/v) which continuously provided for several days in the experiment apiary, and the sample stored in dark at room temperature (25C°).

The melissopalynological characteristics, both qualitative and quantitative, were examined following by [10, 20]. The identification of each pollen grain in the honey sample was performed with the aid of a pollen atlas [21]. The frequency classes of pollen grains are given as predominant pollen (>45%) [20].

Spectrum Acquisition, all samples of honey were diluted with two different solvents, distilled water and ethanol (80%) till a 10% solution. The samples were centrifuged for about 10 min at 4000 rpm/min. the clear supernatant were separated for the spectroscopic analysis. Samples in two different solutions were scanning for absorbance from 200 nm to 900 nm with a spectrophotometer (UV/VIS spectrophotometer, Libra S12, Biochrom, UK) ranging from 190 to 900 nm at 1 nm sampling space. Three ranges of spectrum (UV, VIS, and

UV-VIS) were acquired as follows. UV spectra were taken from 200 nm to 400 nm, VIS spectra were from 400 nm to 900 nm and UV-VIS ranged from 200 nm to 900 nm at 1 nm sampling space. All spectra ranges were recorded for statistical analysis.
Statistical method have been used for processing and evaluation of spectral data obtained by spectroscopy. To ensure full representation and exploration of the dataset, we began analyzing the results by Principal component analysis (PCA), PCA is very useful when there is a large amount of quantitative data to be processed and interpreted. Method aims that the reduction of the dimensionality of multivariate data (e.g., wavelengths) while preserving as much as possible all the relevant information. Principal Components Analysis performs on data (observations of possibly correlated variables) a linear transformation producing a new coordinate system where the new set of variables, the principal
components, are represented as linear functions of the original variables. The data under study were
computing by the covariance matrix for the full dataset, then computing the eigenvectors and eigenvalues of the covariance matrix, and sorting these in a descending order with respect to the eigenvalue [11, 22]. All principal components were used by the following calibration method for developing a discrimination model, Canonical discrimination. The statistical tools such as PCA and Canonical discrimination were analyzed by using IBM SPSS Statistics 26.

3. RESULTS AND DISCUSSION

In the present study, melissopalynological analysis were done in many honey samples to select the clover and citrus honey that had dominated clover or citrus pollen grain. All samples selected were classified as clover honey or citrus honey by dominant pollen grains and the other pollen grains were reported in table (1). The pollen of this species is dominant, suggesting that this plant is the chief source of pollen and nectar in bee foraging [23].
Figure (1) shows the absorbance spectra of the three types of honey under study in the wavelengths range from 200 nm to 900 nm. The spectra presented two major peaks in all honey samples, one peak was in UV region and the other was between the end of UV region and VIS region and low absorbance were
recorded after ~ 450nm. all the spectra showed their similarity in spectral shape in clover and citrus honey, but on the other hand, the sugar fed honey showed low absorbance intensity compared to clover and citrus honey, in addition the water dilution of honey varied between ethanolic dilution and water dilution and the ethanolic dilution showed more intensity in absorbance than the water dilution.
Absorbance spectra of the four brands of honey with wavelengths ranging from 480 to 940 nm present peaks in the band of 400 ~ 450 nm and low absorbance in the NIR range above 760 nm. All the spectra showed a similarity in spectral shape and absorbance. Thus, it is necessary to apply appropriate multivariate analysis methods to build calibration models for honey discrimination [17].
Using a multivariate analysis method to build calibration models for honey discrimination to properly explore the spectral dataset, the study applied some chemometrics methods such as principal component analysis (PCA) and discrimination analysis. PCA was firstly applied based on spectral data, and spectra have been divided into three categories UV region (200nm-400nm), VIS region (400nm to 900nm) and UV-VIS region (200nm to 900nm) for water and ethanol dilution honey samples Fig (2).

Table (1): Number of pollen grains /10 grams of honey samples under the study.

sample	Samples type	Number of pollen count / 10 g honey	Eucalyptus	*Citrus*	Broad bean	Clover	genus Prunus	casuarina	*Corn*	*Coriander*	others	Dominant pollen%
1	Clover honey	15375	375	-	-	8375	125	3125	-	-	3375	54
2		19125	125	-	-	13375	-	1750	-	-	3875	69
3		530000	6000	-	-	428000	2000	22000	-	-	72000	81
4		5625	125	-	-	2625	-	750	-	250	1875	47
5		24000	-	125	-	15250	125	1125	-	250	7125	64
6		55500	9500	-	-	32250	-	3125	-	375	10250	58
7		17125	375	250	125	11000	-	1250	-	-	4125	64
8		50625	750	375	125	39500	-	3000	-	-	6875	78
9		22000	500	-	-	12250	-	625	-	500	8125	56
10		58500	250	-	-	47250	625	2875	-	-	7500	81
11		65000	750	375	-	50000	-	875	-	2000	11000	77
12		28125	375	-	-	21750	-	500	-	1625	3875	77
13		26000	-	-	-	19125	-	1875	-	-	5000	74
14		16625	125	-	-	8375	-	625	-	375	7125	50
15		35625	1125	-	875	25125	-	875	-	250	7375	71
16		14500	250	125	-	9375	-	1125	-	-	3625	65
17		52000	-	-	1125	43500	-	0	375	-	7000	84
18		19625	1500	250	-	14000	-	1125	125	-	2625	71
1	Citrus honey	20500	-	14375	-	-	250	625	-	-	5250	69
2		6250	1375	2875	-	-	125	375	-	1375	125	46
3		15375	250	8375	-	-	125	1125	-	1000	4500	54
4		16625	-	12375	-	250	-	-	-	-	4000	73
5		7250	-	4000	-	-	-	-	-	-	3250	53
6		21000	1125	13875	-	-	500	500	-	125	4875	66
7		21125	-	12000	-	-	-	-	-	-	9125	57
8		16375	1000	7750	-	-	1750	750	-	1125	4000	47
9		17625	375	9000	-	-	500	-	-	1250	6500	51
10		79625	2125	54875	-	-	1125	125	-	4500	16875	68
11		24125	875	9000	-	-	1125	2250	-	2000	8875	37
12		32125	125	20875	-	-	250	-	-	-	10875	65
13		11250	-	7000	-	-	-	1375	-	-	2875	62
14		29000	125	18125	-	-	2000	2500	-	-	6250	61
1	Sugar feeding honey	1000	-	-	-	-	-	500	-	500	-	-
2		1500	-	-	-	-	-	-	-	-	1500	-
3		1750	250	-	-	-	-	500	-	375	625	-

- Undetected

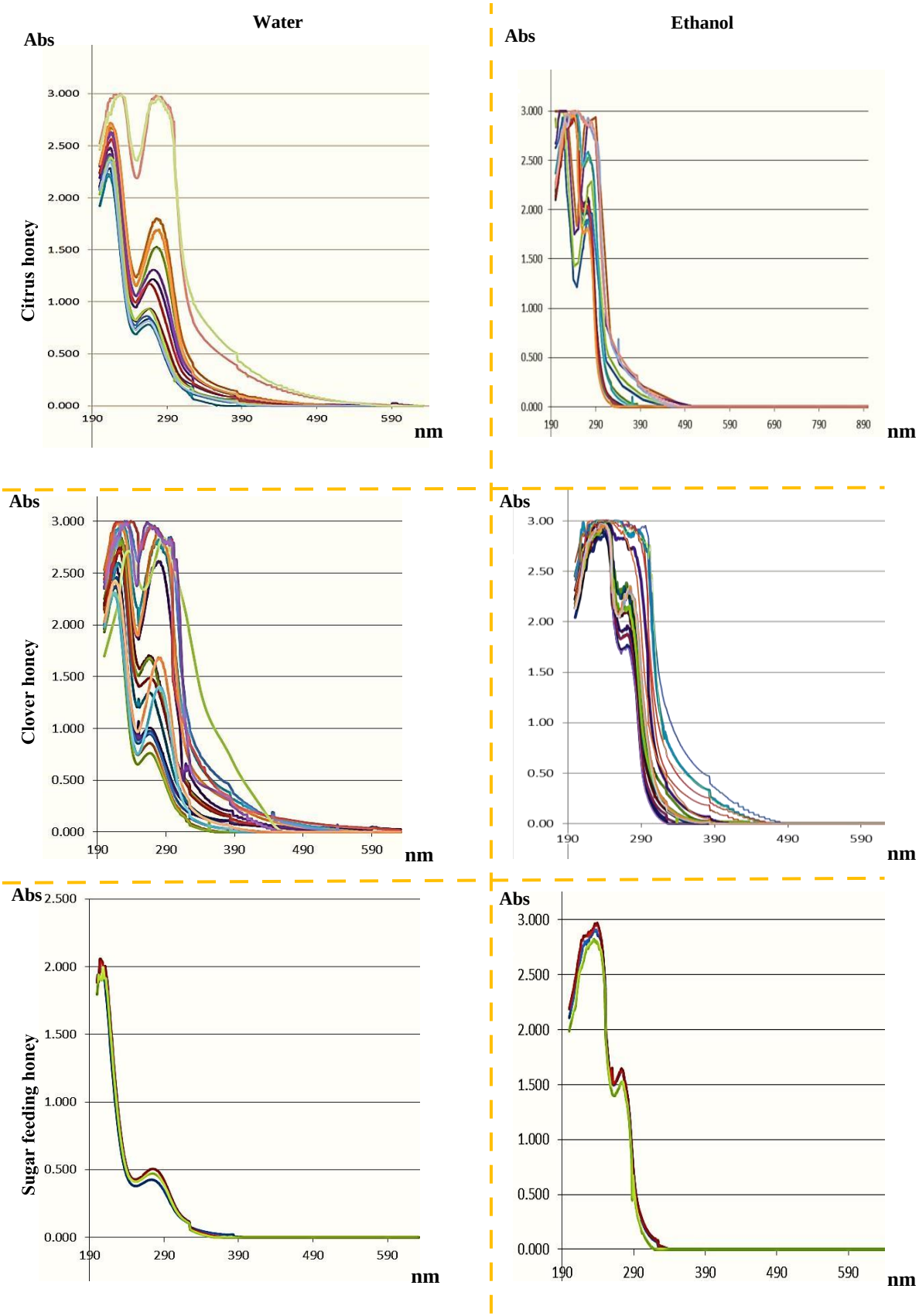

Figure 1: Absorbance spectra of the three types of honey samples with wavelengths from 200 to 900 nm with two different

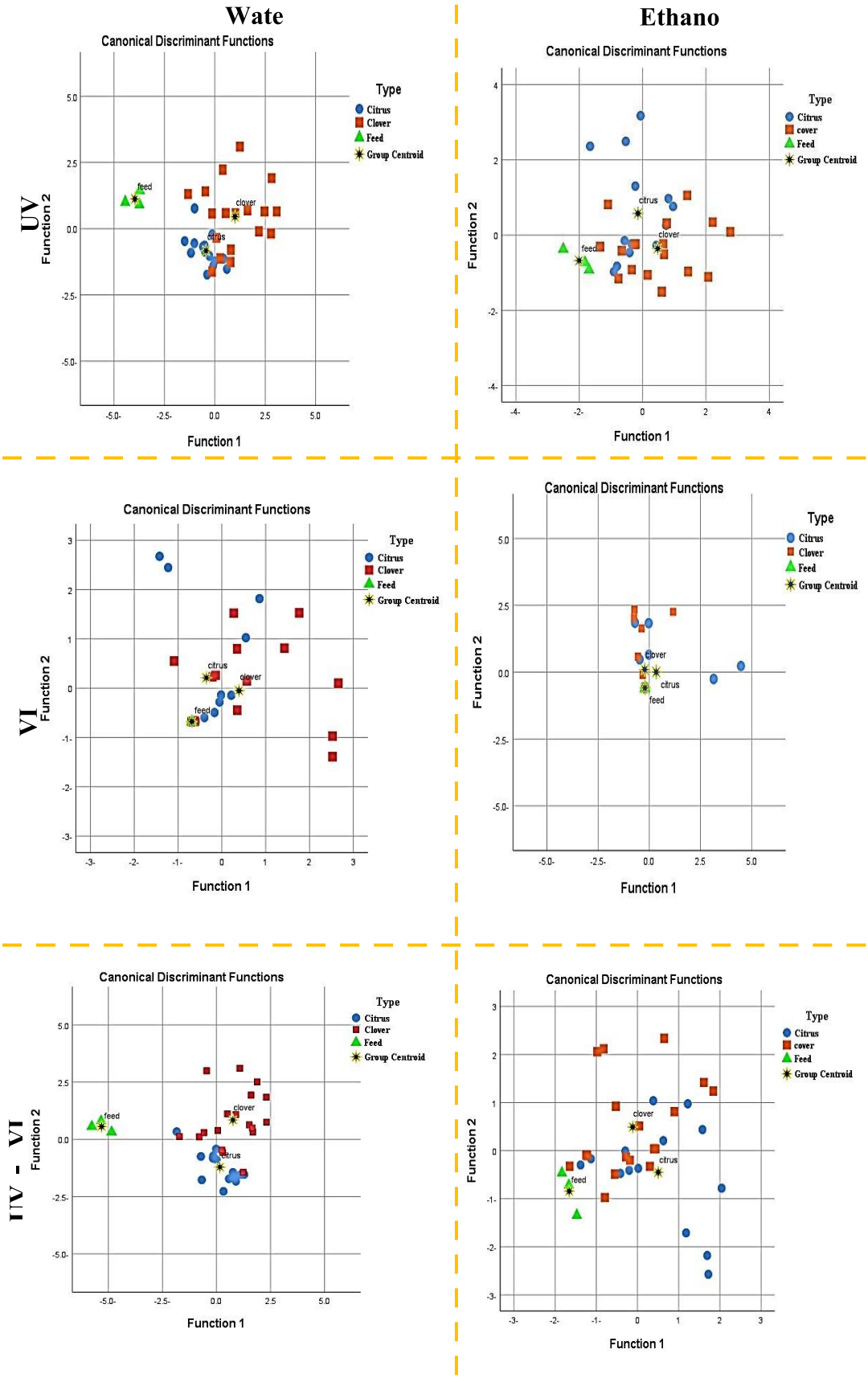

Figure 2: Discriminant analysis scatter plots for principal components of spectrophotometric scanning (200nm-900nm) for the citrus, clover and sugar feeding honey samples.

The application of PCA showed that the first two components represent (85.15,11.15%), (70.65,23.23%), (64.14,23.8%) for UV, VIS and UV-VIS region in water dilution, respectively, in addition (72.73,17.47%), (77.94,17.72%), (68.13,15.46%) for UV, VIS and UV-VIS region in ethanol dilution, of the total variability of the data and the discrimination analysis of PCA components recorded a strong discrimination between the sugar feeding honey group and clover or citrus groups of honey in UV region and UV-VIS region by water dilution, this discrimination is ensured essentially by the main components.

The key factors in determining honey structure was the concentration of honey macromolecules. This finding came from both UV spectroscopy and dynamic light scattering. UV spectroscopy provided a fast, qualitative and quantitative account of levels of UV absorbing compounds in different honeys based on the shape and absorbance intensity of UV spectra obtained from scans in the 200- 400 nm range [24]. UV absorbance spectra of the three types of honey are in the wavelengths range of 190 nm to 380 nm. PCA method of multivariate analysis was applied to distinguish honey. The calculations and visualization were carried out in a MATLAB environment. the scattered plot of the first and second PCs of UV spectral characteristics. As evident, the three types of honey are well distinguished from PCA in the UV spectra. VIS absorbance spectra of the three types of honey in the wavelengths range from 380 nm to 780 nm and PCA of the VIS characteristics. The spectra present a wide peak in the band of (400-500) nm that can be connected with the colour of the samples [25].

The three types of honey are well distinguished from PCA of the VIS spectra and it can be reported that the most appropriate values for the discrimination between a linden honey and an acacia honey are the refractive indices values in the range (400-550) nm while the n values (the refractive indices(n) measured by the method of the diapering diffraction pattern using a laser refractometer at wavelengths 405,532 and 635nm) for acacia and honeydew are almost the same and there was a good separation between acacia honey and honeydew, but not between acacia honey and linden honey can be made by measuring the n in the range (700-800) nm. The honeydew and the linden honey are well separated by their refractive indices in the whole visible range. The results showed that the refractive indices give a good opportunity for distinguishing the botanical origin of honey and separation can be done only by PCA of UV and visible spectra and PCA of EC characteristics. It should be noted that the honeys cannot be divided by group according to its botanical origin by using only peaks in the visible and UV spectra. This can be obtained by PCA. [26]. the graph (Fig,2) also makes it possible to separate two groups adulterated honey (sugar-feeding honey) and non- adulterated floral honey (clover and citrus honey).

Spectral-based HCA (Hierarchical Clustering Analysis) assumes that honey samples with similar spectral profiles are chemically related and should be assigned to a single group [27]. With the application of HCA and PCA techniques in the MIR (Mid Infra-Red) spectra of honey, it was possible to separate and differentiate pure honey from adulterated honey, enabling clear discrimination between the two groups of honey according to their purity.

Its strong discrimination is explained by the spectral difference between adulterated and non-adulterated honey, where it is observed that there is a difference in the spectral intensity of the bands between 3090 cm^{-1} and 3513 cm^{-1} and also between 600 cm^{-1} and 1456 cm^{-1}, which can be explained by the difference in the composition of the samples [28]. the discrimination figure (2) indicted that there was interference between clover and citrus honey and not clearly discriminate from each other, it may be due to the fact that the clover honey may contaminated by the residue of citrus honey in the hive because the citrus honey extraction flowed by clover honey extraction from the same hive and it can be contaminated from other plant nectars.

The chemical compositions of honeys will vary according to the contaminant nectars, which in consequence are influenced by the variation in the chemical composition of the same plant species growing at different altitudes [6]. PCA revealed acceptable scores for the two principal components (PC1 and PC2), providing a 53.82% percentage of predicted membership according to the honey floral origin (28.50% PC1 and 25.32% PC2). PCA allows the reduction of the data dimension, showing the clustering into two main groups, the dendrogram showed the clustering of the honeys in four separate groups. class 1 includes three polyfloral honeys, a honeydew honey produced from coniferous forest, two rape honeys and contaminated rape honeys with sea buckthorn and fruit trees, indicating the polyfloral source of contaminated rape honeys. Class 2 includes the majority of honeydew honeys and one polyfloral honey, while class 3 includes six rape honeys, two acacia honeys, one polyfloral honey and rape contaminated with acacia honey. Class 4 contained the majority of acacia honeys, two rape honeys and contaminated acacia honeys with thyme and rape. It can be concluded that honeys can be classified and according to the major floral source [29].

There was a clearly dilution solution effect on the spectra data and that reflected on the PCA and discrimination analysis and water dilution showed a greater discrimination effect on the honey types. It can be explained this effect is due to the chemical

properties of different dilution solvents are important parameters for extraction compounds and are correlated with absorbance and chemical composition.

Due to a high concentration of UV absorbing compounds. The most characteristic feature of the elaborate spectral profile in the honeys was the presence of double absorption peaks at 240-250 nm wavelengths. In contrast, light- color honeys, such as blueberry or clover produced mostly two-peak UV spectra demonstrating a much smaller number of UV absorbing compounds. UV spectral profiles depend on the concentration of UV absorbing compounds and predict honeys conformational stability upon dilution.

The interpretation of UV spectra of honeys posed a challenge because of the high concentration of UV absorbing compounds. In contrast, the intensity of UV spectra of light honeys was sensitive to water dilution and showed a gradual decrease over the entire dilution range. Thus, high concentrations of UV-absorbing compounds in medium and dark honeys provided increased conformational stability against dilution with water and showed a gradual decrease over the entire dilution range. where's, high concentrations of UV-absorbing compounds in medium and dark honeys provided increased conformational stability against dilution with water [24].

The UV spectral profiling was more effect on PCA and discriminate analysis, the sugar feeding honey demonstrating much fewer number of UV absorbing compounds than the floral honey (citrus and clover honey) because the source of these compounds was plants, UV spectral profiling was applied to quantify honeys compounds such as proteins, polyphenols, free amino acid, phenolic compounds, flavonoids, sugar, organic acids have absorbance in UV region which present in high content in floral honey more than sugar feeding honey. UV spectral profiling was applied to quantify many compounds such as proteins, polyphenols and Maillard reaction products. Proteins, peptides and free amino acids have absorbance maxima in the 200-230 nm and 250-290 nm range, which overlap with the absorption of hydroxybenzoate and hydrocinnamate class of phenolic acids that absorb in the 200 to 290 nm and at 270 to 360 nm ranges, respectively [30,31]. The early stage Maillard reaction products showed a broad absorption range from 220 nm to 350 nm [32]. The oligo- and polysaccharides have absorbance maxima at 230 nm, while honey monosaccharides, glucose and fructose, absorb UV light at 180 to 200 nm and contribute little to the UV profiles [24].

The UV absorption bands of the samples are usually associated with the presence of different chromophores exemplified in various components as phenolics, flavonoids, and conjugated systems as well as another UV- absorbing systems (Andersen and Markham, 2006) and recently these compounds have been used as markers for the determination of the botanical origin of honey [19].

The work presented in this study aims at developing and establishing a simple model, based on chemometric analysis of UV-VIS spectroscopic data, to designed method will allow for the detection of adulteration resulting from sugar feeding with sucrose, as well as the authentication of clover and citrus honey. This way may also serve as a basis for implementing this technique for other honey types worldwide. Further studies are needed in order to include in the proposed methodology other markers that could improve the criteria for traceability and authentication. The development of databases based on analytical information obtained through standardized analytical methods along with the use of appropriate statistical tools could be helpful in establishing clear criteria for honey traceability [29].

4. Conclusion

The authentication of honey is important to protect the industry and consumers from such adulterated honey. Techniques based on the analysis of honey composition as reference methods, are typically time-consuming, require the use of expensive and environmentally polluting reagents, and can only be carried out by qualified technicians. As a result, spectroscopic techniques, combined with appropriate chemometrics multivariate methods, have gained importance in food analysis and these methods have been recognized as a rapid and non-destructive alternative to detect and quantify the presence of adulterants since they provide important information on the presence of certain functional groups. It is regarded as well a "fingerprinting technique" and demonstrated to be useful tool for accurately classifying, authenticating and detecting adulterating honey. The created model offers a quick and effective method of examination and might be used to authenticate various kinds of honey from around the world.

5. Acknowledgment

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6. Conflict of Interest

None

7. Formatting of funding sources

None

8. References

[1] **Codex Alimentarius.** Draft revised standard for honey (at step 10 of the codex procedure). Alinorm,2001.

[2] **EU Council, Council Directive 2001/110/EC of 20 December** Relating to honey. *Official journal of the european communities.* 10:47–52,2021.

[3] **E. Boffo, L.A. Tavares, A.C.T. Tobias, M.M.C. Ferreira, And A.G. Ferreira,** Identification of components of brazilian honey by 1h nmr and classification of its botanical origin by chemometric methods, *Lwt.*49:55–63,2012.

[4] **D. Tsankova, And S. Lekova,** UV- Vis Spectroscopy and chemometrics analysis in distinguishing different types of bulgarian honey, *Big Data, Knowledge and Control Systems Engineering,*1-4,2019.

[5] **T. Cajka, J. Hajslova, F. Pudil, And K. Riddellova,** Traceability of honey origin based on volatiles pattern processing by artificial neural networks, *J Chromatogr A.* 1216:1458–462,2009.

[6] E. **Anklam,** A Review of the analytical methods to determine the geographical and botanical origin of honey, *Food Chem*. **63**: 549–562,1998.

[7] **I. Hermosín, R.M. Chicón, And M.D. Cabezudo,** Free Amino Acid Composition and Botanical Origin of Honey, *Food Chem*. **83**: 263–268,2003.

[8] **I. Arvanitoyannis, C. Chalhoub, P. Gotsiou, N. Lydakis-Simantiris, And P. Kefalas,** Novel quality control methods in conjunction with chemometrics (multivariate analysis) for detecting honey authenticity, *Crit Rev Food Sci Nutr. 45:193–203,2005.*

[9] **J. Louveaux, A. Maurizio, And G. Vorwohl,** Methods of melissopalynology, **59**:139-157,1987.

[10] **W. Von Der Ohe, L. Oddo, M. Piana, M. Morlot, P. Martin, W. Von Der Ohe, L. Persano Oddo, And M. Lucia PIANA,** Harmonized Methods of Melissopalynology, *Apidologie. 35: S18-S25, 2004.*

[11] **M. Vasconcelos, L. Coelho, A. Barros, And J.M.M.M. De Almeida,** Study of adulteration of extra virgin olive oil with peanut oil using FTIR spectroscopy and chemometrics, Cogent Food & Agriculture, 1:1,2015.

[12] **M. Ferreiro-González, E. Espada-Bellido, L. Guillén-Cueto, M. Palma, C.G. Barroso, And G.F. Barbero,** Rapid Quantification of Honey Adulteration by Visible-Near Infrared Spectroscopy Combined with Chemometrics, *Talanta. 188: 288–292,2018.*

[13] **K. Ruoff, W. Luginbühl, S. Bogdanov, J.-O. Bosset, V. Kilchenmann, B. Estermann, T. Ziolko, S. Kheradmandan, And R. Amadò,** Potential of Near Infrared Spectroscopy for Authenticity Testing of Unifloral Honey, *Eur. Congr. For Authenticity of Food, Nyon,2003.*

[14] **A. Davies, B. Radovic, T. Fearn, And E. Anklam**, A Preliminary Study on The Characterisation of Honey By Near Infrared Spectroscopy, *Journal of Near Infrared Spectroscopy,*10:121-135,2002.

[15] **D. Tsankova, L. Svetla, N. Krastena, And T. Georgi,** Vis Spectroscopy-Based Chemometric Analysis of Honey with Respect to Discrimination of Its Botanical Origin, *Annals of Faculty Engineering Hunedoara –International Journal of Engineering*. 275–279,2015.

[16] **D. Tsankova, S. Lekova, And K. Ohridski,** Botanical Origin-Based Honey Discrimination Using Vis- Nir Spectroscopy and Statistical Cluster Analysis, Journal of Chemical Technology and Metallurgy, 50: 638-642, 2015.

[17] **Y. Li, And H. Yang,** Honey Discrimination Using Visible and Near-Infrared Spectroscopy, *ISRN Spectroscopy*. 2012:1–4, 2015.

[18] **J. Reed, D. Devlin, S.R.R. Esteves, R. Dinsdale, And A.J. Guwy,** Performance Parameter Prediction for Sewage Sludge Digesters Using Reflectance FT-NIR Spectroscopy, *Water Res.*45: 2463–2472, 2011.

[19] **J. Bertoncelj, T. Golob, U. Kropf, And M. Korošec,** Characterisation of Slovenian Honeys on The Basis of Sensory and Physicochemical Analysis with A Chemometric Approach, *Int J Food Sci Technol*. 46: 1661–1671,2011.

[20] **J. Louveaux, A. Maurizio, And G. Vorwohl,** Methods of melissopalynology, **59**:139–157,1978.

[21] **Bernard J, And Reille M, Nouvelles,** Analyses Polliniques Dans Atlas De Marrakech, *Maroc. Pollen Spores.* **29**: 225–240,1987.

[22] **T. Dramićanin, L. Lenhardt Acković, I. Zeković, And M.D. Dramićanin,** Detection of Adulterated Honey by Fluorescence Excitation-Emission Matrices, *Journal of Spectroscopy*, vol. 2018, Article ID 8395212, 6 pages, 2018.

[23] **S. Nair, B. Meddah, And A. Aoues,** Melissopalynological characterization of north algerian honeys, *Foods*. 2: 83, 2013.

[24] **K. Brudzynski, D. Miotto, L. Kim, C. Sjaarda, L. Maldonado-Alvarez, And H. Fukś,** Active macromolecules of honey form colloidal particles essential for honey antibacterial activity and hydrogen peroxide production, *Scientific Reports,7:1-15,2017.*

[25] **E. Olga, F.G. María, And S.M. Carmen,** Differentiation of blossom honey and honeydew honey from northwest spain, *Agriculture,* 2: 25-37,2012.

[26] **I. Vlaeva, K. Nikolova, I. Bodurov, M. Marudova, D. Tsankova, S. Lekova, A. Viraneva, And T. Yovcheva,** Using differential scanning calorimetry, laser refractometry, electrical conductivity and spectrophotometry for discrimination of different types of bulgarian honey, *J Phys Conf Ser, 794, 012034,* 2017.

[27] **S. Chanana, C.S. Thomas, F. Zhang, S.R. Rajski, And T.S. Bugni,** Hcapca: Automated hierarchical clustering and principal component analysis of large metabolomic datasets in r, *Metabolites,*10:297,2020.

[28] **A. Siddiqui, S.G. Musharraf, M.I. Choudhary, And A. Ur Rahman,** Application of analytical methods in authentication and adulteration of honey, *Food Chem*. 217: 687-698,2017.

[29] **C. Ciucure, And E.I. Geană,** Phenolic compounds profile and biochemical properties of honeys in relationship to the honey floral sources, *Phytochemical Analysis*. 30:481-492,2019.

[30] **K. Markham,** Techniques of flavonoid identification., *academic press, london. Newyork,1982*.

[31] **J. Harborne, H. Mabry, And T.J. Mabry,** The Flavonoids, Springer-Verlag, Berlin-Heidelberg-New York,1975.

[32] **J. Kim, And Y.S. Lee,** Study of Maillard reaction products derived from aqueous model systems with different peptide chain lengths, *Food Chem.* 11, 846-853,2009.